10th International Conference on Environmental Mutagens

39th Annual Meeting of the European Environmental Mutagen Society

18th Annual Meeting of the Italian Environmental Mutagen Society

Firenze, Italy
August 20-25, 2009

The Renaissance of Environmental Mutagenesis

PROGRAM AND ABSTRACT BOOK

EEMS

SIMA

The Renaissance of Environmental Mutagenesis - Firenze, Italy August 20-25, 2009

X INTERNATIONAL CONFERENCE ON ENVIRONMENTAL MUTAGENS

FIRENZE, ITALY - AUGUST 20-25, 2009

FINAL PROGRAM

Thursday, August 20

8.30-13.00 **Pre Conference Courses**

Angelico Hall
MOLECULAR EPIDEMIOLOGY FOR CHRONIC DISEASES

Bronzino Hall
MICRONUCLEUS CYTOME ASSAY: LYMPHOCYTES AND BUCCAL EXFOLIATED CELLS

Botticelli Hall
18.00 **Opening Ceremony**

11th ICEM Announcement
L. Ribeiro, C.F. Menck

18.45 IN001 **Keynote Lecture**
Introduction: E. Zamorano-Ponce, President Asociación Latinoamericana de Mutagénesis, Carcinogénesis y Teratogénesis Ambiental (ALAMCTA)

LESION SENSING AND DECISION POINTS IN THE DNA DAMAGE RESPONSE
P. C. Hanawalt, Stanford University, Stanford, CA, USA

19.30 **Welcome Cocktail**

Friday, August 21

Botticelli Hall
8.30 IN002 **Plenary Lecture**
Introduction: T. Nohmi, President Asian Association of Environmental Mutagen Societies

CANCER RISK FROM EXPOSURE TO URBAN AIR POLLUTION
HRH Princess M. Chulabhorn, Chulabhorn Research Institute, Bangkok**,** Thailand

9.15 IN003 **Plenary Lecture**
Introduction: D. Kirkland, President-Elect European EMS

SEVEN DEADLY SINS OF ENVIRONMENTAL RESEARCH

P. Grandjean, University of Southern Denmark, Odense, Denmark

10.00 **Coffee break**

10.30-12.30 **Parallel Symposia**

Michelangelo Hall
Mutational and epigenetic mechanisms
EPIGENETICS: THE NEW FRONTIER IN ENVIRONMENTAL MUTAGENESIS
Chairs: R. Feil, M. Hanson

10.30 IN004 EPIGENETIC MECHANISMS AND THE REGULATION OF GENOMIC IMPRINTING IN MAMMALS
R. Feil, CNRS Montpellier, France

10.55 IN005 DEVELOPING HUMAN EMBRYONIC STEM CELLS TO MODEL ENVIRONMENTAL EFFECTS ON THE DEVELOPING EPIGENOME
L.E. Young, University of Nottingham, UK

11.20 IN006 EPIGENETIC REGULATION OF AGING
M.F. Fraga, CNB-CSIC, Madrid, Spain

11.45 IN007 EPIGENETIC PROCESSES IN DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE (DOHaD)
M. Hanson, University of Southampton, UK

12.10 ME021 HYPERMETHYLATION OF TUMOUR SUPPRESSOR GENES IN LUNG TUMOURS FROM NEVER-SMOKERS WITH AND WITHOUT SECOND-HAND TOBACCO SMOKE EXPOSURE
S. *Jarmalaite*, Vilnius University, Vilnius, Lithuania

12.20 EM153 PERSISTENT DYSREGULATION OF DNA METHYLATION IN CELLS WITH ARSENIC-INDUCED GENOMIC INSTABILITY
M. Mauro, New York University, Tuxedo, NY, USA and University of Palermo, Italy

Lippi Hall
DNA damage responses
MECHANISMS OF CELL DEATH AND SURVIVAL
Chairs: B. Kaina, A. Antoccia

10.30 IN008 SURVIVAL AND DEATH STRATEGIES IN CELLS EXPOSED TO GENOTOXINS
B. Kaina, University Medical Center Mainz, Germany

10.55 IN009 TRANSCRIPTIONAL INHIBITION BY DNA DAMAGE AS A TRIGGER OF CELL DEATH
M. Ljungman, University of Michigan, Ann Arbor, MI, USA

11.15 IN010 DIFFERENT MODES OF CELL DEATH INDUCED BY DNA DAMAGE
B. Zhivotovsky, Karolinska Institutet, Stockholm, Sweden

11.35 IN011 ROLE OF DNA-PKcs-PIDDosome IN DNA DAMAGE RESPONSE
C. Du, The University of Cincinnati, OH, USA

11.55 IN012 CELL-CYCLE BLOCKAGE AFFECTS DNA DAMAGE RESPONSES THAT LEAD TO DEATH IN HUMAN PRIMARY FIBROBLASTS.
C.F. Menck, University of Sao Paulo, Brazil

12.15 DD30 ROLE OF THE APOPTOSIS-MODULATORY MOLECULE NF-κB IN DNA REPAIR
M. Volcic, Ulm University, Ulm, Germany

Bronzino Hall
Environmental Mutagenesis
NANOTOXICOLOGY: MECHANISMS AND EFFECTS
(This Symposium is partially supported by ECETOC)
Chairs: G. Oberdörster, L. Tran

10.30 IN013 TOXICITY ASSESSMENT OF NANOPARTICLES
G. Oberdörster, University of Rochester, NY, USA

11.00 IN014 PHYSICO-CHEMICAL FEATURES IN THE TOXICITY OF ENGINEERED NANOPARTICLES
B. Fubini, University of Torino, Italy

11.20 IN015 DISTRIBUTION AND EFFECTS OF NANOMATERIALS AFTER INHALATION AND *I.V.* INJECTION IN RATS
R. Landsiedel, BASF SE, Ludwigshafen, Germany

11.40 IN016 MECHANISMS OF NANOMATERIALS GENOTOXICITY
M. Kirsch-Volders, Vrije Universiteit Brussels, Belgium

12.00 IN017 POTENTIAL PULMONARY EFFECTS OF SINGLE-WALLED CARBON NANOTUBE (SWCNT) EXPOSURE: IN VITRO GENOTOXIC EFFECTS
V. Castranova, National Institute for Occupational Safety and Health, Morgantown, WV, USA

12.20 CONCLUSIONS
L. Tran, Institute of Occupational Medicine, Edinburgh, UK

Angelico Hall
Mutagenesis and health effects
HEALTH EFFECTS OF NUCLEOTIDE POOL DAMAGE
Chairs: T. Nohmi, M. Bignami

10.30 IN018 ERRONEOUS INCORPORATION OF OXIDIZED NUCLEOTIDES BY Y-FAMILY DNA POLYMERASES
T. Nohmi, National Institute of Health Sciences, Tokyo, Japan

10.55 IN019 PROGRAMMED CELL DEATH TRIGGERED BY NUCLEOTIDE POOL DAMAGE
Y. Nakabeppu, Kyushu University, Fukuoka, Japan

11.15	IN020	MULTIPLE ROLES OF THE MTH1 HYDROLASE: PROTECTION AGAINST NEURODEGENERATION AND CONTROL OF LIFE SPAN ***M. Bignami***, Istituto Superiore di Sanità, Roma, Italy
11.35	IN021	INCORPORATION OF EXTRACELLULAR 8-oxodG INTO DNA AND RNA REQUIRES PURINE NUCLEOSIDE PHOSPHORYLASE IN CULTURED MAMMALIAN CELLS AND MICE. ***P.T. Henderson***, University of California, Sacramento, CA, USA
11.55	IN022	MUTAGENICITY OF OXIDIZED DNA PRECURSORS IN LIVING CELLS: ROLES OF NUCLEOTIDE POOL SANITIZATION AND DNA REPAIR ENZYMES, AND Y-FAMILY DNA POLYMERASES ***H. Kamiya***, Hokkaido University, Sapporo, Japan
12.15	DD44	GENOTOXIC ACTIVITY INDUCED BY VARIOUS *H. pylori* STRAINS IS ASSOCIATED WITH A DOWNREGULATION OF DNA MISMATCH REPAIR GENES EXPRESSION ***E. Touati***, Institut Pasteur, Paris, France

Botticelli Hall

Risk assessment

CURRENT ISSUES IN MODE OF ACTION ANALYSIS AND THEIR USE IN CANCER RISK ASSESSMENT

Chairs: N. Keshawa, D. Eastmond

10.30	IN023	AN OVERVIEW OF CURRENT ISSUES IN MODE OF ACTION ANALYSIS AND THEIR USE IN CANCER RISK ASSESSMENT ***N. Keshava***, U.S. Environmental Protection Agency, Washington DC, USA
10.50	IN024	ASSESSING IN VITRO DOSE-RESPONSE RELATIONSHIPS FOR ANEUGENS ***D.A. Eastmond***, University of California, Riverside, CA, USA
11.10	IN025	THE MUTAGENIC POTENTIAL OF FORMALDEHYDE AND ITS RELEVANCE FOR CARCINOGENESIS ***G. Speit***, Universität Ulm, Germany
11.30	IN026	EMS IN VIRACEPT - A LESSON ON MUTATION THRESHOLDS FOR ALKYLATING AGENTS ***L. Müller***, F. Hoffmann-La Roche, Basel, Switzerland
11.50	IN027	FOOD CADMIUM AND THE RISK OF HORMONE-RELATED CANCERS: A POPULATION-BASED PROSPECTIVE COHORT STUDY ***A. Åkesson***, Karolinska Institutet, Stockholm, Sweden
12.10	IN028	ANALYSIS AND INCORPORATION OF MECHANISTIC DATA IN DECISION-MAKING ON SEVERAL CARCINOGENS AT IARC

R. Baan, International Agency for Research on Cancer, Lyon, France

12.30-14.30 **Lunch and poster viewing**
ME001-011; DD001-060; EM001-060;
MH001-032; PD001-013; RA001-037

14.30-16.30 **Parallel Symposia**

Lippi Hall
Mutational and epigenetic mechanisms
EPIGENOME AND THE ENVIRONMENT: FROM UNDERSTANDING THE MECHANISMS TO RISK ASSESSMENT
Chairs: O. Kovalchuk, J. Trosko

14.30 IN029 ROLE OF EPIGENETIC DEREGULATION IN RADIATION-INDUCED GENOME INSTABILITY AND CARCINOGENESIS
O. Kovalchuk, University of Lethbridge, AB, Canada

14.55 IN030 ROLE OF EPIGENETIC EVENTS IN GENOTOXIC LIVER CARCINOGENESIS
I.P. Pogribny, Food and Drug Administration, Jefferson, AR, USA

15.15 IN031 DNA METHYLATION AND PERSISTENT BYSTANDER EFFECT: MEMORY OF AN INSULT
B.P. Engelward, Massachusetts Institute of Technology, Cambridge, MA, USA

15.35 IN032 EPIGENETIC CHANGES UNDERLIE ORGANISMAL ADAPTATION TO CHANGING ENVIRONMENTS
I. Kovalchuk, University of Lethbridge, AB, Canada

15.55 IN033 SYSTEMS INTEGRATION OF HUMAN STEM CELLS, EPI-TOXICOGENOMICS, CELL-CELL COMMUNICATION: THE BARKER HYPOTHESIS AND CHRONIC HUMAN DISEASES.
J.E. Trosko, Michigan State University, East Lansing, MI, USA

16.15 ME025 THE ASSOCIATION OF METHYLATION PATTERN AND PRENATAL POLYCYCLIC AROMATIC HYDROCARBONS (PAHs) EXPOSURE
D. Tang, Columbia University, New York, NY, USA

Botticelli Hall
DNA damage responses
NOVEL INSIGHTS INTO DNA DAMAGE SIGNALLING AND REPAIR
Chairs: J. Jiricny, P. Menichini

14.30 IN034 BASE- AND MISMATCH REPAIR INTERFERENCE DURING SOMATIC HYPERMUTATION
J. Jiricny, University of Zurich, Switzerland

14.50 IN035 MRE11 INTERACTIONS WITH DNA AND RAD50 ATPase PLUS NBS1 INTERACTIONS WITH CTIP CONNECT dsDNA REPAIR MACHINERY AND BREAK SIGNALING
J.A. Tainer, Lawrence Berkeley National Lab, La Jolla, CA, USA

15.10 IN036 HUMAN ELG1 REGULATES THE LEVEL OF UBIQUITINATED PCNA THROUGH INTERACTIONS WITH PCNA AND USP1
K.J. Myung, National Institute of Health, Bethesda, MD, USA

15.30 IN037 DNA BASE EXCISION REPAIR IN (EPI)GENOME MAINTENANCE
P. Schär, University of Basel, Switzerland

15.50 IN038 PROPERTIES OF NEIL3 IN PROLIFERATION AND DIFFERENTIATION OF STEM/PROGENITOR CELLS
M. Bjørås, University of Oslo, Norway

16.10 IN039 ESTABLISHMENT OF REPORTER ASSAY YEASTS RESPONDING TO LIGANDS OF VARIOUS HUMAN NUCLEAR RECEPTORS, AND ROLES OF AHR LIGANDS TO INDUCE OR PROTECT FROM DNA DAMAGE FORMATION
T. Yagi, Osaka Prefecture University, Osaka, Japan

Bronzino Hall
Environmental mutagenesis
NEW DEVELOPMENTS IN THE GENOTOXICITY OF SOIL AND WATER
Chairs: D. DeMarini, R. Marcos

14.30 IN040 THE GENOTOXIC HAZARDS AND CARCINOGENIC RISKS OF PAH CONTAMINATED SOILS
P.A. White, Health Canada, Ottawa, QC, Canada

14.55 IN041 POTENTIAL IMPLICATIONS OF SOIL POLLUTION WITH MUTAGENS IN LUNG CANCER
T. Watanabe, Kyoto Pharmaceutical University, Kyoto, Japan

15.20 IN042 GENOTOXICITY AND CARCINOGENICITY OF DRINKING WATER DISINFECTION BY-PRODUCTS
D.M. DeMarini, U.S. Environmental Protection Agency, Research Triangle Park, NC, USA

15.45 EM109 GENOTOXICITY STUDIES WITH DISINFECTION BYPRODUCTS (DBPs)
R. Marcos, Universitat Autònoma de Barcelona, Bellaterra, Spain

16.10 EM135 AMES II AND HIGH-THROUGHPUT COMET ASSAY FOR EFFICIENT SCREENING OF DRINKING WATER (SOURCES) FOR GENOTOXIC CONTAMINANTS
M.B. Heringa, KWR Watercycle Research Institute, Nieuwegein, The Netherlands

Angelico Hall
Mutagenesis and health effects
CHILDREN CANCER RISK
Chairs: J. Kleinjans, C.P. Wild

14.30		INTRODUCTION ***C.P. Wild***, International Agency for Research on Cancer, Lyon, France
14.40	IN043	ACTIVATION OF INFLAMMATION/NF-κB SIGNALING IN INFANTS BORN TO ARSENIC-EXPOSED MOTHERS ***R. Fry***, Massachusetts Institute of Technology, Cambridge, MA, USA
15.05	IN044	TRANSCRIPTOMIC ANALYSIS IN UMBILICAL CORD BLOOD OF CHILDREN EXPOSED TO GENOTOXIC COMPOUNDS THROUGH THEIR MOTHERS DIET ***D.M. van Leeuwen,*** Maastricht University, Maastricht, The Netherlands
15.30	IN045	GENETIC AND ENVIRONMENTAL RISK FACTORS OF CHILDHOOD LEUKEMIA ***K. Hemminki***, German Cancer Research Center, Heidelberg, Germany
15.55	IN046	THE INTERNATIONAL CHILD CANCER COHORT CONSORTIUM ***T. Dwyer***, Royal Children's Hospital, Parkville, Australia

Michelangelo Hall
Risk assessment
NEW DEVELOPMENTS IN REGULATORY GENETIC TOXICOLOGY
Chairs: E. Lorge, V. Thybaud

14.30	IN047	INTEGRATION OF GENOTOXICITY TESTS INTO ROUTINE TOXICITY STUDIES ***C. Priestley***, AstraZeneca, UK ***A. Czich***, Sanofi Aventis, Germany ***A. Rothfuss***, Bayer Schering Pharma AG, Germany
15.30	IN048	THE ILSI-HESI PROJECT COMMITTEE ON THE RELEVANCE AND FOLLOW-UP OF POSITIVE RESULTS IN *IN VITRO* GENETIC TOXICITY (IVGT) TESTING: INTRODUCTION AND FOLLOW-UP OF POSITIVE RESULTS *IN VITRO* ***V. Thybaud***, Sanofi-Aventis, Vitry sur Seine, France
15.50	IN049	THE ILSI-HESI PROJECT COMMITTEE ON THE RELEVANCE AND FOLLOW-UP OF POSITIVE RESULTS IN *IN VITRO* GENETIC TOXICITY TESTING (IVGT): QUANTITATIVE ASPECTS OF GENOTOXICITY RISK ASSESSMENT ***J.T. MacGregor***, Toxicology Consulting Services, Arnold, MD, USA

16.10 IN050 THE ILSI-HESI PROJECT COMMITTEE ON THE RELEVANCE AND FOLLOW-UP OF POSITIVE RESULTS IN *IN VITRO* GENETIC TOXICITY TESTING (IVGT): EMERGING TECHNOLOGIES FOR THE IMPROVEMENT OF GENOTOXICITY RISK ASSESSMENT
J. Sasaki, Johnson & Johnson Pharmaceutical Research and Development, Raritan, NJ, USA

16.30 **Coffee break**

Botticelli Hall

17.00 IN051 **Plenary Lecture**
Introduction: H.W. Chung, Past-President Korean EMS

CANCER STEM CELLS FROM SOLID TUMORS
R. De Maria, Istituto Superiore di Sanità, Roma, Italy

6th ICEMHP Announcement
W. Au

18.00-19.30 **Parallel Forum**

Bronzino Hall

FOR01 **ANTIMUTAGENESIS AND CHEMOPREVENTION IN A U-SHAPED WORLD**
Chair: D.J. Waters

Promoting health in a U-shaped world
D.J. Waters, Purdue University, West Lafayette, IN, USA

Cancer and aging: U-shaped response to vitamin D
P. Tuohimaa, University of Tampere, Finland

U-shaped dose response of anti-angiogenic agents
A.R. Reynolds, Institute of Cancer Research, London, UK

FOR02 Defining the optimal dose of selenium for prostate cancer risk reduction
D.J. Waters, Purdue University, West Lafayette, IN, USA

Michelangelo Hall

FOR03 **MUTAGENIC MODE OF ACTION FOR CARCINOGENS: HOW HIGH IS THE BURDEN OF PROOF?**
Chair: M. Moore

Overview of Mode of Action (MOA); Framework for determining a mutagenic MOA; case study on cyclophosphamide
R. Schoeny, U.S. Environmental Protection Agency, Washington, DC, USA

Case study on dichloroacetic acid
M. Moore, U.S. Food and Drug Administration, Jefferson, AR, USA

Case study on acrylamide
L. Haber, Toxicology Excellence for Risk Assessment, Cincinnati, OH, USA

Botticelli Hall

FOR04 **RATIONALE OF GENOTOXICITY TESTING OF NANOMATERIALS**
(This Forum is partially supported by ECETOC)
Chairs: H. Greim, H. Norppa

FOR05 Regulatory requirements and appropriateness of available test systems
D. Warheit, DuPont Haskell Global Centers for Health & Environmental Sciences, Newark, DE, USA

FOR06 Possible genotoxic mechanisms: Criteria for improved test strategies
K. Donaldson, University of Edinburgh, Scotland, UK

Angelico Hall

FOR07 **ECOGENOTOXICOLOGY: PAST SUCCESSES AND FUTURE NEEDS**
Chairs: C. Bolognesi, D.M. DeMarini

FOR08 Evaluating the mutagenicity of air, water, and soil by the Salmonella assay: where are we after 40 years?
D. M. DeMarini, US Environmental Protection Agency, Research Triangle Park, NC, USA

FOR09 Ecogenotoxicity applied to environmental quality control
V.M.F. Vargas, Fundação Estadual de Proteção Ambiental Henrique Luís Roessler, Porto Alegre, RS, Brazil

FOR10 Industrial solid waste leachates induced genotoxicity: models and assays
D.K. Chowdhuri, Indian Institute of Toxicology Research, Lucknow, India

FOR11 An approach to validate genotoxicity biomarkers in environmental animals: the example of the micronucleus test
C. Bolognesi, National Cancer Research Institute, Genova, Italy

FOR12 Applications of biomarkers for the monitoring of the aquatic environment: challenges and new trends
A. Jha, University of Plymouth, UK

Presentation of some selected posters

Lippi Hall

PHILOSOPHY, SCIENCE AND ART IN RENAISSANCE ITALY
Chair: P. Dolara

FOR13 Renaissance medicine between typification and direct observation
O. Catanorchi, Scuola Normale Superiore, Pisa, Italy

FOR14 Art, science and nature in the Renaissance from Botticelli to Leonardo
A. Perissa Torrini, Gallerie dell'Accademia di Venezia, Italia

Saturday, August 22

Botticelli Hall

8.30 IN052 **Plenary Lecture**
Introduction: T. Yagi, President Japanese EMS

ENVIRONMENTAL EXPOSURE ASSESSMENT: COLLATERAL DAMAGE IN THE GENOMIC REVOLUTION?
C. P. Wild, International Agency for Research on Cancer, Lyon, France

9.15 IN053 **Plenary Lecture**
Introduction: W. Anwar, President-Elect Pan-African EMS

UNDERSTANDING THE MUTAGENIC CONSEQUENCES OF BASE LESION DNA REPAIR
S.H. Wilson, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA

10.00 **Coffee break**

10.30-12.30 **Parallel Symposia**

Michelangelo Hall
Mutational and epigenetic mechanisms
CHROMOSOME SEGREGATION AND GENOMIC INSTABILITY
Chair: F. Degrassi, U. Eichenlaub-Ritter

10.30 IN054 USING MULTI-DIMENSIONAL PROTEOMICS TO DEFINE THE COMPLETE PROTEIN COMPOSITION OF MITOTIC CHROMOSOMES
W. Earnshaw, University of Edinburgh, UK

10.55 IN055 LIVE CELL STUDIES ON TAXOL AND THE MITOTIC CHECKPOINT IN HUMANS
C.L. Rieder, NYS Dept. of Health, Albany, NY, USA

11.20 IN056 MECHANISMS OF CHROMOSOME MIS-SEGREGATION IN CANCER CELLS

D. Cimini, Virginia Tech, Blacksburg, VA, USA

11.45 IN057 HIGH-RESOLUTION IMAGING OF MITOTIC CHROMOSOME INSTABILITY
D. Gisselsson, University Hospital, Lund, Sweden

12.10 DD117 TRANSPLACENTALLY-INDUCED CENTROSOMAL AMPLIFICATION AND ANEUPLOIDY IN PRIMATES EXPOSED IN UTERO TO ANTIRETROVIRAL DRUGS
O. Olivero, National Cancer Institute, Bethesda, MD, USA

Angelico Hall
DNA damage responses
CELL TYPE AND TIME SPECIFICITY OF DNA DAMAGE RESPONSE
Chairs: E. Dogliotti, G.T. van der Horst

10.30 IN058 **DNA DAMAGE AND CELL DIFFERENTIATION**
E. Dogliotti, Istituto Superiore di Sanità, Roma, Italy

10.55 IN059 RECIPROCAL LINK BETWEEN THE CIRCADIAN CLOCK AND THE DNA DAMAGE RESPONSE
G.T.J. van der Horst, Erasmus University Medical Center, Rotterdam, The Netherlands

11.20 IN060 CELL AND TISSUE-SPECIFIC REQUIREMENTS FOR DNA STRAND BREAK REPAIR DURING NEUROGENESIS
P.J. McKinnon, St. Jude Children's Research Hospital, Memphis, TN, USA

11.45 IN061 CELL CYCLE CHECKPOINTS AND DNA REPAIR PATHWAYS VARY BETWEEN DIFFERENT CELL TYPES FOLLOWING EXPOSURE TO IONIZING RADIATION
P.J. Stambrook, University of Cincinnati, OH, USA

12.05 IN062 SOMATIC MUTATIONS AS A MOLECULAR RATIONAL OF DISEASE IN COMPLEX CONGENITAL HEART DISEASE
J. Borlak, Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany

12.20 DD104 DNA DAMAGE AND CHECKPOINT RESPONSES IN HUMAN PROSTATE EPITHELIUM
T.M. Hällström, University of Helsinki, Finland

Bronzino Hall
Environmental mutagenesis
ENVIRONMENTAL POLLUTION: THE ROLE OF SENTINEL SPECIES AND BIOMARKERS
Chairs: D. Waters, C. Bolognesi

10.30 IN063 PET DOGS AS SENTINELS OF ENVIRONMENTAL CANCER RISK
D. Waters, Purdue University, West Lafayette, IN, USA

10.55 IN064 TOXICOPATHIC LIVER LESIONS AND OTHER BIOMARKERS OF CHEMICAL EXPOSURE AND EFFECT IN SENTINEL FISH SPECIES IN PUGET SOUND, WASHINGTON AND OTHER COASTAL AREAS OF THE UNITED STATES
M.S. Myers, NOAA Fisheries, Seattle, WA, USA

11.20 IN065 GENOTOXICITY OF POLLUTED SOILS: RESPONSE OF BIOLOGICAL INDICATORS
P. Vasseur, University of Metz, France

11.45 IN066 TRANSCRIPTOMICS AND PROTEOMICS IN *Mus spretus*: NEW TOOLS FOR ENVIRONMENTAL POLLUTION ASSESSMENT
C. Pueyo, Córdoba University, Spain

12.05 IN067 ENHANCED *IN VIVO* MUTATIONS IN THE LUNG OF PHASE II ENZYME-SUPPRESSED MICE
Y. Aoki, National Institute for Environmental Studies, Tsukuba, Japan

12.20 EM045 THE COPEPOD TIGRIOPUS: A PROMISING MARINE MODEL ORGANISM FOR ECOTOXICOLOGY AND ENVIRONMENTAL GENOMICS
J.-S. Lee, Hanyang University, Seoul, Korea

Botticelli Hall
Mutagenesis and health effects
IS THE ERA OF GENOME WIDE ASSOCIATIONS ALREADY OVER?
Chair: P. Vineis, Z. Herceg

10.30 IN068 IS THE ERA OF GENOME WIDE ASSOCIATIONS ALREADY OVER?
P. Vineis, Imperial College, London, UK

11.10 IN070 GENOME-WIDE ASSOCIATION STUDIES: STATISTICAL DEVELOPMENTS
D. Balding, Imperial College, London, UK

11.40 IN071 APPLICATION OF EPIGENOMICS IN CANCER RESEARCH
Z. Herceg, International Agency for Research on Cancer, Lyon, France

12.10 MH011 p73 G4C14-to-A4T14 GENE POLYMORPHISM AND INTERACTION WITH p53 EXON 4 Arg72Pro ON CANCER SUSCEPTIBILITY: A META-ANALYSIS OF THE LITERATURE
E. De Feo, Università Cattolica del Sacro Cuore, Roma, Italy

Lippi Hall
Prevention of mutation-related diseases
BIOLOGY, EPIDEMIOLOGY AND PREVENTION OF CANCER-ASSOCIATED MICROBIAL DISEASES
Chairs: P. Bonanni, P.J. Farrell

10.30 IN072 EPIDEMIOLOGY AND PRIMARY PREVENTION OF HPV-RELATED PRE-CANCEROUS AND CANCEROUS LESIONS
P. Bonanni, University of Firenze, Italy

11.05 IN074 DEVELOPMENT OF VACCINES AGAINST *Helicobacter pylori*
G. Del Giudice, Novartis Vaccines and Diagnostics, Siena, Italy

11.35 IN075 EPSTEIN-BARR VIRUS INFECTION, MUTATIONS AND CANCER
P.J. Farrell, Imperial College, London, UK

12.05 PD025 HUMAN PAPILLOMA VIRUS IN BARRETT'S OESOPHAGUS
E. Snow, University of Tasmania, Launceston, Tasmania, Australia

12.30-14.30 **Lunch and poster viewing**
ME012-021; DD061-120; EM061-120;
MH033-064; PD014-026; RA038-075

14.30-16.30 **Parallel Symposia**

Michelangelo Hall
Mutational and epigenetic mechanisms
MicroRNAs AND THEIR REGULATION
Chairs: G.A. Calin, M. Negrini

14.30 IN076 TOWARD A NON-CODING RNA REVOLUTION IN THE CANCER SOCIETY
G.A. Calin, University of Texas, Houston, TX, USA

15.00 IN077 MicroRNAs IN CELL DIFFERENTIATION AND CANCER
F.J. Slack, Yale University, New Haven, CT, USA

15.30 IN078 MicroRNA ONCOGENIC PATHWAYS DERAILED IN HEPATOCELLULAR CARCINOMA
M. Negrini, Università di Ferrara, Italy

15.55 IN079 POST-TRANSCRIPTIONAL REGULATION OF microRNA EXPRESSION IN HUMAN TUMORS AND CANCER CELL LINES
T.D. Schmittgen, Ohio State University, Columbus, OH, USA

16.20 RA070 IDENTIFICATION OF miRNA WITH TOXICOLOGICAL POTENTIAL AFTER BENZO[a]PYRENE EXPOSURE
D. Lizarraga, Maastricht University, Maastricht, The Netherlands

Botticelli Hall
Environmental mutagenesis
CHALLENGING ENVIRONMENTAL HEALTH PROBLEMS AROUND THE WORLD
Chairs: W. Au, R. Sram

14.30 IN080 TRADITIONAL AND FUNCTIONAL BIOMARKERS FOR MONITORING EXPOSED POPULATIONS FOR HEALTH RISK ASSESSMENT
W.W. Au, University of Texas, Galveston,TX, USA

14.50 IN081 ENVIRONMENTAL AIR POLLUTION AND ASSESSMENT OF HEALTH RISK IN VARIOUS POPULATIONS
M. Ruchirawat, Chulabhorn Research Institute, Bangkok, Thailand

15.10 IN082 GENOTOXICITY OF AIR POLLUTANTS – IMPACT TO CHILDREN HEALTH
R.J. Sram, Institute of Experimental Medicine AS CR, Prague, Czech Republic

15.30 IN083 ARSENIC IN DRINKING WATER: GENETIC AND GENOMIC APPROACHES FOR IDENTIFYING ARSENIC SUSCEPTIBILITY AND HEALTH EFFECTS
A.K. Giri, Indian Institute of Chemical Biology, Calcutta, India

15.50 IN084 ENVIRONMENTAL HEALTH PRIORITIES AND CHALLENGES AROUND THE WORLD FOR THE NEXT DECADES
J. Pronczuk, World Health Organization, Geneva, Switzerland

16.10 IN085 ARISTOLOCHIC ACID NEPHROPATHY: AN ENVIRONMENTAL AND IATROGENIC DISEASE
A.P. Grollman, Stony Brook University, New York, NY, USA

Angelico Hall
Mutagenesis and health effects
DNA DAMAGE, REPAIR AND AGING
(This Symposium is supported by The Ellison Medical Foundation)
Chairs: L.J. Niedernhofer, P.L. Opresko

14.30 IN086 HUMAN PREMATURE AGING PROTEINS PARTICIPATE IN DNA REPAIR
V.A. Bohr, National Institute on Aging, Baltimore, MD, USA

14.50 IN087 ENVIRONMENTAL CAUSES OF TELOMERE DEFECTS
P.L. Opresko, University of Pittsburgh, PA, USA

15.10 DD157 THE WERNER SYNDROME PROTEIN PARTICIPATES IN THE RESPONSE TO ONCOGENE-INDUCED REPLICATION STRESS
P. Pichierri, Istituto Superiore di Sanità, Roma, Italy

15.25 IN088 EVIDENCE THAT DNA DAMAGE PLAYS A CAUSAL ROLE IN AGING AND AGE-RELATED DISEASE
L.J. Niedernhofer, University of Pittsburgh, PA, USA

15.50 IN089 TRANSCRIPTION-BLOCKING DNA LESIONS: AT THE CROSSROAD OF AGING AND LONGEVITY
G.A. Garinis, Institute of Molecular Biology and Biotechnology, Heraklion, Greece

16.10 IN090 CELLULAR SENESCENCE AS A DNA DAMAGE RESPONSE
F. D'Adda di Fagagna, IFOM-IEO, Milano, Italy

Bronzino Hall
Prevention of mutation-related diseases
NUTRIGENOMICS AND PUBLIC HEALTH
Chairs: M. Fenech, P. Dolara

14.30 IN091 PERSONALISED AND POPULATION-BASED STRATEGIES FOR DIAGNOSIS OF DNA DAMAGE AND ITS PREVENTION VIA NUTRITIONAL AND LIFE-STYLE INTERVENTION
M. Fenech, CSIRO Human Nutrition, Adelaide, Australia

15.00 IN092 NUTRITIONAL SYSTEMS BIOLOGY: FROM INTEGRATING MECHANISMS TO PREVENTION
B. van Ommen, TNO-Quality of Life, Zeist, The Netherlands

15.25 IN093 BACTERIA-HOST INTERACTION IN CHRONIC DISEASE: INFLAMMATION MEETS METABOLISM
D. Haller, Technical University of Munich, Germany

15.50 IN094 PROTEOMIC BIOMARKERS OF VULNERABILITY TO CANCER IN THE ALIMENTARY TRACT
I.T. Johnson, Norwich Research Park, Norwich, UK

16.15 RA065 A STEP FORWARD ON THE ROAD TO PREVENT CERVICAL CARCINOMA: A NUTRIGENETIC APPROACH
A. Agodi, University of Catania, Italy

Lippi Hall
Risk assessment
MOLECULAR EPIDEMIOLOGY AND ETHICS
Chairs: K. Vähäkangas, D. Palli

14.30 IN095 HOW TO PROTECT INTEGRITY OF SCIENTIFIC RESEARCH
K. Vähäkangas, University of Kuopio, Finland

15.00 IN096 OBTAINING, SHIPPING, BIOBANKING AND USING OF HUMAN SPECIMENS: LOGISTICAL AND ETHICAL CHALLENGES
P. Hainaut, International Agency for Research on Cancer, Lyon, France

15.30 IN097 CHILDREN AS RESEARCH SUBJECTS: TODAY'S RESEARCH FOR A BETTER FUTURE
D.F. Merlo, National Cancer Research Institute, Genova, Italy

16.00 IN098 RECENT TRENDS AND CHALLENGES IN ENVIRONMENTAL HEALTH RESEARCH
S.H. Wilson, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA

16.20 RA103 THE CYTOKINESIS BLOCKED MICRONUCLEUS CYTOME ASSAY AND RISK PREDICTION OF LUNG CANCER

R. El-Zein, The University of Texas M.D. Anderson Cancer Center, Houston, TX, USA

16.30 **Coffee break**

Botticelli Hall

17.00 IN099 **Plenary Lecture**

Introduction: A. Guevara, President Philippines EMS

THE DNA DAMAGE PROBLEM IN THE CONTEXT OF CANCER, AGING AND LONGEVITY

J.H.J. Hoeijmakers, Erasmus Medical Center, Rotterdam, The Netherlands

18.00-19.30 **Parallel Forum**

Botticelli Hall

FOR15 **WORKSHOP ON CYTOTOXICITY MEASURES IN THE *IN VITRO* MICRONUCLEUS TEST**

Chair: D. Kirkland

Brief introduction and rationale
D. Kirkland, Covance, Harrogate, UK

Summary of L5178Y cell results
E. Lorge, Servier, Fleury-les-Aubrais, France

Summary of TK6 results
A. Elhajouji, Novartis, Basel, Switzerland

Summary of hamster cell results
J. Whitwell, Covance, Harrogate, UK

Summary of US data
M. Schuler, Pfizer, Groton, CT, USA

Overall conclusions
D. Kirkland, Covance, Harrogate, UK

Angelico Hall

FOR16 **RISK ASSESSMENT OF GENOTOXIC TRACE SUBSTANCES IN FOOD**

Chair: R. Crebelli

FOR17 The margin of exposure approach to substances in food that are genotoxic and carcinogenic
D. Benford, Food Standard Agency, London, UK

FOR18 Possible mechanisms underlying practical thresholds for genotoxic carcinogens

T. Nohmi, National Institute of Health Sciences, Tokyo, Japan

FOR19 US FDA safety assessment of genotoxic food contact substances
C.W. Sheu, Food and Drug Administration, Washington, DC, USA

Michelangelo Hall

FOR20 **PREPARING THE NEXT GENERATION OF SCIENTISTS THROUGH EDUCATION AND RESEARCH**
Chair: J. Gentile

J. Gentile, Research Corporation for Science Advancement, Tucson, AZ, USA

D. DeMarini, US Environmental Protection Agency, Research Triangle Park, NC, USA

D. Tweats, University of Wales, Swansea, UK

Sunday, August 23

Botticelli Hall

8.30 IN100 **Plenary Lecture**
Introduction: M. Chulasiri, President Thai EMS

COMPLEX CELLULAR RESPONSES TO DNA DAMAGING AGENTS
L. Samson, Massachusetts Institute of Technology, Cambridge, MA, USA

9.15 IN101 **Plenary Lecture**
Introduction: J. Gentile, Past-President IAEMS

NOVEL STRATEGIES IN THE PREVENTION OF MUTATION-RELATED DISEASES
S. De Flora, University of Genova, Italy

10.00 **Coffee break**

10.30-12.30 **Parallel Symposia**

Michelangelo Hall
Mutational and epigenetic mechanisms
NEW INSIGHTS IN GERM CELL MUTAGENESIS
Chairs: F. Marchetti, C.L. Yauk

10.30 IN102 DNA DOUBLE STRAND BREAK REPAIR IN PARENTAL CHROMOSOMES OF MOUSE ZYGOTES
P. de Boer, Radboud University, Nijmegen, The Netherlands

11.00	IN103	PATERNAL EXPOSURES AFFECT SPERM CHROMATIN AND DISTURB EPIGENETIC PROGRAMMING DURING EARLY EMBRYO DEVELOPMENT ***B. Robaire***, McGill University, Montreal, QC, Canada
11.30	IN104	EFFECTS OF TOBACCO SMOKE ON MALE GERM CELLS AND EARLY EMBRYONIC DEVELOPMENT ***F. Marchetti***, Lawrence Berkeley National Laboratory, Berkeley, CA, USA
11.50	IN105	HERITABLE EFFECTS OF EXPOSURE TO COMBUSTION DERIVED PARTICLES ***C.L. Yauk***, Health Canada, Ottawa, QC, Canada
12.10	EM102	3,3'-DINTRITRO-BISPHENOL A SIGNIFICANTLY DISTURBS MATURATION AND SPINDLE FORMATION, AND CHROMATIN INTEGRITY IN MOUSE OOCYTES ***U. Eichenlaub-Ritter,*** University Bielefeld, Germany

Angelico Hall

DNA damage responses

CELLULAR DEFENSES AGAINST OXIDATIVE DAMAGE

Chairs: T. Lindahl, B. Demple

10.30	IN106	ROLES OF THE FTO AND TREX1 ENZYMES IN REMOVAL OF DAMAGED OR DISPLACED DNA ***T. Lindahl***, CR-UK London Research Institute, South Mimms, UK
10.50	IN107	INTEGRATING CELLULAR FUNCTION THROUGH A BASE EXCISION DNA REPAIR PROTEIN ***B. Demple***, Harvard School of Public Health, Boston, MA, USA
11.10	IN108	REGULATION OF BASE EXCISION REPAIR IN RESPONSE TO DNA DAMAGE ***G. Dianov***, University of Oxford, UK
11.30	IN109	MUTYH AND DNA POLYMERASE λ COOPERATE IN A NOVEL LONG PATCH BASE EXCISION REPAIR OF 8-oxo-GUANINE ***U. Hübscher***, University of Zurich, Switzerland
11.50	IN110	DNA REPAIR ENZYME NEIL1, METABOLIC SYNDROME AND CANCER ***M. Dizdaroglu***, National Institute of Standards and Technology, Gaithersburg, MD, USA
12.10	IN111	INTERACTION OF PROTEINS INVOLVED IN DNA REPAIR WITH AP SITE CONTAINING DNA ***O. Lavrik***, Russian Academy of Sciences, Novosibirsk, Russia

Lippi Hall
Environmental mutagenesis
CRITICAL ISSUES ON ENVIRONMENTAL GENOTOXICITY IN LATIN AMERICA
Chairs: L.R. Ribeiro, E. Zamorano-Ponce

10.30	IN112	INTRODUCTION TO SIMPOSIUM: CRITICAL ISSUES ON ENVIRONMENTAL GENOTOXICITY IN LATIN AMERICA ***E. Zamorano-Ponce***, Universidad del Bío-Bío, Chillán, Chile
10.40	IN113	DNA DAMAGE, OXIDATIVE BALANCE, AND EXPOSURE BIOMARKERS IN A RURAL POPULATION EXPOSED TO PESTICIDES. ***M.A. Carballo***, Universidad de Buenos Aires, Argentina
11.10	IN114	ATMOSPHERIC POLLUTION BY MUTAGENIC AGENTS IN AREAS OF INDUSTRIAL IMPACT: HUMAN BIOMONITORING ***V.M.F. Vargas***, Fundação Estadual de Proteção Ambiental, FEPAM/Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil
11.40	IN115	POLYCYCLIC AROMATIC HYDROCARBON (PAH)-DNA ADDUCTS, CHROMOSOMAL ABERRATIONS, AND *CYP1A*, *CYP1B1* AND *GSTM1* RISK VARIANTS IN PERIPHERAL BLOOD LYMPHOCYTES FROM YOUNG ADULTS LIVING IN MEXICO CITY. ***M.E. Gonsebatt***, UNAM, Mexico City, DF, Mexico
12.05	IN116	USE OF AGROCHEMICALS IN ARGENTINA: GENOTOXIC AND CYTOTOXIC COMPARISONS BETWEEN PURE AND FORMULATED PRODUCTS ***M.L. Larramendy***, University of La Plata, Argentina

Botticelli Hall
Mutagenesis and health effects
CANCER MODELS AND MECHANISMS
Chairs: K. Tanaka, Y. Pommier

10.30	IN117	NOVEL FUNCTION OF NUCLEOTIDE EXCISION REPAIR FACTOR AND ITS RELEVANCE TO XERODERMA PIGMENTOSUM AND COCKAYNE SYNDROME ***K. Tanaka***, Osaka University, Osaka, Japan
10.55	IN118	CHROMOSOMAL INSTABILITY IN CANCER PATHOGENESIS AND TREATMENT ***A. Venkitaraman***, University of Cambridge, UK
11.20	IN119	ROLE OF TOPOISOMERASE I IN GENOMIC STABILITY ***Y. Pommier***, National Institutes of Health, Bethesda, MD, USA
11.40	IN120	OXIDATIVE STRESS-INDUCED TUMORIGENESIS IN THE SMALL INTESTINES OF VARIOUS TYPES OF DNA REPAIR-DEFICIENT MICE ***T. Tsuzuki***, Kyushu University, Fukuoka, Japan

12.00 IN121 ROLE OF MICROENVIRONMENT ON TUMOR PROGRESSION: ENDOTHELIUM, ANGIOGENESIS AND INFLAMMATION
A. Albini, IRCCS Multimedica, Sesto San Giovanni, Milano, Italy

12.20 MH069 AN ATTEMPT TO IDENTIFY GENES INVOLVED IN PROGRESSION OF TOBACCO SMOKE ASSOCIATED CANCER
K. Szyfter, Polish Academy of Sciences, Poznan, Poland

Bronzino Hall
Prevention of mutation-related diseases
PHARMACOLOGICAL PREVENTION OF MUTATION AND CANCER
Chairs: V.E. Steele, T.W. Kensler

10.30 IN122 ANTIMUTAGENIC STRATEGIES APPLIED TO CHEMOPREVENTIVE DRUG DEVELOPMENT
V.E. Steele, National Cancer Institute, Bethesda, MD, USA

10.55 IN123 CLINICAL STRATEGIES FOR DEVELOPING ANTIMUTAGENIC CHEMOPREVENTIVE DRUGS
G.J. Kelloff, National Cancer Institute, Bethesda, MD, USA

11.20 IN124 TARGETING KEAP1-NRF2 SIGNALING WITH DRUGS
T.W. Kensler, Johns Hopkins University, Baltimore, MD, USA

11.45 IN125 CHEMOPREVENTION OF CIGARETTE SMOKE GENOTOXICITY AND CARCINOGENICITY
R. Balansky, National Center of Oncology, Sofia, Bulgaria

12.10 PD002 PHARMACOLOGICAL ANTIOXIDANTS PROTECT FROM RADIATION INDUCED DNA DAMAGE AS WELL AS GENETIC INSTABILITY AND LYMPHOMA IN ATM DEFICIENT MICE
R.H. Schiestl, University of California, Los Angeles, CA, USA

12.30 **Lunch**

13.30-15.30 **Parallel Symposia**

Michelangelo Hall
Mutational and epigenetic mechanisms
***IN VIVO* MUTAGENESIS: RECENT ADVANCES AND FUTURE PROSPECTS**
Chairs: G.R. Douglas, J.H. Bielas

13.30 IN126 LIKE FATHER LIKE SON: TRANSGENERATIONAL GENOMIC INSTABILITY IN MAMMALS
Y.E. Dubrova, University of Leicester, UK

14.00 IN127 THE MECHANISM AND CLINICAL UTILITY OF SOMATIC MITOCHONDRIAL MUTAGENESIS IN CANCER
J.H. Bielas, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

14.30 IN128 CROSS-SPECIES, ENDOGENOUS MUTATION ASSAY BASED ON THE *PIG-A* GENE
J.C. Bemis, Litron Laboratories, Rochester, NY, USA

15.00 IN129 VALIDATION AND UTILITY OF TRANSGENIC RODENT GENE MUTATION ASSAYS
G.R. Douglas, Health Canada, Ottawa, ON, Canada

Botticelli Hall
DNA damage responses
DNA DAMAGE RESPONSE AS THERAPEUTIC TARGET
Chairs: A. Sarasin, P. Karran

13.30 IN130 OVEREXPRESSION OF SOME DNA REPAIR PATHWAYS ARE ASSOCIATED WITH METASTASIS RISK IN MELANOMA PATIENTS
A. Sarasin, CNRS, Villejuif, France

13.55 IN131 DNA DAMAGE AND DNA DAMAGE RESPONSES AFTER THIOPURINE/UVA TREATMENT
P. Karran, Cancer Research UK London Research Institute, South Mimms, UK

14.20 IN132 GENOTOXIC STRESS RESPONSE: MECHANISMS AND RELEVANCE TO CANCER
J. Bartek, Danish Cancer Society, Copenhagen, Denmark

14.40 IN133 PROCESSING OF DNA ADDUCTS INTO DOUBLE STRAND BREAKS
A.K. Larsen, INSERM, Université Paris 6, Paris, France

15.00 IN134 IMPLICATION OF THE NUCLEOTIDE EXCISION REPAIR MACHINERY ON THE RESPONSE TO DOXORUBICIN TREATMENT IN HUMAN FIBROBLASTS
J. Saffi, Lutheran University of Brazil, Canoas, Brazil

15.20 DD003 A MRN/TIP60 COMPLEX INVOLVED IN DNA DOUBLE STRAND BREAKS REPAIR
Y. Canitrot, LBCMCP, CNRS UMR 5088 and University of Toulouse, France

Angelico Hall
Environmental mutagenesis
SYSTEMS BIOLOGY APPROACHES TO ENVIRONMENTAL CARCINOGENESIS AND RELATED BIOMARKERS
(This Symposium is supported by ECNIS)
Chairs: A. Hirvonen, S. Kyrtopoulos

13.30 INTRODUCTION
A. Hirvonen, Finnish Institute of Occupational Health, Helsinki, Finland

13.40 IN135 INTOGEN: A NOVEL FRAMEWORK FOR INTEGRATION AND DATA-MINING OF MULTIDIMENSIONAL ONCOGENOMIC DATA
N. Lopez-Bigas, Universitat Pompeu Fabra, Barcelona, Spain

14.05 IN136 THE COMPARATIVE TOXICOGENOMICS DATABASE: A DISCOVERY TOOL FOR IDENTIFYING CHEMICAL-GENE-DISEASE NETWORKS
C.J. Mattingly, The Mount Desert Island Biological Laboratory, Salisbury Cove, ME, USA

14.30 IN137 METABOLIC PROFILING AS A TOOL IN BIOMARKER RESEARCH AND SYSTEMS BIOLOGY
H.C. Keun, Imperial College, London, UK

14.55 IN138 BLOOD TRANSCRIPTOMICS AND EXPOSURE BIOMARKERS IN A POPULATION-BASED COHORT – THE NOWAC POSTGENOME STUDY
V. Dumeaux, University of Tromsø, Norway

15.20 CONCLUSION
S. Kyrtopoulos, National Hellenic Research Foundation, Athens, Greece

Lippi Hall
YOUNG SCIENTIST SESSION I
Chairs: M. Kirsch-Volders, A.Zijno

13.30 DD040 DNA POLYMERASES BETA AND LAMBDA AS A POTENTIAL PARTICIPANTS OF TLS DURING GENOMIC DNA REPLICATION ON THE LEADING AND LAGGING STRANDS
E.A. Belousova, Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russia

13.45 EM053 BIOLOGICAL ACTIVITIES OF ENDOGENOUS MUTAGENS/CARCINOGENS, AMINOPHENYLNORHARTMAN AND *N*-NITROSO BILE ACID CONJUGATES
Y. Totsuka, National Cancer Center Research Institute, Tokyo, Japan

14.00 DD096 DNA DAMAGE RESPONSES IN IRRADIATED GLIOBLASTOMA CELL LINES
P.R.D.V. Godoy, University of São Paulo, Ribeirão Preto, SP, Brazil

14.15 EM140 DNA DAMAGE ASSESSMENT OF HUMAN POPULATIONS EXPOSED TO AIRBORNE POLLUTANTS FROM INDUSTRIAL AND URBAN SOURCES
M.V. Coronas, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

14.30 RA071 MOLECULAR GENETIC ANALYSIS OF C677T AND A1298C POLYMORPHISMS OF THE GENE OF METHYL TETRA HYDRO FOLATE REDUCTASE AS A RISK FACTOR FOR DEVELOPMENT OF CHRONIC MALNUTRITION IN

CHILDREN UNDER 3 YEARS OF THE MUNICIPALITY OF LURIBAY, EXPOSED TO PESTICIDES
R.E. Montaño Arrieta, Universidad Mayor de San Andres UMSA, La Paz, Bolivia

14.45 ME033 HYPOXIA UPREGULATES microRNA-210 AND CONTRIBUTES TO CANCER CELL SURVIVAL VIA MODULATING MITOCHONDRIAL ACTIVITY
M.E. Crosby, Yale University School of Medicine, New Haven, CT, USA

Bronzino Hall
Risk assessment
NEW DATA INITIATIVES AND PREDICTIVE APPROACHES FOR MUTAGENICITY AND CARCINOGENICITY
Chairs: R. Benigni, A. Richard

13.30 IN139 EXPERIMENTAL TESTS AND MODELING APPROACHES: GETTING THE BEST FROM BOTH
R. Benigni, Istituto Superiore di Sanità, Roma, Italy

13.55 IN140 NEW CHEMICAL/BIOLOGICAL PROFILING AND INFORMATICS APPROACHES FOR EXPLORING MUTAGENICITY AND CARCINOGENICITY: UPDATES OF EPA TOXCAST^{TM} AND TOX21 PROGRAMS
A. Richard, U.S. Environmental Protection Agency, Research Triangle Park, NC, USA

14.20 IN141 A KNOWLEDGE-BASE APPROACH TO IDENTIFY SIGNATURES FOR BIOLOGICAL AND CHEMICAL PAIRS IN THE RISK ASSESSMENT PROCESS
C. Yang, US Food and Drug Administration, College Park, MD, USA

14.45 IN142 THE USE OF (Q)SAR IN FOOD SAFETY ASSESSMENT
E. Lo Piparo, Institute for Health and Consumer Protection, Ispra, Varese, Italy

15.10 RA021 ENVIRONMENTAL RISK INDEX (iERICA): A NEW PROPOSAL FOR RISK ASSESSMENT INTEGRATING *IN VITRO* TOOLS
D. Baderna, Istituto di Ricerche Famacologiche “Mario Negri”, Milano, Italy

15.20 RA082 DIRECTING GENOTOXICITY TESTING TO ASSIST IN THE DEVELOPMENT OF *IN SILICO* MODELS
R.V. Williams, Lhasa Limited, Leeds, UK

16.00-19.30 **Optional tours**

20.30 **Social dinner**

Monday, August 24

Botticelli Hall

8.30 IN143 **Plenary Lecture**
Introduction: A.B. Prasad, President EMS India

THE RATE OF SOMATIC MUTATIONS AND HUMAN CANCER
L. Luzzatto, Istituto Toscano Tumori, Firenze, Italy

9.15 IN144 **Plenary Lecture**
Introduction: E. Snow, President EMS Australia-New Zealand (MEPSA)

CAUSES AND MECHANISMS OF COLON CANCER DEVELOPMENT, AND STRATEGIES FOR ITS PREVENTION
K. Wakabayashi, National Cancer Center Research Institute, Tokyo, Japan

10.00 **Coffee break**

10.30-12.30 **Parallel Symposia**

Michelangelo Hall
Mutational and epigenetic mechanisms
INDUCED MUTAGENESIS: A NETWORK OF INTERPLAYING PROCESSES
Chairs: J.M. Essigmann, A. Abbondandolo

10.30 IN145 AN INTEGRATED VIEW OF INDUCED MUTAGENESIS IN *E. coli*
R.P. Fuchs, CNRS, Marseille, France

10.55 IN146 INTERPLAY OF DNA REPAIR, DNA POLYMERASES AND TRANSCRIPTION IN THE PROCESS OF SPONTANEOUS MUTAGENESIS IN YEAST
S. Boiteux, CNRS & CEA, Fontenay aux Roses, France

11.20 IN147 OXIDATIVE STRESS, DNA ALKYLATION AND MUTAGENESIS
J.M. Essigmann, Massachusetts Institute of Technology, Cambridge, MA, USA

11.45 IN148 MUTAGENIC OR ACCURATE OUTCOME OF TRANSLESION DNA SYNTHESIS IS DETERMINED BY SPECIFIC TWO-POLYMERASE MECHANISMS IN MAMMALIAN CELLS
Z. Livneh, Weizmann Institute of Science, Rehovot, Israel

12.10 DD025 LESION RECOGNITION AND CATALYSIS IN BER PATHWAY INVOLVE MULTIPLE CONFORMATIONAL CHANGES IN ENZYMES AND DNA
O.S. Fedorova, Russian Academy of Sciences, Novosibirsk, Russia

12.20 DD056 NEIL GLYCOSYLASES: NOVEL ACTIVITIES AND REGULATION

I.R. Grin, Russian Academy of Sciences, Novosibirsk, Russia

Botticelli Hall
DNA damage responses
LOOKING INTO THE FUTURE: GENOME WIDE TECHNOLOGIES IN TOXICOLOGY
Chairs: L. Mullenders, E. Prosperi

10.30 IN149 EPISTASIS ANALYSIS OF THE DNA DAMAGE RESPONSE
H. van Attikum, Leiden University, Leiden, The Netherlands
(EEMS Young Scientist Award 2009)

10.55 IN150 TRANSLATIONAL RESPONSES TO DNA DAMAGE
T.J. Begley, University at Albany, Rensselaer, NY, USA

11.20 IN151 GLOBAL ANALYSIS OF SIGNALING NETWORKS BY HIGH-RESOLUTION MASS SPECTROMETRY-BASED QUANTITATIVE PHOSPHOPROTEOMICS
J.V. Olsen, University of Copenhagen, Denmark

11.45 IN152 QUANTITATIVE IMAGING-BASED FUNCTIONAL GENOMICS SCREENING TO UNRAVEL TOXICITY RELEVANT SIGNALING PATHWAYS
B. van de Water, Leiden University, Leiden, The Netherlands

12.10 IN153 CHIP-SEQ APPROACH TO STUDY THE CELLULAR RESPONSE TO DAMAGE INDUCED TRANSCRIPTION INTERFERENCE
M. Fousteri, Leiden University, Leiden, The Netherlands

12.25 DD147 PROTEOMICS AND SPR IMAGING APPLIED TO PLATINATED DNA INTERACTOME STUDIES
J. Breton, Commissariat à l'Énergie Atomique, Grenoble, France

Angelico Hall
DNA damage responses
REGULATION OF DNA DAMAGE RESPONSE: LESSONS LEARNED FROM DOUBLE STRAND BREAK REPAIR PATHWAYS
Chairs: J. Surralles, P. Mosesso

10.30 IN154 FANCONI ANEMIA: OMIC APPROACHES AND THERAPEUTIC APPLICATIONS
J. Surralles, Universitat Autònoma de Barcelona, Spain

11.00 IN155 FANCM CONNECTS THE TWO GENOME INSTABILITY DISORDERS BLOOM'S SYNDROME AND FANCONI ANEMIA
A.J. Deans, London Research Institute, Cancer Research UK, South Mimms, UK

11.30 IN156 GENETIC PATHWAYS REQUIRED FOR TEMPLATE-SWITCH MEDIATED DAMAGE BYPASS REPLICATION
D. Branzei, FIRC Institute of Molecular Oncology, Milano, Italy

12.00 DD150 POLO-LIKE KINASE REGULATION OF DNA REPLICATON UNDER STRESSED CONDITIONS
K. Trenz, Institute of Cancer Research, Sutton, UK

12.10 DD091 REPLICATION FORK STABILIZATION PROTEINS TIMELESS AND TIMELESS-INTERACTING PROTEIN (TIPIN) MAINTAIN GENOMIC STABILITY
S.L. Smith-Roe, University of North Carolina at Chapel Hill, NC, USA
(US-EMS Young Scientist award)

Bronzino Hall
Environmental mutagenesis
BIOLOGICAL RISKS FROM SPACE RADIATION ENVIRONMENTS
Chairs: F.A. Cucinotta, M. Durante

10.30 IN158 ATM AND TGFβ PATHWAY SIGNALING FOLLOWING X-RAY AND HEAVY IONS EXPOSURE
F.A. Cucinotta, NASA, Houston, TX, USA

11.00 IN159 CYTOGENETIC EFFECTS OF HEAVY IONS
M. Durante, GSI, Darmstadt, Germany

11.30 IN160 DNA DAMAGE AND REPAIR FROM SPACE RADIATION
M.A. Tabocchini, Istituto Superiore di Sanità and INFN, Roma, Italy

11.55 IN161 POPULATION ACTION AS A MODIFIER OF RADIATION-INDUCED CARCINOGENESIS
L. Hlatky, Tufts University, Boston, MA, USA

12.20 EM007 THE COMPARISON STUDY ON CHROMOSOMAL ABERRATIONS IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES INDUCED BY 18.8 MeV PROTON AND ^{60}Co-γ RADIATION
Y. Chen, Beijing Institute of Radiation Medicine, Beijing, China

Lippi Hall
Prevention of mutation-related diseases
PUBLIC HEALTH GENOMICS
Chairs: W. Ricciardi, A. Brand

10.30 IN162 THE EUROPEAN AND INTERNATIONAL AGENDA OF PUBLIC HEALTH GENOMICS
A. Brand, Maastricht University, Maastricht, The Netherlands

10.55 IN163 PUBLIC HEALTH AND GENOMIC EPIDEMIOLOGY
S. Boccia, Università Cattolica del Sacro Cuore, Roma, Italy

11.20 IN206 HOW TO DELIVER QUALITY STANDARDS IN PUBLIC HEALTH GENOMICS? THE EXAMPLE OF GENETIC SERVICES IN EUROPE
D. Coviello, Policlinico Foundation, Milano, Italy

11.45 IN165 TRANSLATIONAL RESEARCH IN GENOMICS

C. Janssens, Erasmus University Medical Center Rotterdam, The Netherlands

12.10 IN166 PRACTICAL IMPLEMENTATION OF PUBLIC HEALTH GENOMICS: THE CASE OF GENAR INSTITUTE
***B.S. Savaş*, **GENAR Institute for Public Health and Genomics Research**,** Istanbul**,** Turkey

12.30-14.30 **Lunch and poster viewing**
ME022-033; DD121-180; EM121-180; MH065-095; PD027-039; RA076-114

14.30-16.30 **Parallel Symposia**

Michelangelo Hall
Mutational and epigenetic mechanisms
PHYSIOLOGICAL MUTAGENESIS IN IMMUNITY
Chair: M.S. Neuberger, J. Miller

14.30 IN167 THE FUNCTIONS OF AID AND OTHER DNA DEAMINASES IN IMMUNITY
M.S. Neuberger, MRC, Cambridge, UK

15.00 IN168 INTENTIONAL MUTAGENESIS IN B CELLS: PCNA-UBIQUITYLATION CONTROLS SOMATIC HYPERMUTATION
H. Jacobs, The Netherlands Cancer Institute, Amsterdam, The Netherlands

15.25 IN169 DIVERSITY-GENERATING RETROELEMENTS
J. Miller, UCLA School of Medicine, Los Angeles, CA, USA

15.50 IN170 ERROR-PRONE REPAIR PATHWAYS MOBILIZING TLS DNA POLYMERASES IN IMMUNOGLOBULIN GENE HYPERMUTATION
C.A. Reynaud, INSERM, Paris, France

16.15 IN171 EDITING DEAMINASES: STORY OF A MULTI-TALENTED DOMAIN
S. Conticello, Istituto Toscano Tumori, Firenze, Italy

Botticelli Hall
Mutational and epigenetic mechanisms
TOLERANCE OF DNA DAMAGE: TRANSLESION DNA SYNTHESIS
Chair: A.R. Lehmann, G. Villani

14.30 IN172 REGULATION OF DNA POLYMERASE ETA IN HUMAN CELLS
A.R. Lehmann, University of Sussex, Falmer, UK

15.00 IN174 HUMAN DNA POLYMERASE NU (POLN), A UNIQUE A-FAMILY DNA POLYMERASE WHICH CAN BYPASS DNA DAMAGE
K. Takata, The University of Texas, Smithville, TX, USA

15.25 IN175 REPAIR AND TOLERANCE MECHANISMS OF DNA-PROTEIN CROSSLINK DAMAGE
H. Ide, Hiroshima University, Hiroshima, Japan

15.50 IN176 NEW FUNCTIONAL ROLES OF THE HUMAN DNA POLYMERASES ETA AND KAPPA DURING GENOMIC DNA REPLICATION
J.S. Hoffmann, CNRS, University of Toulouse, France

16.15 DD130 K63-LINKED UBIQUITIN CHAINS CONJUGATED TO PCNA CONTROL RECOMBINATION REPAIR OF ss-GAPS
G.I. Karras, Max Planck Institute of Biochemistry, Martinsried, Germany

Lippi Hall
YOUNG SCIENTIST SESSION II
Chairs: F. Pacchierotti, H.-J. Martus

14.30 EM130 A NEW PARADIGM FOR MOLECULAR TOXICOLOGY: INHIBITION OF P53-MEDIATED DNA REPAIR BY HEAVY METAL NICKEL
Young R. Seo, Kyung Hee University, Seoul, Korea

14.45 DD028 BIOCHEMICAL EVIDENCES INVOLVE DNA POLYMERASE BETA OF *Trypanosoma cruzi* IN REPAIR OF OXIDATIVE LESIONS IN MITOCHONDRIAL DNA
B.L.F. Schamber-Reis, Federal University of Minas Gerais, Brazil

15.00 MH013 POSTGENOMIC ALTERATIONS IN AICARDI-GOUTIÈRES SYNDROME, A RARE NEURODEGENERATIVE DISEASE OF MUTATIONAL ORIGIN
M. Longobardi, Department of Health Sciences, University of Genova, Italy

15.15 MH031 GENETIC MARKERS OF SUSCEPTIBILITY INVOLVED IN PROSTATE CANCER
H. Kuasne, Londrina State University, Londrina, PR, Brazil

15.30 MH074 BIOCHEMICAL AND GENOTOXIC EFFECTS IN PESTICIDE SPRAYERS: PRELIMINARY RESULTS
M.F. Simoniello, Universidad Nacional del Litoral, Santa Fe, Argentina

15.45 EM029 THE USE OF GENOTOXIC BIOASSAYS TO EVALUATE THE ENVIRONMENTAL QUALITY IN A REGION UNDER THE INFLUENCE OF URBAN WASTE IN GUAÍBA LAKE BASIN (BRAZIL)

I.V. Villela, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

Angelico Hall
Mutagenesis and health effects
GENE-ENVIRONMENT INTERACTIONS IN NONCANCER DISEASES
Chairs: A. Izzotti, F.J. van Schooten

14.30	IN177	GENE-ENVIRONMENT INTERACTIONS AS COMMON DETERMINANTS OF NONCANCER-DEGENERATIVE DISEASES ***A. Izzotti***, University of Genova, Italy
14.55	IN178	GENETIC INFLUENCES ON SMOKING BEHAVIOR AND PREVENTION OF CHRONIC DEGENERATIVE DISEASES ***F.J. van Schooten***, Maastricht University, Maastricht, The Netherlands
15.20	IN179	THE IMPACT OF GENETIC AND ENVIRONMENTAL FACTORS IN NEURODEGENERATION: THE EMERGING ROLE OF EPIGENETICS ***L. Migliore***, University of Pisa, Italy
15.45	IN180	POLYMORPHISMS OF CYTOCHROME P4501A1, CIGARETTE SMOKING AND RISK OF CORONARY ARTERY DISEASE ***C.-C.Yeh***, China Medical University, Taichung, Taiwan
16.10	MH095	MITOCHONDRIAL DNA HAPLOGROUPS AND ETHNICAL ORIGINS IN PATIENTS WITH MULTIPLE SCLEROSIS ***H. Groot***, Universidad de los Andes, Bogotà, Colombia
16.20	MH066	OXIDATIVE DAMAGE AND TRANSCRIPT DOWN-REGULATION OF DNA REPAIR GENES IN LYMPHOCYTES FROM PATIENTS WITH ALZHEIMER DISEASE, FRAILTY SYNDROME AND DIABETES MELLITUS TYPE-2 ***E.T. Sakamoto-Hojo***, Universidade de São Paulo, Ribeirão Preto, SP, Brazil

Bronzino Hall
Prevention of mutation-related diseases
DIETARY PREVENTION OF MUTATION AND CANCER
Chairs: S. Knasmüller, N. Loprieno

14.30	IN181	USE OF THE COMET ASSAY FOR THE DETECTION OF DNA PROTECTIVE CONSTITUENT IN THE HUMAN DIET ***S. Knasmüller***, Medical University of Vienna, Austria
14.55	IN182	TRANSIENT GENERATION OF REACTIVE OXYGEN SPECIES AS AN IMPORTANT SIGNALLING MECHANISM IN CANCER CHEMOPREVENTION ***C. Gerhäuser***, German Cancer Research Center, Heidelberg, Germany

15.20 IN183 IN SEARCH OF A MECHANISM: FOOD POLYPHENOLS FROM ANTIOXIDANTS TO MODULATORS OF GENE EXPRESSION
P. Dolara, University of Firenze, Italy

15.45 IN184 NON-ANTIOXIDANT EFFECTS OF PHYTOCHEMICALS: DNA REPAIR
A.R. Collins, University of Oslo, Norway

16.10 EM023 COMPARISON OF MUTAGENICITIES AND GENE EXPRESSION PROFILES OF COMFREY AND RIDDELLIINE IN RAT LIVER
M.G. Manjanatha, National Center for Toxicological Research, Jefferson, AR, USA

16.20 EM097 PRENATAL EXPOSURE TO FLAVONOIDS: IMPLICATION FOR DEVELOPMENT OF LEUKEMIA
K. Vanhees, Maastricht University, Maastricht, The Netherlands

16.30 **Coffee break**

Botticelli Hall

17.00 IN185 **Plenary Lecture**
Introduction: P. Cooper, President EMS North-America

CELLULAR ADAPTIVE SURVIVAL RESPONSE TO OXIDATIVE, NITROSATIVE AND INFLAMMATORY STRESSES: ROLES OF REDOX-SENSITIVE TRANSCRIPTION FACTORS
Y.-J. Surh, Seoul National University, Seoul, Korea

18.00 **General Assembly of the International Association of Environmental Mutagen Societies (IAEMS)**

19.30-20.30 **Assemblies of Regional Mutagen Societies**

<u>Tuesday, August 25</u>

8.30-10.30 **Parallel Symposia**

Michelangelo Hall
Mutational and epigenetic mechanisms
MECHANISMS OF UNTARGETED MUTAGENESIS
Chairs: L.A. Loeb, T.A. Kunkel

8.30 IN186 INVOLVEMENT OF MUTATOR DNA POLYMERASES IN CARCINOGENESIS
L.A. Loeb, University of Washington, Seattle, WA, USA

9.00	IN187	THE EFFICIENCY OF DNA MISMATCH REPAIR IN *Saccharomyces cerevisiae* ***T.A. Kunkel***, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA
9.30	IN188	PATHWAYS SUPPRESSING SPONTANEOUS MUTATION AND CANCER IN MICE ***B.D. Preston***, University of Washington, Seattle, WA, USA
9.55	IN189	GENOMIC INSTABILITY AND CANCER IN MOUSE DNA REPLICATION MUTANTS ***J. Schimenti***, Cornell University, Ithaca, NY, USA
10.20	ME007	HIGH FREQUENCY OF GENOMIC DELETIONS INDUCED BY ME-LEX, A SEQUENCE SELECTIVE N3-ADENINE METHYLATING AGENT, AT THE HPRT LOCUS IN CHINESE HAMSTER OVARY CELLS ***P. Menichini***, National Cancer Research Institute (IST), Genova, Italy

Botticelli Hall
Environmental mutagenesis
ADVANCES IN THE ASSESSMENT OF EXPOSURE AND EARLY BIOLOGICAL EFFECTS
Chairs: S. Bonassi, D.H. Phillips

8.30	IN190	CHALLENGES IN THE DESIGN AND STATISTICAL ANALYSIS OF POPULATION STUDIES WITH HIGH-THROUGHPUT ASSAYS ***S. Bonassi***, National Cancer Research Institute (IST), Genova, Italy
8.55	IN191	CLUES TO CANCER AETIOLOGY AND CARCINOGENIC MECHANISMS DERIVED FROM DNA ADDUCTS ***D.H. Phillips***, Institute of Cancer Research, Sutton, UK
9.20	IN192	THE USE OF DNA ADDUCTS IN RISK ASSESSMENT ***H. Autrup***, University of Aarhus, Denmark
9.45	IN193	GENOMIC ALTERATIONS AS EARLY INDICATORS OF ADVERSE EFFECTS OF EXPOSURES ***R.S. Paules***, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA
10.10	IN194	MASS SPECTRAL DETECTION OF DNA ADDUCTS PRODUCED BY EXPOSURES TO CARCINOGENS ***P.B. Farmer***, University of Leicester, UK

Angelico Hall
Mutagenesis and health effects
NUCLEOTIDE EXCISION REPAIR AND TRANSCRIPTION: MECHANISMS AND CLINICAL IMPLICATIONS

Chairs: J.M. Egly, M. Stefanini

8.30 IN195 THE NUCLEOTIDE EXCISION REPAIR: TFIIH AND Co
J.M. Egly, CNRS, Strasbourg, France

9.00 IN196 INSIGHTS INTO GENOTYPE-PHENOTYPE RELATIONSHIPS IN THE REPAIR/TRANSCRIPTION SYNDROME TRICHOTHIODYSTROPHY
D. Orioli, Institute of Molecular Genetics, CNR, Pavia, Italy

9.25 IN197 TRANSCRIPTION STALLING AND CELLULAR RESPONSES
L.H. Mullenders, Leiden University, Leiden, The Netherlands

9.50 IN198 INTERSECTING DNA REPAIR PATHWAYS AND COORDINATION WITH TRANSCRIPTION
P. Cooper, Lawrence Berkeley National Laboratory, Berkeley, CA, USA

10.15 DD052 SILENCING OF OXIDATIVELY-DAMAGED GENE IN MAMMALIAN CELLS
A. Khobta, University of Mainz, Germany

Lippi Hall
Prevention of mutation-related diseases
DIETARY FACTORS, MUTATION AND CANCER
Chairs: L. Ferguson, R. Barale

8.30 IN199 DIETARY FACTORS, MUTATION AND CANCER
L.R. Ferguson, The University of Auckland, New Zealand

8.55 IN200 BASE EXCISION REPAIR, OXIDATIVE STRESS AND CANCER
B. Tudek, University of Warsaw, Poland

9.20 IN201 DIETARY INTAKE OF ARISTOLOCHIC ACID AS A RISK FACTOR FOR BALKAN ENDEMIC NEPHROPATHY-ASSOCIATED UROTHELIAL CANCER
V.M. Arlt, Institute of Cancer Research, Sutton, UK

9.45 IN202 THE ROLE OF GENETIC AND NON-GENETIC MECHANISMS IN FURAN RISK
A. Mally, University of Würzburg, Germany

10.10 PD032 MECHANISTIC ASPECTS OF GENOTOXICITY OF FURAN AND ITS KEY METABOLITE CIS-2-BUTENE-1,4-DIAL IN MAMMALIAN CELLS *IN VITRO*
P. Mosesso, Tuscia University, Viterbo, Italy

10.20 EM060 URINARY FUMONISIN B1 AS A BIOMARKER OF FUMONISIN EXPOSURE AND ITS APPLICATION IN INTERVENTION STUDIES
Y.Y. Gong, University of Leeds, UK

Bronzino Hall
Risk assessment
REPORTS FROM THE 5th INTERNATIONAL WORKSHOP ON GENOTOXICITY TESTING
Chairs: D. Kirkland, L. Müller

	IN203	**REPORTS FROM THE 5th INTERNATIONAL WORKSHOP ON GENOTOXICITY TESTING**
8.30		***D. Kirkland***, Covance, Harrogate, UK
8.45		***S. Galloway***, Merck, West Point, PA, USA
9.00		***M. Moore***, Jefferson, AR, USA
9.15		***P. Kasper***, Federal Institute for Drugs and Medical Devices, Bonn, Germany
9.35		***S. Pfuhler***, Procter & Gamble, Marly, Switzerland
9.55		***M. Hayashi,*** National Institute of Health Sciences, Tokyo, Japan
10.10		***V. Thybaud***, Sanofi-Aventis, Vitry sur Seine, France
10.30		**Coffee break**

Botticelli Hall

11.00	IN204	**Plenary Lecture** Introduction: E. Dogliotti, President European EMS THE MOLECULAR BASIS OF LIFE'S ROBUSTNESS ***M. Radman***, University of Paris 5, Paris, France
11.45	IN205	**Plenary Lecture** Introduction: J. Yang, Representative Chinese EMS MUTATIONS IN microRNA PRECURSORS IN HEMATOPOIETIC MALIGNANCIES ***C.M. Croce***, Ohio State University, Columbus, OH, USA
12.30		**Closing Ceremony**

X INTERNATIONAL CONFERENCE ON ENVIRONMENTAL MUTAGENS

Program at a glance

	Hall	BOTTICELLI	ANGELICO	LIPPI	BRONZINO	MICHELANGELO
Thu, August 20	8:30		**Pre Conference course Molecular Epidemiology for Chronic Diseases**		**Pre Conference course Micronucleus Cytome Assay: lymphocytes and buccal exfoliated cells**	
	18:45	**PC Hanawalt,** *Stanford University, Stanford, CA, USA*				
Fri, August 21	8:30	**HRH Princess M Chulabhorn,** *Chulabhorn Research Institute, Bangkok, Thailand* **P Grandjean,** *University of Southern Denmark, Odense, Denmark*				
	10:30	**Current issues in mode of action analysis and their use in cancer risk assessment** *(N Keshava; D Eastmond; G Speit; L Müller; A Akesson; R Baan)*	**Health effects of nucleotide pool damage** *(T Nohmi; Y Nakabeppu; M Bignami; P Henderson; H Kamiya; E Touati)*	**Mechanisms of cell death and survival** *(B Kaina; M Ljungman; B Zhivotovsky; C Du; CF Menck; M Volcic)*	**Nanotoxicology: mechanisms and effects** *(G Oberdörster; B Fubini; R Landsiedel; M Kirsch-Volders; V Castranova; L Tran)*	**Epigenetics: the new frontier in environmental mutagenesis** *(R Feil; LE Young; MF Fraga; M Hanson; S Jarmalaite; M Mauro)*
	14:30	**Novel insights into DNA damage signalling and repair** *(J Jiricny; JA Tainer; KJ Myung; P Schär; M. Bjorås; T Yagi)*	**Children cancer risk** *(CP Wild; R Fry; DM van Leeuwen; K Hemminki; T Dwyer)*	**Epigenome and environment: from mechanisms to risk assessment** *(O Kovalchuk; IP Pogribny; BP Engelward; I Kovalchuk; JE Trosko; D Tang)*	**New developments in the genotoxicity of soil and water** *(PA White; T Watanabe; DM DeMarini; R Marcos; MB Heringa)*	**New developments in regulatory genetic toxicology** *(C Priestley; A Czich; A Rothfuss; V Thybaud; JT MacGregor; J Sasaki)*
	17:00	**R De Maria,** *Istituto Superiore di Sanità, Roma, Italy*				
	18:00	**Rationale of genotoxicity testing of nanomaterials** *(Chairs: H Greim, H Norppa)*	**Ecogenotoxicology: past successes and future needs** *(Chairs: C Bolognesi, DM DeMarini)*	**Philosophy, science and art in Renaissance Italy** *(Chair: P Dolara)*	**Antimutagenesis and chemoprevention in a U-shaped world** *(Chair: DJ Waters)*	**Mutagenic mode of action for carcinogens: how high is the burden of proof?** *(Chair: M Moore)*
Sat, August 22	8:30	**CP Wild** *International Agency for Research on Cancer, Lyon, France* **SH Wilson,** *National Institute of Environmental Health Sciences, Research Triangle Park, USA*				
	10:30	**Is the era of genome-wide associations already over?** *(P Vineis; T Manolio; D Balding; Z Herceg; E De Feo)*	**Cell-type and time specificity of the DNA damage response** *(E Dogliotti; GTJ van der Horst; PJ McKinnon; PJ Stambrook; J Borlak; TM Hällström)*	**Biology, epidemiology and prevention of cancer-associated microbial diseases** *(P Bonanni; D Shouval; G Del Giudice; PJ Farrell; E Snow)*	**Environmental pollution: the role of sentinel species and biomarkers** *(D Waters; MS Myers; P Vasseur; C Pueyo; Y Aoki; J-S Lee)*	**Chromosome segregation and genomic instability** *(W Earnshaw; CL Rieder; D Cimini; D Gisselsson; O Olivero)*
	14:30	**Challenging environmental health problems around the world** *(W Au; M Ruchirawat; RJ Sram; AK Giri; J Pronczuk; AP Grollman)*	**DNA damage, repair and aging** *(VA Bohr; P Opresko; P Pichierri; LJ Niedernhofer; GA Garinis; F D'Adda di Fagagna)*	**Molecular epidemiology and ethics** *(K Vähäkangas; P Hainaut; DF Merlo; T Manolio; S Wilson; R. El-Zein)*	**Nutrigenomics and public health** *(M Fenech; B van Ommen; D Haller; IT Johnson; A Agodi)*	**MicroRNAs and their regulation** *(GA Calin; FJ Slack; M Negrini; TD Schmittgen; D Lizarraga)*
	17:00	**JHJ Hoeijmakers,** *Erasmus Medical Center, Rotterdam, The Netherlands*				
	18:00	**Workshop on cytotoxicity measures in the *in vitro* micronucleus test** *(Chair: D Kirkland)*	**Risk assessment of genotoxic trace substances in food** *(Chair: R Crebelli)*			**Preparing the next generation of scientists through education and research** *(Chair: J Gentile)*
Sun, August 23	8:30	**L Samson,** *Massachusetts Institute of Technology, Cambridge, USA* **S De Flora,** *University of Genova, Italy*				
	10:30	**Cancer models and mechanisms** *(K Tanaka; A Venkitaraman; Y Pommier; T Tsuzuki; A Albini; K Szyfter)*	**Cellular defences against oxidative damage** *(T Lindahl; B Demple; G Dianov; U Hübscher; M Dizdaroglu; O Lavrik)*	**Critical issues on environmental genotoxicity in Latin America** *(E Zamorano-Ponce; MA Carballo; VM Ferrão Vargas; ME Gonsebatt; ML Larramendy)*	**Pharmacological prevention of mutation and cancer** *(VE Steele; GJ Kelloff; TW Kensler; R Balansky; RH Schiestl)*	**New insights in germ cell mutagenesis** *(P De Boer; B Robaire; F Marchetti; CL Yauk; U Eichenlaub-Ritter)*
	13:30	**DNA damage response as therapeutic target** *(A Sarasin; P Karran; J Bartek; Ak Larsen; J Saffi; Y Canitrot)*	**Systems biology approaches to environmental carcinogenesis and related biomarkers** *(A Hirvonen; N Lopez-Bigas; CJ Mattingly; HC Keun; V Dumeaux; S Kyrtopoulos)*	**Young Scientist Session I** *(EA Belousova; AK Sundaram; Y Totsuka; PRDV Godoy; B Manshian; MV Coronas; RE Montaño Arrieta; ME Crosby)*	**New data initiatives and predictive approaches for mutagenicity and carcinogenicity** *(R Benigni; A Richard; C Yang; E Lo Piparo; D Badema; RV Williams)*	**In vivo mutagenesis: recent advances and future prospects** *(YE Dubrova; JH Bielas; JC Bernis; GR Douglas)*
Mon, August 24	8:30	**L Luzzatto,** *Istituto Toscano Tumori, Firenze, Italy* **K Wakabayashi** *National Cancer Center Research Institute, Tokyo, Japan*				
	10:30	**Looking into the future: genome wide technologies in toxicology** *(H van Attikum; TJ Begley; JV Olsen; B van de Water; M Fousteri; J Breton)*	**Regulation of DNA damage response: lessons learned from double strand break repair pathways** *(J Surralles; AJ Deans; D Branzei; E Soutoglou; K Trenz; SL Smith-Roe)*	**Public Health Genomics** *(A Brand; S Boccia; P Boffetta; C Janssens; BS Savas)*	**Biological risks from space radiation environments** *(FA Cucinotta; M Durante; MA Tabocchini; L Hlatky; Y Chen; N Yu Vorobyova)*	**Induced mutagenesis: a network of interplaying processes** *(RP Fuchs; S Boiteux; JM Essigmann; Z Livneh; OS Fedorova; IR Grin)*
	14:30	**Tolerance of DNA damage: translesion DNA synthesis** *(AR Lehmann; EC Friedberg; K Takata; H Ide; JS Hoffmann; GI Karras)*	**Gene-environment interactions in noncancer diseases** *(A Izzotti; FJ van Schooten; L Migliore; C-C Yeh; H Groot; ET Sakamoto-Hojo)*	**Young Scientist Session II** *(SH Doak; Young R Seo; BLF Schamber-Reis; M Longobardi; H Kuasne; MF Simoniello; IV Villela)*	**Dietary prevention of mutation and cancer** *(S Knasmüller; C Gerhäuser; P Dolara; AR Collins; MG Manjanatha; K Vanhees)*	**Physiological mutagenesis in immunity** *(MS Neuberger; H Jacobs; J Miller; CA Reynaud; S Conticello)*
	17:00	**Y-J Surh,** *Seoul National University, Seoul, Korea*				
	18:00	**IAEMS General Assembly**				
Tue, August 25	8:30	**Advances in the assessment of exposure and early biological effects** *(S Bonassi; DH Phillips; H Autrup; RS Paules; PB Farmer)*	**Nucleotide excision repair and transcription: mechanisms and clinical implications** *(JM Egly; D Orioli; L Mullenders; P Cooper; A Khobta)*	**Dietary factors, mutation and cancer** *(L Ferguson; B Tudek; VM Arlt; A Mally; P Mosesso; YY Gong)*	**Reports from the 5th International Workshop on Genotoxicity Testing** *(D Kirkland; S Galloway; M Moore; P Kasper; S Pfuhler; M Hayashi; V Thybaud)*	**Mechanisms of untargeted mutagenesis** *(LA Loeb; TA Kunkel; BD Preston; J Schimenti; P Menichini)*
	11:00	**M Radman,** University of Paris 5, Paris, France **CM Croce** Ohio State University, Columbus, OH, USA				

Mutational and epigenetic mechanisms	***DNA damage responses***	***Environmental mutagenesis***
Mutagenesis and health effects	***Prevention of mutation-related diseases***	***Risk assessment***

10th International Conference on Environmental Mutagens (ICEM)

39th Annual Meeting of the European Environmental Mutagen Society (EEMS)
18th Annual Meeting of the Italian Environmental Mutagen Society (SIMA)

The Renaissance of Environmental Mutagenesis

Firenze, Italy, August 20-25, 2009

Scientific Program and Abstract Book

Under the auspices of

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INDEX

COMMITTEES	4
WELCOME	7
GENERAL INFORMATION	8
FLORENCE MAP	12
ACKNOWLEDGEMENTS	13
EXHIBITORS' LIST AND MAP	15
CONGRESS AREA MAP	16
PROGRAMME AT A GLANCE	18
SCIENTIFIC PROGRAM	
Thursday, August 20	21
Friday, August 21	21
Saturday, August 22	29
Sunday, August 23	35
Monday, August 24	41
Tuesday, August 25	47
ABSTRACTS	
Plenary lectures and Symposium abstracts	51
Forum abstracts	107
Abstracts of posters and selected oral presentations	115
Authors' index	302

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WELCOME

The International Association of Environmental Mutagen Societies (IAEMS), founded in 1973, includes regional and national member Societies spread throughout all continents. The official congress of IAEMS, called ICEM (International Conference on Environmental Mutagens), are held every 4 years. In 2009, a new ICEM event occurs. It is the tenth in a series of successful conferences, recognized for the high scientific standard, their broad attendance from all over the world, and the catalytic role in the area of environmental mutagenesis.

The 10th ICEM will be held in Firenze (Florence, Italy) on August 20-25, 2009. We entitled it "The Renaissance of Environmental Mutagenesis" first because Firenze was the heart of the Renaissance period, one of the most fascinating period of time in human history that was characterized by a huge development in the arts. Thus, Firenze is a living art museum. The second reason for that denomination is our hope that the 10th ICEM may give a new impulse and precise physiognomy to the vast and multidisciplinary area of environmental mutagenesis and related fields. The program will embrace a broad variety of subjects in basic and applied research, from the traditional themes to the fascinating novelties of the postgenomic era. The Conference Committees have done their best to offer an important scientific event and an appealing social program. The venue will be the International Conference Center, located in the city center, at walking distance from the railway station and almost 300 hotels, closed to the most famous museums and monuments. The main building is the Palazzo dei Congressi, a 19th century villa, hosting an auditorium that accomodates 1000 seats. In the beautiful garden, other buildings contain several conference rooms for parallel sessions.

According to the ICEM tradition, satellite meetings will be held in Italy and neighboring countries before and after the main conference: the 5th International Workshop on Genotoxicity Tests (IWGT), held in Basel, Switzerland on August 17-19, the 8th International Comet Assay Workshop, organized in Perugia, Italy on August 27-30 and the Workshop on Genomics in Cancer Risk Assessment held in Venice on August 27-28.

We enthusiastically look forward to welcoming you to the 10th ICEM in Firenze.

Silvio De Flora, Chair

Eugenia Dogliotti, Program Committee Chair

GENERAL INFORMATION

Congress venue
Palazzo dei Congressi and Palazzo degli Affari
Piazza Adua, 1
50123 Florence, Italy
www.firenzefiera.it
Telephone: +39/055/49.72.1
Fax: +39/055/49.73.237

The congress venue is located in the city centre just in front of the "Santa Maria Novella" main railway station in Florence and is within walking distance of the hotels reserved downtown.
The "A. Vespucci" airport of Florence is only 5 km away from the city centre and is connected with the most important European cities. Alternatively the "G. Galilei" airport of Pisa is situated 90 km from Florence offering confortable direct shuttle trains and buses operating almost every hour during the day.

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The registration desk is located in the basement of Palazzo dei Congressi building (see map) and is open with the following time schedule during the congress:

Thursday,	August 20	7.30-19.30
Friday,	August 21	7.30-19.30
Saturday,	August 22	7.30-19.30
Sunday,	August 23	7.30-17.00
Monday,	August 24	7.30-19.30
Tuesday,	August 25	7.30-13.00

On site registrations (€, VAT included)	**On site registration fees**
Standard	**€ 675,00**
Student	**€ 475,00**
Course N. 1 - August 20 (8.30-13.00) **Title "Micronuclei cytome assay:** **lymphocytes and buccal exfoliated cells"**	**€ 50,00**
Course N. 2 – August 20 (8.30-13.00) **Title "Molecular Epidemiology for Chronic Diseases"**	**€ 50,00**
Daily registration *(maximum one day)*	**€ 190,00**
Accompanying person	**€ 225,00**
Social dinner - reduced fee for fully registered participants only	**€ 35,00**
Social dinner – standard fee for one-day registrants and non-registered persons	**€ 80,00**

The standard and student registration fees include: access to the scientific sessions and exhibition/catering areas, the congress kit and name badge, the program and abstract book, the certificate of attendance, coffee breaks and working lunches, invitation to the opening ceremony scheduled for August 20 at 18.00 in the main hall (Botticelli room) followed by the welcome cocktail.

The course registration fee includes: only access to the meeting room of the selected course with an appropriate pre-paid ticket. As room capacities are limited, on site requests for both courses are accepted on first-come-first-served basis at the registration desk upon availability.

The daily registration fee includes: access to the scientific sessions of the selected day and exhibition/catering areas, the congress kit and name badge, the program and abstract book, the certificate of attendance, coffee breaks and the working lunch of the selected day.
This fee is applicable ***for one day only*** starting from August 21, more days imply a standard registration. In case of request for the social dinner the full price is charged (€ 80,00).

The accompanying person registration fee includes: the welcome cocktail, the half-day city tour of Florence and the social dinner. Non-registered accompanying persons can buy full price tickets for the social dinner (€ 80,00) at the social program desk upon availability on first-come-first-served basis.

Social program

Pre-registered delegates and accompanying persons are kindly invited to the opening ceremony scheduled for August 20 at 18.00 in the main hall (Botticelli room) followed by the welcome cocktail.
The social dinner, scheduled for August 23 at 20.30, takes place in a selected area of the Fortezza da Basso, one of the most visible and important historical venues in Florence of the Medicean period, a significant example in the history of military architecture design by Antonio da Sangallo and finished in 1535. The Fortezza is located at about 5 minutes walking distance from the congress venue and from most hotels downtown.
The cost is not included in the registration fee except for pre-registered accompanying persons.
Tickets can be bought separately by fully registered delegates at the cost of € 35,00, by one-day registrants and non-registered accompanying persons at the full price of € 80,00 upon availability.
Pre-booked tickets are required to obtain admission to the Fortezza da Basso and are included in the personal congress kit, that must be collected at the registration desk by pre-registered participants.
Due to organizational reasons and limited space of this social venue early reservations were recommended on first-come-first-served basis, as on site availability cannot be guaranteed.
In case of need for extra tickets please check availability at the social program desk at Palazzo dei Congressi basement floor - see map page 16.

Optional excursions

A program of optional excursions is managed by the official housing bureau and travel agency (O.I.C. Way Srl). Pre-booked tickets are included in the personal congress kit, that must be collected at the registration desk by pre-registered participants.
Staff members at the social program desk are available to give information on excursions and sell tickets for the tours on first-come first-served basis.

Official language

English is the official language of the congress. No simultaneous translation is provided for the scientific sessions.

Personal badge

A name badge is included in the congress kit and must be collected at the registration desk by pre-registered participants.
This badge is the official congress document for delegates and must be worn at all times throughout the congress and evening functions.

Slide preview centre

A computerized slide centre is managing all the presentations and scientific sessions of the congress through a central network.
This office is located in the Palazzo dei Congressi building basement floor (see map page 17) and is available for speakers to deliver their powerpoint presentations with the following timetable:

Thursday,	August	20	7.30-18.30	Sunday,	August	23	7.30-16.00
Friday,	August	21	7.30-18.00	Monday,	August	24	7.30-18.00
Saturday,	August	22	7.30-18.00	Tuesday,	August	25	7.30-12.00

Only computer projection is available. No slide projectors are at disposal in the meeting rooms and it is not possible to directly use personal laptops at the podium. Powerpoint presentations (Windows or MacIntosh/Apple) on USB pens, memory sticks or personal laptops must be delivered to the slide centre at least one hour before the beginning of the session or the day before in case of early morning presentations. This timing allows a smooth uploading of all presentations to the central computerized network and a swift transmission to the assigned meeting rooms. Staff members at the slide centre are also available to help those speakers wishing to rehearse their presentations.

Instructions for oral and poster presentations

Oral presentations: as the scientific program is made of parallel sessions, speakers are recom-

mended to respect the allotted time given for their presentations to contribute to the smooth running of all scheduled sessions.
The computer slides must be written in English, the presentations must be prepared on powerpoint and delivered to the slide centre at least one hour before the beginning of the session to be uploaded and transferred to the PC of the podium in the assigned meeting room.
Presenters must follow the technical indications included in the above chapter (slide preview centre).
Registration at the congress is absolutely necessary for oral presenters.

Poster presentations: posters must be written in English and no bigger than 90 cm wide and 120 cm high. The title of the presentation and the authors' names must be indicated at the top of the poster.
Double-sided tape is available in the poster area and at the secretariat desk to fix the posters to the boards. It is absolutely forbidden to use drawing-pins, nails or similar.
The poster display area is located in the Palazzo dei Congressi building underground level along the galleries above the exhibition (see map page 17).
Each poster has the opportunity to be on display one day during the conference either on August 21, 22 or 24 and is uniquely identified by a code number reported in the scientific program/abstract book, on site distributed with the congress kit to registered participants, where the daily display shifts of all accepted posters are also included.

Posters will be displayed according to the following rotation schedule:

Day 12.30-14.30	Mutational and epigenetic mechanisms	DNA damage responces	Environmental mutagenesis	Mutagenesis and health effects	Prevention of mutation-related diseases	Risk assessment
August 21	ME001-011	DD001-060	EM001-060	MH001-032	PD001-013	RA001-037
August 22	ME012-021	DD061-120	EM061-120	MH033-064	PD014-026	RA038-075
August 24	ME022-032	DD121-180	EM121-179	MH065-095	PD027-039	RA076-113

Posters must be put up at 8.30 in the morning on the assigned day and numbered board. They must be removed by presenters the same day of the presentation at the end of the session no later than 17.30.
Posters left on the boards after the above deadline will be destroyed.
The presenting authors wearing the personal badges are expected to be present in the poster area close to their display boards during the assigned viewing sessions and are encouraged to bring some handouts of their posters to take the best opportunity to communicate their data to viewers.
Registration at the congress is absolutely necessary for poster presenting authors.

Exhibition
An exhibition area, located in the Palazzo dei Congressi basement floor (see map page 16), is scheduled from August 20 at 16.00 to August 25 until 13.00 following the same timetable as the scientific sessions.
Pharmaceutical and biotechnology companies, medical publishers and scientific societies are represented in the exhibition. Exhibitors may refer to the registration desk located in the nearby area for any technical need. See the exhibitors' list and detailed map (pages 15).

Registration – cancellations and refunds
In case of cancellation or name change written requests were to be sent to the organizing secretariat prior to the congress start.
The paid amount for registration fee is refunded with a deduction of € 25,00 that is invoiced as penalty.
No refund is processed for cancellations received after the congress end.
All authorized refunds are processed only after the event.

Hotel – cancellations and refunds
In case of cancellation or change written requests were to be sent to the organizing secretariat prior to the congress. **Until June 30** the paid amount was refunded with a deduction of 50% that was invoiced as penalty. No refund is processed for cancellations received after June 30.
All authorized refunds are made only after the congress. Hotel reservations are automatically cancelled after one night of no-show. No-shows and booking fees cannot be refunded in any way.

Relax area and internet point
A comfortable relax area equipped with a free of charge internet point is available for delegates to check and send personal e-mails on a reasonable basis from August 21 to 25 during the congress, located in the venue garden between the two congress buildings (Limonaia room - see map page 17).

Catering services
Coffee tickets and working lunches are included in the registration fees according to the scientific program daily schedule; the catering services are provided in selected areas within the two congress buildings near to the meeting rooms. Different bars serving beverages and snacks are also available in the venue and outside downtown Florence.

Insurance
The congress organizers are not liable for personal injuries or loss of/ damage to property belonging to delegates (or their accompanying persons), occurring either during or as a result of the congress. Participants are required to make own arrangements for health and travel insurance in the countries of origin.

Mobile phones
Participants are kindly requested to keep the mobile phones turned off in the meeting rooms during the scientific sessions.

Smoking policy
In accordance with the Italian regulations this congress follows a strict no-smoking policy. Therefore participants are kindly requested to refrain from smoking in any area within the congress venue.

Travel agency
The official travel agency O.I.C. Way Srl is at the participants' direct disposal at the registration desk and available for hotel reservations, train and/or flight bookings from August 20 to 25.

Weather
At the end of August the weather in Florence is usually quite warm during the day with temperatures that can exceed 30°C and more pleasant in the evening. Rainfall during this period should be at its lowest, but raincoats and umbrellas are always recommended for occasional showers.

Means of transportation in Florence
From the airport of Florence: a bus service (Linea Vola-in-bus shuttle) is operating every 20-25 minutes from the "A. Vespucci" airport to the "Santa Maria Novella" main railway station in Florence with an average travel time of 30 minutes. Tickets can be bought directly on the bus.
For detailed timetable check www.ataf.net
From the airport of Pisa: direct trains and buses to the "Santa Maria Novella" railway station are available every day from the "G. Galilei" airport with an average travel time of 60 minutes.
For detailed timetable check www.trenitalia.it
From the "Santa Maria Novella" railway station of Florence: it takes only 5 minutes to reach the congress venue on foot.
Taxis: in Florence they can be ordered by phone (Tel. 055/4242 or 055/4390) or found at the official taxi stands downtown. Taxis cannot be hailed on the street.
Buses: Florence has a comprehensive network of local bus routes. Most buses stop at the "Santa Maria Novella" railway station near to the congress venue. Tickets can be purchased at coffee bars, tobacconists and kiosks downtown. The minimum fare for a multiple trip ticket valid for 70 minutes is €1,20.
For detailed information check www.ataf.net

Car parking
Different fee parking lots are available near to the congress venue where delegates may park the car. A detailed city map is included in the congress kit.

Useful telephone numbers in Florence

Medical emergency	118	Florence airport – information	055/30.61.300
Fire emergency	115	Florence airport – lost luggage	055/30.61.302
Police	113	For international calls from Italy	00 + country code + number
Taxi call	055/4242 or 4390	For international calls to Italy	+39 + city code + number

FLORENCE MAP

****

ADLER CAVALIERI A3
ALBANI B3
ANGLO AMERICAN A3
ATLANTIC PALACE B3
BAGLIONI B3
BERCHIELLI B4
BRUNELLESCHI C4
CROCE DI MALTA B3
EMBASSY A2
KRAFT A3
LONDRA A3
MINERVA B3
PALAZZO OGNISSANTI A3
PALAZZO RICASOLI D2
ROMA B4
SANTA MARIA NOVELLA B3
STROZZI B4

**

BELLETTINI C3
DECO B3
FIORITA B2
LOMBARDI B3
LORENA B3
MEDICI C4
NUOVA ITALIA B3
ROMAGNA B3

***

AURORA B3
BEACCI TORNABUONI B4
BEATRICE B3
BOCCACCIO B3
BOTTICELLI C3
CARAVAGGIO C2
CELLAI C2
CORONA D'ITALIA B3
IL GUELFO BIANCO C3
MACHIAVELLI PALACE B3
MALASPINA C2
PALAZZO VECCHIO B3
PARIS B4
PITTI PALACE B5
REX B3
ROSSO B3
SAN GIORGIO B3
UNIVERSO B3

*

B&B
A TEATRO D4
IL GHIRO B2
LA RESIDENZA DEL PROCONSOLO C4
RESIDENZA DEI PUCCI C3
RESIDENZA GIULIA A2
RESIDENZA IL MAGGIO A3

ACKNOWLEDGEMENTS

The Organizers wish to thank the following companies and societies that have contributed to the 10th International Conference on Environmental Mutagens:

Basf Italia

Bayer Schering Pharma

Biopredic International

Brazilian Environmental Mutagen Society SBMCTA

Cassa di Risparmio di Firenze

Carl Zeiss

Compucyte Corporation

Covance

Dow AgroSciences Italia

Du Pont De Nemours Italiana

DuPont Haskell Global Centers for

Health and Environmental Science

ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals)

ECNIS (Environmental Cancer Risk, Nutrition and Individual Susceptibility Network of Excellence)

The Ellison Medical Foundation

Elsevier

Florence Convention Bureau

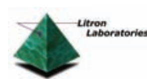
Harlan Laboratories

Istituto Italo-Latino Americano

Imstar

Institute of Hygiene - Università Cattolica del Sacro Cuore

LC Sciences



Litron Laboratories

Metasystems

Microptic Cytogenetic Services

Nestlè Research Centre

EU Integrated Project NewGeneris

Novartis Pharma

Novartis Vaccines and Diagnostics

EU Network of Excellence NuGO (The European nutrigenomics organization)

Oxford University Press

Perceptive Instruments

Procter & Gamble

Regione Toscana

Research Toxicology Centre

Sanofi Aventis

Solvay

Società di Scienze Farmacologiche Applicate

Università degli Studi di Roma Tor Vergata
Dipartimento di Biopatologia e Diagnostica per Immagini

US National Institute of Health

Wiley-Blackwell

Xenometrix

EXHIBITORS' LIST

BIOPREDIC INTERNATIONAL, FRANCE – ***Booth No.. 10***

BRAZILIAN ENVIRONMENTAL MUTAGEN SOCIETY, BRAZIL – ***Booth No. 13***

CARL ZEISS, ITALY - ***Booth No. 4***

COMPUCYTE CORPORATION, USA – ***Booth No. 16***

COVANCE CAPS, UNITED KINGDOM – ***Booth No. 6-7***

ELSEVIER, THE NETHERLANDS – ***Booth No. 5***

HARLAN LABORATORIES, SWITZERLAND – ***Booth No. 1***

IMSTAR, FRANCE – ***Booth No. 8***

LC SCIENCES, USA – ***Booth No. 11***

METASYSTEMS, GERMANY – ***Booth No. 3***

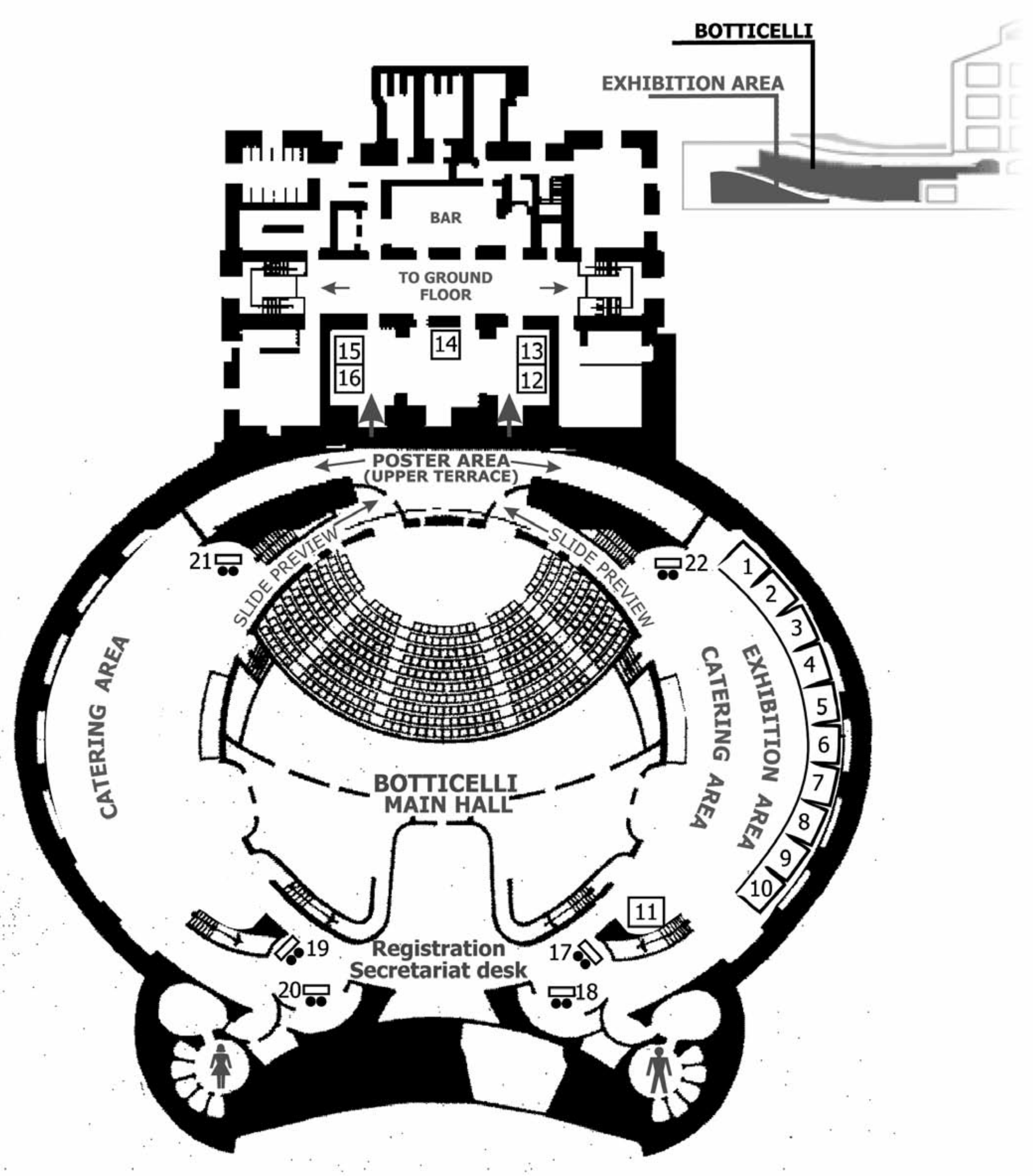
OXFORD UNIVERSITY PRESS, UNITED KINGDOM – ***Booth No. 12***

PERCEPTIVE INSTRUMENTS, UNITED KINGDOM – ***Booth No. 9***

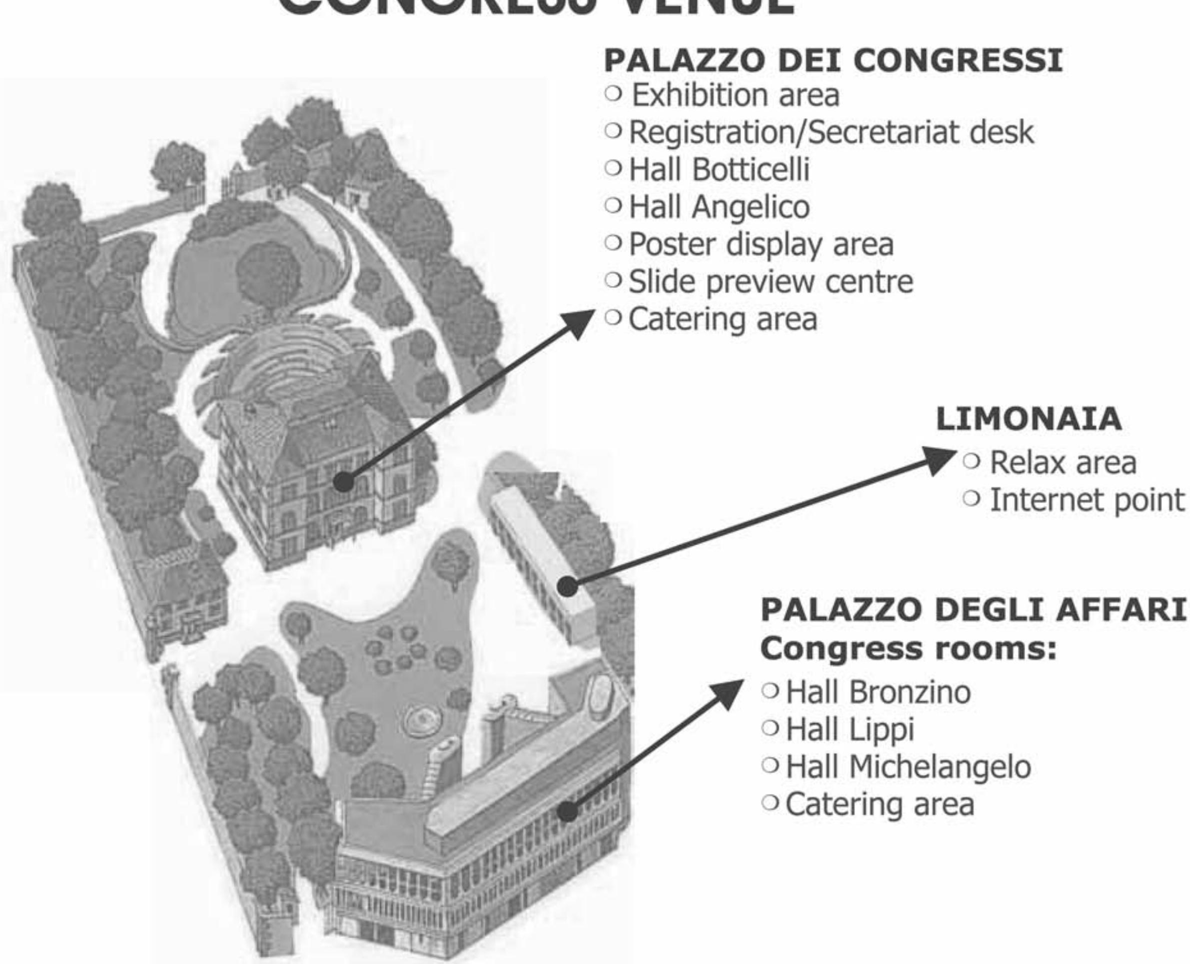
WILEY - BLACKWELL, USA – ***Booth No. 22***

XENO.METRIX, SWITZERLAND - ***Booth No. 2***

EXHIBITION MAP



CONGRESS AREA MAP



PROGRAM AT A GLANCE

HALL	BOTTICELLI	ANGELICO	LIPPI	BRONZINO	MICHELANGELO
Thursday, August 20					
18.00	*Opening Ceremony* PC Hanawalt				
19.30	*Welcome reception*				
Friday, August 21					
8.30	HRH Princess Chulaborn P Grandjean				
10.30	CURRENT ISSUES IN MODE OF ACTION ANALYSIS AND THEIR USE IN CANCER RISK ASSESSMENT	HEALTH EFFECTS OF NUCLEOTIDE POOL DAMAGE	MECHANISMS OF CELL DEATH AND SURVIVAL	NANOTOXICOLOGY: MECHANISMS AND EFFECTS	EPIGENETICS: THE NEW FRONTIER IN ENVIRONMENTAL MUTAGENESIS
12.30	Lunch and poster viewing (Poster ME001-011, DD001-060, EM001-060, MH001-032, PD001-013, RA001-037)				
14.30	NOVEL INSIGHTS INTO DNA DAMAGE SIGNALLING AND REPAIR	CHILDREN CANCER RISK	EPIGENOME AND THE ENVIRONMENT: FROM UNDERSTANDING THE MECHANISMS TO RISK ASSESSMENT	NEW DEVELOPMENTS IN THE GENOTOXICITY OF SOIL AND WATER	NEW DEVELOPMENTS IN REGULATORY GENETIC TOXICOLOGY
17.00	R De Maria				
18.00	RATIONALE OF GENOTOXICITY TESTING OF NANOMATERIALS	ECOGENOTOXICOLOGY: PAST SUCCESSES AND FUTURE NEEDS	PHILOSOPHY, SCIENCE AND ART IN RENAISSANCE ITALY	ANTIMUTAGENESIS AND CHEMOPREVENTION IN A U-SHAPED WORLD	MUTAGENIC MODE OF ACTION FOR CARCINOGENS: HOW HIGH IS THE BURDEN OF PROOF?
Saturday, August 22					
8.30	CP Wild SH Wilson				
10.30	IS THE ERA OF GENOME WIDE ASSOCIATION ALREADY OVER?	CELL TYPE AND TIME SPECIFICITY OF DNA DAMAGE RESPONSE	BIOLOGY, EPIDEMIOLOGY, AND PREVENTION OF CANCER-ASSOCIATED MICROBIAL DISEASES	ENVIRONMENTAL POLLUTION: THE ROLE OF SENTINEL SPECIES AND BIOMARKERS	CHROMOSOME SEGREGATION AND GENOMIC INSTABILITY
12.30	Lunch and poster viewing (Poster ME012-021, DD061-120, EM061-120, MH033-064, PD014-026, RA038-075)				
14.30	CHALLENGING ENVIRONMENTAL HEALTH PROBLEMS AROUND THE WORLD	DNA DAMAGE, REPAIR AND AGING	MOLECULAR EPIDEMIOLOGY AND ETHICS	NUTRIGENOMICS AND PUBLIC HEALTH	MICRORNAS AND THEIR REGULATION
17.00	JHJ Hoeijmakers				
18.00	WORKSHOP ON CYTOTOXICITY MEASURES IN THE IN VITRO MICRONUCLEUS TEST	RISK ASSESSMENT OF GENOTOXIC TRACE SUBSTANCES IN FOOD			PREPARING THE NEXT GENERATION OF SCIENTISTS THROUGH EDUCATION AND RESEARCH

PROGRAM AT A GLANCE

HALL	BOTTICELLI	ANGELICO	LIPPI	BRONZINO	MICHELANGELO
Sunday, August 23					
8.30	L Samson S De Flora				
10.30	CANCER MODELS AND MECHANISMS	CELLULAR DEFENCES AGAINST OXIDATIVE DAMAGE	CRITICAL ISSUES ON ENVIRONMENTAL GENOTOXICITY IN LATIN AMERICA	PHARMACOLOGICAL PREVENTION OF MUTATION AND CANCER	NEW INSIGHTS IN GERM CELL MUTAGENESIS
13.30	DNA DAMAGE RESPONSE AS THERAPEUTIC TARGET	SYSTEMS BIOLOGY APPROACHES TO ENVIRONMENTAL CARCINOGENESIS AND RELATED BIOMARKERS	YOUNG SCIENTIST SESSION I	NEW DATA INITIATIVES AND PREDICTIVE APPROACHES FOR MUTAGENICITY AND CARCINOGENICITY	*IN VIVO* MUTAGENESIS: RECENT ADVANCES AND FUTURE PROSPECTS
16.00	*Free afternoon (Optional tours)*				
20.30	*Social dinner*				
Monday, August 24					
8.30	L Luzzatto K Wakabayashi				
10.30	LOOKING INTO THE FUTURE: GENOME WIDE TECHNOLOGIES IN TOXICOLOGY	REGULATION OF DNA DAMAGE RESPONSE: LESSONS LEARNED FROM DOUBLE STRAND BREAK REPAIR PATHWAYS	PUBLIC HEALTH GENOMICS	BIOLOGICAL RISKS FROM SPACE RADIATION ENVIRONMENTS	INDUCED MUTAGENESIS: A NETWORK OF INTERPLAYING PROCESSES
12.30	Lunch and poster viewing (Poster ME022-032, DD121-180, EM121-179, MH065-095, PD027-039, RA076-113)				
14.30	TOLERANCE OF DNA DAMAGE: TRANSLESION DNA SYNTHESIS	GENE-ENVIRONMENT INTERACTIONS IN NONCANCER DISEASES	YOUNG SCIENTIST SESSION II	DIETARY PREVENTION OF MUTATION AND CANCER	PHYSIOLOGICAL MUTAGENESIS IN IMMUNITY
17.00	Y-J Surh				
18.00	IAEMS General Assembly				
Tuesday, August 25					
8.30	ADVANCES IN THE ASSESSMENT OF EXPOSURE AND EARLY BIOLOGICAL EFFECTS	NUCLEOTIDE EXCISION REPAIR AND TRANSCRIPTION: MECHANISMS AND CLINICAL IMPLICATIONS	DIETARY FACTORS, MUTATION AND CANCER	REPORTS FROM THE 5TH INTERNATIONAL WORKSHOP ON GENOTOXICITY TESTING	MECHANISMS OF UNTARGETED MUTAGENESIS
11.00	M Radman C Croce Closing ceremony				

Science For A Better Life

There are approximately 30,000 different diseases known today. A satisfactory form of therapy is only available for one-third of them.

Bayer HealthCare, a subgroup of Bayer AG, develops innovative products to improve health and quality of life for both humans and animals. In addition to activities in the fields of Animal Health, Consumer Care and Diabetes Care, the company also focuses on specialty pharmaceuticals. Bayer HealthCare is an international leader in this sector.

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SCIENTIFIC PROGRAM

Thursday, August 20

8.30-13.00 **Pre Conference Courses**

Angelico Hall
MOLECULAR EPIDEMIOLOGY FOR CHRONIC DISEASES

Bronzino Hall
MICRONUCLEUS CYTOME ASSAY: LYMPHOCYTES AND BUCCAL EXFOLIATED CELLS

Botticelli Hall
18.00 **Opening Ceremony**

11th ICEM Announcement
L. Ribeiro, E. Zamorano-Ponce

18.45 IN001 **Keynote Lecture**
Introduction: E. Zamorano-Ponce, President Asociación Latinoamericana de Mutagénesis, Carcinogénesis y Teratogénesis Ambiental (ALAMCTA)

LESION SENSING AND DECISION POINTS IN THE DNA DAMAGE RESPONSE
P. C. Hanawalt*,* Stanford University, Stanford, CA, USA

19.30 **Welcome Reception**

Friday, August 21

Botticelli Hall
8.30 IN002 **Plenary Lecture**
Introduction: D.M. DeMarini, President IAEMS

CANCER RISK FROM EXPOSURE TO URBAN AIR POLLUTION
HRH Princess M. Chulabhorn, Chulabhorn Research Institute, Bangkok**,** Thailand

9.15 IN003 **Plenary Lecture**
Chair: D. Kirkland, President-Elect European EMS

SEVEN DEADLY SINS OF ENVIRONMENTAL RESEARCH
P. Grandjean, University of Southern Denmark, Odense, Denmark

10.00 **Coffee break**

10.30-12.30 **Parallel Symposia**

Michelangelo Hall
Mutational and epigenetic mechanisms
EPIGENETICS: THE NEW FRONTIER IN ENVIRONMENTAL MUTAGENESIS
Chairs: R. Feil, M. Hanson

10.30 IN004 EPIGENETIC MECHANISMS AND THE REGULATION OF GENOMIC IMPRINTING IN MAMMALS
R. Feil, CNRS Montpellier, France

10.55 IN005 DEVELOPING HUMAN EMBRYONIC STEM CELLS TO MODEL ENVIRONMENTAL EFFECTS ON THE DEVELOPING EPIGENOME
L.E. Young, University of Nottingham, UK.

11.20 IN006 Epigenetic Regulation of Aging
M.F. Fraga, CNB-CSIC, Madrid, Spain

11.45 IN007 EPIGENETIC PROCESSES IN DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE (DOHaD)
M. Hanson, University of Southampton, UK

12.10 ME021 HYPERMETHYLATION OF TUMOUR SUPPRESSOR GENES IN LUNG TUMOURS FROM NEVER-SMOKERS WITH AND WITHOUT SECOND-HAND TOBACCO SMOKE EXPOSURE
S. *Jarmalaite*, Vilnius University, Vilnius, Lithuania

12.20 EM153 PERSISTENT DYSREGULATION OF DNA METHYLATION IN CELLS WITH ARSENIC-INDUCED GENOMIC INSTABILITY
M. Mauro, New York University, Tuxedo, NY, USA and University of Palermo, Italy

Lippi Hall
DNA damage responses
MECHANISMS OF CELL DEATH AND SURVIVAL
Chairs: B. Kaina, A. Antoccia

10.30 IN008 SURVIVAL AND DEATH STRATEGIES IN CELLS EXPOSED TO GENOTOXINS
B. Kaina, University Medical Center Mainz, Germany

10.55 IN009 TRANSCRIPTIONAL INHIBITION BY DNA DAMAGE AS A TRIGGER OF CELL DEATH
M. Ljungman, University of Michigan, Ann Arbor, MI, USA

11.15 IN010 DIFFERENT MODES OF CELL DEATH INDUCED BY DNA DAMAGE
B. Zhivotovsky, Karolinska Institutet, Stockholm, Sweden

11.35 IN011 ROLE OF DNA-PKcs-PIDDosome IN DNA DAMAGE RESPONSE
C. Du, The University of Cincinnati, OH, USA

11.55 IN012 CELL-CYCLE BLOCKAGE AFFECTS DNA DAMAGE RESPONSES THAT LEAD TO DEATH IN HUMAN PRIMARY FIBROBLASTS.
C.F. Menck, University of Sao Paulo, Brazil

12.15 DD30 ROLE OF THE APOPTOSIS-MODULATORY MOLECULE NF-kB IN DNA REPAIR
M. Volcic, Ulm University, Ulm, Germany

Bronzino Hall
Environmental Mutagenesis
NANOTOXICOLOGY: MECHANISMS and EFFECTS
(This Symposium is partially supported by ECETOC)
Chairs: G. Oberdörster, L. Tran

10.30 IN013 TOXICITY ASSESSMENT OF NANOPARTICLES
G. Oberdörster, University of Rochester, NY, USA

11.00 IN014 PHYSICO-CHEMICAL FEATURES IN THE TOXICITY OF ENGINEERED NANOPARTICLES
B. Fubini, University of Torino, Italy

11.20 IN015 DISTRIBUTION AND EFFECTS OF NANOMATERIALS AFTER INHALATION AND *I.V.* INJECTION IN RATS
R. Landsiedel, BASF SE, Ludwigshafen, Germany

11.40 IN016 MECHANISMS OF NANOMATERIALS GENOTOXICITY
M. Kirsch-Volders, Vrije Universiteit Brussels, Belgium

12.00 IN017 POTENTIAL PULMONARY EFFECTS OF SINGLE-WALLED CARBON NANOTUBE (SWCNT) EXPOSURE: IN VITRO GENOTOXIC EFFECTS
V. Castranova, National Institute for Occupational Safety and Health, Morgantown, WV, USA

12.20 CONCLUSIONS
L. Tran, Institute of Occupational Medicine, Edinburgh, UK

Angelico Hall
Mutagenesis and health effects
HEALTH EFFECTS OF NUCLEOTIDE POOL DAMAGE
Chairs: T. Nohmi, M. Bignami

10.30 IN018 ERRONEOUS INCORPORATION OF OXIDIZED NUCLEOTIDES BY Y-FAMILY DNA POLYMERASES
T. Nohmi, National Institute of Health Sciences, Tokyo, Japan

10.55 IN019 PROGRAMMED CELL DEATH TRIGGERED BY NUCLEOTIDE POOL DAMAGE
Y. Nakabeppu, Kyushu University, Fukuoka, Japan

11.15 IN020 MULTIPLE ROLES OF THE MTH1 HYDROLASE: PROTECTION AGAINST NEURODEGENERATION AND CONTROL OF LIFE SPAN
M. Bignami, Istituto Superiore di Sanità, Roma, Italy

11.35 IN021 INCORPORATION OF EXTRACELLULAR 8-oxodG INTO DNA AND RNA REQUIRES PURINE NUCLEOSIDE PHOSPHORYLASE IN CULTURED MAMMALIAN CELLS AND MICE.
P.T. Henderson, University of California, Sacramento, CA, USA

11.55 IN022 MUTAGENICITY OF OXIDIZED DNA PRECURSORS IN LIVING CELLS: ROLES OF NUCLEOTIDE POOL SANITIZATION AND DNA REPAIR ENZYMES, AND Y-FAMILY DNA POLYMERASES
H. Kamiya, Hokkaido University, Sapporo, Japan

12.15 DD44 GENOTOXIC ACTIVITY INDUCED BY VARIOUS *H. Pylori* STRAINS IS ASSOCIATED WITH A DOWNREGULATION OF DNA MISMATCH REPAIR GENES EXPRESSION
E. Touati, Institut Pasteur, Paris, France

Botticelli Hall
Risk assessment
CURRENT ISSUES IN MODE OF ACTION ANALYSIS AND THEIR USE IN CANCER RISK ASSESSMENT
Chairs: N. Keshawa, D. Eastmond

10.30 IN023 AN OVERVIEW OF CURRENT ISSUES IN MODE OF ACTION ANALYSIS AND THEIR USE IN CANCER RISK ASSESSMENT
N. Keshava, U.S. Environmental Protection Agency, Washington DC, USA

10.50 IN024 ASSESSING IN VITRO DOSE-RESPONSE RELATIONSHIPS FOR ANEUGENS
D.A. Eastmond, University of California, Riverside, CA, USA

11.10 IN025 THE MUTAGENIC POTENTIAL OF FORMALDEHYDE AND ITS RELEVANCE FOR CARCINOGENESIS
G. Speit, Universität Ulm, Germany

11.30 IN026 EMS IN VIRACEPT - A LESSON ON MUTATION THRESHOLDS FOR ALKYLATING AGENTS
L. Müller, F. Hoffmann-La Roche, Basel, Switzerland

11.50 IN027 FOOD CADMIUM AND THE RISK OF HORMONE-RELATED CANCERS: A POPULATION-BASED PROSPECTIVE COHORT STUDY
A. Åkesson, Karolinska Institutet, Stockholm, Sweden

12.10 IN028 ANALYSIS AND INCORPORATION OF MECHANISTIC DATA IN DECISION-MAKING ON SEVERAL CARCINOGENS AT IARC
R. Baan, International Agency for Research on Cancer, Lyon, France

12.30-14-30 **Lunch and Poster viewing**
ME001-011; DD001-060; EM001-060;
MH001-032; PD001-013; RA001-037

14.30-16.30 **Parallel symposia**

Lippi Hall
Mutational and epigenetic mechanisms
EPIGENOME AND THE ENVIRONMENT: FROM UNDERSTANDING THE MECHANISMS TO RISK ASSESSMENT
Chairs: O. Kovalchuk, J. Trosko

14.30 IN029 ROLE OF EPIGENETIC DEREGULATION IN RADIATION-INDUCED GENOME INSTABILITY AND CARCINOGENESIS
O. Kovalchuk, University of Lethbridge, AB, Canada

14.55 IN030 ROLE OF EPIGENETIC EVENTS IN GENOTOXIC LIVER CARCINOGENESIS
I.P. Pogribny, Food and Drug Administration, Jefferson, AR, USA

15.15 IN031 DNA METHYLATION AND PERSISTENT BYSTANDER EFFECT: MEMORY OF AN INSULT
B.P. Engelward, Massachusetts Institute of Technology, Cambridge, MA, USA

15.35 IN032 EPIGENETIC CHANGES UNDERLIE ORGANISMAL ADAPTATION TO CHANGING ENVIRONMENTS
I. Kovalchuk, University of Lethbridge, AB, Canada

15.55 IN033 SYSTEMS INTEGRATION OF HUMAN STEM CELLS, EPI-TOXICOGENOMICS, CELL-CELL COMMUNICATION: THE BARKER HYPOTHESIS AND CHRONIC HUMAN DISEASES.
J.E. Trosko, Michigan State University, East Lansing, MI, USA

16.15 ME025 THE ASSOCIATION OF METHYLATION PATTERN AND PRENATAL POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) EXPOSURE
D. Tang, Columbia University, New York, NY, USA

Botticelli Hall
DNA damage responses
NOVEL INSIGHTS INTO DNA DAMAGE SIGNALLING AND REPAIR
Chairs: J. Jiricny, P. Menichini

14.30 IN034 BASE- AND MISMATCH REPAIR INTERFERENCE DURING SOMATIC HYPERMUTATION
J. Jiricny, University of Zurich, Switzerland

14.50 IN035 MRE11 INTERACTIONS WITH DNA AND RAD50 ATPASE PLUS NBS1 INTERACTIONS WITH CTIP CONNECT DSDNA REPAIR MACHIN ERY AND BREAK SIGNALING
J.A. Tainer, Lawrence Berkeley National Lab, La Jolla, CA, USA

15.10 IN036 HUMAN ELG1 REGULATES THE LEVEL OF UBIQUITINATED PCNA THROUGH INTERACTIONS WITH PCNA AND USP1
K.J. Myung, National Institute of Health, Bethesda, MD, USA

15.30 IN037 DNA BASE EXCISION REPAIR IN (EPI)GENOME MAINTENANCE
P. Schär , University of Basel, Switzerland

15.50 IN038 PROPERTIES OF NEIL3 IN PROLIFERATION AND DIFFERENTIATION OF STEM/PROGENITOR CELLS
M. Bjørås, University of Oslo, Norway

16.10 IN039 ESTABLISHMENT OF REPORTER ASSAY YEASTS RESPONDING TO LIGANDS OF VARIOUS HUMAN NUCLEAR RECEPTORS, AND ROLES OF AHR LIGANDS TO INDUCE OR PROTECT FROM DNA DAMAGE FORMATION
T. Yagi, Osaka Prefecture University, Osaka, Japan

Bronzino Hall
Environmental Mutagenesis
NEW DEVELOPMENTS IN THE GENOTOXICITY OF SOIL AND WATER
Chairs: D. DeMarini, R. Marcos

14.30 IN040 THE GENOTOXIC HAZARDS AND CARCINOGENIC RISKS OF PAH CONTAMINATED Soils
P.A. White, Health Canada, Ottawa, QC, Canada.

14.55 IN041 POTENTIAL IMPLICATIONS OF SOIL POLLUTION WITH MUTAGENS IN LUNG CANCER
T. Watanabe, Kyoto Pharmaceutical University, Kyoto, Japan

15.20 IN042 GENOTOXICITY AND CARCINOGENICITY OF DRINKING WATER DISINFECTION BY-PRODUCTS
D.M. DeMarini, U.S. Environmental Protection Agency, Research Triangle Park, NC, USA

15.45 EM109 GENOTOXICITY STUDIES WITH DISINFECTION BYPRODUCTS (DBPs)
R. Marcos, Universitat Autònoma de Barcelona, Bellaterra, Spain

16.10 EM135 AMES II AND HIGH-THOUGHPUT COMET ASSAY FOR EFFICIENT SCREENING OF DRINKING WATER (SOURCES) FOR GENOTOXIC CONTAMINANTS
M.B. Heringa, KWR Watercycle Research Institute, Nieuwegein, The Netherlands

Angelico Hall
Mutagenesis and health effects
CHILDREN CANCER RISK
Chairs: J. Kleinjans, C.P. Wild

14.30 INTRODUCTION
C.P. Wild, International Agency for research on Cancer, Lyon, France

14.40 IN043 ACTIVATION OF INFLAMMATION/NF-kB SIGNALING IN INFANTS BORN TO ARSENIC-EXPOSED MOTHERS
R. Fry, Massachusetts Institute of Technology, Cambridge, MA, USA

15.05 IN044 TRANSCRIPTOMIC ANALYSIS IN UMBILICAL CORD BLOOD OF CHILDREN EXPOSED TO GENOTOXIC COMPOUNDS THROUGH
THEIR MOTHERS DIET
D.M. van Leeuwen, Maastricht University, Maastricht, The Netherlands

15.30 IN045 GENETIC AND ENVIRONMENTAL RISK FACTORS OF CHILDHOOD LEUKEMIA
K. Hemminki, German Cancer Research Center, Heidelberg, Germany

15.55 IN046 THE INTERNATIONAL CHILD CANCER COHORT CONSORTIUM
T. Dwyer, Royal Children’s Hospital, Parkville, Australia

Michelangelo Hall
Risk assessment
NEW DEVELOPMENTS IN REGULATORY GENETIC TOXICOLOGY
Chairs: E. Lorge, V. Thybaud

14.30 IN047 INTEGRATION OF GENOTOXICITY TESTS INTO ROUTINE TOXICITY STUDIES
C. Priestley, AstraZeneca, UK
A. Czich, Sanofi Aventis, Germany
A. Rothfuss, Bayer Schering Pharma AG, Germany

15.30 IN048 THE ILSI-HESI PROJECT COMMITTEE ON THE RELEVANCE AND FOLLOW-UP OF POSITIVE RESULTS IN *IN VITRO* GENETIC TOXICITY (IVGT) TESTING: INTRODUCTION AND FOLLOW-UP OF POSITIVE RESULTS *IN VITRO*
V. Thybaud, Sanofi-Aventis, Vitry sur Seine, France

15.50 IN049 THE ILSI-HESI PROJECT COMMITTEE ON THE RELEVANCE AND FOLLOW-UP OF POSITIVE RESULTS IN IN VITRO GENETIC TOXICITY TESTING (IVGT): QUANTITATIVE ASPECTS OF GENOTOXICITY RISK ASSESSMENT
J.T. MacGregor, Toxicology Consulting Services, Arnold, MD, USA

16.10 IN050 THE ILSI-HESI PROJECT COMMITTEE ON THE RELEVANCE AND FOLLOW-UP OF POSITIVE RESULTS IN IN VITRO GENETIC TOXICITY TESTING (IVGT): EMERGING TECHNOLOGIES FOR THE IMPROVEMENT OF GENOTOXICITY RISK ASSESSMENT
J. Sasaki, Johnson & Johnson Pharmaceutical Research and Development, Raritan, NJ, USA

16.30 **Coffee break**

Botticelli Hall

17.00 IN051 **Plenary Lecture**
Introduction: H.W. Chung, Past-President Korean EMS

CANCER STEM CELLS FROM SOLID TUMORS
R. De Maria, Istituto Superiore di Sanità, Roma, Italy

6ht ICEMHP Announcement
W. Au

18.00-19.30 **Parallel Forum**

Bronzino Hall

FOR01 **ANTIMUTAGENESIS AND CHEMOPREVENTION IN A U-SHAPED WORLD**
Chair: D.J. Waters

Promoting health in a U-shaped world
D.J. Waters, Purdue University, West Lafayette, IN, USA

Cancer and aging: U-shaped response to vitamin D
P. Tuohimaa, University of Tampere, Finland

U-shaped dose response of anti-angiogenic agents
A.R. Reynolds, Institute of Cancer Research, London, UK

FOR02 Defining the optimal dose of selenium for prostate cancer risk reduction
D.J. Waters, Purdue University, West Lafayette, IN, USA

Michelangelo Hall

FOR03 **MUTAGENIC MODE OF ACTION FOR CARCINOGENS: HOW HIGH IS THE BURDEN OF PROOF?**
Chair: M. Moore

Overview of Mode of Action (MOA); Framework for determining a mutagenic MOA; case study on cyclophosphamide
R. Schoeny, U.S. Environmental Protection Agency, Washington, DC, USA

Case study on dichloroacetic acid
M. Moore, U.S. Food and Drug Administration, Jefferson, AR, USA

Case study on acrylamide
L. Haber, Toxicology Excellence for Risk Assessment, Cincinnati, OH, USA

Botticelli Hall

FOR04 **RATIONALE OF GENOTOXICITY TESTING OF NANOMATERIALS**
(This forum is partially supported by ECETOC)
Chairs: H. Greim, H. Norppa

FOR05 Regulatory requirements and appropriateness of available test systems
D. Warheit, DuPont Haskell Global Centers for Health & Environmental Sciences, Newark, DE, USA

FOR06 Possible genotoxic mechanisms: Criteria for improved test strategies
K. Donaldson, University of Edinburgh, Scotland, UK

Angelico Hall

FOR07 **ECOGENOTOXICOLOGY: PAST SUCCESSES AND FUTURE NEEDS**
Chairs: C. Bolognesi, D.M. DeMarini

FOR08 Evaluating the mutagenicity of air, water, and soil by the Salmonella assay: where are we after 40 years?
D. DeMarini, US Environmental Protection Agency, Research Triangle Park, NC, USA

FOR09 Ecogenotoxicity applied to environmental quality control
V.M.F. Vargas, Fundação Estadual de Proteção Ambiental Henrique Luís Roessler, Porto Alegre, RS, Brazil

FOR10 Industrial solid waste leachates induced genotoxicity: models and assays
D.K. Chowdhuri, Indian Institute of Toxicology Research, Lucknow, India

FOR11 An approach to validate genotoxicity biomarkers in environmental animals: the example of the micronucleus test
C. Bolognesi, National Cancer Research Institute, Genova, Italy

FOR12 Applications of biomarkers for the monitoring of the aquatic environment: challenges and new trends
A. Jha, University of Plymouth, UK

Presentation of some selected posters

Lippi Hall

PHILOSOPHY, SCIENCE AND ART IN RENAISSANCE ITALY
Chair: P. Dolara

FOR13 Renaissance medicine between typification and direct observation
O. Catanorchi, Scuola Normale Superiore, Pisa, Italy

FOR14 Art, science and nature in the Renaissance from Botticelli to Leonardo
A. Perissa Torrini, Gallerie dell’Accademia di Venezia, Italia

Saturday, August 22

Botticelli Hall

8.30 IN052 **Plenary Lecture**
Introduction: T. Yagi, President Japanese EMS

ENVIRONMENTAL EXPOSURE ASSESSMENT: COLLATERAL DAMAGE IN THE GENOMIC REVOLUTION?
C. P. Wild, International Agency for Research on Cancer, Lyon, France

9.15 IN053 **Plenary Lecture**
Introduction: W. Anwar, President-Elect Pan-African EMS

UNDERSTANDING THE MUTAGENIC CONSEQUENCES OF BASE LESION DNA REPAIR
S.H. Wilson, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA

10.00 **Coffee break**

10.30-12.30 **Parallel Symposia**

Michelangelo Hall
Mutational and epigenetic mechanisms
CHROMOSOME SEGREGATION AND GENOMIC INSTABILITY
Chair: F. Degrassi, U. Eichenlaub-Ritter

10.30 IN054 USING MULTI-DIMENSIONAL PROTEOMICS TO DEFINE THE COMPLETE PROTEIN COMPOSITION OF MITOTIC CHROMOSOMES
W. Earnshaw, University of Edinburgh, UK

10.55 IN055 LIVE CELL STUDIES ON TAXOL AND THE MITOTIC CHECKPOINT IN HUMANS
C.L. Rieder, NYS Dept. of Health, Albany, NY, USA

11.20 IN056 MECHANISMS OF CHROMOSOME MIS-SEGREGATION IN CANCER CELLS
D. Cimini, Virginia Tech, Blacksburg, VA, USA

11.45 IN057 HIGH-RESOLUTION IMAGING OF MITOTIC CHROMOSOME INSTABILITY
D. Gisselsson, University Hospital, Lund, Sweden

12.10 DD117 TRANSPLACENTALLY-INDUCED CENTROSOMAL AMPLIFICATION AND ANEUPLOIDY IN PRIMATES EXPOSED IN UTERO TO ANTIRETROVIRAL DRUGS
O. Olivero, National Cancer Institute, Bethesda, MD, USA

Angelico Hall
DNA damage responses
CELL TYPE AND TIME SPECIFICITY OF DNA DAMAGE RESPONSE
Chairs: E. Dogliotti, G.T. van der Horst

10.30 IN058 DNA DAMAGE AND CELL DIFFERENTIATION
E. Dogliotti, Istituto Superiore di Sanità, Roma, Italy

10.55 IN059 RECIPROCAL LINK BETWEEN THE CIRCADIAN CLOCK AND THE DNA DAMAGE RESPONSE
G.T.J. van der Horst, Erasmus University Medical Center, Rotterdam, The Netherlands

11.20 IN060 CELL AND TISSUE-SPECIFIC REQUIREMENTS FOR DNA STRAND BREAK REPAIR DURING NEUROGENESIS
P.J. McKinnon, St. Jude Children's Research Hospital, Memphis, TN, USA

11.45 IN061 CELL CYCLE CHECKPOINTS AND DNA REPAIR PATHWAYS VARY BETWEEN DIFFERENT CELL TYPES FOLLOWING EXPOSURE TO IONIZING RADIATION
P.J. Stambrook, University of Cincinnati, OH, USA

12.05 IN062 SOMATIC MUTATIONS AS A MOLECULAR RATIONAL OF DISEASE IN COMPLEX CONGENITAL HEART DISEASE
J. Borlak, Fraunhofer Institute for Toxicology and Experimental Medicine, Hannover, Germany

12.20 DD104 DNA DAMAGE AND CHECKPOINT RESPONSES IN HUMAN PROSTATE EPITHELIUM
T.M. Hällström, University of Helsinki, Finland

Bronzino Hall
Environmental Mutagenesis
ENVIRONMENTAL POLLUTION: THE ROLE OF SENTINEL SPECIES AND BIOMARKERS
Chairs: D. Waters, C. Bolognesi

10.30 IN063 PET DOGS AS SENTINELS OF ENVIRONMENTAL CANCER RISK
D. Waters, Purdue University, West Lafayette, IN, USA

10.55 IN064 TOXICOPATHIC LIVER LESIONS AND OTHER BIOMARKERS OF CHEMICAL EXPOSURE AND EFFECT IN SENTINEL FISH SPECIES IN PUGET SOUND, WASHINGTON AND OTHER COASTAL AREAS OF THE UNITED STATES
M.S. Myers, NOAA Fisheries, Seattle, WA, USA

11.20 IN065 GENOTOXICITY OF POLLUTED SOILS: RESPONSE OF BIOLOGICAL INDICATORS
P. Vasseur, University of Metz, France

11.45 IN066 TRANSCRIPTOMICS AND PROTEOMICS IN *Mus spretus*: NEW TOOLS FOR ENVIRONMENTAL POLLUTION ASSESSMENT
C. Pueyo, Córdoba University, Spain

12.05 IN067 ENHANCED IN VIVO MUTATIONS IN THE LUNG OF PHASE II ENZYMESUPPRESSED MICE
Y. Aoki, National Institute for Environmental Studies, Tsukuba, Japan

12.20 EM045 THE COPEPOD TIGRIOPUS: A PROMISING MARINE MODEL ORGAN ISM FOR ECOTOXICOLOGY AND ENVIRONMENTAL GENOMICS
J.-S. Lee, Hanyang University, Seoul, Korea

Botticelli Hall
Mutagenesis and health effects
IS THE ERA OF GENOME WIDE ASSOCIATIONS ALREADY OVER?
Chairs: P. Vineis, T. Manolio

10.30	IN068	IS THE ERA OF GENOME WIDE ASSOCIATIONS ALREADY OVER? ***P. Vineis***, Imperial College, London, UK
11.00	IN069	GENOME-WIDE ASSOCIATION STUDIES: OUTSTANDING ADVANCES, EXCITING CHALLENGES ***T. Manolio***, National Human Genome Research Institute, Bethesda, MD, USA
11.25	IN070	GENOME-WIDE ASSOCIATION STUDIES: STATISTICAL DEVELOPMENTS ***D. Balding***, Imperial College, London, UK
11.50	IN071	APPLICATION OF EPIGENOMICS IN CANCER RESEARCH ***Z. Herceg***, International Agency for Research on Cancer, Lyon, France
12.15	MH011	p73 G4C14-to-A4T14 GENE POLYMORPHISM AND INTERACTION WITH p53 EXON 4 Arg72Pro ON CANCER SUSCEPTIBILITY: A META-ANALYSIS OF THE LITERATURE ***E. De Feo***, Università Cattolica del Sacro Cuore, Roma, Italy

Lippi Hall
Prevention of mutation-related diseases
BIOLOGY, EPIDEMIOLOGY AND PREVENTION OF CANCER-ASSOCIATED MICROBIAL DISEASES
Chairs: P. Bonanni, D. Shouval

10.30	IN072	EPIDEMIOLOGY AND PRIMARY PREVENTION OF HPV-RELATED PRE-CANCEROUS AND CANCEROUS LESIONS ***P. Bonanni***, University of Firenze, Italy
11.00	IN073	RECENT PROGRESS IN PREVENTION AND TREATMENT OF HEPATOCELLULAR CARCINOMA (HCC) ***D. Shouval***, Hadassah-Hebrew University Hospital, Jerusalem, Israel
11.25	IN074	DEVELOPMENT OF VACCINES AGAINST *HELICOBACTER PYLORI* ***G. Del Giudice***, Novartis Vaccines and Diagnostics, Siena, Italy
11.50	IN075	EPSTEIN-BARR VIRUS INFECTION, MUTATIONS AND CANCER ***P.J. Farrell***, Imperial College, London, UK
12.15	PD025	HUMAN PAPILLOMA VIRUS IN BARRETT'S OESOPHAGUS ***E. Snow***, University of Tasmania, Launceston, Tasmania, Australia
12.30-14.30		**Lunch and poster viewing** *(ME012-021; DD061-120; EM061-120; MH033-064; PD014-026; RA038-075*)
14.30-16-30		**Parallel Symposia**

Michelangelo Hall
Mutational and epigenetic mechanisms
MicroRNAs AND THEIR REGULATION
Chairs: G.A. Calin, M. Negrini

14.30	IN076	TOWARD A NON-CODING RNA REVOLUTION IN THE CANCER SOCIETY ***G.A. Calin***, University of Texas, Houston, TX, USA
15.00	IN077	MicroRNAs IN CELL DIFFERENTIATION AND CANCER ***F.J. Slack,*** Yale University, New Haven, CT, USA
15.30	IN078	MicroRNA ONCOGENIC PATHWAYS DERAILED IN HEPATOCELLULAR CARCINOMA ***M. Negrini***, Università di Ferrara, Italy
15.55	IN079	POST-TRANSCRIPTIONAL REGULATION OF MicroRNA EXPRESSION IN HUMAN TUMORS AND CANCER CELL LINES ***T.D. Schmittgen***, Ohio State University, Columbus, OH, USA
16.20	RA070	IDENTIFICATION OF miRNA WITH TOXICOLOGICAL POTENTIAL AFTER BENZO[a]PYRENE EXPOSURE ***D. Lizarraga***, Maastricht University, Maastricht, The Netherlands

Botticelli Hall
Environmental Mutagenesis
CHALLENGING ENVIRONMENTAL HEALTH PROBLEMS AROUND THE WORLD
Chairs: W. Au, R. Sram

14.30	IN080	TRADITIONAL AND FUNCTIONAL BIOMARKERS FOR MONITORING EXPOSED POPULATIONS FOR HEALTH RISK ASSESSMENT ***W.W. Au***, University of Texas, Galveston,TX, USA
14.50	IN081	ENVIRONMENTAL AIR POLLUTION AND ASSESSMENT OF HEALTH RISK IN VARIOUS POPULATIONS ***M. Ruchirawat***, Chulabhorn Research Institute, Bangkok, Thailand
16.10	IN082	GENOTOXICITY OF AIR POLLUTANTS – IMPACT TO CHILDREN HEALTH ***R.J. Sram***, Institute of Experimental Medicine AS CR, Prague, Czech Republic
16.30	IN083	ARSENIC IN DRINKING WATER: GENETIC AND GENOMIC APPROACHES FOR IDENTIFYING ARSENIC SUSCEPTIBILITY AND HEALTH EFFECTS ***A.K. Giri***, Indian Institute of Chemical Biology, Calcutta, India
16.50	IN084	ENVIRONMENTAL HEALTH PRIORITIES AND CHALLENGES AROUND THE WORLD FOR THE NEXT DECADES ***J. Pronczuk***, World Health Organization, Geneva, Switzerland
17.10	IN085	ARISTOLOCHIC ACID NEPHROPATHY: AN ENVIRONMENTAL AND IATROGENIC DISEASE ***A.P. Grollman***, Stony Brook University, New York, NY, USA

Angelico Hall
Mutagenesis and health effects
DNA DAMAGE, REPAIR AND AGING
(This Symposium is supported by The Ellison Medical Foundation)
Chairs: L.J. Niederhofer, P.L. Opresko

14.30 IN086 HUMAN PREMATURE AGING PROTEINS PARTICIPATE IN DNA REPAIR
V.A. Bohr, National Institute on Aging, Baltimore, MD, USA

14.50 IN087 ENVIRONMENTAL CAUSES OF TELOMERE DEFECTS
P.L. Opresko, University of Pittsburgh, PA, USA

15.10 DD157 THE WERNER SYNDROME PROTEIN PARTICIPATES IN THE RESPONSE TO ONCOGENE-INDUCED REPLICATION STRESS
P. Pichierri, Istituto Superiore di Sanità, Roma, Italy

15.25 IN088 EVIDENCE THAT DNA DAMAGE PLAYS A CAUSAL ROLE IN AGING AND AGE-RELATED DISEASE
L.J. Niederhofer, University of Pittsburgh, PA, USA

15.50 IN089 TRANSCRIPTION-BLOCKING DNA LESIONS: AT THE CROSSROAD OF AGING AND LONGEVITY
G.A. Garinis, Institute of Molecular Biology and Biotechnology, Heraklion, Greece

16.10 IN090 CELLULAR SENESCENCE AS A DNA DAMAGE RESPONSE
F. D'Adda di Fagagna, IFOM-IEO, Milano, Italy

Bronzino Hall
Prevention of mutation-related diseases
NUTRIGENOMICS AND PUBLIC HEALTH
Chairs: M. Fenech, P. Dolara

14.30 IN091 PERSONALISED AND POPULATION-BASED STRATEGIES FOR DIAGNOSIS OF DNA DAMAGE AND ITS PREVENTION VIA NUTRITIONAL AND LIFE-STYLE INTERVENTION.
M. Fenech, CSIRO Human Nutrition, Adelaide, Australia

15.00 IN092 NUTRITIONAL SYSTEMS BIOLOGY: FROM INTEGRATING MECHANISMS TO PREVENTION
B. van Ommen, TNO-Quality of Life, Zeist, The Netherlands

15.25 IN093 BACTERIA-HOST INTERACTION IN CHRONIC DISEASE: INFLAMMATION MEETS METABOLISM
D. Haller, Technical University of Munich, Germany

15.50 IN094 PROTEOMIC BIOMARKERS OF VULNERABILITY TO CANCER IN THE ALIMENTARY TRACT
I.T. Johnson, Norwich Research Park, Norwich, UK

16.15 RA065 A STEP FORWARD ON THE ROAD TO PREVENT CERVICAL CARCINOMA: A NUTRIGENETIC APPROACH
A. Agodi, University of Catania, Italy

Lippi Hall
Risk assessment
MOLECULAR EPIDEMIOLOGY AND ETHICS
Chairs: K. Vähäkangas, D. Palli

14.30 IN095 HOW TO PROTECT INTEGRITY OF SCIENTIFIC RESEARCH
K. Vähäkangas, University of Kuopio, Finland

14.55 IN096 OBTAINING, SHIPPING, BIOBANKING AND USING OF HUMAN SPECIMENS: LOGISTICAL AND ETHICAL CHALLENGES
P. Hainaut, International Agency for Research on Cancer, Lyon, France

15.20 IN097 CHILDREN AS RESEARCH SUBJECTS: TODAY'S RESEARCH FOR A BETTER FUTURE
D.F. Merlo, National Cancer Research Institute, Genova, Italy

15.45 TAKING OUR OBLIGATIONS TO RESEARCH PARTICIPANTS SERIOUSLY: DISCLOSING INDIVIDUAL RESULTS OF GENETIC RESEARCH
T. Manolio, National Human Genome Research Institute, Bethesda, MD, USA

16.00 IN098 RECENT TRENDS AND CHALLENGES IN ENVIRONMENTAL HEALTH RESEARCH
S.H. Wilson, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA

16.20 RA103 THE CYTOKINESIS BLOCKED MICRONUCLEUS CYTOME ASSAY AND RISK PREDICTION OF LUNG CANCER
R. El-Zein, The University of Texas M.D. Anderson Cancer Center, Houston, TX, USA

16.30 **Coffee break**

Botticelli Hall
17.00 IN099 **Plenary Lecture**
Introduction: A. Guevara, President Philippines EMS

The DNA damage problem in the context of cancer, aging and longevity
J.H.J. Hoeijmakers, Erasmus Medical Center, Rotterdam, The Netherlands

18.00-19.30 **Parallel Forum**

Botticelli Hall
FOR15 **Workshop on cytotoxicity measures in the *in vitro* micronucleus test**
Chair: D. Kirkland

Brief Introduction and rationale
D. Kirkland, Covance, Harrogate, UK

Summary of L5178Y cell results
E. Lorge, Servier, Fleury-les-Aubrais, France

Summary of TK6 results
A. Elhajouji, Novartis, Basel, Switzerland

Summary of hamster cell results
J. Whitwell, Covance, Harrogate, UK

Summary of US data
M. Schuler, Pfizer, Groton, CT, USA

Overall conclusions
D. Kirkland, Covance, Harrogate, UK

Angelico Hall

FOR16 **Risk assessment of genotoxic trace substances in food**
Chair: R. Crebelli

FOR17 The margin of exposure approach to substances in food that are genotoxic and carcinogenic
D. Benford, Food Standard Agency, London, UK

FOR18 Possible mechanisms underlying practical thresholds for genotoxic carcinogens
T. Nohmi, National Institute of Health Sciences, Tokyo, Japan

FOR19 US FDA Safety Assessment of Genotoxic Food Contact Substances
C.W. Sheu, Food and Drug Administration, Washington, DC, USA

Michelangelo Hall

FOR20 **PREPARING THE NEXT GENERATION OF SCIENTISTS THROUGH EDUCATION AND RESEARCH**
Chair: J. Gentile

J. Gentile, Research Corporation for Science Advancement, Tucson, AZ, USA

D. DeMarini, US Environmental Protection Agency, Research Triangle Park, NC, USA

D. Tweats, University of Wales, Swansea, UK

Sunday, August 23

Botticelli Hall

8.30 IN100 **Plenary Lecture**
Introduction: M. Chulasiri, President Thai EMS

COMPLEX CELLULAR RESPONSES TO DNA DAMAGING AGENTS
L. Samson, Massachusetts Institute of Technology, Cambridge, MA, USA

9.15 IN101 **Plenary Lecture**
Introduction: J. Gentile, Past-President IAEMS

NOVEL STRATEGIES IN THE PREVENTION OF MUTATION-RELATED DISEASES
S. De Flora, University of Genova, Italy

10.00 **Coffee break**

10.30-12.30 **Parallel Symposia**

Michelangelo Hall
Mutational and epigenetic mechanisms
New insights in germ cell mutagenesis
Chairs: F. Marchetti, C.L. Yauk

10.30 IN102 DNA DOUBLE STRAND BREAK REPAIR IN PARENTAL CHROMO-SOMES OF MOUSE ZYGOTES
P. de Boer, Radboud University, Nijmegen, The Netherlands

11.00 IN103 PATERNAL EXPOSURES AFFECT SPERM CHROMATIN AND DISTURB EPIGENETIC PROGRAMMING DURING EARLY EMBRYO DEVELOPMENT
B. Robaire, McGill University, Montreal, QC, Canada

11.30 IN104 EFFECTS OF TOBACCO SMOKE ON MALE GERM CELLS AND EARLY EMBRYONIC DEVELOPMENT
F. Marchetti, Lawrence Berkeley National Laboratory, Berkeley, CA, USA

11.50 IN105 HERITABLE EFFECTS OF EXPOSURE TO COMBUSTION DERIVED PARTICLES
C.L. Yauk, Health Canada, Ottawa, QC, Canada

12.10 EM102 3,3'-DINTRITRO-BISPHENOL A SIGNIFICANTLY DISTURBS MATURATION AND SPINDLE FORMATION, AND CHROMATIN INTEGRITY IN MOUSE OOCYTES
U. Eichenlaub-Ritter, University Bielefeld, Germany.

Angelico Hall
DNA damage responses
CELLULAR DEFENSES AGAINST OXIDATIVE DAMAGE
Chairs: T. Lindahl, B. Demple

10.30 IN106 ROLES OF THE FTO AND TREX1 ENZYMES IN REMOVAL OF DAMAGED OR DISPLACED DNA
T. Lindahl, CR-UK London Research Institute, South Mimms, UK

10.50 IN107 INTEGRATING CELLULAR FUNCTION THROUGH A BASE EXCISION DNA REPAIR PROTEIN
B. Demple, Harvard School of Public Health, Boston, MA, USA

11.10 IN108 REGULATION OF BASE EXCISION REPAIR IN RESPONSE TO DNA DAMAGE
G. Dianov, University of Oxford, UK

11.30 IN109 MUTYH AND DNA POLYMERASE λ COOPERATE IN A NOVEL LONG PATCH BASE EXCISION REPAIR OF 8-oxo- Guanine
U. Hübscher, University of Zurich, Switzerland

11.50 IN110 DNA REPAIR ENZYME NEIL1, METABOLIC SYNDROME AND CANCER
M. Dizdaroglu, National Institute of Standards and Technology, Gaithersburg, MD, USA

12.10 IN111 INTERACTION OF PROTEINS INVOLVED IN DNA REPAIR WITH AP SITE CONTAINING DNA
O. Lavrik, Russian Academy of Sciences, Novosibirsk, Russia

Lippi Hall

Environmental Mutagenesis

CRITICAL ISSUES ON ENVIRONMENTAL GENOTOXICITY IN LATIN AMERICA

Chairs: L.R. Ribeiro, E. Zamorano-Ponce

10.30	IN112	INTRODUCTION TO SIMPOSIUM: CRITICAL ISSUES ON ENVIRONMENTAL GENOTOXICITY IN LATIN AMERICA ***E. Zamorano-Ponce***, Universidad del Bío-Bío, Chillán, Chile
10.40	IN113	DNA DAMAGE, OXIDATIVE BALANCE, AND EXPOSURE BIOMARKERS IN A RURAL POPULATION EXPOSED TO PESTICIDES. ***M.A. Carballo***, Universidad de Buenos Aires, Argentina
11.10	IN114	ATMOSPHERIC POLLUTION BY MUTAGENIC AGENTS IN AREAS OF INDUSTRIAL IMPACT: HUMAN BIOMONITORING ***F.V.M. Vargas***, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil
11.40	IN115	POLYCYCLIC AROMATIC HYDROCARBON (PAH)-DNA ADDUCTS, CHROMOSOMAL ABERRATIONS, AND *CYP1A*, *CYP1B1* AND *GSTM1* RISK VARIANTS IN PERIPHERAL BLOOD LYMPHOCYTES FROM YOUNG ADULTS LIVING IN MEXICO CITY. ***M.E. Gonsebatt***, UNAM, Mexico City, DF, Mexico
12.05	IN116	USE OF AGROCHEMICALS IN ARGENTINA: GENOTOXIC AND CYTOTOXIC COMPARISONS BETWEEN PURE AND FORMULATED PRODUCTS ***M.L. Larramendy***, University of La Plata, Argentina.

Botticelli Hall

Mutagenesis and health effects

CANCER MODELS AND MECHANISMS

Chairs: K. Tanaka, Y. Pommier

10.30	IN117	NOVEL FUNCTION OF NUCLEOTIDE EXCISION REPAIR FACTOR AND ITS RELEVANCE TO XERODERMA PIGMENTOSUM AND COCKAYNE SYNDROME ***K. Tanaka***, Osaka University, Osaka, Japan
10.55	IN118	CHROMOSOMAL INSTABILITY IN CANCER PATHOGENESIS AND TREATMENT ***A. Venkitaraman***, University of Cambridge, UK
11.20	IN119	ROLE OF TOPOISOMERASE I IN GENOMIC STABILITY ***Y. Pommier***, National Institutes of Health, Bethesda, MD, USA
11.40	IN120	OXIDATIVE STRESS-INDUCED TUMORIGENESIS IN THE SMALL INTESTINES OF VARIOUS TYPES OF DNA REPAIR-DEFICIENT MICE ***T. Tsuzuki***, Kyushu University, Fukuoka, Japan
12.00	IN121	ROLE OF MICROENVIRONMENT ON TUMOR PROGRESSION: ENDOTHELIUM, ANGIOGENESIS AND INFLAMMATION ***A. Albini***, IRCCS Multimedica, Sesto San Giovanni, Milano, Italy
12.20	MH069	AN ATTEMPT TO IDENTIFY GENES INVOLVED IN PROGRESSION OF TOBACCO SMOKE ASSOCIATED CANCER ***K. Szyfter***, Polish Academy of Sciences, Poznan, Poland

Bronzino Hall
Prevention of mutation-related diseases
PHARMACOLOGICAL PREVENTION OF MUTATION AND CANCER
Chairs: V.E. Steele, T.W. Kensler

10.30 IN122 ANTIMUTAGENIC STRATEGIES APPLIED TO CHEMOPREVENTIVE DRUG DEVELOPMENT
V.E. Steele, National Cancer Institute, Bethesda, MD, USA

10.55 IN123 CLINICAL STRATEGIES FOR DEVELOPING ANTIMUTAGENIC CHEMOPREVENTIVE DRUGS
G.J. Kelloff, National Cancer Institute, Bethesda, MD, USA

11.20 IN124 TARGETING KEAP1-NRF2 SIGNALING WITH DRUGS
T.W. Kensler, Johns Hopkins University, Baltimore, MD, USA

11.45 IN125 CHEMOPREVENTION OF CIGARETTE SMOKE GENOTOXICITY AND CARCINOGENICITY
R. Balansky, National Center of Oncology, Sofia, Bulgaria

12.10 PD002 PHARMACOLOGICAL ANTIOXIDANTS PROTECT FROM RADIATION INDUCED DNA DAMAGE AS WELL AS GENETIC INSTABILITY AND LYMPHOMA IN ATM DEFICIENT MICE
R.H. Schiestl, University of California, Los Angeles, CA, USA

12.30 **Lunch**

13.30-15.30 **Parallel Symposia**

Michelangelo Hall
Mutational and epigenetic mechanisms
***IN VIVO* MUTAGENESIS: RECENT ADVANCES AND FUTURE PROSPECTS**
Chairs: G.R. Douglas, J.H. Bielas

13.30 IN126 LIKE FATHER LIKE SON: TRANSGENERATIONAL GENOMIC INSTABILITY IN MAMMALS
Y.E. Dubrova, University of Leicester, UK

14.00 IN127 THE MECHANISM AND CLINICAL UTILITY OF SOMATIC MITOCHONDRIAL MUTAGENESIS IN CANCER
J.H. Bielas, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

14.30 IN128 CROSS-SPECIES, ENDOGENOUS MUTATION ASSAY BASED ON THE *PIG-A* GENE
J.C. Bemis, Litron Laboratories, Rochester, NY, USA

15.00 IN129 VALIDATION AND UTILITY OF TRANSGENIC RODENT GENE MUTATION ASSAYS
G.R. Douglas, Health Canada, Ottawa, ON, Canada

Botticelli Hall
DNA damage responses
DNA DAMAGE RESPONSE AS THERAPEUTIC TARGET
Chairs: A. Sarasin, P. Karran

13.30	IN130	OVEREXPRESSION OF SOME DNA REPAIR PATHWAYS ARE ASSOCIATED WITH METASTASIS RISK IN MELANOMA PATIENTS ***A. Sarasin***, CNRS, Villejuif, France
13.55	IN131	DNA DAMAGE AND DNA DAMAGE RESPONSES AFTER THIOPURINE/UVA TREATMENT ***P. Karran***, Cancer Research UK London Research Institute, South Mimms, UK
14.20	IN132	GENOTOXIC STRESS RESPONSE: MECHANISMS AND RELEVANCE TO CANCER ***J. Bartek***, Danish Cancer Society, Copenhagen, Denmark
14.40	IN133	PROCESSING OF DNA ADDUCTS INTO DOUBLE STRAND BREAKS ***A.K. Larsen***, INSERM, Université Paris 6, Paris, France
15.00	IN134	IMPLICATION OF THE NUCLEOTIDE EXCISION REPAIR MACHINERY ON THE RESPONSE TO DOXORUBICIN TREATMENT IN HUMAN FIBROBLASTS ***J. Saffi***, Lutheran University of Brazil, Canoas, Brazil
15.20	DD003	A MRN/TIP60 COMPLEX INVOLVED IN DNA DOUBLE STRAND BREAKS REPAIR ***Y. Canitrot***, LBCMCP, CNRS UMR 5088 and University of Toulouse, France

Angelico Hall
Environmental Mutagenesis
SYSTEMS BIOLOGY APPROACHES TO ENVIRONMENTAL CARCINOGENESIS AND RELATED BIOMARKERS
(This Symposium is supported by ECNIS)
Chairs: A. Hirvonen, S. Kyrtopoulos

13.30		INTRODUCTION ***A. Hirvonen,*** Finnish Institute of Occupational Health, Helsinki, Finland.
13.40	IN135	INTOGEN: A NOVEL FRAMEWORK FOR INTEGRATION AND DATA-MINING OF MULTIDIMENSIONAL ONCOGENOMIC DATA ***N. Lopez-Bigas***, Universitat Pompeu Fabra, Barcelona, Spain
14.05	IN136	THE COMPARATIVE TOXICOGENOMICS DATABASE: A DISCOVERY TOOL FOR IDENTIFYING CHEMICAL-GENE-DISEASE NETWORKS ***C.J. Mattingly***, The Mount Desert Island Biological Laboratory, Salisbury Cove, ME, USA
14.30	IN137	METABOLIC PROFILING AS A TOOL IN BIOMARKER RESEARCH AND SYSTEMS BIOLOGY ***H.C. Keun***, Imperial College, London, UK
14.55	IN138	BLOOD TRANSCRIPTOMICS AND EXPOSURE BIOMARKERS IN A POPULATION-BASED COHORT – THE NOWAC POSTGENOME STUDY ***V. Dumeaux***, University of Tromsø, Norway
15.20		CONCLUSION ***S. Kyrtopoulos***, National Hellenic Research Foundation, Athens, Greece

Lippi Hall
YOUNG SCIENTIST SESSION I
Chairs: M. Kirsch-Volders, A.Zijno

13.30 DD040 DNA POLYMERASES BETA AND LAMBDA AS A POTENTIAL PARTICIPANTS OF TLS DURING GENOMIC DNA REPLICATION ON THE LEADING AND LAGGING STRANDS
E.A. Belousova, Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russia

13.45 MH089 ANTIOXIDANT ENZYME ACTIVITIES AND mRNA LEVELS IN THE KIDNEY OF PRE-HYPERTENSIVE SPONTANEOUSLY HYPERTENSIVE RATS (SHR) AND WISTAR-KYOTO RATS (WKY)
AK Sundaram, University Sains Malaysia, Kelantan, Malaysia

14.00 EM053 BIOLOGICAL ACTIVITIES OF ENDOGENOUS MUTAGENS/ / CARCINOGENS, AMINOPHENYLNORHARTMAN AND *N*-NITROSO BILE ACID CONJUGATES
Y. Totsuka, National Cancer Center Research Institute, Tokyo, Japan

14.15 DD096 DNA DAMAGE RESPONSES IN IRRADIATED GLIOBLASTOMA CELL LINES
P.R.D.V. Godoy, University of São Paulo, Ribeirão Preto, SP, Brazil

14.30 DD092 POTENTIAL GENOTOXIC RISKS OF SINGLE WALLED CARBON NANOTUBES
B. Manshian, Swansea University, Swansea, Wales, UK

14.45 EM140 DNA DAMAGE ASSESSMENT OF HUMAN POPULATIONS EXPOSED TO AIRBORNE POLLUTANTS FROM INDUSTRIAL AND URBAN SOURCES
M.V. Coronas, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

15.00 RA071 MOLECULAR GENETIC ANALYSIS OF C677T AND A1298C POLYMORPHISMS OF THE GENE OF METHYL TETRA HYDRO FOLATE REDUCTASE AS A RISK FACTOR FOR DEVELOPMENT OF CHRONIC MALNUTRITION IN CHILDREN UNDER 3 YEARS OF THE MUNICIPALITY OF LURIBAY, EXPOSED TO PESTICIDES
R.E. Montaño Arrieta, Universidad Mayor de San Andres UMSA, La Paz, Bolivia

Bronzino Hall
Risk assessment
NEW DATA INITIATIVES AND PREDICTIVE APPROACHES FOR MUTAGENICITY AND CARCINOGENICITY
Chairs: R. Benigni, A. Richard

13.30 IN139 EXPERIMENTAL TESTS AND MODELING APPROACHES: GETTING THE BEST FROM BOTH
R. Benigni, Istituto Superiore di Sanità, Roma, Italy

13.55 IN140 NEW CHEMICAL/BIOLOGICAL PROFILING AND INFORMATICS APPROACHES FOR EXPLORING MUTAGENICITY AND CARCINOGENICITY: UPDATES OF EPA TOXCAST™ AND TOX21 PROGRAMS
A. Richard, U.S. Environmental Protection Agency, Research Triangle Park, NC, USA

14.20	IN141	A KNOWLEDGE-BASE APPROACH TO IDENTIFY SIGNATURES FOR BIOLOGICAL AND CHEMICAL PAIRS IN THE RISK ASSESSMENT PROCESS ***C. Yang***, US Food and Drug Administration, College Park, MD, USA
14.45	IN142	THE USE OF (Q)SAR IN FOOD SAFETY ASSESSMENT ***E. Lo Piparo***, Institute for Health and Consumer Protection, Ispra, Varese, Italy
15.10	RA021	ENVIRONMENTAL RISK INDEX (ERI) : A NEW PROPOSAL FOR RISK ASSESSMENT INTEGRATING *IN VITRO* TOOLS ***D. Baderna***, Istituto di Ricerche Famacologiche “Mario Negri”, Milano, Italy
15.20	RA082	DIRECTING GENOTOXICITY TESTING TO ASSIST IN THE DEVELOPMENT OF *IN SILICO* MODELS ***R.V. Williams***, Lhasa Limited, Leeds, UK
16.00-19.30		**Optional tours**
20.30		**Social Dinner**

Monday, August 24

Botticelli Hall

8.30	IN143	**Plenary Lecture** Introduction: A.B. Prasad, President EMS India THE RATE OF SOMATIC MUTATIONS AND HUMAN CANCER ***L. Luzzatto***, Istituto Toscano Tumori, Firenze, Italy
9.15	IN144	**Plenary Lecture** Introduction: E. Snow, President EMS Australia-New Zealand (MEPSA) CAUSES AND MECHANISMS OF COLON CANCER DEVELOPMENT, AND STRATEGIES FOR ITS PREVENTION ***K. Wakabayashi***, National Cancer Center Research Institute, Tokyo, Japan
10.00		**Coffee break**
10.30-12.30		**Parallel Symposia**

Michelangelo Hall
Mutational and epigenetic mechanisms
INDUCED mutagenesis: A NETWORK OF INTERPLAYING PROCESSES
Chairs: J.M. Essigmann, A. Abbondandolo

10.30	IN145	AN INTEGRATED VIEW OF INDUCED MUTAGENESIS IN *E. coli* ***R.P. Fuchs***, CNRS, Marseille, France
10.55	IN146	INTERPLAY OF DNA REPAIR, DNA POLYMERASES AND TRANSCRIPTION IN THE PROCESS OF SPONTANEOUS MUTAGENESIS IN YEAST ***S. Boiteux***, CNRS & CEA, Fontenay aux Roses, France
11.20	IN147	OXIDATIVE STRESS, DNA ALKYLATION AND MUTAGENESIS ***J.M. Essigmann***, Massachusetts Institute of Technology, Cambridge, MA, USA

11.45 IN148 MUTAGENIC OR ACCURATE OUTCOME OF TRANSLESION DNA SYNTHESIS IS DETERMINED BY SPECIFIC TWO-POLYMERASE MECHANISMS IN MAMMALIAN CELLS
Z. Livneh, Weizmann Institute of Science, Rehovot, Israel

12.10 DD025 LESION RECOGNITION AND CATALYSIS IN BER PATHWAY INVOLVE MULTIPLE CONFORMATIONAL CHANGES IN ENZYMES AND DNA
O.S. Fedorova, Russian Academy of Sciences, Novosibirsk, Russia

12.20 DD056 NEIL GLYCOSYLASES: NOVEL ACTIVITIES AND REGULATION
I.R. Grin, Russian Academy of Sciences, Novosibirsk, Russia

Botticelli Hall
DNA damage responses
LOOKING INTO THE FUTURE: GENOME WIDE TECHNOLOGIES IN TOXICOLOGY
Chairs: L. Mullenders, E. Prosperi

10.30 IN149 EPISTASIS ANALYSIS OF THE DNA DAMAGE RESPONSE
H. van Attikum, Leiden University, Leiden, The Netherlands
(EEMS Young Scientist Award 2009)

10.55 IN150 TRANSLATIONAL RESPONSES TO DNA DAMAGE
T.J. Begley, University at Albany, Rensselaer, NY, USA

11.20 IN151 GLOBAL ANALYSIS OF SIGNALING NETWORKS BY HIGH-RESOLUTION MASS SPECTROMETRY-BASED QUANTITATIVE PHOSPHOPROTEOMICS
J.V. Olsen, University of Copenhagen, Denmark

11.45 IN152 QUANTITATIVE IMAGING-BASED FUNCTIONAL GENOMICS SCREENING TO UNRAVEL TOXICITY RELEVANT SIGNALING PATHWAYS.
B. van de Water, Leiden University, Leiden, The Netherlands

12.10 IN153 CHIP-SEQ APPROACH TO STUDY THE CELLULAR RESPONSE TO DAMAGE INDUCED TRANSCRIPTION INTERFERENCE
M. Fousteri, Leiden University, Leiden, The Netherlands

12.25 DD147 PROTEOMICS AND SPR IMAGING APPLIED TO PLATINATED DNA INTERACTOME STUDIES
J Breton, Commissariat à l'Énergie Atomique, Grenoble, France

Angelico Hall
DNA damage responses
REGULATION OF DNA DAMAGE RESPONSE: LESSONS LEARNED FROM DOUBLE STRAND BREAK REPAIR PATHWAYS
Chairs: J. Surralles, P. Mosesso

10.30 IN154 FANCONI ANEMIA: OMIC APPROACHES AND THERAPEUTIC APPLICATIONS
J. Surralles, Universitat Autònoma de Barcelona, Spain

10.55 IN155 FANCM CONNECTS THE TWO GENOME INSTABILITY DISORDERS BLOOM'S SYNDROME AND FANCONI ANEMIA
A.J. Deans, London Research Institute, Cancer Research UK, South Mimms, UK

11.20 IN156 GENETIC PATHWAYS REQUIRED FOR TEMPLATE-SWITCH MEDIATED DAMAGE BYPASS REPLICATION
D. Branzei, FIRC Institute of Molecular Oncology, Milano, Italy

11.45 IN157 ACTIVATION OF THE CELLULAR DNA DAMAGE RESPONSE IN THE ABSENCE OF DNA LESIONS
E. Soutoglou, CNRS INSERM, Illkirch, France

12.10 DD150 POLO-LIKE KINASE REGULATION OF DNA REPLICATON UNDER STRESSED CONDITIONS
K. Trenz, Institute of Cancer Research, Sutton, UK

12.20 DD091 REPLICATION FORK STABILIZATION PROTEINS TIMELESS AND TIMELESS-INTERACTING PROTEIN (TIPIN) MAINTAIN GENOMIC STABILITY
S.L. Smith-Roe, University of North Carolina at Chapel Hill, NC, USA

Bronzino Hall
Environmental Mutagenesis
BIOLOGICAL RISKS FROM SPACE RADIATION ENVIRONMENTS
Chairs: F.A. Cucinotta, M. Durante

10.30 IN158 ATM AND TGF β PATHWAY SIGNALING FOLLOWING X-RAY AND HEAVY IONS EXPOSURE
F.A. Cucinotta, NASA, Houston TX, USA

10.55 IN159 CYTOGENETIC EFFECTS OF HEAVY IONS
M. Durante, GSI, Darmstadt, Germany

11.20 IN160 DNA DAMAGE AND REPAIR FROM SPACE RADIATION
M.A. Tabocchini, Istituto Superiore di Sanità and INFN, Roma, Italy

11.45 IN161 POPULATION ACTION AS A MODIFIER OF RADIATION-INDUCED CARCINOGENESIS
L. Hlatky, Tufts University, Boston, MA, USA

12.10 EM007 THE COMPARISON STUDY ON CHROMOSOMAL ABERRATIONS IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES INDUCED BY 18.8 MeV PROTON AND 60Co-γ RADIATION
Y. Chen, Beijing Institute of Radiation Medicine, Beijing, China

12.20 DD078 ASSESSING OF RADIOSENSITIVITY OF PERSONS EXPOSED TO IONIZING RADIATION DURING FLIGHTS
N.Yu Vorobyova, Institute of Chemistry Physics, Russian Academy of Sciences, Moscow, Russia

Lippi Hall
Prevention of mutation-related diseases
PUBLIC HEALTH GENOMICS
Chairs: W. Ricciardi, A. Brand

10.30 IN162 THE EUROPEAN AND INTERNATIONAL AGENDA OF PUBLIC HEALTH GENOMICS
A. Brand, Maastricht University, Maastricht, The Netherlands

10.55 IN163 PUBLIC HEALTH AND GENOMIC EPIDEMIOLOGY
S. Boccia, Università Cattolica del Sacro Cuore, Roma, Italy

11.20 IN164 ASSESSING GENE-ENVIRONMENT INTERACTIONS IN CANCER
P. Boffetta, International Agency for Research on Cancer, Lyon, France

11.45 IN165 TRANSLATIONAL RESEARCH IN GENOMICS
C. Janssens, Erasmus University Medical Center Rotterdam, The Netherlands

12.10 IN166 PRACTICAL IMPLEMENTATION OF PUBLIC HEALTH GENOMICS: THE CASE OF GENAR INSTITUTE
***BS. Savaş*, **GENAR Institute for Public Health and Genomics Research**,** Istanbul**,** Turkey

12.30-14.30 **Lunch and poster viewing**
(ME022-032; DD121-180; EM121-179;
MH065-095; PD027-039; RA076-113)

14.30-16.30 **Parallel Symposia**

Michelangelo Hall
Mutational and epigenetic mechanisms
Physiological mutagenesis in immunity
Chairs: M.S. Neuberger, J. Miller

14.30 IN167 THE FUNCTIONS OF AID AND OTHER DNA DEAMINASES IN IMMUNITY
M.S. Neuberger, MRC, Cambridge, UK

15.00 IN168 INTENTIONAL MUTAGENESIS IN B CELLS: PCNA-UBIQUITYLATION CONTROLS SOMATIC HYPERMUTATION
H. Jacobs, The Netherlands Cancer Institute, Amsterdam, The Netherlands

15.25 IN169 DIVERSITY-GENERATING RETROELEMENTS
J. Miller, UCLA School of Medicine, Los Angeles, CA, USA

15.50 IN170 ERROR-PRONE REPAIR PATHWAYS MOBILIZING TLS DNA POLYMERASES IN IMMUNOGLOBULIN GENE HYPERMUTATION
C.A. Reynaud, INSERM, Paris, France

16.15 IN171 EDITING DEAMINASES: STORY OF A MULTI-TALENTED DOMAIN
S. Conticello, Istituto Toscano Tumori, Firenze, Italy

Botticelli Hall
Mutational and epigenetic mechanisms
TOLERANCE OF DNA DAMAGE: TRANSLESION DNA SYNTHESIS
Chairs: A.R. Lehmann, E.C. Friedberg

14.30 IN172 REGULATION OF DNA POLYMERASE ETA IN HUMAN CELLS
A.R. Lehmann, University of Sussex, Falmer, UK

14.55 IN173 DNA POLYMERASE KAPPA-DEFECTIVE MICE ARE SPONTANEOUS MUTATORS
E.C. Friedberg, University of Texas, Dallas, TX, USA

15.20 IN174 HUMAN DNA POLYMERASE NU (POLN), A UNIQUE A-FAMILY DNA POLYMERASE WHICH CAN BYPASS DNA DAMAGE
K. Takata, The University of Texas, Smithville, TX, USA.

15.40 IN175 REPAIR AND TOLERANCE MECHANISMS OF DNA-PROTEIN CROSSLINK DAMAGE
H. Ide, Hiroshima University, Hiroshima, Japan

16.00 IN176 NEW FUNCTIONAL ROLES OF THE HUMAN DNA POLYMERASES ETA AND KAPPA DURING GENOMIC DNA REPLICATION
J.S. Hoffmann, CNRS, University of Toulouse, France

16.20 DD130 K63-LINKED UBIQUITIN CHAINS CONJUGATED TO PCNA CONTROL RECOMBINATION REPAIR OF SS-GAPS
G.I. Karras, Max Planck Institute of Biochemistry, Martinsried, Germany

Lippi Hall
YOUNG SCIENTIST SESSION II
Chairs: F. Pacchierotti, H.-J. Martus

14.30 DD040 GENOTOXIC AND CELLULAR SIGNALLING RESPONSES FOLLOWING EXPOSURE TO ULTRAFINE SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES
S.H. Doak, Swansea University, Swansea, Wales, UK
(EEMS Young Scientist Award 2009)

15.00 EM130 A NEW PARADIGM FOR MOLECULAR TOXICOLOGY: INHIBITION OF P53-MEDIATED DNA REPAIR BY HEAVY METAL NICKEL
Young R. Seo, Kyung Hee University, Seoul, Korea

15.15 DD028 BIOCHEMICAL EVIDENCES INVOLVE DNA POLYMERASE BETA OF *Trypanosoma cruzi* IN REPAIR OF OXIDATIVE LESIONS IN MITOCHONDRIAL DNA
B.L.F. Schamber-Reis, Federal University of Minas Gerais, Brazil

15.30 MH013 POSTGENOMIC ALTERATIONS IN AICARDI-GOUTIÈRES SYNDROME, A RARE NEURODEGENERATIVE DISEASE OF MUTATIONAL ORIGIN
M. Longobardi, Department of Health Sciences, University of Genova, Italy

15.45 MH031 GENETIC MARKERS OF SUSCEPTIBILITY INVOLVED IN PROSTATE CANCER
H. Kuasne, Londrina State University, Londrina, PR, Brazil

16.00 MH074 BIOCHEMICAL AND GENOTOXIC EFFECTS IN PESTICIDE SPRAYERS: PRELIMINARY RESULTS
M.F. Simoniello, Universidad Nacional del Litoral, Santa Fe, Argentina

16.15 EM029 THE USE OF GENOTOXIC BIOASSAYS TO EVALUATE THE ENVIRONMENTAL QUALITY IN A REGION UNDER THE INFLUENCE OF URBAN WASTE IN GUAÍBA LAKE BASIN (BRAZIL)
I.V. Villela, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

Angelico Hall

Mutagenesis and health effects

GENE-ENVIRONMENT INTERACTIONS IN NONCANCER DISEASES

Chairs: A. Izzotti, F.J. van Schooten

14.30 IN177 GENE-ENVIRONMENT INTERACTIONS AS COMMON DETERMINANTS OF NONCANCER-DEGENERATIVE DISEASES
A. Izzotti, University of Genova, Italy

14.55 IN178 GENETIC INFLUENCES ON SMOKING BEHAVIOR AND PREVENTION OF CHRONIC DEGENERATIVE DISEASES
F.J. van Schooten, Maastricht University, Maastricht, The Netherlands

15.20 IN179 THE IMPACT OF GENETIC AND ENVIRONMENTAL FACTORS IN NEURODEGENERATION: THE EMERGING ROLE OF EPIGENETICS
L. Migliore, University of Pisa, Italy

15.45 IN180 POLYMORPHISMS OF CYTOCHROME P4501A1, CIGARETTE SMOKING AND RISK OF CORONARY ARTERY DISEASE
C.-C.Yeh, China Medical University, Taichung, Taiwan

16.10 MH095 MITOCHONDRIAL DNA HAPLOGROUPS AND ETHNICAL ORIGINS IN PATIENTS WITH MULTIPLE SCLEROSIS
H. Groot, Universidad de los Andes, Bogotà, Colombia

16.20 MH066 OXIDATIVE DAMAGE AND TRANSCRIPT DOWN-REGULATION OF DNA REPAIR GENES IN LYMPHOCYTES FROM PATIENTS WITH ALZHEIMER DISEASE, FRAILTY SYNDROME AND DIABETES MELLITUS TYPE-2
E.T. Sakamoto-Hojo, Universidade de São Paulo, Ribeirão Preto, SP, Brazil

Bronzino Hall

Prevention of mutation-related diseases

DIETARY PREVENTION OF MUTATION AND CANCER

Chairs: S. Knasmüller, N. Loprieno

14.30 IN181 USE OF THE COMET ASSAY FOR THE DETECTION OF DNA PROTECTIVE CONSTITUENT IN THE HUMAN DIET
S. Knasmüller, Medical University of Vienna, Austria

14.55 IN182 TRANSIENT GENERATION OF REACTIVE OXYGEN SPECIES AS AN IMPORTANT SIGNALLING MECHANISM IN CANCER CHEMOPREVENTION
C. Gerhäuser, German Cancer Research Center, Heidelberg, Germany

15.20 IN183 IN SEARCH OF A MECHANISM: FOOD POLYPHENOLS FROM ANTIOXIDANTS TO MODULATORS OF GENE EXPRESSION
P. Dolara, University of Firenze, Italy

15.45 IN184 NON-ANTIOXIDANT EFFECTS OF PHYTOCHEMICALS: DNA REPAIR
A.R. Collins, University of Oslo, Norway

16.10 EM023 COMPARISION OF MUTAGENICITIES AND GENE EXPRESSION PROFILES OF COMFREY AND RIDDELLIINE IN RAT LIVER
M.G. Manjanatha, National Center for Toxicological Research, Jefferson, AR, USA

16.20 EM097 PRENATAL EXPOSURE TO FLAVONOIDS: IMPLICATION FOR DEVELOPMENT OF LEUKEMIA
K Vanhees, Maastricht University, Maastricht, The Netherlands

16.30 **Coffee break**

Botticelli Hall

17.00 IN185 **Plenary Lecture**
Introduction: P. Cooper, President EMS North-America

CELLULAR ADAPTIVE SURVIVAL RESPONSE TO OXIDATIVE, NITROSATIVE AND INFLAMMATORY STRESSES: ROLES OF REDOX-SENSITIVE TRANSCRIPTION FACTORS
Y.-J. Surh, Seoul National University, Seoul, Korea

18.00 **General Assembly of the International Association of Environmental Mutagen Societies (IAEMS)**

19.30-20.30 **Assemblies of Regional Mutagen Societies**

Tuesday, August 25

8.30-10.30 **Parallel Symposia**

Michelangelo Hall
Mutational and epigenetic mechanisms
MECHANISMS OF UNTARGETED MUTAGENESIS
Chairs: L.A. Loeb, T.A. Kunkel

8.30 IN186 INVOLVEMENT OF MUTATOR DNA POLYMERASES IN CARCINOGENESIS
L.A. Loeb, University of Washington, Seattle, WA, USA

9.00 IN187 THE EFFICIENCY OF DNA MISMATCH REPAIR IN *SACCHAROMYCES CEREVISIAE*
T.A. Kunkel, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA

9.30 IN188 PATHWAYS SUPPRESSING SPONTANEOUS MUTATION AND CANCER IN MICE
B.D. Preston, University of Washington, Seattle, WA, USA

9.55 IN189 GENOMIC INSTABILITY AND CANCER IN MOUSE DNA REPLICATION MUTANTS
J. Schimenti, Cornell University, Ithaca, NY, USA

10.20 ME007 HIGH FREQUENCY OF GENOMIC DELETIONS INDUCED BY ME-LEX, A SEQUENCE SELECTIVE N3-ADENINE METHYLATING AGENT, AT THE HPRT LOCUS IN CHINESE HAMSTER OVARY CELLS
P. Menichini, National Cancer Research Institute (IST), Genova, Italy

Botticelli Hall
Environmental Mutagenesis
ADVANCES IN THE ASSESSMENT OF EXPOSURE AND EARLY BIOLOGICAL EFFECTS
Chairs: S. Bonassi, D.H. Phillips

8.30 IN190 CHALLENGES IN THE DESIGN AND STATISTICAL ANALYSIS OF POPULATION STUDIES WITH HIGH-THROUGHPUT ASSAYS
S. Bonassi, National Cancer Research Institute (IST), Genova, Italy

8.55 IN191 CLUES TO CANCER AETIOLOGY AND CARCINOGENIC MECHANISMS DERIVED FROM DNA ADDUCTS
D.H. Phillips, Institute of Cancer Research, Sutton, UK

9.20 IN192 THE USE OF DNA ADDUCTS IN RISK ASSESSMENT
H. Autrup, University of Aarhus, Denmark

9.45 IN193 GENOMIC ALTERATIONS AS EARLY INDICATORS OF ADVERSE EFFECTS OF EXPOSURES
R.S. Paules, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA

10.10 IN194 MASS SPECTRAL DETECTION OF DNA ADDUCTS PRODUCED BY EXPOSURES TO CARCINOGENS
P.B. Farmer, University of Leicester, UK

Angelico Hall
Mutagenesis and health effects
NUCLEOTIDE EXCISION REPAIR AND TRANSCRIPTION: MECHANISMS AND CLINICAL IMPLICATIONS
Chairs: J.M. Egly, M. Stefanini

8.30 IN195 THE NUCLEOTIDE EXCISION REPAIR: TFIIH AND Co
J.M. Egly, CNRS, Strasbourg, France

9.00 IN196 INSIGHTS INTO GENOTYPE-PHENOTYPE RELATIONSHIPS IN THE REPAIR/TRANSCRIPTION SYNDROME TRICHOTHIODYSTROPHY
D. Orioli, Institute of Molecular Genetics, CNR, Pavia, Italy

9.25 IN197 TRANSCRIPTION STALLING AND CELLULAR RESPONSES
L.H. Mullenders, Leiden University, Leiden, The Netherlands

9.50 IN198 INTERSECTING DNA REPAIR PATHWAYS AND COORDINATION WITH TRANSCRIPTION
P. Cooper, Lawrence Berkeley National Laboratory, Berkeley, CA, USA

10.15 DD052 SILENCING OF OXIDATIVELY-DAMAGED GENE IN MAMMALIAN CELLS
A. Khobta, University of Mainz, Germany

Lippi Hall
Prevention of mutation-related diseases
DIETARY FACTORS, MUTATION AND CANCER
Chairs: L. Ferguson, R. Barale

8.30 IN199 DIETARY FACTORS, MUTATION AND CANCER
L.R. Ferguson, The University of Auckland, New Zealand

8.55	IN200	BASE EXCISION REPAIR, OXIDATIVE STRESS AND CANCER ***B. Tudek***, University of Warsaw, Poland
9.20	IN201	DIETARY INTAKE OF ARISTOLOCHIC ACID AS A RISK FACTOR FOR BALKAN ENDEMIC NEPHROPATHY-ASSOCIATED UROTHELIAL CANCER ***V.M. Arlt***, Institute of Cancer Research, Sutton, UK
9.45	IN202	THE ROLE OF GENETIC AND NON-GENETIC MECHANISMS IN FURAN RISK ***A. Mally***, University of Würzburg, Germany
10.10	PD032	MECHANISTIC ASPECTS OF GENOTOXICITY OF FURAN AND ITS KEY METABOLITE CIS-2-BUTENE-1,4-DIAL IN MAMMALIAN CELLS IN VITRO ***P. Mosesso***, Tuscia University, Viterbo, Italy
10.20	EM060	URINARY FUMONISIN B1 AS A BIOMARKER OF FUMONISIN EXPOSURE AND ITS APPLICATION IN INTERVENTION STUDIES ***Y.Y. Gong***, University of Leeds, UK

Bronzino Hall
Risk assessment
REPORTS FROM THE 5TH INTERNATIONAL WORKSHOP ON GENOTOXICITY TESTING
Chairs: D. Kirkland, L. Müller

	IN203	**Reports from the 5th International Workshop on Genotoxicity Testing**
8.30		***D. Kirkland***, Covance, Harrogate, UK
8.45		***S. Galloway***, Merck, West Point, PA, USA
9.00		***M. Moore***, Jefferson, AR, USA
9.15		***P. Kasper***, Federal Institute for Drugs and Medical Devices, Bonn, Germany
9.35		***S. Pfuhler***, Procter & Gamble, Marly, Switzerland
9.55		***M. Hayashi,*** National Institute of Health Sciences, Tokyo, Japan
10.10		***V. Thybaud***, Sanofi-Aventis, Vitry sur Seine, France
10.30		**Coffee break**

Botticelli Hall

11.00	IN204	**Plenary Lecture** Introduction: E. Dogliotti, President European EMS THE MOLECULAR BASIS OF LIFE'S ROBUSTNESS ***M. Radman***, University of Paris 5, Paris, France
11.45	IN205	**Plenary Lecture** Introduction: J. Yang, Representative Chinese EMS MUTATIONS IN microRNA PRECURSORS IN HEMATOPOIETIC MALIGNANCIES ***C.M. Croce***, Ohio State University, Columbus, OH, USA
12.30		**Closing Ceremony**

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ABSTRACTS

Plenary lectures and Symposium abstracts

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IN001

LESION SENSING AND DECISION POINTS IN THE DNA DAMAGE RESPONSE

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Replication and maintenance of cellular genomes are absolute requirements for life. Proliferating cells must duplicate their DNA with incredible precision in the face of a barrage of genotoxic chemicals and radiations. Transcription of DNA-encoded instructions is also crucial, as are the epigenetic modulations of those instructions. Cell function and survival are threatened by alterations in DNA structure that interfere with or compromise the fidelity of replication and transcription. We strive to minimize our exposures to mutagens/carcinogens in the environment while utilizing some of the very same genotoxins in therapeutic protocols for treating cancer and other human diseases.
Some types of DNA damage are more serious than others; one unrepaired double-strand break can be sufficient to preclude the generation of viable daughter cells. A compounded threat may arise when a replication fork encounters an arrested transcription complex. Non-canonical DNA structures in naturally occurring nucleotide sequences can also block transcription and replication. Different parts of the genome (e.g. telomeres) may be more at risk than others. The DNA damage response must be highly versatile to sense a multiplicity of different sorts of lesions in various sequence contexts and relegate them to appropriate pathways for repair.
We have learned about many DNA repair pathways as well as tolerance modes for persisting damage. The most versatile schemes are based upon excision repair, in which the faulty segment of a DNA strand is replaced by repair replication, using the undamaged complementary strand as template. We are finding many instances of crosstalk and overlap between different repair pathways; the various modes for processing damage may compete with each other. Each step in a repair pathway generates an intermediate that may be susceptible to intervention by enzymes from another pathway. It may be instructive for us to view overall damage processing, and the allocation of cellular resources in terms of a series of layers in the stages of repair, in which there are "decision points" at each level until DNA integrity is finally reestablished. The outcome for the cell and for the organism of which it is a part may depend upon which protein encounters the lesion first.

IN002

CANCER RISK FROM EXPOSURE TO URBAN AIR POLLUTION

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Polycyclic aromatic hydrocarbons (PAHs), benzene and 1,3-butadiene are among the major carcinogenic compounds found in urban air pollution from motor vehicle emissions. PAHs such as benzo[a]pyrene are believed to be associated with lung cancer, benzene is known to cause leukemia, and 1,3-butadiene has been shown to cause lymphoma and hematopoietic cancer. In major cities in Asia, the levels of PAHs and benzene are relatively high compared with those in Europe or in the United States. People living in such cities, therefore, are exposed to high levels of these carcinogenic pollutants. Work in our laboratory has included monitoring of PAHs and benzene and 1,3-butadiene exposure in many susceptible groups of the population, such as in traffic policemen. In addition to monitoring both ambient as well as personal exposure through collection of air samples, we have also measured the internal dose as well as the potential health effects through the use of various biomarkers associated with the different toxicants. Through these biomarkers, a cause and effect relationship can be established. People who spend most of their time close to the traffic source are exposed to higher levels of these carcinogens. The traffic police were exposed to a 20-fold higher level of total PAHs than the office police. Consequently, urinary-1-hydroxy pyrene (a metabolite of PAH) level and PAHs albumin adduct level were also higher in the traffic police group. PAH-DNA adduct which is a biomarker of biologically effective dose and is predictive of the risk of cancer development was also significantly higher in the traffic police group. Exposure to benzene and 1,3-butadiene are significantly higher in traffic police than in their office counterparts. The potential health risks from exposure to carcinogenic substances were assessed through DNA-damage levels and DNA repair capacity. DNA strand breaks and 8-OHdG levels were significantly higher, whereas DNA repair capacity was significantly reduced in traffic policemen. This indicates an increased health risk of the development of cancer in traffic police due to exposure to genotoxic substances in air pollution.

IN003

SEVEN DEADLY SINS OF ENVIRONMENTAL RESEARCH

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The evidence from environmental mutagen research is an imperfect tool to further public health. Although even ivory-tower scientists are only rarely indifferent to the welfare of others, modern-day research is organised particularly to favour an appetite for credentials and publications. Further, scientific tradition demands replication and verification, so that the majority of published papers in environmental science journals focus on a limited, rather stable list of mutagens and pollutants. Worse, science frequently does not appear as impartial as it should be. The Vatican recently deemed pollution a sin, so would it not be fair to ask whether science can be sinful? I have compiled a list of vices in environmental health science as suggested by the traditional seven deadly sins. They include (with examples of sins common in science) Pride (preoccupation with methodology), Envy (failure to recognize achievements by others), Wrath (self-righteous intimidation of competitors), Lust (desire for academic honours), Gluttony (excessive craving for publications), Greed (benefit from vested interests), and Sloth (callousness to injustice). Inspired by the precautionary principle, I wish to highlight (see Epidemiology 2008; 19: 158-62), some specific virtues that are needed to counter the vices: Humility, Fairness, Empathy, Restraint, Innovation, Transparency, and Compassion. These virtues relate to some overall values that need to be introduced or revived in research. Resources should no longer be spent chasing a formal proof. Instead, uncertainties should be explored and their implications characterised. We should therefore pay more attention to the range of the confidence interval and less to the p value. As part of the scientific discourse, we must ask ourselves what could possibly be known given our (limited) research insights and opportunities. Absence of evidence should of course not be misunderstood as evidence of absence of a hazard. Science planning and reporting therefore ought to be recognized as a social activity that forms part of a dynamic interface with policymaking and intervention. Our research deserves an open discussion to explore the wider perspectives of our findings and the implications of the uncertainties.

IN004

EPIGENETIC MECHANISMS AND THE REGULATION OF GENOMIC IMPRINTING IN MAMMALS

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The term 'epigenetic' is used to refer to heritable phenotypes that occur without changes in the DNA sequence. Epigenetic regulation of gene expression plays an important role in animal and plant development, for example, to achieve stable repression in specific cell types and at defined developmental stages. In placental mammals, the maternally and paternally inherited genomes are functionally not equivalent. They are both required for the embryo's development and well-being, throughout gestation, and also after birth. This functional asymmetry between the parental genomes is a consequence of differential marking of the DNA in the egg versus the sperm. The differential methylation marks placed onto the parental genomes (the imprints) persist in the developing embryo, and after birth, and convey the allelic expression of genes from either their maternal or their paternal copy. At least a

hundred genes are controlled by this epigenetic phenomenon called genomic imprinting. Many of the known imprinted genes play key roles in foetal development and growth, others influence behaviour after birth. Not surprisingly, therefore, pathological perturbation of genomic imprinting gives rise to growth-related and behavioural diseases in humans, and is associated with cancer as well. In addition, environmental stress, including in vitro culture and manipulation, can readily lead to perturbation of imprints and this may have long-lasting phenotypic consequences. After a brief introduction of epigenetics and the importance of DNA methylation, I will present the developmental regulation of imprinting. During my talk, I will present some of our group's recent work on the role of histone methyltransferases in the unusual organisation of chromatin at the key regulatory sequences involved imprinted gene expresion, the 'imprinting control regions'. Recently, we have started to explore the importance of histone methylation in the regulation of tissue-specific imprinting. I will present several examples of how lysine methylation on histone H3 controls imprinted gene expression in the placenta and brain, and will describe the enzymatic machineries involved.

IN005

DEVELOPING HUMAN EMBRYONIC STEM CELLS TO MODEL ENVIRONMENTAL EFFECTS ON THE DEVELOPING EPIGENOME

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The formation of the pluripotent cells of the preimplantation embryo, giving rise to all tissues of the fetus, is a rapid process that requires extensive remodelling of the highly specialised and diverse oocyte and sperm cells post-fertilisation. In addition to structural changes, this remodelling phase encompasses reprogramming of the wide range of epigenetic modifications to DNA and their associated histone proteins that are key to specifying cell lineage. The interest of our laboratory is the degree of plasticity that can be conferred on the embryonic epigenome by environmental factors such as maternal nutrition and *in vitro* embryo culture and the relevant phenotypic consequences of such early "programming" events. In particular, we are examining the hypothesis that plasticity in the addition of epigenetic modifications to DNA during early development can alter subtly the trajectory of fetal development in a manner that can predispose both healthy and diseased functioning of adult tissues. Our focus is on the cellular methyl and folate cycles that determine the provision of methyl groups required to methylate both cytosine residues of DNA (DNA methylation) and histone proteins (histone methylation). In addition to animal studies, we are also developing human embryonic stem cells to investigate the effects of dietary methyl group contributors on embryonic cells directly within the species of clinical interest. Although this *in vitro* model has required some development in order to address the experimental goals outlined above, the ability to perform a wide range of dose-response experiments assessing epigenetic effects on the undifferentiated cells, gene-nutrient interactions using cell lines with different genotypes and ability to form specific disease-relevant human fetal cell types makes this an exciting novel model to answer fundamental developmental questions. Our latest findings will be presented and used to illustrate how both environmental influences and nutrient-gene interactions can impact on the embryonic epigenome with more important later health consequences than is often recognised.

IN006

EPIGENETIC REGULATION OF AGING

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Many age-associated conditions, such as the decrease in regenerative capacity of tissues, appear to be determined by a decline in the function of specific somatic stem cells. Although it is obvious that the genotype determines the average lifespan of different species, the variation in lifespan of individuals within a species seems to be more affected by the accumulation over time of molecular errors that compromise adult stem cell function. These molecular alterations can occur at both the genetic and epigenetic levels and depend on hereditary, environmental and stochastic factors. This complex multifactorial mixture determines characteristics, such as longevity and a healthy life, that are central concerns of human existence.

IN007

EPIGENETIC PROCESSES IN DEVELOPMENTAL ORIGINS OF HEALTH AND DISEASE (DOHaD)

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The heritable or familial components of susceptibility to chronic non-communicable diseases such as type 2 diabetes, obesity and cardiovascular disease were thought to be largely genetic (from GWAS) or environmental (shared lifestyle risk factors). There is now increasing evidence that some elements of such heritability are transmitted non-genomically and can have effects beyond a single generation[1]. The underlying processes operate through epigenetic mechanisms involving regulation of either imprinted or non-imprinted genes, via changes in DNA methylation, histone structure and miRNAs. These affect offspring phenotype, especially response to environmental challenges[2]. Other processes include parental physiology or behaviour, e.g. utero-placental blood flow or suckling. DOHaD is a maladaptive consequence of an ancestral mechanism of developmental plasticity, with adaptive value in the evolution the generalist species *Homo sapiens*. However exposure to high energy and fat content foods, and low levels of physical activity, occurring within a generation, leaves today's children and adolescents mismatched[3]. The risks of chronic disease are increased, and these in turn increase risk in the next generation[4]. The effects are exacerbated by demographic and reproductive changes, e.g. the tendency for women to have children at the extremes of reproductive age, more primiparous pregnancies, ART. The social consequences of DOHaD will be great in economic terms, particularly in developing countries[5]. Understanding the underlying epigenetic processes will aid prediction of those at risk and design and monitoring of interventions.
MAH and GB are supported by the BHF.
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IN008

SURVIVAL AND DEATH STRATEGIES IN CELLS EXPOSED TO GENOTOXINS

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Genotoxins induce a plethora of DNA damages that are usually considered to be harmful for the cell. However, not every type of DNA damage is mutagenic and not each of them is cytotoxic and has the ability to activate the DNA damage response (DDR). Therefore, it is important to identify critical lesions that trigger specific responses. This has been successfully accomplished for alkylating agents, which are powerful mutagens and carcinogens and, moreover, are being used in cancer therapy. Repair of alkylation lesions is executed by ABH proteins, base excision repair and MGMT, which contribute to survival of exposed cells to different degree. Furthermore, translesion polymerases such as Rev3 may also be involved in defence against alkylation damage. If repair is lacking, saturated or down-regulated, non-repaired lesions can activate complex cellular responses. Thus, in MGMT lacking cells O^6-methylguanine (O^6MeG) mispairs with thymine that activates the mismatch repair system. This generates DNA double-strand breaks (DSBs) which in turn activate MRN, ATR, ATM, Chk1, Chk2 dependent pathways as well as MAP kinase and p53 driven functions. Other lesions are tolerated or, upon blocking replication, generate DSBs, which are considered to be most important downstream genotoxic lesions activating DDR. Therefore, mechanisms repairing DSBs such as homologous recombination and non-homologous end joining appear to play a decisive role for many genotoxins as well. Both survival and death functions can be activated by DNA damage. Survival strategies include activation of DNA repair, autophagy and antiapotiotic functions, whereas death strategies rest on the activation of death receptor and/or mitochondrial apoptotic functions and mitotic catastrophe. We have shown that in some cells the efficiency of specific lesions such as O^6MeG to trigger the p53 dependent death receptor (Fas) pathway is much higher than the p53 independent endogenous mitochondrial pathway, which has strong impact on the sensitivity of p53 mutated tumor cells to anticancer drugs such as temozolomide. An opposite effect of p53 was found for agents inducing bulky lesions, such as chloroethylnitrosourea and UV light, thus protecting against apoptosis and necrosis. This is due to p53 controled upregulation of repair genes, notably *ddb2* and *xpc*. Data will be shown to dissect the role of WRN, NBN, XRCC2 and BRCA-2 as well as DNA-PK_{CS} in the O^6MeG response, which support a role for DSBs as most critical downstream apoptosis-triggering lesions and homologous recombination as a key player in the cell's survival strategy towards monofunctional alkylating agents. For genotoxins inducing bulky lesions and interstrand crosslinks transcriptional inhibition comes into play as well. Data will be shown that genotoxin provoked block of transcription attenuates the expression of MKP1, which leads to sustained activation of the MAP kinase pathway that finally triggers cell death.

Work was supported by DFG KA724/13, German Cancer Foundation and Stiftung Rheinland-Pfalz.

IN009

TRANSCRIPTIONAL INHIBITION BY DNA DAMAGE AS A TRIGGER OF CELL DEATH

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It is becoming increasingly clear that transcription is not limited to just protein-coding genes but rather, most of the genome is traversed in both directions by RNA polymerase II. While many of the noncoding RNAs generated have regulatory functions, it is still surprising that the genome is so heavily transcribed. One potential role of this pervasive transcription of the genome is that RNA polymerase II may act as a damage scanning device alerting the cell when transcription-blocking lesions are ecountered. Indeed, we have shown that blockage of RNA polymerase II triggers the induction p53 (Oncogene, 18:583, 1999) and that this induction is due to both the loss of RNA-mediated export of p53 and by phosphorylation of p53 in an ATR and RPA-dependent manner (PNAS, 104:12778, 2007). Importantly, if transcription-blocking lesions are not removed in a timely fashion, cells undergo apoptosis (Oncogene, 13:823, 1996). In proliferating cells, transcription blockage by DNA-damaging agents such as UV light, cisplation or photo-activated psoralen induces apoptosis predominantelly in cells moving through S-phase. Presumably a tug-a-war between the replication and transcription machineries for the same piece of DNA leads to apoptosis. In stationary cells, apoptosis may be induced by the loss of expression of gene products important for survival and we are currently using a new technique to explore the effect that UV light has on the synthesis and stability of mRNAs from apoptosis-regulating genes.

IN010

DIFFERENT MODES OF CELL DEATH INDUCED BY DNA DAMAGE

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In dividing cells DNA damage caused by genotoxic insults results in the activation of cell cycle checkpoints followed by DNA repair to ensure the integrity of the transcribed genome. p53, being a "guardian of genome" is activated by stress and, depending on the severity of damage, it triggers either G1 or G2 arrest or cell death. DNA damage-induced apoptotic pathway includes caspase-2, which is activated within the PIDDosome complex, and causes cytochrome *c* release and caspase activation. Hence, PIDDosome-mediated caspase-2 activation might be an important link between DNA damage and the engagement of the mitochondria-mediated apoptotic pathway. In addition to PIDDosome, caspase-2 is able to use the CD95 DISC as a platform and the recruitment of caspase-8 to this complex is required for activation of both enzymes. Investigation of the contribution of p53 and caspase-2 to apoptosis and mitotic catastrophe (MC) induced by DNA damage in carcinoma cells revealed that both functional p53 and caspase-2 are required for the apoptotic response, which was preceded by translocation of caspase-2 to the cytoplasm. In the absence of functional p53, DNA damage resulted in caspase-2-independent MC followed by necrosis. In these cells apoptotic functions could be restored by transient expression of wt-p53. Hence, in this experimental model p53 appeared to act as a switch between apoptosis and MC followed by necrosis-like lysis. It seems that the final mode of cell death triggered by DNA damage in cancer cells is determined by the profile of proteins involved in the regulation of the cell cycle.

IN011

ROLE OF DNA-PKcs-PIDDosome IN DNA DAMAGE RESPONSE

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Apoptosis (programmed cell death) is an essential process for eliminating unwanted cells in development and homeostasis. It is executed through precisely orchestrated biochemical and genetic pathways. Dysregulation of apoptosis can lead to human diseases including cancers. In addition, DNA repair and cell cycle checkpoint control is a genome surveillance mechanism to prevent the passage of damaged genetic material to daughter cells and antagonize tumorigenesis. The crosstalk between apoptosis and DNA repair and checkpoints is of importance since they are both related to tumorigenesis. Caspase-2 plays a pro-apoptotic role in apoptosis. The identification of the protein complex PIDDosome has accelerated our understanding of caspase-2 activation apoptosis in response to genotoxic stress. Caspase-2 is unique among all the mammalian caspases in that it is the only caspase that is present constitutively in the cell nucleus, in addition to other cellular compartments. However, the functional significance of this nuclear localization is unknown. We have used a combination of biochemistry, proteomics, and cell biology to investigate the role of caspase-2 in the cell nucleus in response to DNA damage induction. We have found that DNA damage induced by gamma-radiation triggers the phosphorylation of nuclear caspase-2, leading to its cleavage and activation. This phosphorylation is carried out by the nuclear serine/threonine protein kinase DNA-PKcs and is promoted by the death-domain protein PIDD within a large nuclear protein complex consisting of DNA-PKcs, PIDD, and caspase-2, which we have named DNA-PKcs-PIDDosome. In contrast to PIDDosome that activates caspase-2

for apoptosis, characterization of this DNA-PKcs-PIDDosome has revealed unexpected novel functions of it in DNA damage response pathway. Data will be presented to show the molecular mechanism of this protein complex in the cellular response to DNA damage. The ability of caspase-2 to participate in both pro-apoptotic and pro-survival processes means that caspase-2 may stand at the crossroads of a DNA-damage response network, connecting apoptosis and cell survival to determine cell fate.

IN012

CELL-CYCLE BLOCKAGE AFFECTS DNA DAMAGE RESPONSES THAT LEAD TO DEATH IN HUMAN PRIMARY FIBROBLASTS.

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Unrepaired DNA lesions promote blockage of RNA transcription and DNA replication, which trigger signals that eventually lead to cell death. This results in higher sensitivity of cells that are deficient in DNA repair pathways, such as those from human patients suffering from the xeroderma pigmentosum (XP) and Cockayne's (CS) syndromes. In this work, we will present the differences in UVB-induction of apoptosis in synchronized human primary XP and CS cells, and correlate it with cell cycle progression, as well as the activation of p53 and MDM2 proteins. These two proteins play key roles in the signaling events that lead to cell choice of DNA repair or cell death. In these experiments, we employed UVB doses which were either high just enough to trigger apoptosis or sufficiently low to induce only changes in cell responses, but with almost no apoptosis. As cells have different DNA repair capabilities, these doses were not the same, but were equitoxic. The results indicated that low UVB doses caused only delays in cell cycle progression, while higher UVB doses resulted in consistent cell blockage, in G1 or early S-phase. The accumulation of p53 protein corresponded to the levels of apoptosis induction, however, the induction of MDM2 protein was similar in DNA repair deficient (XP-C) or proficient cells, and the levels of this protein did not correlate with apoptosis. Normally MDM2 acts as a negative regulator of p53, but the data show that the induction of this protein is not the main factor determining cell evasion from apoptosis induced by UVB, as could be expected. In fact, curiously, XP-C cells die despite of high levels of this protein, indicating this is not enough for the cells' choice for survival. The results are also in agreement with a role of DNA replication blockage (at the beginning of S-phase) in triggering cell death events by UVB DNA lesions in primary human cells.

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IN013

TOXICITY ASSESSMENT OF NANOPARTICLES

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Serious concerns have been expressed about risks posed by nanomaterials (NMs) their potential to cause undesirable effects, to contaminate the environment and specifically adversely affect susceptible parts of the population when they are exposed. Information about the toxicity of nanomaterials combined with the knowledge of potential human and environmental exposure will be necessary to determine real or perceived risks of nanomaterials. Key concepts of nanotoxicology are addressed, including significance of dose, dose rate, biokinetics which are exemplified by specific findings of NM toxicity, and by a discussion of the importance of a detailed physicochemical characterization of nanoparticles, specifically surface properties, that influence their biological/toxicological properties and their biokinetics. Examples demonstrate that, on the one hand, we need to be aware of possible acute adverse effects and potential long-term consequences; on the other hand, they show the intriguing possibilities that nanoparticles offer for beneficial industrial applications and in nanomedicine. In addition to potential effects induced at the portal of entry, the respiratory tract, effects in secondary organs have to be considered, e.g., in the brain (neuro-degeneration?) or the pleural cavity (mesothelioma, from fibrous NMs). A thorough evaluation of desirable vs. adverse effects is required for the safe use of engineered NMs, and major challenges lie ahead to answer key questions of nanotoxicology. Foremost are the assessment of human and environmental exposure, the identification of potential hazards, and the biopersistence in cells and subcellular structures in order to perform meaningful risk assessments.

IN014

PHYSICO-CHEMICAL FEATURES IN THE TOXICITY OF ENGINEERED NANOPARTICLES

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With the development of nanotoxicology the crucial role of some chemical aspects in the study of the health effect of particles has been recently emphasized, for two main reasons: (i) Most adverse effects are the consequence of molecular events taking place at the interface between the particle surface and body fluids, cells and tissues. Nanoparticles, by respect to bigger particles, offer a much larger specific surface, and, in some cases, an enhanced or new surface reactivity. (ii) Nanoparticles, because of their size, may move from one to the other biological compartment. However the smaller the particles, the stronger the interparticle forces which determine agglomeration, followed sometimes by aggregation (chemical bonds between the particles). Often in aqueous suspensions there are few free primary particles and very large agglomerate/aggregates. Moreover agglomeration varies with the ionic and molecular content of the solution. Under these circumstances, one needs to measure the nature and the extent of all the reactive surface sites - e.g. free radical generating centres, structural defects, poorly coordinated ions – usually involved in the toxicity mechanisms in order to understand particle/ living matter interactions at the molecular level and predict the potential toxicity of a given material. However the real impact of such features depends upon the extent of surface or number of particles which are really in contact with the target cell or molecule. Dosimetry is becoming one of the most difficult aspects in the design of experimental studies on the toxicity of nanoparticles. So far several proposal have been made, but the question on how to plan experiments and measure the doses - in weight, in surface exposed, in number of particles, in oxidant potential – is still unresolved. Nanoparticles are not all hazardous just because of their size as some media erroneously report. The chemical nature of the solid structure determines surface reactivity, hence toxicity. The physico-chemical features most involved in the adverse responses will be discussed in detail.

IN015

DISTRIBUTION AND EFFECTS OF NANOMATERIALS AFTER INHALATION AND *I.V.* INJECTION IN RATS

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The deposition of TiO_2 nanomaterial in the body was investigated after inhalation and after *i.v.* injection in rats. Deposition, clearance and effects of inhaled TiO_2 nanomaterial was compared with micron-scaled TiO_2 and quartz. Rats were exposed by head/nose inhalation of aerosols [1] on 5 consecutive days [2]. In another study dispersions [3] of TiO_2 nanomaterial in serum were injected into the tail vein of rats [4]. The amount of the test materials in the blood, lung, lymph nodes, liver, kidney, spleen and basal brain with olfactory bulb was determined by ICP-AES and by TEM. The examinations were carried out shortly after the exposure and at different times within three weeks after the first inhalation or *i.v.* exposure and additionally 90 days after *i.v.* injection. After inhalation all three materials deposited similarly due

to their similar aerodynamic particle size). The majority of the deposited TiO_2 nanomaterial was retained in the lung (extracellularly and in macrophages); the particles were mostly agglomerates of about the same size as found in the atmosphere; there were no signs of desagglomeration in the lung. Clearance from the lungs and a translocation to the mediastinal lymph nodes was noted, though in smaller amount than those exposed to micron-scale TiO_2 or quartz. There was no indication of a translocation into other organs. All inhaled materials caused inflammation in the lung, but no systemic effects. Whereas the effects for both nano- and micron-scale-materials were reversible, those of quartz were progressive. Systemically available TiO_2 nanomaterial, as simulated by *i.v.* injection, was trapped mainly in the liver, followed by spleen, lung and kidney. There were no detectable levels of Ti in blood cells, plasma, brain, or lymph nodes. No clearance was observed and no excretion of Ti was detectable in the urine or feces. There were no clinical changes and no changes in blood parameters, indicating that there was no detectable inflammatory response or organ toxicity. The highest concern of inhaled TiO_2 nanomaterial is the (local) effect in the lung, but no translocation or effects in other organs were noted. Systemically available TiO_2 nanomaterial showed no obvious signs of toxicity; the missing clearance from the body is, however, a concern. *This work was supported by the integrated EU (FP6) project NanoSafe2*

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IN016

MECHANISMS OF NANOMATERIALS GENOTOXICITY

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Background/Aims: Because of the growing industrial applications of engineered nanomaterials (ENMs), evaluation of their genotoxic potential and of their mode of action (MoA) is a necessity to conduct adequate hazard/risk assessment and to produce safer and sustainable ENMs. In a recent review published by us (Gonzalez et al. 2008) we aimed (1) at providing an evaluation of in vitro and in vivo genotoxicity data available for ENM and (2) at proposing minimal criteria for conducting nanogenotoxicity assays. The possible MoA of ENM (i.e. reactive oxygen species generation and mechanical interference with cellular components) and the potential cellular targets were discussed. The available studies were evaluated on the basis of the proposed criteria. Results and conclusions: We found that no definitive conclusion can be drawn concerning the genotoxic activity of ENMs, because of the limited number of data, incomplete physico-chemical characterization of ENMs examined and shortcomings in experimental approaches. This evaluation revealed gaps to be considered in future studies (e.g. one-sided approach focusing mainly on ROS as mode of action) and the need to develop adequate positive controls for genotoxicity assays when conducted with nanomaterials. We applied these criteria, when considering amorphous silica nanoparticles (SNPs) of different sizes. The in vitro CBMN assay showed an induction of MN frequencies after treatment with 16 and 60 nm SNPs, with a higher fold induction after treatment with the smallest SNPs, without clear dose-response. When considering the cellular dose, expressed as particle number or as surface area, a quasi-linear relationship with the fold MN induction is observed, indicating that these dose metrics are determinants for genotoxic effects. The alkaline comet assay with addition of FPG revealed the induction of oxidative DNA damage by 16 and 60 nm SNPs. Besides oxidative damage, these SNPs induced other genotoxic effects, such as chromosome loss, metaphase block and mitotic slippage, suggesting interference with the mitotic spindle. In conclusion, in future studies it is crucial to consider (1) appropriate metrics, (2) cellular uptake kinetics and (3) different possible mechanisms of ENM-induced genotoxicity.

IN017

POTENTIAL PULMONARY EFFECTS OF SINGLE-WALLED CARBON NANOTUBE (SWCNT) EXPOSURE: IN VITRO GENOTOXIC EFFECTS

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Pharyngeal aspiration of mice to SWCNT (10-40 µg 1 mouse) resulted in a rapid but transient oxidant stress and inflammatory response as well as diffuse alveolar interstitial fibrosis of early onset (7 days post-exposure) which progressed through 56 days post-exposure. Preliminary histological evaluation of lung tissue 1 yr post-exposure noted epithelial cell hyperplasia and the presence of multinucleated cells. Inhalation exposure (5 mg/m^3, 5 hr 1 day, 4 days) demonstrated similar pulmonary reactions to SWCNT. In addition, inhalation of SWCNT resulted in mutation of the K-ras gene at 1–28 days post-exposure. In vitro exposure of fibroblasts to SWCNT (24 $\mu g/cm^2$) was not cytotoxic and failed to cause significant DNA damage. However, there was a significant elevation in micronucleated cells after a 24 hr exposure. In vitro exposure of human airway epithelial cells to SWCNT (24 $\mu g/cm^2$) was also non-cytotoxic and did not induce apoptosis. However, SWCNT caused abnormalities (non-bipolar) of the mitotic spindles, centrosome fragmentation, anaphase bridges of cytokinesis, and aneuploidy. Since exposure to SWCNT can cause genotoxic effects in vitro and in vivo, long-term evaluation of lung tumors in mice exposed to SWCNT is warranted.

IN018

ERRONEOUS INCORPORATION OF OXIDIZED NUCLEOTIDES BY Y-FAMILY DNA POLYMERASES

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Excess oxidation is a property of many cancer cells. Oxidation of nucleotide pool as well as DNA is a source of mutagenesis, carcinogenesis and cellular senescence. Recent evidence suggests that Y-family DNA polymerases (Pols), a novel family of Pols involved in translesion DNA synthesis, may play important roles in erroneous incorporation of oxidized dNTPs into DNA, thereby contributing to genome instability (Shimizu et al., EMBO Rep., 4, 269-273, 2003). In *Escherichia coli*, spontaneous mutation frequencies of A:T-to-C:G and G:C-to-T:A are extremely elevated in *sod fur* strains and oxidized dNTPs contribute to the mutagenesis. Pol IV (DinB) incorporates 8-oxo-dGTP, an oxidized form of dGTP, exclusively opposite template dA and 2-hydroxy-dATP, an oxidized form of dATP, opposite template dG or dT *in vitro*. The mutator phenotypes are greatly diminished by deletion of *dinB* and *umuDC* encoding Pol V. Thus, Pol IV (DinB) and Pol V (UmuDC), members of Y-family, appear to be involved in the oxidized-dNTP-dependent mutagenesis in the strains (Yamada et al., J. Bacteriol., 188, 4992-4995, 2006). In humans, two of the Y-family Pols, i.e., Polη and κ, incorporate 8-oxo-dGTP opposite template dA and 2-hydroxy-dATP opposite template dG, dT or dC (Shimizu et al., Biochem., 46, 5515-5522, 2007). In particular, Polη incorporates 8-oxo-dGTP opposite template dA at almost the same efficiency as incorporation of normal dTTP and the incorporation into M13 phage DNA results in mainly A-to-C and deletion mutations *in vitro* (Hidaka et al., DNA Repair, 7, 497-506, 2008). To gain insights into the erroneous incorporation by Polη,

amino acids that might modulate the specificity incorporating 8-oxo-dGTP into DNA were substituted. Arg61, which is unique to Polη, appears crucial to the erroneous incorporation because substitution of Arg61 with Lys (R61K) altered the ratio of incorporation opposite template dA:dC from 660:1 (wild-type Pol) to 7:1 (R61K). R61 may alter the conformation of 8-oxo-dGTP from the anti to the syn form in the active site of Polη and therefore direct the erroneous incorporation.

IN019

PROGRAMMED CELL DEATH TRIGGERED BY NUCLEOTIDE POOL DAMAGE

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Intracellular nucleotides suffer chemical modifications caused by endogenous reactive molecules such as reactive oxygen species, or by exogenous factors such as chemicals and ionizing irradiation. Some modified nucleotides are incorporated by DNA/RNA polymerases and accumulate in newly synthesized DNA or RNA, thus prevent DNA replication, transcription or translation, resulting in mutagenesis or cell death. Normal functions of nucleotides, other than DNA/RNA synthesis, may also be adversely affected by modified nucleotides. In human and rodent cells, MTH1, an oxidized purine nucleoside triphosphatase, efficiently hydrolyzes oxidized purine nucleoside triphosphates such as 8-oxo-(d)GTP and 2-OH-(d)ATP, thereby avoiding their incorporation into DNA or RNA. MTH1-null cells exhibit a two-fold increased spontaneous mutation rate and MTH1-null mice exhibit a several-fold increased incidence of spontaneous tumorigenesis in the liver, lung and stomach, demonstrating that accumulation of oxidized purine nucleoside triphosphates is indeed mutagenic and carcinogenic.
We have reported that MTH1-null mice exhibited a greater buildup of 8-oxoG in mitochondrial DNA of striatal nerve terminals of dopamine neurons accompanied by dopamine neuron loss after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine administration, compared with wild-type mice, and that in MTH1-null cells, the buildup of 8-oxoG in both nuclear and mitochondrial DNA was induced by exposure to nitric oxide donor, resulting in mitochondrial dysfunction and finally cell death. These facts indicate that oxidized nucleotides may be one of the major causes of such cell dysfunction or death under oxidative stress. We demonstrated that incorporation of 8-oxo-dGTP into mitochondrial DNA in MTH1-null cells exposed to nitric oxide donor causes a programmed cell death dependent on MUTYH which excises adenine inserted opposite 8-oxoG in DNA. Moreover, we found that exposure of MTH1-null cells to 2-OH-ATP or 2-OH-adenosine causes p38-MAPK activation, resulting in growth arrest and delayed cell death which is efficiently suppressed by p38-MAPK inhibitor as well as by expression of MTH1. We thus concluded that nucleotide pool damage triggers programmed cell death through divergent pathways.

IN020

MULTIPLE ROLES OF THE MTH1 HYDROLASE: PROTECTION AGAINST NEURODEGENERATION AND CONTROL OF LIFE SPAN

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Background: We have previously shown that high level of the human MTH1 hydrolase in the brain of a transgenic mice (hMTH1-Tg) conferred robust protection against neurodegeneration, and significantly improved the neurobehavioural phenotype in a chemical model of Huntington disease (De Luca et al., 2008). Aim of this study was to assess the hypothesis that expression of hMTH1 influences the aging process and the associated behavioural changes by reducing the detrimental consequences of oxidative stress. Methods: To determine whether hMTH1 expression significantly increased lifespan, hMTH1-Tg mice and their wild-type littermates were maintained until death. Levels of oxidized guanine in DNA and RNA were also measured by HPLC/EC in different brain areas. hMTH1-Tg mice and their corresponding wild-type controls were assessed at different ages (from day 11 to 2 years) for exploration and anxiety levels, learning and memory functions and motor coordination. In parallel with the behavioural analysis, serum total anti-oxidant capacity was also evaluated. Finally we investigated whether the low levels of oxidized purine nucleotides in the soluble pool provided by hMTH1 over-expression affected senescence of early-passage mouse embryo fibroblasts (MEFs) derived from hMTH1-Tg animals. Results: compared to wild-type mice, hMTH1-Tg animals show increased lifespan accompanied by a strong decrease in the levels of oxidation of nucleic acids in striatum, hippocampus and cortex. hMTH1-Tg mice showed increased curiosity towards environmental and social cues and this more explorative phenotype became significant with old age. These findings indicate that oxidized nucleotides are involved in senescence *in vivo*, and suggest that hMTH1 exerts a crucial protective role against degenerative process associated with aging. In addition the observation that early passage MEFs from hMTH1-Tg mice are prevented from entrying into senescence supports a major role of hMTH1 as a general protective factor against *in vitro* senescence.

IN021

INCORPORATION OF EXTRACELLULAR 8-OXODG INTO DNA AND RNA REQUIRES PURINE NUCLEOSIDE PHOSPHORYLASE IN CULTURED MAMMALIAN CELLS AND MICE.

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7,8-Dihydro-8-oxo-2'-deoxyguanosine (8-oxodG) is a well-known marker of oxidative stress. We report a mechanistic analysis of several pathways by which 8-oxodG is converted to nucleotide triphosphates and incorporated into both DNA and RNA. Exposure of MCF-7 cells to [^{14}C]8-oxodG combined with specific inhibitors of several nucleotide salvage enzymes followed with accelerator mass spectrometry provided precise quantitation of the resulting radiocarbon-labeled species. Concentrations of exogenously dosed nucleobase portion of 8-oxodG in RNA reached one per 10^6 nucleotides, 5–6-fold higher than the maximum observed in DNA. Radiocarbon incorporation into DNA and RNA was abrogated by Immucillin H, an inhibitor of human purine nucleoside phosphorylase (PNP). Inhibition of ribonucleotide reductase (RR) decreased the radiocarbon content of the DNA, but not in RNA, indicating an important role for RR in the formation of 8-oxodG-derived deoxyribonucleotides. Inhibition of deoxycytidine kinase had little effect on radiocarbon incorporation in DNA, which is in contrast to the known ability of mammalian cells to phosphorylate dG. Exogenous 8-oxodG could also be incorporated into the DNA of normal tissues and tumors in a mouse model of human breast cancer. Our data indicate that PNP and RR enable nucleotide salvage of 8-oxodG in MCF-7 cells, a recently characterized pathway that may contribute to mutagenesis and carcinogenesis.

IN022

MUTAGENICITY OF OXIDIZED DNA PRECURSORS IN LIVING CELLS: ROLES OF NUCLEOTIDE POOL SANITIZATION AND DNA REPAIR ENZYMES, AND Y-FAMILY DNA POLYMERASES

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An increasing body of evidence suggests that endogenous oxidation of DNA and DNA precursors by reactive oxygen species seems to induce spontaneous mutations, various diseases including cancer and neurodegeneration, and normal aging. 8-Hydroxy-2'-deoxyguanosine 5'-

triphosphate (8-OH-dGTP, also known as 7,8-dihydro-8-oxo-dGTP) is an oxidized form of dGTP, and its presence in the mitochondrial nucleotide pool was recently shown. DNA polymerases that incorporate 8-OH-dGTP, hydrolyzing enzymes specific for the oxidized dGTP (nucleotide pool sanitization enzymes), and DNA repair proteins would be involved in enhancement/suppression of the mutagenesis induced by 8-OH-dGTP. To assess roles of these proteins in the mutagenesis induced by 8-OH-dGTP, the oxidized dGTP and reporter plasmid containing the supF gene were introduced into human 293T cells in which the nucleotide pool sanitization and DNA repair enzymes, and Y-family DNA polymerases were knocked-down by siRNAs. We found that the knock-downs of DNA polymerases η and ζ, and REV1 by siRNAs reduced the induced A:T→C:G substitution mutations. Moreover, the knock-down of MYH (MUTYH) decreased the mutations induction by 8-OH-dGTP. On the other hand, the knock-downs of the three nucleotide pool sanitization enzymes, MTH1, MTH2, and NUDT5, seemed to enhance the induced mutation. These results suggest that DNA polymerases η and ζ, and REV1 are involved in the misincorporation of 8-OH-dGTP opposite A and that the MYH protein fixes the induced mutation. The three nucleotide pool sanitization enzymes are suggested to prevent the mutagenesis by 8-OH-dGTP, by hydrolyzing the oxidized dGTP or the diphosphate derivative.

IN023

AN OVERVIEW OF CURRENT ISSUES IN MODE OF ACTION ANALYSIS AND THEIR USE IN CANCER RISK ASSESSMENT

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Advances in the scientific understanding of mode(s) and mechanism(s) by which chemicals in the environment induce cancer in humans present a new challenge to incorporate this information into human health risk assessment. This challenge is complicated by issues including the human relevance of experimental animal data (in vivo and in vitro), extrapolation to low levels of environmental exposures, and use of biologically-based dose response models in risk assessment. Elucidation of mode of action (MOA) for a carcinogenic response in animals or humans can inform human health risk assessments in a number of ways. For instance, MOA information helps inform the extrapolation of laboratory animal-based toxicity study results to humans. However, the increasing availability of the MOA data raises several important issues as to its application to risk assessment. For example, (1) characterizing the extent to which a hypothesized MOA for a particular response to a specific chemical exposure is sufficiently supported in in vitro and in vivo experimental animals; (2) evaluating the degree to which there are inter- and intra-species differences in MOA; and (3) determining whether a MOA supports particular approaches to low dose extrapolation. These issues will be discussed in the symposium presentations using case examples. For instance, dose response relationships among aneugens and their implications to MOA analysis and risk assessment, use of MOA information for inter- and intra-species differences, toxicokinetic modeling of cadmium-induced carcinogenicity will be discussed. Lastly, International Agency for Research on Cancer's new approach for using mechanistic data in the classification of carcinogens will be presented.
Disclaimer: The views expressed are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA.

IN024

ASSESSING IN VITRO DOSE-RESPONSE RELATIONSHIPS FOR ANEUGENS.

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Mode of action information, combined with a determination of whether or not a chemical is mutagenic, is playing an increasingly important role in determining how cancer risks should be estimated at low doses. Both gene and chromosomal mutations are considered important precursor events for cancer, and information on these events may be useful in informing decisions in the risk assessment process. One of the key decisions that must be made is whether the shape of the dose response curve in the low dose region is linear, curvilinear or exhibits a threshold. Obtaining this information for agents that are believed to act through chromosomal mechanisms has been problematic as experimental methods have restricted the numbers of cells and doses that could feasibly be scored. Recently, Muehlbauer and Schuler have developed a flow cytometric method for rapidly measuring numerical chromosome aberrations in human lymphocytes. We have used this method with human TK6 lymphoblastoid cells to examine the shapes of the dose-response curves for hypodiploidy, hyperdiploidy and polyploidy induced by a series of model aneuploidy-inducing agents including vincristine sulfate, paclitaxel and noscapine. The experiments were performed with closely spaced doses and by evaluating approximately 2000 mitotic cells per test concentration for each replicate experiment. Initial results indicate that, while nonlinear increases were occasionally seen for polyploidy and hyperdiploidy, the increases in hypodiploidy induced by these three aneugens exhibited linear or curvilinear responses, particularly in the low dose region, with no evidence of a distinct threshold. Additional studies and analyses are ongoing but these initial results are consistent with other reports and emphasize the difficulties of demonstrating the existence of thresholds using empirical approaches.

IN025

THE MUTAGENIC POTENTIAL OF FORMALDEHYDE AND ITS RELEVANCE FOR CARCINOGENESIS

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The mutagenic potential of formaldehyde (FA) has been well characterized in numerous in vitro tests. It is generally accepted that the primary FA-induced DNA alterations are DNA-protein cross-links (DPX). Incompletely repaired DPX can lead to the formation of mutations. Chromosomal effects (chromosome aberrations and micronuclei) seem to be most efficiently induced. In vivo, local genotoxic effects at the site of contact (induction of DPX in nasal mucosa cells) have been demonstrated in experimental animals after FA inhalation. Due to its high reactivity and efficient metabolic inactivation, systemic mutagenic effects of FA are unlikely. However, contradictory results regarding distant site genotoxicity have been published. In the context of a comprehensive in vivo study on toxic effects of FA inhalation, we investigated local and distant site genotoxicity in rats exposed to FA (0.5 to 15 ppm) by inhalation for 28 days. Using the comet assay for the detection of DPX, the SCE-test and the micronucleus test (MNT), no indications for distant site genotoxicity (lung, blood) were found. The lack of systemic mutagenic effects excludes a direct action of FA on bone marrow as a potential mechanism for the induction of leukemia. The contribution of FA-induced local mutagenicity to carcinogenesis is less clear. Local mutagenic effects may contribute to the formation of nasal tumors. However, dose-response investigations and mode of action considerations suggest a threshold mechanism for FA-induced mutagenicity and carcinogenicity. Although FA induces mutations in directly exposed proliferating cells, it is unlikely that local genotoxic effects can lead to cell transformation at distant sites. In vitro experiments have shown that DPX are efficiently repaired in all cell types studied so far. The induction of mutations seems to require high DPX levels that also cause cytotoxic effects. Cocultivation experiments with primary human nasal epithelial cells suggest that FA that has entered a cell reacts within the cell but is not released and does not damage other cells (e.g., lymphocytes). Taken together, mutagenicity data for FA do not support a hypothesis assuming induction of leukemia by damaging progenitor cells in the nasal tissue or peripheral blood.

IN026

EMS IN VIRACEPT - A LESSON ON MUTATION THRESHOLDS FOR ALKYLATING AGENTS

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The accidental contamination of Viracept, an HIV medication with high levels of the mutagen, teratogen and carcinogen ethyl methanesulfonate (EMS) in 2007 led to a suspected exposure of several thousand patients with likely several mg EMS per day for a period of ~3 months. The marketing authorization holder, F. Hoffmann La-Roche subsequently designed a series of non-clinical in vitro and in vivo studies to better define quantitatively the risk for mutations emerging from exposure to EMS. These studies yielded clear and statistically unequivocal evidence for a threshold of mutation induction in pivotal tissues by EMS. In vitro and in vivo studies on metabolic stability, bioavailability, and exposure of EMS facilitated an quantitative human risk assessment for mutagenesis by EMS in humans. This risk assessment indicated that there was highly likely no risk for affected patients beyond their background risk. These studies can become model type studies for risk assessment for genotoxic carcinogens. For the first time, reliable evidence was generated for a mutational threshold of an alkylating agent in vivo. The studies do also facilitate the proposal for a "permitted daily exposure" value for such alkylating agents, e.g. if they occur as impurities in drug products. This PDE is much higher than currently enforced levels of control.

IN027

FOOD CADMIUM AND THE RISK OF HORMONE-RELATED CANCERS: A POPULATION-BASED PROSPECTIVE COHORT STUDY

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It has been suggested that environmental pollutants mimicking the effects of estrogen contribute to the disruption of the reproductive system of animals and to the high incidence of hormone-related cancers in Western populations. Even though this hypothesis has received extensive media attention, data have hitherto been sparse in supporting such associations in humans. The food-contaminant cadmium was recently, and unexpectedly, discovered to possess hormone disruption properties. The metal was proposed to increase ER alpha-mediated cell proliferation. Environmentally relevant doses of cadmium induced several well-characterized estrogenic responses in vivo, including increased uterine weight, hyperplasia and hypertrophy of the endometrial lining and increased mammary epithelial density in animals. The possible human health consequences of such effects were not known. The overall aim of this project is to prospectively examine the association between food cadmium exposure and incidence of hormone-related cancers in a large population-based cohort on Swedish women. A food-cadmium database on the cadmium content in all foods available on the Swedish market was created. The estimated dietary cadmium intake for each subject in the cohort was assessed based on the consumption (a food questionnaire completed at baseline in 1987). The average estimated dietary cadmium intake was 15 µg/day, of which plant food contributed to more than 80%. Among 30,000 postmenopausal women, free of cancer diagnose at baseline (1987), during average 15 years of follow-up, we ascertained 378 incident cases of endometrioid adenocarcinoma and 1200 incident cases of breast cancer. Cadmium intake was statistically significantly associated with increased risk of endometrial cancer. Specifically, we observed a 2.9-fold increased risk (95% confidence interval, 1.05-7.79) associated with long-term cadmium intake consistently above the median ass assessed in at baseline 1987 and in 1997 in never-smoking women with low estrogen. Although these results need to be confirmed by experimental as well as further epidemiological studies, our results support the hypothesis that cadmium may exert estrogenic effects and thereby increase the risk of hormone-related cancers.

IN028

ANALYSIS AND INCORPORATION OF MECHANISTIC DATA IN DECISION-MAKING ON SEVERAL CARCINOGENS AT IARC

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The aim of the *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans* is to critically review and evaluate the published scientific evidence on carcinogenic hazards to which humans are exposed. These include chemicals, complex mixtures, physical agents, biological agents, occupational exposures, and lifestyle factors. International, interdisciplinary Working Groups of expert scientists develop the critical reviews and evaluations, which are published in the *IARC Monographs* series. Over time, the structure of a *Monograph* has evolved to include sections on exposure, epidemiology of cancer in humans, studies of cancer in experimental animals, and mechanistic and other relevant data, followed by a summary, and a section with evaluations and a rationale. The Preamble to the *IARC Monographs* describes the objective and scope of the programme, the scientific principles and procedures used in developing a *Monograph*, the types of evidence considered, and the scientific criteria that guide the evaluations. The recent revision of the Preamble was motivated by the shift in cancer research from mostly 2-year bioassays in animals and epidemiological studies with cancer as the endpoint to an increasing prevalence of studies in molecular epidemiology and on mechanisms of carcinogenesis. Mechanistic data can now play a role in the classification into any Group, and several agents (e.g., benzo[*a*]pyrene) were newly classified as *carcinogenic to humans* (Group 1) with a contribution from strong mechanistic evidence in exposed humans, and in the absence of specific epidemiological data. The forthcoming Volume 100 of the *Monographs* will review the more than 100 agents that had been placed in Group 1 in Volumes 1-99. In updating previous evaluations, Volume 100 is identifying cancer hazards that were unknown at the time of the first review. So far, several significant new consensus findings have emerged, e.g., linking estrogen-only menopausal therapy with ovarian cancer, infection with hepatitis-C virus with non-Hodgkin lymphoma, and exposure to asbestos with ovarian cancer. Volume 100 will provide the foundation for future cancer assessments, in which molecular epidemiology and information about mechanisms will play a larger role. To this end, Volume 100 is also summarizing new information on the multiple mechanisms of action for the known human carcinogens. This will give insight into how other agents may cause cancer in humans and will be useful in future assessments of new chemicals, for which 2-year bioassays and epidemiological studies are likely to be unavailable. Thus the *IARC Monographs Programme* is preparing for the way that carcinogens will be identified in the future, when mostly mechanistic information is likely to be available for newly introduced chemicals.

IN029

ROLE OF EPIGENETIC DEREGULATION IN RADIATION-INDUCED GENOME INSTABILITY AND CARCINOGENESIS

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In recent decades, improvements in treatment have led to significant increases in survival and cure rates. In Canada, cancer is the leading cause of post-neonatal death in children and young adults. One in 400 Canadian adults are survivors of childhood cancer. More than two-thirds of them experience late-occurring health problems, such as treatment-related secondary tumors. Most cancer patients undergo radiation diagnostics and are treated with radiotherapy. These modalities are mutagenic and genotoxic. There is well-documented evidence that radiation exposure leads to transgenerational genome instability in the offspring of exposed parents. The exact molecular mechanisms of this phenomenon have yet to be defined; however recent evidence suggests that it may be epigenetic in nature. Epigenetic changes include alterations in DNA methylation, histone modification, and small RNA - associated silencing. To fully understand transgenerational genome instability, it is important to define: (i) what happens in the carcinogenesis-target-organs of the progeny; and (ii) what happens in the germline of exposed parents. Therefore, we dissect the epigenetic mechanisms of transgenerational changes in unexposed progeny from exposed parents. In parallel, we will dissect the molecular changes in the germline of exposed animals. We have confirmed that epigenetic changes, especially altered global DNA methylation and changed miRNA expression, are important determinants of radiation-induced transgenerational genome instability. Specifically, we proved that whole body and local-

ized body part exposure causes transgenerational genome instability in the offspring of irradiated parents; (ii) IR-induced transgenerational genome instability manifests in the organs that are main targets of carcinogenesis: hematopoietic tissues, such as thymus, spleen and bone marrow; (iii) transgenerational effects in the progeny of exposed parents are associated with global changes in DNA methylation and microRNA expression; and (iv) paternal IR exposure leads to DNA damage, changes in DNA methylation and altered short RNA pools in the germline of exposed fathers. The epigenetic model of radiation-induced genome instability will be presented and discussed. The study was supported by ACRI, CIHR and NSERC

IN030

ROLE OF EPIGENETIC EVENTS IN GENOTOXIC LIVER CARCINOGENESIS

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The need for the rapid identification and appropriate regulation of carcinogens before their dissemination into society is of foremost importance for the primary prevention of neoplasia in humans. Historically to assess safety, toxicologists have focused on the measurement of DNA damage, DNA adduct formation, and mutations induced by any given factor as the most appropriate indicator of carcinogenic potential. The recognition of the role of epigenetic mechanisms in carcinogenesis has challenged the current approach to carcinogenicity testing and indicated the need for a new generation of exposure biomarkers. We have conducted experiments to examine the role and contribution of epigenetic alterations in rodent liver carcinogenesis induced by genotoxic carcinogens. Long-term exposure of rats to the genotoxic carcinogens tamoxifen and 2-acetylaminofluorene resulted in the accumulation of carcinogen-specific DNA adducts and the induction of profound epigenetic alterations, including aberrant DNA methylation, histone modifications, altered gene expression, and miRNA expression. Our data demonstrate that stages of multistage carcinogenesis following the initiation are driven primarily by carcinogen-induced epigenetic alterations, emphasizing the significance of epigenetic events in genotoxic liver carcinogenesis. These findings are particularly significant because they demonstrate that different carcinogenic agents induce similar epigenetic alterations that occur in the target organ only. The remarkable feature of carcinogen-induced epigenetic changes is their early appearance and correspondence to the changes frequently found in tumor cells suggesting that these alterations may be used as biomarkers for the carcinogen exposure and as a novel approach for carcinogenicity testing and cancer risk assessment. Furthermore, the potential reversibility of epigenetic alterations makes them promising targets for chemoprevention strategies.

IN031

DNA METHYLATION AND PERSISTENT BYSTANDER EFFECT: MEMORY OF AN INSULT

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Abstract not available at the time of publication.

IN032

EPIGENETIC CHANGES UNDERLIE ORGANISMAL ADAPTATION TO CHANGING ENVIRONMENTS

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Plants are capable of rapidly reprogramming patterns of gene expression, allowing fast acclimation and adaptation in response to specific environmental stress. We analyzed whether these conditions are associated with genome instability and whether the response is regulated epigenetically. For the experiments we used transgenic *Arabidopsis thaliana* plants carrying in the genome the substrate for the analysis of homologous recombination frequency (HRF). To analyze the mechanisms of epigenetic regulation we used Dicer-like mutants, *dcl2*, *dcl3*, *dcl4*. We found that various environmental stresses, such as UV, salinity, heavy metals and pathogens destabilize the genome of somatic cells and result in inheritance of genome instability in the progeny. The changes persist in the following generatinos only when plants were propagated in the presence of stress. The progeny of the stressed plants had hypermethylated genomes and showed increased tolerance to stress. The progeny exhibited differential pattern of small RNA expression and locus-specific changes in methylation. While analyzing the response of *dcl2*, *dcl3 and dcl4* mutants to stress, we found these plants to differentially respond to all stresses and to be impaired in transgenerational response. Pretreatment of plants with 5-azaC, methylation inhibitor, prevented establishment of stress tolerance and genome instability, indicating impotant role of methylation in the process. We thus hypothesize that stress adaptation requires function of small RNAs and establishment of new pattern of genome methylation and chromatin structure leading to transgenerational changes in stress tolerance and genome stability.

IN033

SYSTEMS INTEGRATION OF HUMAN STEM CELLS, EPI-TOXICOGENOMICS, CELL-CELL COMMUNICATION: THE BARKER HYPOTHESIS AND CHRONIC HUMAN DISEASES.

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The human being should be viewed "as greater than the sum of its parts". Homeostatic control of the "emergent properties" of the human hierarchy, needed to maintain human health, requires complex integration of endogenous and exogenous signaling molecules that control cell proliferation, differentiation, apoptosis and senescence of stem, progenitor and differentiated cells. Currently, in vitro toxicity assays (mutagenesis, cytotoxicity, epigenetic modulation), done on 2-dimensional primary rodent or human cells (which are always mixtures of cells), on immortalized or tumorigenic rodent or human cell lines do not represent normal human cells in vivo[which do not grow on plastic and which are in micro-environments representing 3 dimensions and constantly interacting factors]. In addition, with the known genetic, gender, and developmental state of cells in vivo, any in vitro toxicity assay will need to mimic these conditions in vitro. More specifically, while tissues contain a few stem cells, many progenitor/transit cells and terminally differentiated cells, it should be obvious that both embryonic and adult stem cells would be critical "target" cells for toxicity testing. The ultimate potential for in vitro testing of human stem cells will to try to mimic a 3-D in vitro microenvironment on multiple "organ-specific and multiple genotypic/gender " adult stem cells. The role of stem cells in many chronic diseases, such as cancer, birth defects, and possibly adult diseases after pre-natal and early post-natal exposures (Barker hypothesis), demands toxicity studies of stem cells. While alteration of gene expression ("toxico-epigenomics") is a legitimate endpoint of these toxicity studies, alteration of the quantity of stem cells during development by genetic, environmental, pharmaceutical, dietary, nutritional factors must be seriously considered. If the future utility of human stem cells proves to be valid, particularly for the screening of epigenetic toxicants, the elimination of less relevant, expensive and time-consuming rodent and 2-D human in vitro assays will be eliminated.

IN034

BASE- AND MISMATCH REPAIR INTERFERENCE DURING SOMATIC HYPERMUTATION

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Somatic hypermutation of immunoglobulin genes is initiated by activa-

tion-induced deaminase (AID). This enzyme is induced in antigen-stimulated B-cells, where it converts cytosines downstream from transcription start sites within the immunoglobulin locus to uracils. In most cells, this process is generally error-free, due to the efficient replacement of deoxyuridine in DNA with deoxycytidine by the base excision repair system. Interestingly, in antigen-stimulated proliferating B-cells, AID induction is associated with substantial mutagenesis. Roughly one half of these mutations arise at C/G base pairs, most likely through incomplete or inefficient AID-induced uracil repair. However, the second class of mutations arises at A/T base pairs, and these cannot be caused by the direct action of AID. Because the occurrence of these mutations is genetically associated with the mismatch repair genes *MSH2*, *MSH6* and *EXO1*, as well as with polymerase-□, it has been proposed that they arise through the error-prone processing by the mismatch repair system. We now show that in substrates containing several U/G mispairs, uracil residues can act as initiation sites for a MSH2/MSH6 and EXO1-dependent strand displacement reaction, which can replace several hundred nucleotides of the uracil-containing strand. In the subset of cases where the strand displacement reaction is catalyzed by the error-prone pol-□, mutations might arise at A/T base pairs. Our work provides the first mechanistic insight into the interplay of the base- and mismatch repair pathways in somatic hypermutation. We also show that a similar mechanism can give rise to double-strand breaks, which trigger class switch recombination, a second AID-induced phenomenon in B-cells.

IN035

MRE11 INTERACTIONS WITH DNA AND RAD50 ATPASE PLUS NBS1 INTERACTIONS WITH CTIP CONNECT DSDNA REPAIR MACHINERY AND BREAK SIGNALING

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At DNA double-strand breaks (DSBs) the Mre11/Rad50/Nbs1 (MRN) complex promotes central architectural, structural, enzymatic, sensing, and signaling functions in DSB responsesMRN's three-protein multidomain composition promotes its central architectural, structural, enzymatic, sensing, and signaling functions in DSB responses. To organize the MRN complex, the Mre11 exonuclease directly binds Nbs1, DNA and Rad50. Mre11 dimers bind and distinguish two-ended DSBs and collapsed replication forks (Williams et al., 2008). Rad50 employs its ATP-binding cassette (ABC) ATPase, Zn-hook and coiled-coils to bridge DSBs and facilitate DNA end processing by Mre11. The Nijmegen breakage syndrome 1 (Nbs1) subunit coordinates DSB repair and checkpoint signaling through interactions with ATM, MDC1 and Sae2/Ctp1/CTIP via its fused, extended, FHA-BRCT1-BRCT2 domains flexibly linked to C-terminal Mre11- and ATM-binding motifs (Williams et al., 2009). Structural and biological evidence suggests MRN has three coupled critical roles in DSB sensing, stabilization, signaling and effector scaffolding: 1) expeditious establishment of protein-nucleic acid tethering scaffolds for the recognition and stabilization of DSBs; 2) initiation of DSB sensing, cell cycle checkpoint signaling cascades, and establishment of epigenetic marks via the ATM kinase; and 3) functional regulation of chromatin remodeling at DSBs. Our results also help suggest how MRN mutations cause the human cancer predisposition diseases Nijmegen breakage syndrome and ataxia telangiectasia-like disorder (ATLD).

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IN036

HUMAN ELG1 REGULATES THE LEVEL OF UBIQUITINATED PCNA THROUGH INTERACTIONS WITH PCNA AND USP1

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The level of ubiquitinated PCNA in chromatin is closely linked with DNA damage bypass during DNA replication. However, it remains unclear how the level of ubiquitinated PCNA in chromatin is regulated. Here, we demonstrate that human ELG1 decreases the level of ubiquitinated PCNA in chromatin presumably by interactions with PCNA and the deubiquitinating enzyme, USP1. The level of ELG1 is induced during recovery from DNA damage and ELG1 forms distinct foci at stalled DNA replication forks where PCNA foci exist. Targeted gene knockdown of ELG1 resulted in genomic instabilities including chromosome instability and hypersensitivity to DNA damaging agents. Knockdown of ELG1 by siRNA reduced homologous recombination frequency in I-SceI induced double strand break-dependent assay similar to PCNA or USP1 knockdown. Taken together, ELG1 suppresses genomic instability by reducing ubiquitinated PCNA from chromatin through its interaction with USP1.

IN037

DNA BASE EXCISION REPAIR IN (EPI)GENOME MAINTENANCE

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The human thymine DNA glycosylase (TDG) first attracted attention because of its ability to remove thymine, i.e. a normal DNA base, from G•T mispairs. This implicated a function in the restoration of G•C base pairs at sites of 5-methylcytosine (5-meC) deamination. TDG then turned out to be the founding member of a family of mismatch-directed uracil DNA glycosylases that act on a broad spectrum of DNA base lesion, including G•U mispairs as well as lesions generated by the anticancer drug 5-Fluorouracil. 5-meC DNA glycosylase activity has also been associated with TDG, thrusting the enzyme into limelight as a possible DNA demethylase. Last but not least, TDG was found to interact with transcription factors, nuclear receptors as well as with DNA methyltransferases, implicating a function in gene regulation, which appears to be critically important in developmental processes. We have been pursuing biochemical, molecular, and genetic approaches to determine the biological function of this multifaceted DNA repair enzyme. We will present data from genome-wide analyses of TDG-chromatin interactions, DNA cytosine methylation patterns and gene expression profiles in TDG proficient and deficient mouse embryonic stem cells, before and after *in vitro* differentiation to neuronal progenitor cells. The data implicate novel and non-redundant functions of TDG dependent BER in (epi)genome maintenance and regulation of gene expression, providing a conceptual framework for a mechanistic explanation of a developmental phenotype associated with the loss of TDG.

IN038

PROPERTIES OF NEIL3 IN PROLIFERATION AND DIFFERENTIATION OF STEM/PROGENITOR CELLS

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Mammalian cells encode three homologues of the Fpg/Nei superfamily of DNA glycosylases, Neil1, Neil2 and Neil3. Neil1 and Neil2 remove formamidopyrimidines and a broad spectra of oxidized pyrimidines, including 5-hydroxycytosine, 5-hydroxyuracil, 5,6-dihydrothymine and thyminglycol. In contrast, no DNA glycosylase activity is found for Neil3 and its molecular function is unknown. Nothern blot analysis and *in situ* hybridization revealed that Neil3 exhibited a completely different expression pattern than Neil1 and Neil2. High expression of *Neil3* was observed in the subventricular zone of hilus of the hippocampus and rostral migratory stream in 3 day old mice brain, which contain most of the neural stem cells. However, with age this expression declined and in adult mouse brain Neil3 was almost exclusively distributed in layer 4 of the neocortex. In contrast, *Neil1* and *Neil2* transcripts were ubiquitously expressed and increased with age. To determine the impact of Neil3 on neural progenitor cells (NPC) on proliferation and differentiation, we employed the neurospheres assay, which identifies NPC according to their multipotency and self-renewal capacity. The pool of NPC in *Neil3* knock-out animals was strongly impaired. Moreover, differentiation of neurospheres into glia cells was blocked in cells overexpressing Neil3. Finally, we showed that formation of cardiospheres, which identifies cardiac progenitor cells, was impaired in *Neil3* animals. Our data suggest an important role of Neil3 in renewal and differentiation of adult stem/progenitor cells.

IN039

ESTABLISHMENT OF REPORTER ASSAY YEASTS RESPONDING TO LIGANDS OF VARIOUS HUMAN NUCLEAR RECEPTORS, AND ROLES OF AHR LIGANDS TO INDUCE OR PROTECT DNA DAMAGE FORMATION

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Nuclear receptors (NRs) are a class of cellular proteins that are responsible for sensing the ligand substances such as hormones and certain other molecules. In response to the ligands, NRs work in concert with other proteins to regulate the expression of specific genes as transcription factors, thereby controlling the development, homeostasis, and metabolism of higher organisms. Some ligand substances, regardless of their DNA-damaging activity, binds to NRs and are transported into nucleus, e.g., estradiols, bile acids and aryl hydrocarbons bind to estrogen receptor (ER), farnesoid X receptor (FXR) and aryl hydrocarbon receptor (AhR), respectively. Some receptors such as AhR, CAR, PXR and PPAR appear to function as xenobiotic sensors for expression of cytochrome P450 enzymes (CYP 1, 2, 3 and 4 families, respectively) that metabolize these xenobiotics. We established fast and conventional bioassays using yeasts (Saccharomyces cerevisiae) that respond to ligand substances of these human NRs. These yeasts express one of human NRs and their coactivator protein SRC-1, and have a β-galactosidase reporter gene downstream the NR-binding sequence. These assay yeasts are valuable to investigate DNA-damaging pathways of NR-mediated substances, and also to explore the environmental contaminants that influence animals through the NRs, i.e., endocrine-disturbing substances. Benzo[a]pyrene (BP) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) are carcinogenic environmental contaminants, which strongly respond to the AhR assay yeast as its ligands. Both substances therefore induce the large amount of CYP1A1 protein in human cells. The CYP1A1 metabolically activates BP to the reactive form, benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE), but does not activate TCDD. Hence, TCDD is thought to enhance the mutagenic and carcinogenic potential of BP due to increasing the CYP1A1 level. However, our experiment unexpectedly showed that TCDD pretreatment protects BP-caused DNA adduct-formation in human cells. This phenomenon can be explained that TCDD-induced CYP1A1 further decomposes the reactive BPDE to a non-reactive form. Our study suggests that TCDD may have a beneficial function, that is, protection of aryl hydrocarbon-induced mutagenesis and carcinogenesis.

IN040

THE GENOTOXIC HAZARDS AND CARCINOGENIC RISKS OF PAH CONTAMINATED SOILS

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The intentional and accidental discharge of toxic pollutants into the lithosphere contributes to soil contamination. In some cases (e.g., wood preserving wastes, coal-tar), the contaminated soil constitutes a genotoxic and/or carcinogenic hazard. Many industrialized countries contain tens of thousands of contaminated sites, and regulatory agencies are required to assess the hazards and risks posed by these sites, and determine the most prudent course of action (e.g., access restriction, remediation). Routine hazard and risk assessment of contaminated sites is hampered by a host of assumptions and uncertainties. The most troublesome uncertainties relate to the hazards of contaminants in complex mixtures, the bioaccessibility and bioavailability of soil contaminants, the efficacy of remediation techniques, and the per diem rates of soil exposure. Our recent research, which has focused on a series of PAH contaminated sites in Canada and Sweden, is addressing each of these issues, assumptions and uncertainties. For example, using the Salmonella mutagenicity assay and the lacZ mutation assay in cultured cells derived from the transgenic Muta™Mouse, we have shown that the mutagenic hazard of complex PAH mixtures in contaminated soils is far lower than that calculated using the standard risk assessment paradigm (i.e., total hazard is the sum of that contributed by the identified priority components). lacZ mutant frequency results in Muta™Mouse FE1 cells suggests that the carcinogenic risks posed by complex PAH mixtures in contaminated soils is less than 10% of that calculated using the traditional chemical-specific method that assumes additivity. Analyses of PAH-contaminated soils treated using bench-scale bioreactors revealed that despite a decline in the concentration of noteworthy PAHs, the mutagenic hazard of the soils increased over the course of the treatment. Changes in Salmonella mutagenicity across treatment time and activation conditions suggest a pattern of formation and transformation of mutagenic compounds. Additional analyses demonstrated that mutagenic PAHs are enriched in the fraction of soil (e.g., <45μm) that is ingested by humans. Moreover, the bioaccessibility of the ingested mutagenic PAHs in contaminated soils is less than 10%.

IN041

POTENTIAL IMPLICATIONS OF SOIL POLLUTION WITH MUTAGENS IN LUNG CANCER

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Many epidemiological studies have shown that air pollution is associated with the incidence of lung cancer. Diverse genotoxic compounds are released into ambient air from anthropogenic sources such as engines of motor vehicles and municipal incinerators. Compounds released into the air eventually deposit on the ground and can be accumulated in surface soil; therefore, surface soil is thought to be a promising material for monitoring environmental pollution with genotoxic compounds. In addition, adequate amount of soil samples can be collected for biological assays and chemical analysis without any special equipment or power source. To clarify contamination of surface soil with mutagens, we collected at residential sites in five regions of Japan, i.e. Hokkaido, Kanto, Tokai, Kinki, and Kyushu. Most of the organic extracts from soil samples were mutagenic toward S.typhimurium TA98 and TA100, and potent mutagenicity was observed for the samples from several prefectures in the Kinki region, Aichi prefecture in the Tokai region and Hokkaido, which have high mortality ratios of lung cancer. 1,6-Dinitropyrene (DNP), 1,8-DNP, 1,3,6-trinitropyrene, 3,9-dinitrofluoranthene (DNF), and 3,6-dinitrobenzo[e]pyrene

(DNBeP) were commonly identified as major mutagenic constituents in five highly mutagenic soils from Osaka and Kyoto prefectures in the Kinki region and Aichi prefecture. These nitroarenes are extremely mutagenic in TA98 without S9 mix. The total percent contributions of these nitroarenes to these five soil samples were from 39 to 45%. 1,6-DNP, 1,8-DNP, and 3,9-DNF are carcinogenic in experimental animals and are classified as possible human carcinogens (Group 2B) by the IARC. In addition, 3-nitrobenzanthrone, which is a representative air pollutant and carcinogen, was also detected in other soil samples from the Kinki and Tokai regions. 3,6-DNBeP is a novel chemical and was identifies as a soil mutagen at first. Recently 3,6-DNBeP was detected in airborne and diesel engine exhaust particles and municipal incinerator ashes at much higher levels than DNP. Latterly 3,6-DNBeP was found to be genotoxic in vivo, and its carcinogenicity in mice by intratracheal administration is under investigation.

IN042

GENOTOXICITY AND CARCINOGENICITY OF DRINKING WATER DISINFECTION BY-PRODUCTS

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This talk will review the literature on the mutagenicity and carcinogenicity of disinfection by-products (DPBs) as identified and regulated by the U.S. EPA. Also, future research is proposed to resolve remaining health concerns regarding drinking water. Among the 11 DBPs regulated by the U.S. EPA, (a) 2 DBPs (chloroacetic acid and chlorite) are not carcinogenic—each in 2 species; (b) chlorite is not carcinogenic in 3 rodent assays and has never been tested for genotoxicity; (c) 1 DBP (bromoacetic acid) has never been tested for carcinogenicity; (d) 2 DBPs, chloroform and trichloroacetic acid, are carcinogenic via nongenotoxic mechanisms; (e) 6 DBPs have significant genotoxicity data gaps; and (f) 5 DBPs have been assessed as possible or probable human carcinogens. Among 74 unregulated DBPs, 29 that occur at sub-low μg/L levels are genotoxic; and another 14 that occur at this level have no toxicological data except for 2, which are carcinogenic. The toxicity of DBPs is iodo > bromo > chloro, and 50% of the organic carbon and organic halogens of drinking water are unknown, i.e., not chemically characterized. Approximately 30% of the municipal water suppliers in the U.S. have changed from chlorination to chloramination, which has resulted in the formation of new DBPs, such as the halonitromethanes and brominated forms of DBPs. Although more toxic than the regulated DBPs, these newly identified DBPs are generally present at much lower concentrations than those that are regulated. Nonetheless, alternative disinfection practices result in drinking water in which extracted organic material is less mutagenic than extracts of chlorinated water. Recent molecular epidemiology indicates that an increased risk for bladder cancer is associated with dermal/inhalation exposure to drinking water (from bathing/showering and/or swimming) rather than to drinking the water and that risk is enhanced in people carrying the *GSTT1*-1 gene, which is present in 75% of the U.S. population. Further studies are needed to clarify if dermal and inhalation exposures are more important than oral exposure to trihalomethanes for increased risk for bladder cancer.

[Abstract does not necessarily reflect the policy of the U.S. EPA.]

IN043

ACTIVATION OF INFLAMMATION/NF-KB SIGNALING IN INFANTS BORN TO ARSENIC-EXPOSED MOTHERS

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The long-term health outcome of prenatal exposure to arsenic has been associated with increased mortality in human populations. In this study, the extent to which maternal arsenic exposure impacts gene expression in the newborn was addressed. We monitored gene expression profiles in a population of newborns whose mothers experienced varying levels of arsenic exposure during pregnancy. Through the application of machine learning–based two-class prediction algorithms, we identified expression signatures from babies born to arsenic-unexposed and -exposed mothers that were highly predictive of prenatal arsenic exposure in a subsequent test population. Furthermore, 11 transcripts were identified that captured the maximal predictive capacity to classify prenatal arsenic exposure. Network analysis of the arsenic-modulated transcripts identified the activation of extensive molecular networks that are indicative of stress, inflammation, metal exposure, and apoptosis in the newborn. Exposure to arsenic is an important health hazard both in the United States and around the world, and is associated with increased risk for several types of cancer and other chronic diseases. These studies clearly demonstrate the robust impact of a mother's arsenic consumption on fetal gene expression as evidenced by transcript levels in newborn cord blood.

IN044

TRANSCRIPTOMIC ANALYSIS IN UMBILICAL CORD BLOOD OF CHILDREN EXPOSED TO GENOTOXIC COMPOUNDS THROUGH THEIR MOTHERS DIET

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Background/aim: Over the last decades, an increased incidence of childhood cancers, such as leukemia is noted. A risk factor for the development of these diseases may be maternal exposure to carcinogenic compounds during pregnancy. Molecular epidemiology studies the causation of diseases with the use of biomarkers which may also provide information at a mechanistic level. Gene expression profiling forms a promising tool for the development of such new biomarkers. The objective of our research is to develop novel genomics-based biomarkers for genotoxic risks in newborns within the EU FP6 project NewGeneris, based on genome wide transcriptomic analyses in umbilical cord blood samples. Methods: Umbilical cord blood samples were selected from the Norwegian NewGeneris BraMat cohort, which resides under the Norwegian Mother and Child cohort (MoBa). Dietary exposure of the mothers to carcinogenic compounds was assessed using food frequency questionnaires. Upon blood sampling, RNA was conserved and isolated using the RNAlater/RiboPure system. Whole genome transcriptomic profiles were generated using microarrays (n=116). Furthermore, internal measurements of persistent organic pollutants (ER-, DR- and AR-CALUX) and micronuclei frequencies in PBMC of the participants are available which were analyzed by NewGeneris partners. Results: Data are analyzed for differential expression between groups with high versus low exposure to specific genotoxic/carcinogenic compounds as well as an integral exposure level. Correlations are investigated between gene expression and exposures, CALUX data and micronuclei frequencies. Conclusion: The generated profiles will lead to a selection of the most predictive set of genes for genotoxic risks of maternal dietary exposure in newborns, which will be used to develop and apply a high-throughput system to screen different NewGeneris cohorts across Europe.

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IN045

GENETIC AND ENVIRONMENTAL RISK FACTORS OF CHILDHOOD LEUKEMIA

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Acute lymphoblastic leukaemia (ALL) is the commonest malignancy affecting children. Despite substantial progress in the treatment, the underlying causes are largely unknown. Childhood ALL is heterogeneous with respect to underlying cellular and molecular biology, acquired genetic abnormalities and associated clinical responses to combination chemotherapy. Epidemiology data are compatible with transplacental carcinogen exposure as a basis for infant leukaemia associated with MLL gene fusion. Moreover, a dysregulated immune response to common infection is strong candidate for childhood ALL but the role of environmental carcinogenesis in ALL remain largely undefined. It is, however probable that the risk of ALL from environmental exposure is influenced by genetic variation. Data from the Swedish Family-Cancer Database lends support to a small familial risk of ALL, independent of the high concordance in monozygotic twins (which has a genetic, in-utero explanation). Although rare (<5% of ALL) direct evidence for an inherited genetic predisposition to ALL is provided by the high risk associated with Bloom's syndrome, neurofibromatosis, ataxia telangiectasia and constitutional trisomy 21. The heritable basis of susceptibility to ALL outside these syndromes is presently undefined but it is likely that the co-inheritance of multiple low-risk variants contribute to disease risk. We examined 399 pediatric ALL samples using the 50k SNP-chip platform and a two-fold design: somatic event were compared between disease and remission samples (molecular allelokaryotyping) and germline changes were compared between remission samples and a healthy control group. While the germline analyses are ongoing, the somatic analyses allowed identification of ALL-related genes that were amplified, deleted or have allelic imbalance. Three common genetic alterations were found: deletion of ETV6, deletion of p16INK4A and hyperdiploidy. Uniparental disomy was a frequent event in ALL, especially affecting chromosome 9. Children with hyperdiploid ALL without gain of chromosomes 17 and 18 had a poor prognosis. A novel candidate tumor suppressor gene for ALL, SNAP91 on 6q, was frequently inactivated in ALL. PAX5 gene was frequently rearranged to a variety of partner genes.

IN046

THE INTERNATIONAL CHILD CANCER COHORT CONSORTIUM

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Dramatic advances have been made in the treatment of childhood Acute Lymphoblastic Leukaemia (ALL), yet approximately 25% of patients in the developed world still succumb to the disease. Therefore, the development of preventive strategies becomes a priority. Despite more than five decades of epidemiologic research on childhood ALL, the only established risk factors are ionizing radiation, certain chemotherapy agents, and specific genetic syndromes, which together account for a small fraction of occurrence. [1] The evidence base for the identification of environmental risk factors has been largely obtained from retrospective case-control studies, with just a few modest sized cohort studies that generally have collected in depth data on only a small number of exposures. [1] The exposure data obtained from retrospective studies is likely to be affected by recall bias, [2-4] so examining prospective data may play an important role in identifying key risk factors. However, given the rarity of ALL, a very large cohort is needed to provide sufficient power to obtain valid risk estimates. This has precluded prospective investigations to this point. The International Childhood Cancer Cohort Consortium (I4C) presents a new and unique opportunity to obtain prospective data to test environmental and genetic hypotheses relating to the causes of childhood leukaemia. The idea of establishing the I4C was first put forward during the planning of the National Children's Study (NCS), a large childhood cohort study in the USA recruiting 100,000 mothers and babies. [5] Due to the relative rarity of childhood leukaemia, the NCS alone did not have sufficient power to detect associations of relevant exposures with childhood leukaemia but it was recognised that sufficient power could be achieved if a collaboration between existing and planned large childhood cohort studies could be initiated. The I4C was established for this purpose. It presently has eleven collaborating cohorts. [6]. Initial data pooling will incorporate data from the following cohorts: Avon Longitudinal Study of Parents and Children (ALSPAC), Birth Defects surveillance System for the Collaborative Project-China (CPBDDP-China), Tasmanian Infant Health Survey (TIHS), Danish National Birth Cohort (DNBC), the Norwegian Mother & Child Cohort Study (MoBa) and the NCS, USA. Pooled data from these six cohorts will be available on 571,309 infants and their mothers. This will provide 5,962,856 person-years of follow-up data on the infants by the end of 2012. The first two hypotheses to be tested will be those concerning periconceptional folate intake by mothers and paternal age.

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IN047

INTEGRATION OF GENOTOXICITY TESTS INTO ROUTINE TOXICITY STUDIES

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Integration of genotoxicity tests into routine toxicity studies, such as the pivotal 4 week rat toxicity study, is a central concept in the revision of ICHS2. A collaborative industry initiative on integration was recently conducted which aimed at investigating the sensitivity, specificity and feasibility of determining Comet Assay and Micronucleus effects after repeated-dosing over 14-28 days. Furthermore, a dedicated working group on Integration of genotoxicity tests will take place at the 5th IWGT Workshop in Basel, Switzerland.

Three presentations in this session will discuss the main aspects on the Integration of Genotoxicity Tests into Standard Toxicity Tests.

An introduction to the topic will be given by Andreas Rothfuss (Bayer Schering Pharma AG, Germany) who will report on the IWGT working group "Improvement of In Vivo Genotoxicity Assessment – The link to Standard Toxicity Testing"**.**

Catherine Priestley (AstraZeneca, UK) will focus on the Integration of the Comet Assay and report on "Practical Aspects of Integrating the Comet Assay into Pivotal Rat Toxicity Studies"**.**

Andreas Czich (Sanofi Aventis, Germany) will give an insight on the Integration of the Micronucleus Test by talking on "Practical Aspects of Integrating the Micronucleus Assay into Pivotal Toxicity Studies".

IN048

THE ILSI-HESI PROJECT COMMITTEE ON THE RELEVANCE AND FOLLOW-UP OF POSITIVE RESULTS INTHE ILSI-HESI PROJECT COMMITTEE ON THE RELEVANCE AND FOLLOW-UP OF POSITIVE RESULTS IN IN VITRO GENETIC TOXICITY (IVGT) TESTING: INTRODUCTION AND FOLLOW-UP OF POSITIVE RESULTS IN VITRO

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The *in vitro* genotoxicity assays play a key role in the genotoxicity evaluation, whatever the chemical intended use. The relatively high rate of irrelevant positive results, especially the mammalian cell assays, has recently raised concern and led to many worldwide initiatives. The Health and Environmental Sciences Institute (HESI) of the International Life Sciences Institute (ILSI) recently formed a project committee (IVGT) to work on "the relevance and follow-up of positive results in *in vitro* genetic toxicity". IVGT committee identified three main objectives: 1) review of existing assays and development of a flow chart for follow up actions in case of positive results in the *in vitro* assays ("review" sub-group), 2) review of new and emerging technologies in genetic toxicology ("new technology" sub-group) that could be used to replace and improve the existing assays, and as follow assays to better assess the risk for human, and 3) development of a quantitative approach in evaluating genotoxicity findings and their relevance to human ("quantitative" sub-group). The consensus reached and the recommendations made by this tripartite (regulatory, industry and academic scientists) group benefit from the numerous years of experience of the participants in the field. The so-called "review sub- group" has classified the existing genotoxicity assays in four categories based on their strengths and weaknesses, and their ability to contribute to data interpretation; from assays that are robust enough to be used in the standard battery to assays seldom used because better alternatives exist. This sub-group has also developed a flow chart to be considered in case of positive findings in the *in vitro* assays of the standard genotoxicity battery. It attempts to describe how the intended chemical use, the data obtained in the initial genotoxicity battery, the potential confounding factors, and the already available data other than genotoxicity data could be used to define the most appropriate follow-up strategies that could include additional *in vitro* genotoxicity assays, mechanistic studies (e. g. DNA reactive versus non-DNA reactive) and *in vivo* genotoxicity tests, if any, in order to better evaluate the level of concern for human in the intended usage.

IN049

THE ILSI-HESI PROJECT COMMITTEE ON THE RELEVANCE AND FOLLOW-UP OF POSITIVE RESULTS IN IN VITRO GENETIC TOXICITY TESTING (IVGT): QUANTITATIVE ASPECTS OF GENOTOXICITY RISK ASSESSMENT

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The need for quantitative analysis of the relationship between exposure and health risk of genotoxic damage in vivo was recognized when regulatory genetic toxicology testing began in the 1970s. Nonetheless, the current regulatory paradigm remains based mainly on qualitative interpretation of the outcome of in vitro and limited in vivo testing. The Quantitative Working Group of the HESI Committee on Interpretation and Follow-up Testing of Positive In Vitro Genetic Toxicology Results is developing a database of in vitro and in vivo exposure-response relationships in test systems used for genotoxicity testing to use as a basis for analysis of exposure-response relationships, development of recommendations for extrapolation of results across test systems, and approaches to improved exposure-based estimates of in vivo risk. Among the issues being addressed are: 1) whether effects in different systems can be normalized against cellular parameters such as toxicity, adduct levels, or other cellular perturbations to permit extrapolation of risk of specific types of genetic damage across test systems, 2) whether dose-response relationships allow extrapolation of responses to operational threshold exposure levels below which significant damage is not expected, for both DNA-reactive agents and those that induce DNA damage indirectly via cellular perturbations, and 3) evaluation of analytical and statistical procedures for identification of thresholds or operational thresholds in the exposure-response curve. Results are preliminary, but suggest that even well-characterized DNA-reactive genetic toxicants have exposure thresholds below which modification of spontaneous DNA damage rates is not significant. If confirmed, the next step will be to evaluate strategies to define exposures associated with specific levels of concern and associated safety margins.

IN050

THE ILSI-HESI PROJECT COMMITTEE ON THE RELEVANCE AND FOLLOW-UP OF POSITIVE RESULTS IN IN VITRO GENETIC TOXICITY TESTING (IVGT): EMERGING TECHNOLOGIES FOR THE IMPROVEMENT OF GENOTOXICITY RISK ASSESSMENT

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The ILSI- HESI IVGT Project Committee established a New-Emerging Technologies subgroup to identify and review promising approaches that could be used to replace or improve the existing models, and to identify useful follow-up tests in case of in vitro positive results in the initial battery of genotoxicity assays. As part of this initiative, a workshop was convened in May 2008 to discuss mature, maturing and emerging technologies in genetic toxicology. This workshop provided a forum to solicit informal feedback on assay strengths and weaknesses and lessons learned from previous validation efforts were shared. The conclusions that ensued from these discussions included:
-In silico technologies can assist selection of appropriate follow up tests based on identified structural alerts.
- Integration of genotoxicity endpoints in conventional toxicology studies and adoption of automated approaches to enumerate effects are desirable, but must be supported by sufficient validation data.
-Assessment of multiple endpoints such as mutagenicity, clastogenicity, and and gene expression signature in a common platform such as TK6 cells can produce a holistic data set which may be beneficial when determining the risk and relevance of positive findings.
- Platforms such as engineered 3-D tissue systems are attractive in vivo alternatives, although availability/cost may hinder facile development.
-Approaches to interrogate mammalian mutagenic potential that translate across in vitro to in vivo platforms, or cross-species, are urgently needed.
This presentation will provide a brief overview of the above conclusions and identify opportunities in which New/Emerging Technologies in genotoxicity testing could be used to (1) enhance the existing toolkit for management of positive findings and (2) used to generate dose-response data to enable quantitative assessment of positive responses.

IN051

CANCER STEM CELLS FROM SOLID TUMORS

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Cancer stem cells (CSCs) are the rare population of undifferentiated tumorigenic cells that are thought to be responsible for tumor initiation, maintenance and spreading. Their existence might also explain why tumours are resistant to many conventional therapies, which typically target the rapidly proliferating tumor cells but spare the slow dividing tumor stem cell population. The concept of CSCs has profound implications for our understanding of tumor biology and for the development of more effective cancer therapeutics. The selective targeting of these cells offers a potential revolutionary advance in the treatment of cancer, by attacking the roots of the disease. Such cell population should therefore represent the target of new therapies aimed at eradicating the tumor. We developed a technology that allowed us to isolate and expand in vitro CSCs from several solid tumors, including glioblastoma, melanoma, breast, lung, colon, thyroid and ovary cancer. We are currently characterizing these tumorigenic cell populations at

different levels, including genome-wide expression of mRNA, microRNA and proteome profiling. Such extensive characterization may allow the identification of more specific CSC markers, while providing key information on the relevant pathways to be targeted for successful therapies. Moreover, the use of CSC-based xenografts which reproduce the parental tumor, as assessed by morphological and molecular analysis, offers a unique opportunity to test new anticancer treatments and potentially optimize future individualized therapies.

IN052

ENVIRONMENTAL EXPOSURE ASSESSMENT: COLLATERAL DAMAGE IN THE GENOMIC REVOLUTION?

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Environmental exposures, including lifestyle, infections, radiation, chemicals and occupation, are a major cause of human cancer. However, the contribution of specific risk factors and their interaction, both with each other and with genotype, is difficult to elucidate. One limitation is the inability to accurately measure environmental exposure, particularly past exposure, with the consequence of misclassification in epidemiological studies. Molecular epidemiology promises to improve exposure assessment but this area has been relatively undeveloped in comparison to advances in measurement of genetic risk factors. Progress has been made with biomarkers such as carcinogens and their metabolites, DNA and protein adducts and mutations measured in various tissues and body fluids. At the same time many challenges remain, not least the importance of considering the effects of exposure at different stages of life, including the perinatal period. Technological advances and understanding of mechanisms of carcinogenesis offer new opportunities to this field of research. Notably transcriptomics, proteomics and metabonomics may provide a step-change in environmental exposure assessment, providing patterns of alterations that reflect specific exposures. In addition, the increasing recognition of the role of epigenetic changes in carcinogenesis presents a fresh challenge as alterations in DNA methylation, histone modification and microRNA levels in response to environmental exposures demand a new generation of exposure biomarker. Biomarkers of exposure present added value over and above their contribution to understanding etiology. For example, these same biomarkers can contribute to establishing the biological plausibility of associations between exposure and disease and may be valuable endpoints in intervention studies. The overall importance of this area of research is highlighted by the large prospective cohort studies (e.g. UK Biobank) which need accurate exposure measurement in order to shed light on the complex gene:environment interactions underlying cancer and other common chronic disorders. A concerted effort is required to develop and validate the required exposure assessment methodology before these cohorts come to maturity.

IN053

UNDERSTANDING THE MUTAGENIC CONSEQUENCES OF BASE LESION DNA REPAIR

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Mammalian cells have a variety of DNA repair systems that are essential for maintenance of genomic integrity. Of these systems, base excision repair (BER) is critical in removal of base lesions that arise spontaneously and after exposure to environmental toxicants or radiation. If base lesions are not repaired, they can lead to mutations in the next round of replication or to stalling of RNA polymerase. It is well known, however, that the intermediates of BER are both cytotoxic and prone to genomic instability. BER is considered to operate by two broad subpathways, single-nucleotide BER (SN-BER) and multi-nucleotide or long-patch BER (LP-BER), differentiated by the "repair patch" size as well as the enzymes and co-factors involved. There are many reports suggesting roles for DNA polymerase ß (Pol ß) in SN-BER and LP-BER: For example, purified Pol ß has BER-related enzymatic activities; BER activity is decreased in extracts from Pol ß null cells or when Pol ß activity is inhibited in wild-type cell extracts; in addition, Pol ß is concentrated at sites of focal base damage in living cells. Experiments with cultured fibroblasts mouse cells also have pointed to a BER role for Pol ß: For example, treatment of cells with DNA base damaging agents have revealed a protective effect of Pol ß against cell death, chromosomal aberrations, and persistence of strand breaks. Nevertheless, point mutations can arise during BER as well as frameshift mutations and insertions/deletions. Insight on molecular mechanisms of such mutagenic consequences of BER has been provided through crystal structures of Pol ß in complex with various premutagenic intermediates. Recent results using this structural approach will be summarized and integrated with other approached toward understanding the biology of base lesions and their repair.

IN054

USING MULTI-DIMENSIONAL PROTEOMICS TO DEFINE THE COMPLETE PROTEIN COMPOSITION OF MITOTIC CHROMOSOMES

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Mitotic chromosomes accomplish the critical segregation of the genome when cells divide, yet despite many decades of study remain poorly characterized with respect to their structure, mechanism of condensation and composition. We have used mass spectrometry to identify 4027 polypeptides in prometaphase chromosomes isolated from chicken DT40 cells. Of these, 124 were previously annotated as centromere proteins and 14 as telomere proteins. Analysis of the spectra enabled us to determine accurate copy numbers for all chromosomal proteins, with surprising implications for the structure of vertebrate kinetochores. Our subsequent analysis utilized three classifiers in a combination of novel SILAC-based approaches to sort proteins into groups. These included the comparison of the abundance of proteins found on chromosomes versus in post-chromosomal supernatants; the exchange of proteins between chromosomes and cytosol; and the dependence of protein association with mitotic chromosomes upon the presence of a functional condensin complex. We used these classifiers to develop a novel multi-dimensional analysis that enabled us to identify which of the >560 novel proteins novel proteins were likely to be important for chromosome structure and function. As a validation of this approach, GFP tagging of 20 previously unknown proteins selected from this "space" revealed 10 to be novel centromere proteins, and another 8 to occupy other chromosomal domains during mitosis. This approach offers a powerful way to define the functional proteome of complex organelles and structures whose composition is not simple or fixed.

IN055

LIVE CELL STUDIES ON TAXOL AND THE MITOTIC CHECKPOINT IN HUMANS

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The idea that concentrations of taxol >100-nM prevent satisfaction of the mitotic checkpoint (MC) has been virtually unchallenged since the drug was clinically introduced in 1984. However, here we provide direct evidence that taxol, over a broad range of concentrations (5nM to 10-µM) does not prevent satisfaction of the MC in mammalian cells. When the MC cannot be satisfied, e.g., when spindle microtubule (MT) assembly is prevented with nocodazole or when centrosome separation is inhibited with Eg5 inhibitors, non-transformed human RPE-1 cells average 20 hrs in mitosis. By contrast in 5 nM, 500 nM, 5-µM or 10-µM taxol they average, respectively, just 2.5, 12.0, 5.0 and 3.5 hrs in mitosis. This accelerated exit from mitosis in taxol concentrations

>500-nM is also seen in normal human BJ fibroblasts, rat kangaroo kidney epithelia (PtK2), HeLa and U2OS cells, and it also occurs in response to the taxol analogue epothilone B. Since increasing taxol concentrations do not accelerate exit form mitosis in cells treated previously with vinblastine to prevent spindle MT assembly, this response to taxol is due to its effects on MTs and not to an off-site target. Indirect Mad2 immuno-fluorescence and live cell studies with Mad2/YFP or cyclin B/GFP, reveal that exit from mitosis coincides over a range of taxol concentrations with satisfaction of the MC in the absence of tension on kinetochores. Stabilizing syntelic kinetochore attachments in RPE-1 cells treated with 500-nM or 10-µM taxol, by inhibiting the aurora B kinase, shortens the duration of mitosis in both to ~1.5-hrs, and under these conditions exit from mitosis correlates with satisfaction of the MC. These data suggest that the progressive decrease in the duration of mitosis in response to increasing taxol concentration is due the progressive stabilization of syntelic kinetochore attachments on spindles formed in taxol. Not only do these results provide a novel conceptual framework for how taxol prolongs mitosis, but they also provide solid evidence that inter-kinetochore tension is not involved in MC signaling.

IN056
MECHANISMS OF CHROMOSOME MIS-SEGREGATION IN CANCER CELLS

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Many cancer cells display a CIN (*C*hromosome *In*stability) phenotype, by which they exhibit high rates of chromosome loss or gain at each cell cycle. Over the years, a number of different mechanisms, including mitotic spindle multipolarity, cytokinesis failure, and merotelic kinetochore orientation, have been proposed as causes of CIN. However, a comprehensive theory of how CIN is perpetuated is still lacking. We used CIN colorectal cancer cells as a model system to investigate the possible cellular mechanism(s) underlying CIN. We found that CIN cells frequently assembled multipolar spindles in early mitosis. However, anaphase multipolar cells were very rare, and live-cell experiments showed that almost all CIN cells divided in a bipolar fashion. Moreover, fixed-cell analysis showed high frequencies of merotelically attached lagging chromosomes in bipolar anaphase CIN cells, and higher frequencies of merotelic attachments in multipolar vs. bipolar prometaphases. Finally, we found that multipolar CIN prometaphases typically possessed γ-tubulin at all spindle poles, and that a significant fraction of bipolar metaphase/early anaphase CIN cells possessed more than one centrosome at a single spindle pole. Taken together, our data suggest a model by which merotelic kinetochore attachments can easily be established in multipolar prometaphases. Most of these cells would then bi-polarize before anaphase onset, and the residual merotelic attachments would produce chromosome mis-segregation due to anaphase lagging chromosomes. We propose this spindle pole coalescence mechanism as a major contributor to chromosome instability in cancer cells.

IN057
HIGH-RESOLUTION IMAGING OF MITOTIC CHROMOSOME INSTABILITY

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Abnormal chromosome segregation is one way by which cells accumulate the genetic abnormalities associated with tumour development. Routine chromatin stains are sufficient to detect many such alterations in chromosome dynamics. However, the precise location of individual chromosomes can be difficult to discern by routine staining. Fluorescence in situ hybridisation (FISH) on mitotic figures is one efficient strategy to allow simultaneous visualisation of several chromosomes at different stages of the mitotic process. In particular, FISH on anaphase or telophase configurations allows the scoring of sister chromatid segregation patterns. FISH can now be efficiently combined with immunofluorescence, making it possible to visualize simultaneously the dynamics of chromosomes and the movement of key proteins in the mitotic machinery. This methodology can be efficiently combined with confocal microscopy and other techniques for studying mitotic topography in three dimensions. There are now also several fluorescence-based and/or laser-enhanced systems available to study the movement of chromosomes in real time. Using these methods, chromosome segregation errors in cancer can be broadly sub-divided into abnormalities in spindle symmetry (spindle multipolarity and size-asymmetry of ana-telophase poles) and abnormalities in sister chromatid segregation (chromosome bridges, chromatid bridges, chromosome lagging, acentric fragment lagging). Often, these categories of errors must be combined to accurately describe the events in a single abnormal mitotic cell. Further characterisation of the molecular alterations leading to abnormal chromosome segregation together with the current developments in nano-level and real-time imaging will undoubtedly lead to an improved understanding of chromosome dynamics in cancer cells.

IN058
DNA DAMAGE AND CELL DIFFERENTIATION

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Proliferating cells respond to DNA damage by activating a complex network signaling that slows down the progression of the cell cycle to permit to the cells to repair the lesions before division occur. Repair should be very efficient to avoid that unrepaired lesions are fixed into mutations when encountering the replication machinery. This last mechanism should not be a problem in post-mitotic cells which no longer replicate but they need to maintain the integrity of the active genes and avoid cell death as required for cells with limited or no regeneration potential. During differentiation nucleotide excision repair is downregulated at genome level but active genes are efficiently repaired (Nouspikel and Hanawalt, Mol Cell Biol., 2000, 20:1562). To compensate for the lack of homologous recombination, nonhomologous DNA end joining is activated during commitment into adipogenosis leading to an increased double-strand break repair (Meulle et al., PLoS ONE, 2008, 3:e3345). At genome level base excision repair is downregulated during skeletal muscle differentiation (Narciso et al., Proc Natl Acad Sci USA, 2007, 104:17010). Upon oxidative stress PARP 1 is hyperactivated in myotubes due to DNA single-strand breaks (SSB) accumulation. This is expected to eventually lead to cellular energy failure and cell death but surprisingly myotubes are resistant to acute treatments with a variety of SSB-inducing agents including hydrogen peroxide, methyl methanesulfonate and camptothecin. P53 has been implicated in the regulation of cell death in response to DNA damage in mitotic as well as in post-mitotic cells. P53 mRNA levels reach a maximum during muscle cell differentiation but then decline strongly in cells undergoing terminal differentiation. Of the two kinase branches involved in DNA damage response, the ATR/Chk1 pathway is inactive in myotubes whereas the ATM/Chk2 module is active. Upon SSB induction the ATM-mediated phosphorylation of H2AX occurs in both myoblasts and myotubes but the activation of the p53 gene response is impaired in myotubes. This phenomenon together with the significant downregulation of Apaf-1, a key component of the apoptosome, along the muscle cell differentiation process might at least partially account for the exceptional resistance of myotubes to SSB-inducing agents. Recently, another type of cell death, autophagy, has been reported to function as novel response to some types of DNA damage. Autophagy is stimulated during muscle cell differentiation as a protective system to guarantee the turnover of cytoplasmic components. P53 null mouse satellite cells do not activate autophagy during differentiation indicating that this process is p53-dependent. The contribution of autophagy to cell death/survival of myoblasts and myotubes upon DNA damage will be discussed.

IN059

RECIPROCAL LINK BETWEEN THE CIRCADIAN CLOCK AND THE DNA DAMAGE RESPONSE

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Most organisms have developed an internal clock that drives circadian rhythms in metabolism, physiology and behavior, and allows them to optimally anticipate the momentum of the day. At the basis of circadian timekeeping lies a molecular oscillator, made up of auto-regulatory transcription/translation feedback loops, in which cyclically expressed clock gene products regulate their own expression with an approximate (circa) 24-hour (dies) periodicity. The mammalian circadian system consists of a light-entrainable master oscillator in the neurons of the suprachiasmatic nucleus (SCN) in the basal hypothalamus, and light-irresponsive peripheral oscillators in the cells of virtually all other tissues. Although the molecular oscillator of non-SCN cells is self-sustaining, SCN-mediated synchronization of individual cellular oscillators is required to maintain a coherent output rhythm in peripheral tissues. Disconnected from the master circadian clock in the brain, the circadian clocks of individual cells in culture rapidly desynchronize. The importance of the circadian clock is further illustrated by the fact that some ten percent of the liver transcriptome displays robust mRNA cycling. Energy metabolism (producing reactive radicals) and xenobiotic metabolism are prominently controlled by the circadian clock, implying that the sensitivity of tissues to geno- and cytotoxic agents may well depend on the phase of the circadian clock. Moreover, the circadian system has been associated with control over DNA damage and cell cycle response pathways, while conversely, we have shown that DNA damage can phase shift the circadian oscillator in an ATM/ATR dependent manner. This presentation will address the mechanism and biological/medical importance of the circadian clock, with emphasis on its impact on mutagenesis, carcinogenesis, and risk assessment of (non)genotoxic carcinogens.

IN060

CELL AND TISSUE-SPECIFIC REQUIREMENTS FOR DNA STRAND BREAK REPAIR DURING NEUROGENESIS.

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Maintaining genomic integrity by DNA repair is fundamental for the survival of an organism. The development of the nervous system requires vigilant DNA repair processes capable of repairing multiple types of damage. DNA repair is especially critical during early neurogenesis when rapid proliferation and progenitor expansion and differentiation generates cellular diversity in the nervous system. Mutations in DNA damage response factors can lead to a variety of human diseases that are characterized by pronounced neuropathology, including neurodegeneration, microcephaly and developmental abnormalities or brain tumors. This presentation will cover our recent studies investigating the spatiotemporal requirements for DNA strand break repair and signaling pathways during neurogenesis. We have focused on delineating the requirement for DNA strand break repair pathways during neurogenesis. Genetic analysis using mouse models in which key DNA repair factors important for DNA double strand break repair (non-homologous end-joining and homologous recombination) or single strand break repair/base excision repair have been inactivated, will be presented to illustrate the differential tissue-specific requirements for these pathways during neural development. Our studies have revealed unexpected cell-type utilization of different strand break repair factors, and DNA damage signalling pathways in the developing nervous system. Understanding the interplay between signaling networks that function to maintain genomic stability in the nervous system will be of paramount importance for the further understanding and treatment of neurological diseases associated with DNA repair deficiency.

IN061

CELL CYCLE CHECKPOINTS AND DNA REPAIR PATHWAYS VARY BETWEEN DIFFERENT CELL TYPES FOLLOWING EXPOSURE TO IONIZING RADIATION

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Exposure of cells to ionizing radiation or a radiomimetic produces double strand DNA breaks. Cellular response to such damage can be variable depending on cell type. We had previously shown that splenic T cells and skin fibroblasts from mice heterozygous at Aprt both showed an 8-fold increase in mutant frequency following radiation. Only skin fibroblasts and not T cells display a concomitant increase in mitotic recombination. We have proposed that embryonic stem (ES) cells, like germ cells must have robust mechanisms not available to somatic cells for maintaining genomic integrity. In this context we have shown that the mutation frequency in mouse ES cells is suppressed more than 100-fold compared with isogenic somatic cells in vivo and in vitro. Unlike somatic cells, mouse ES cells lack a G1 checkpoint and are hypersensitive to external challenge. The absence of the G1 checkpoint allows cells with damaged DNA to enter S phase, exacerbate the damage by replication and undergo apoptosis, thereby maintaining the population pristine. We have now examined ES cells and mouse embryo fibroblasts (MEFs) from two mouse strains for mechanisms of double strand DNA break repair. Our premise is that ES cells preferentially utilize homology mediated repair (HMR) presumably to maintain faithful repair. We are testing this proposition. Proteins involved in (HMR), which uses the sister chromatid or homologous chromosome as a template for high fidelity repair, are highly elevated in the ES cell compared with MEFs. Nonhomologous end-joining (NHEJ), which is inherently error-prone, is the other major double strand break pathway and is predominant in somatic cells. Proteins that participate in this pathway are variably expressed. Functional assays that utilize reporter plasmids that discriminate between HMR and NHEJ support the hypothesis and suggest that HMR predominates in ES cells and that NHEJ is barely detectable. When ES cells are induced to differentiate by exposure to retinoic acid, the activities of the two repair pathways reverses with NHEJ becoming predominant.

IN062

SOMATIC MUTATIONS AS A MOLECULAR RATIONAL OF DISEASE IN COMPLEX CONGENITAL HEART DISEASE.

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Birth defects are the leading cause of infant mortality and malformations in congenital heart disease (CHD) are among the most prevalent and fatal of all birth defects. Yet the molecular mechanisms leading to CHD are complex and the causes for the cardiac malformations observed in humans are still unclear. In recent years, the pivotal role of certain transcription factors in heart development has been demonstrated, and gene-targeting of cardiac-specific transcription factor genes in animal models has provided valuable insights into heart anomalies. Nonetheless results in these models can be species-specific, and in humans, germline mutations in transcription factor genes can only account for some cases of CHD. Furthermore, most patients do not have family history of CHD. There is, therefore, a need for a better understanding of the mechanisms in both normal cardiac development and the formation of malformations. Combining expertise in cardiac anatomy, pathology, and molecular genetics is essential as to adequately comprehend developmental abnormalities associated with CHD. To help elucidate genetic alterations in affected tissues of malformed hearts, we carried out genetic analysis of cardiac-specific transcription factor genes from the Leipzig collection of formalin-fixed malformed hearts. Working with this morphologically well-characterized archival material not only provided valuable genetic information associated

with disease, but enabled us to put forward a hypothesis of somatic mutations as a novel molecular cause of CHD. Knowledge of cause and disease mechanism may allow intervention that could modify the degree of cardiac malformations or development of new approaches for prevention of CHD.

IN063

PET DOGS AS SENTINELS OF ENVIRONMENTAL CANCER RISK

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For decades, dogs in the research laboratory have advanced the understanding of the acute and long-term effects of high doses of cancer-causing chemicals. The goal of this presentation is to introduce an emerging new paradigm to the field of environmental toxicology: utilizing pet dogs living in the same environment as humans as sentinels of environmental cancer risk *(Waters and Wildasin, Cancer Clues From Pet Dogs, Scientific American 2006)*. The rationale for this approach is that: (1) the association between relevant, low-level exposures encountered by humans and spontaneous tumor development can be studied in pet dogs; and (2) the compressed lifespan of dogs compared to humans translates into relatively brief exposure-to-cancer diagnosis intervals. For example, time from asbestos exposure to diagnosis of mesothelioma in humans is up to 30 years; in contrast, the time interval for dogs is usually less than 8 years. Studying pet dogs may have important implications for: (1) remediating environmental sources of toxicants; (2) informing closer monitoring of exposed humans; and (3) guiding mechanistic and biomarker studies. Our research team is committed to unleashing the pet dog population as a one-of-a-kind resource to better understand the factors that influence human cancer risk, cancer resistance, and healthy longevity.

IN064

TOXICOPATHIC LIVER LESIONS AND OTHER BIOMARKERS OF CHEMICAL EXPOSURE AND EFFECT IN SENTINEL FISH SPECIES IN PUGET SOUND, WASHINGTON AND OTHER COASTAL AREAS OF THE UNITED STATES

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For 30 years our laboratory has studied the impact of PAHs and related pollutants on benthic fish, following an interdisciplinary approach involving chemical exposure assessment linked to synoptic detection of various effects at several levels of biological organization. These studies demonstrate a cause-and-effect relationship between the occurrence of neoplastic and neoplasia-related liver lesions in English sole (Parophrys vetulus), and exposure to PAHs, and to a far lesser degree, chlorinated hydrocarbons such as PCBs. In statistical analyses of data from many field studies conducted since 1978, exposure to PAHs measured in various compartments has consistently been identified as a highly significant risk factor for neoplasms and related lesions in this species, explaining up to 54% of the variability in lesion prevalence. A cause-and-effect relationship is further supported by the induction of liver lesions identical to those observed in field-collected fish, in sole experimentally exposed to model carcinogenic PAHs such as BaP or to PAH-rich extracts of sediments from Eagle Harbor, a severely PAH-contaminated site in Puget Sound, WA, USA. Recent field studies have identified significant associations between hepatic cytochrome P4501A (CYP1A) induction and xenobiotic-DNA adducts, and hepatic lesion prevalences in wild sole. Field studies in Eagle Harbor after capping of the most PAH-contaminated region of this harbor with clean dredge spoils (Sept.1993-March 1994) and up through May of 2005, have shown a decline in PAH exposure as assessed by biliary fluorescent aromatic compounds (FACs) and hepatic xenobiotic-DNA adducts, along with a parallel, dramatic decline in occurrence of toxicopathic liver lesions in sole from this Superfund site. Sediment ∑PAH-effects threshold concentrations for these lesions in sole have also been estimated using segmented regression of field data, and range from 54 to 2800 ppb (dw). Collectively, these lab and field findings relating PAH exposure to the occurrence of hepatic neoplasms and neoplasia-related lesions in English sole fulfill the classic criteria for causality in epizootiological or ecological risk assessment studies. Supportive case studies in other fish species in the coastal USA are also discussed.

IN065

GENOTOXICITY OF POLLUTED SOILS : RESPONSE OF BIOLOGICAL INDICATORS

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Industrially-contaminated soils are a major concern of most developed countries which are faced with the problem of historical contamination. Chemical analytical results generally indicate the presence of pollutants of concern such as chemicals known to be genotoxic to vertebrates. Polycyclic aromatic hydrocarbons (PAHs) and metals are often found at high concentrations in polluted soils generated by coal and iron extraction, mining, cokeries and steel industries. The question of the impact of genotoxic pollutants to soil organisms and population dynamics can be answered by parallel studies of genotoxicity and chronic toxicity on terrestrial species. While genotoxicity of leachates and soil extracts is commonly measured using microorganisms and plants, investigations of genotoxicity in terrestrial species exposed to field-contaminated soils are rare. Soil genotoxicity in earthworms can be assessed using the comet assay applied to coelomocytes of the intestinal tract particularly exposed to soil pollutants. Testing the complex environmental matrices is advisable to determine bioavailability of pollutants and their interacting effects. Results of investigations on soils from an industrial wasteland and an industrial site will be used to address the above questions. Both soils were heavily contaminated by PAHs, and by metals at different levels. A follow up of genotoxicity measured in the first days of earthworm exposure was predictive of pollutant bioavailability and chronic toxicity. It indicated also possible DNA repair. Our investigations demonstrated that pollutant bioavailability was dramatically reduced due to ageing and weathering of polluted soils. Therefore pitfalls could result from extrapolating toxicity based on chemical analyses of soil pollutant concentrations. Bioassays are indispensable to measure toxicity of a soil matrix to complete the first diagnostic of contamination given by chemical analyses. Genotoxicity is helpful to highlight the actual toxicity of a soil contaminated by pollutants of concern. Improvements in the approach to link reponses of biological indicators to populational effects will be discussed.

IN066

TRANSCRIPTOMICS AND PROTEOMICS IN MUS SPRETUS: NEW TOOLS FOR ENVIRONMENTAL POLLUTION ASSESSMENT

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Biomarkers are measured in bioindicators for assessing environmental pollution. Omics methodologies are powerful tools with the potential for identifying novel biomarkers and acquiring insight into toxicity mechanisms. Bioindicators are usually poorly represented in gene/protein sequence databases, limiting the use of omics in ecotoxicological studies. Mus spretus is a nonprotected rodent that attains high population densities, typically inhabits marshlands, and feeds on plants, seeds and insects around its burrow. We studied the suitability of high-throughput transcriptomics and proteomics approaches in

assessing the biological effects of polluted terrestrial ecosystems on inhabitant M. spretus mice. Given that the genome of this aboriginal species is unknown, we used as reference the sequence databases of Mus musculus (~1.5 million years of evolutionary divergence). Mice were captured at six sites in SW Spain. Animals dwelling at the Doñana Biological Reserve (SOL) were used as negative control and compared with specimens collected along the lower (DR1), middle (DR4) and upper (DR6) course of the "Domingo Rubio" stream, and at a surrounding industrial settlement (PS) and rice field (MAT). Genome-wide transcriptional profiling was performed with microarrays manufactured from mRNAs of M. musculus (44K Whole Mouse Genome Oligo Microarray, Agilent). Arrays were hybridized with samples derived from pooled livers (nine/site). Statistical analyses (FEWER<0.01) showed that ~2000 sequences were differentially expressed in animals from one or several of the problem sites in comparison with the negative reference, considering the technical variation (four microarrays/group). The mRNA copy numbers of selected genes were quantified by qRT-PCR to validate the microarray results and to further quantify the mRNA profiles in individual mice. Samples were also subjected to proteomic analysis: 2-DE protein separation, MALDI-TOF-PMF identification and peptide matching with M. musculus database. Selected proteomic data were validated by 1-DE and 2-DE immunoblotting. Metal analysis, conventional biochemical assays, and mRNA level quantification were performed to assist the interpretation of proteomic results.

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IN067

ENHANCED IN VIVO MUTATIONS IN THE LUNG OF PHASE II ENZYME-SUPPRESSED MICE

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Polycyclic aromatic hydrocarbons (PAH) and related nitroarenes (eg, dinitropyrene) are recognized to be potent environmental mutagens and carcinogens. These compounds are metabolized by phase I enzymes to reactive intermediates that can form DNA adducts and lead to gene mutations. Phase II enzymes (eg, glutathione S-transferase) catalyze the transformation of reactive PAH intermediates to excretable hydrophilic conjugated metabolites. The expression levels of phase II and related antioxidant enzyme genes are thought to determine the mutagenicity of PAH. Nrf2 is an essential transcription factor for phase II enzymes and the nrf2 knockout mouse has been hypothesized to be an excellent model system for analyzing mutations under phase II enzyme-suppressed conditions. We examined the lungs from nrf2 knockout mice for the production of DNA adducts after exposure for 4 weeks to diesel exhaust (DE) at 3 mg/m3 suspended particulate matter, which is a source of noxious mutagenic and carcinogenic PAH (eg, benzo[a]pyrene (BaP)) in ambient air. The increase in the relative adduct level in the lungs of mice exposed to DE was 2.3-fold higher in nrf2-/- mice than in nrf2+/- mice suggesting that the suppressed activity of phase II enzymes plays a key role in increasing DNA adduct formation. Next, to address whether Nrf2 is also involved in the protection of DNA against mutation, we generated nrf2+/-::gpt and nrf2-/-::gpt mice. In this system, the guanine phosphoribosyltransferase (gpt) gene is integrated into the genome as a target gene for assessing genotoxicity in vivo. The frequency of spontaneous mutation of the gpt gene in the lung was approximately three times higher in nrf2-/- mice than in nrf2+/- or wild-type (nrf2+/+) mice. A single intratracheal instillation of 1 mg BaP increased the frequency of mutation 3.1- and 6.1-fold in nrf2+/- and nrf2-/- mice, respectively, compared with that in untreated nrf2+/- mice, showing that nrf2-/- mice are more susceptible to genotoxic carcinogens. These results suggest that the expression of phase II enzymes protects genomic DNA against mutation, and also highlight the potential of the nrf2 knockout mouse in assessing the carcinogenic risk of environmental mutagens under conditions where protective enzymes are suppressed.

IN068

IS THE ERA OF GWAS ALREADY OVER?

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There are considerable expectations about the ability of genome-wide association (GWA) studies to make exciting discoveries on the role of genes in common diseases. GWA studies may allow researchers to identify causal pathways that have not been unveiled before, thus opening new avenues to disease understanding, prevention and therapy. However, there are still many open challenges. One is how to analyse these studies. The problem of false positives and false negatives provides an interesting methodological stimulus to find optimal solutions. Once main genetic effects have been concretely documented, the next question is how to proceed with the investigation of gene-gene and gene-environment interactions. It is possible that what really counts is not the main effect of genes but complex interactions. Finding and interpreting such interactions is not straightforward. Finally, continuous updated integration of all evidence, from both old studies, current GWA investigations and future replication studies and careful interpretation of the strength of the evidence are crucial to maximize transparency and lead to informative selection of the next steps of research in this field. The present Symposium will contribute to elucidate these and other GWAS-related issues.

IN069

GENOME-WIDE ASSOCIATION STUDIES: OUTSTANDING ADVANCES, EXCITING CHALLENGES

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Genome-wide association (GWA) studies have been remarkably successful in identifying genetic variants associated with common, complex diseases—over 400 such associations have been robustly replicated in the four years since the advent of this technology. Many of these genetic variants lie in genes previously unsuspected of being related to the associated conditions, and nearly half are in regions containing no known genes at all. These findings are shedding new light on the pathophysiology of complex diseases, as well as identifying promising targets for drug development or variants related to drug selection or dosing. Surprisingly, most GWA-defined variants confer small increased risk of disease and taken together explain only a small proportion of the familial clustering of a given trait. Approaches for finding this "missing heritability" include whole-genome sequencing and association testing of resulting rare variants, exploring GWA data for structural variation and evidence of interaction among genes and with the environment, and expanding GWA sample sizes through meta-analyses and consortia, especially in persons of non-European ancestry. Identification of these variants is typically only a first step, with extensive efforts needed to pinpoint the true causal variant, define its function, and modify its effects. Findings from sequencing large numbers of individuals are expected to provide important clues to the impact of GWA-defined variants on genomic function. GWA studies will continue to be critical for determining the role of genomic variation in complex diseases, by seeking associations with other traits in persons with existing GWA data, and by establishing new studies of previously unstudied traits. A relatively under-explored area is the environmental modification of genetic effects, or gene-environment interaction, which will be facilitated by wide availability of GWA data in well-characterized, population-based cohorts with extensive exposure information. The challenge of relating millions of genetic variants to potentially hundreds or thousands of traits and exposures, though computationally and inferentially daunting, presents exciting opportunities for discovery of the joint role of genes and environment in complex diseases.

IN070
GENOME-WIDE ASSOCIATION STUDIES: STATISTICAL DEVELOPMENTS
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Given the small effect sizes of causal genetic variants discovered by genome-wide association studies, enthusiasm for funding new GWAS is beginning to decline. The solution to the problem of the "heritability gap", between high heritability estimates and low variance explained by common causal variants, may lie in structural variants or epigenetic factors, for example, but there is also more information to be gained from SNP-based GWAS, through better statistical modelling to detect, for example, of weak common variants at which the association is due to rarer causal variants of larger effect. I will discuss recent statistical developments for SNP-based GWAS, and also look forward to future sequence-based GWAS.

IN071
APPLICATION OF EPIGENOMICS IN CANCER RESEARCH
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Recent spectacular progress in the field of epigenetics and epigenomics may prove essential for understanding the causes of complex diseases, including cancer, and provide the basis for novel strategies for treatment and prevention. Although the role of epigenetic events is supported by both epidemiological and experimental studies, the precise gene targets of epigenetic alterations in human cancers are largely unknown. Remarkable technological advances in epigenetics and epigenomics now allow powerful screening of large series of samples with unprecedented resolution. These approaches are beginning to reveal a number of cancer-associated genes susceptible to inactivation through epigenetic mechanisms as well as highly specific epigenetic biomarkers. Epigenetic profiling using both genome-wide and candidate-gene approaches in normal tissues, tumour speciments and bodily fluids will help not only to elucidate the mechanism underlying tumourigenesis, but also to identify specific epigenetic targets, environmental, nutritional and lifestyle factors, and the critical windows of vulnerability to environmental epimutagens. Together with the intrinsic reversibility and ubiquity of epigenetic changes in virtually all types of human cancer, these advances should prove instrumental in the development of epigenetics-based strategies for early detection, treatment and prevention. New emerging technologies in epigenomics and approaches to exploit them in the context of cancer research and strategies for cancer control will be discussed.

IN072
EPIDEMIOLOGY AND PRIMARY PREVENTION OF HPV-RELATED PRE-CANCEROUS AND CANCEROUS LESIONS
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Human Papillomaviruses (HPV) have an icosaedric structure and are characterised by a double-stranded circular DNA genome containing 7800-7900 base pairs codifying for 8 different proteins. The two external proteins L1 and L2 form the viral capsid, while non-structural proteins are important for the virus life cycle. In particular, E6 and E7-encoded proteins show a high transforming activity and have a key role in the initiation of the oncogenic process because they interfere with the normal function of onco-suppressive genes (namely, p53, a down-regulator of cellular growth and proliferation, and pRB). Among the over 100 HPV types, at least 13 (plus possibly other 5) have been recognised by the IARC as clearly oncogenic. Types 16 and 18 account for >70% of cervical cancer cases. Other types (especially 6 and 11) are responsible for the development of genital warts. Although HPV infection is extremely frequent in the sexually active population, most infections are spontaneously cleared in few weeks or months. Only persistent infections can lead to the occurrence of cervical pre-cancerous lesions (CIN1-3) and, in the longer term, to the development of cervical cancer. However, other cancers (vulvar, vaginal, penile, anal, etc.) are associated with chronic HPV infection. Until recently, only secondary prevention of cervical cancer was possible based on the identification by cytological abnormalities via periodical screening (Pap Test) and consequent treatment. Since 3 years, two preventive vaccines have been licensed, one containing 4 (16, 18, 6 and 11) and the other 2 (16 and 18) HPV types. Such vaccines were demonstrated to be extremely effective to prevent pre-cancerosus lesions (CIN2+) in clinical trials and in the follow-up of vaccinated women, and are now recommended for universal use especially in pre-adolescent girls. The most recent open questions regard long term protection and cross protection. Being a correlate of protection still unknown, we need to understand the exact role of antibodies and of other possible mechanisms for protection. Of particular importance, is the still undemonstrated ability of natural mucosal infection to boost immunological memory acquired through vaccination.

IN073
RECENT PROGRESS IN PREVENTION AND TREATMENT OF HEPATOCELLULAR CARCINOMA (HCC)
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Epidemiologic studies lead to identification of a large number of risk factors affecting progression of patients with cirrhosis to HCC. These include among others hepatitis B virus infection - HBV (and especially viral load, male sex and age of infection), HCV, HDV and HIV infection or co-infection, inflammatory liver diseases (i.e steato-hepatitis), alcohol and environmental toxins (i.e. aflatoxin).Major advances have been made in the last 2-3 decades in the primary and secondary prevention and treatment of HCC .Universal immunization against HBV in neonates , already introduced in 167 countries worldwide, is leading to a constant reduction in the global prevalence of HBsAg carriers and the incidence of HBV associated HCC. Furthermore, potent anti-viral agents (with low resistance rates) enables excellent control of HBV viral load ,shown to be a major risk factor in development of HCC. In addition, liver transplantation has markedly improved the prognosis of HCC, irrespective of etiology. Efforts are now in progress in identification of new therapeutic targets in HCC cells. Recent introduction of multi-kinase inhibitors such as Sorafenib is one example of the new molecules in development which suppress the complex process of malignant transformation. Chronic inflammation has been suggested as a major risk factor in development of cancer including HCC. NF-kappaB is a family of transcription factors believed to trigger onset and resolution of inflammation which are now being explored as therapeutic anti-inflammatory targets. Blocking of NF-kappaB passways should suppress generation of free radicals, stimulation of cytokines, chemokines, as well as epithelial and vascular growth factors which are all involved in the HCC associated inflammatory process. Another experimental avenue involves blocking of alterations and expression of p53 which may lead to over-activation of anti-apoptotic pathways and resistance to chemotherapeutic agents. In conclusion, universal immunization against HBV is leading to a major reduction in incidence of HCC worldwide. New anti-HCC agents and liver transplantation offer new hope for an improved survival for those patients with already established HCC.

IN074
DEVELOPMENT OF VACCINES AGAINST *HELICOBACTER PYLORI*
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Helicobacter pylori is a gram negative, microaerophilic bacterium adapted to survive in the stomach of humans beings where it causes peptide ulcer and can induce gastric cancer. Although effective antibiotic treatment exists, there is consensus that vaccines are necessary to prevent the complications of this infection. Great progress has been made since its discovery 25 years ago in the understanding of the viru-

lence factors and of several aspects of the pathogenesis of the *H. pylori* gastric diseases. Several key bacterial factors have been identified, such as urease, the vacuolating cytotoxin, the cytotoxin-associated antigen and the pathogenicity island, the neutrophil-activating protein, and others. These proteins, in their native or recombinant forms, have been shown to confer protection against infectious challenge with *H. pylori* in experimental animal models. It is not known, however, through which effector mechanisms this protection is achieved. Nevertheless, a number of clinical trials in healthy volunteers have been conducted mainly using urease given orally as a soluble protein or expressed in bacterial vectors with limited results. More recently, a mixture of *H. pylori* antigens has been reported to be highly immunogenic in *H. pylori*-negative volunteers following intramuscular administration of the vaccine with aluminium hydroxide as an adjuvant. This data show that vaccination against this pathogen is feasible. A successful vaccine approach could pave the way to the prevention of the infection with *H. pylori* and of the major complications of this infection, including gastric cancer.

IN075

EPSTEIN-BARR VIRUS INFECTION, MUTATIONS AND CANCER

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Epstein-Barr virus (EBV) infection efficiently induces human B cell proliferation, causing lymphomas in some immunosuppressed patients. EBV is also involved in several types of human cancer in immunocompetent people, contributing to about 1% of human cancer worldwide. EBV genes expressing EBNA and LMP proteins and several functional RNAs and are thought to contribute to cancer development in combination with cell gene mutations, which are selected in the cancers. The mechanisms, including a potentially directly mutagenic process induced by EBV, will be reviewed. We have devised convenient assays to identify the cell genes regulated by EBV EBNA2, including RUNX3, which we showed to be required for proliferation of EBV infected B cell lines. Investigation of natural sequence variation in EBV has lead to identification of two main EBV types (type 1 and 2) and additional worldwide regional strain variation. Type 1 EBNA2 strains of EBV immortalize human B cells much more efficiently than type 2. We identified a small number of cell genes that are differentially regulated by the two viral types. One of these differentially regulated cell genes is CXCR7, which we showed is required for EBV driven B cell proliferation. The EBV LMP1 gene is also differentially regulated by EBNA2 types. By making chimaeras of the type 1 and type 2 EBNA2 genes, we have mapped the part of type 1 EBNA2 responsible for sustaining proliferation of a human B cell line. The relationship between EBV strain variation and disease will be discussed.

IN076

TOWARD A NON-CODING RNA REVOLUTION IN THE CANCER SOCIETY

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MicroRNA and other short or long non-codingRNAs alterations are involved in the initiation, progression and metastases of human cancer. The main molecular alterations are represented by variations in gene expression, usually mild and with consequences for a vast number of target protein coding genes. The causes of the widespread differential expression of non-codingRNAs in malignant compared with normal cells can be explained by the location of these genes in cancer-associated genomic regions, by epigenetic mechanisms and by alterations in the processing machinery. MicroRNA and other short or long non-codingRNAs expression profiling of human tumors has identified signatures associated with diagnosis, staging, progression, prognosis and response to treatment. In addition, profiling has been exploited to identify non-codingRNAs that may represent downstream targets of activated oncogenic pathways or that are targeting protein coding genes involved in cancer. Recent studies proved that miRNAs and non-coding ultraconserved genes are main candidates for the elusive class of cancer predisposing genes and that other types of non-codingRNAs participate in the genetic puzzle giving rise to the malignant phenotype. These discoveries could be exploited for the development of useful markers for diagnosis and prognosis, as well as for the development of new RNA-based cancer therapies.

IN077

MICRORNAS IN CELL DIFFERENTIATION AND CANCER

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Summary: We are focused on the role of *let-7* and other microRNA (miRNAs) in regulating proto-oncogene expression during development and cancer, and on using miRNAs to diagnose and suppress tumorigenesis. Background: MiRNAs are a large family of small regulatory RNAs found in multicellular eukaryotes, including humans that regulate gene expression to control important aspects of development and metabolism such as cell differentiation, apoptosis and lifespan. MiRNAs, while poorly understood, have been shown to be important regulators of development and even implicated in human cancers. miRNAs control gene expression through complementary elements in the 3'UTRs of target mRNAs. *let-7*, a founding member of the miRNAs is required for timing of the developmental switch from larval to adult cell fates in *C. elegans*. Four closely related miRNAs constitute the *C. elegans let-7* family. Expression of *let-7* RNA is temporally regulated, with robust expression in the fourth larval and adult stages. The *let-7* miRNA is phylogenetically conserved and temporally expressed in many animals. *C. elegans let-7* controls terminal differentiation in a stem cell-like lineage in the hypodermis, while human *let-7* has been implicated in lung cancer. In recent years, many miRNAs have been linked to cancer. Results: To elucidate *let-7*'s role in temporal control of nematode development, we used sequence analysis and reverse genetics to identify candidate *let-7* target genes. Our findings show that *let-7* acts in at least three tissues to regulate different transcription factors, raising the possibility of *let-7* as a master temporal regulator. Additionally we found that one *let-7* family member, *mir-84*, is expressed dynamically in vulval precursor cells at the time that they are specified for distinct roles in vulval morphogenesis. Specifically, *mir-84* is absent when *let-60/ras* is actively specifying the 1^0 vulval fate in P6.p. The 3'UTR of *let-60/ras* contains multiple *mir-84* miRNA complementary sites, and restricts reporter gene expression to P6.p. *mir-84* over-expression suppresses the multivulva phenotype of activating *let-60/ras* mutations. This suggests that *let-60/ras* is a target of *mir-84*, which modulates *ras* signaling leading to vulval cell fate specification. In addition, mammalian *HRAS*, *KRAS* and *NRAS* 3'UTRs contain multiple *let-7* complementary sites, suggesting conservation of gene interactions. We showed that the *let-7* family of miRNAs controls expression of RAS, through sequences in its 3'untranslated region. The 3' UTRs of the human *RAS* genes contain multiple LCSs, allowing *let-7* to regulate *RAS* expression. *let-7* expression is lower in lung tumors than in normal lung tissue while RAS protein is significantly higher in lung tumors, providing a possible mechanism for *let-7* in cancer. The RAS family makes up major oncogenes in various cancers, including lung cancer. We showed that human *let-7* is poorly expressed or deleted in lung cancer, but *let-7* is highly expressed in lung tissue. This work suggests that the level of expression of the *let-7* miRNA might be an important factor in limiting or contributing to oncogenesis. Inhibiting *let-7* function leads to increased cell division in A549 lung cancer cells, providing evidence that *let-7* functions as a tumor suppressor in lung cells. Over-expression of *let-7* in cancer cell lines alters cell cycle progression and reduces cell division. While *let-7* regulates the expression of the *RAS* lung cancer oncogenes. we also showed that multiple genes involved in cell cycle and cell division functions are also directly or indirectly repressed by *let-7.* This work reveals the *let-7* miRNA to be a master regulator of cell proliferation pathways. This was also be the first step in a possible cancer therapy, targeting RAS with a miRNA.
Lung cancers do not respond to current chemotherapy and radiothera-

py and novel therapeutic options are desperately needed. We showed that delivery of a miRNA to lung cancers *in vivo* can slow their growth, the first time any microRNA has been shown to have therapeutic efficacy in vivo in cancer. This opens up the possibility of miRNA replacement therapy for cancer patients. Most lung cancer patients beyond stage 1 have a poor outcome, but very few lung cancers are diagnosed at stage I and screening mechanisms are expensive, demonstrating the desperate need for markers of individuals with a greater risk of developing lung cancer. We identified a single nucleotide polymorphism (SNP) in the *KRAS* 3'UTR that disrupts *let-7* miRNA binding, and is associated with a 2-fold increased risk of developing lung cancer. This is the first solid genetic marker for inherited lung cancer risk, and also suggested that 3'UTRs are an unmined area for variation associated with cancer risk.

IN078

MICRORNA ONCOGENIC PATHWAYS DERAILED IN HEPATOCELLULAR CARCINOMA

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A myriad of studies have conclusively proven the central role of microRNAs in human cancer. Gene expression studies revealed the tens of microRNAs that are deregulated in cancer cells. Among microRNAs deregulated in human hepatocellular carcinoma (HCC), miR-122 and miR-221 emerge. Illustrative of how microRNAs promote cancer, we present studies aimed at clarifying their involvement in molecular and biological oncogenic processes. To this end, the identification of target mRNAs is a key step for assessing the function of aberrantly expressed microRNAs. miR-221 is up-regulated in 70% of HCCs. We found that CDKN1B/p27 and CDKN1C/p57 genes, encoding for cyclin-dependent kinase inhibitors, are targets of miR-221. p27 and p57 proteins, which are down-regulated in 77% of HCCs, exhibit a significant inverse correlation with miR-221. We have shown that miR-221 can also target and inhibit the expression of the pro-apoptotic BH3-only protein BMF. Thus, the up-regulation of miR-221 in HCC can, at the same time, promote cell cycle progression and inhibit apoptosis, thereby favoring tumor formation. miR-122, a liver-specific microRNA, is down-regulated in 70% of HCCs. We found that cyclin G1 is one of its target and is up-regulated in HCC. We found that, by influencing cyclin G1 level, the down-regulation of miR-122 in HCC promotes p53 protein degradation and inhibits its activity. This result establishes a link between miR-122 down-regulation and tumorigenesis. These findings have relevance for HCC prognostic stratification. High miR-221 expression is associated with tumor multifocality and reduced time to recurrence after surgery. Low miR-122 is associated with a shorter time to recurrence, whereas high cyclin G1 expression is correlated with a low survival rate. Moreover, these findings may also be important for the development of systemic liver cancer therapy, at present poorly effective against HCC. Indeed, miR-221 may represent a potential target for non-conventional treatment against HCC. Moreover, the restoration of miR-122 expression, as well as cyclin G1 silencing, increases sensitivity to doxorubicin challenge. Discoveries of mechanisms and molecular functions derailed by microRNA aberrant expression have greatly improved our understanding of the molecular basis of cancer and, more importantly, laid the foundation for the exploitation of microRNAs in cancer therapy.

IN079

POST TRANSCRIPTIONAL REGULATION OF MICRORNA EXPRESSION IN HUMAN TUMORS AND CANCER CELL LINES

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microRNAs (miRNAs) are a class of small noncoding RNAs that regulate translation following binding of the mature miRNA to conserved sequences within the target messenger RNAs. The ~ 21 nt mature miRNA is processed from longer precursor molecules in two subsequent steps; the first step is the processing of the primary precursor miRNA (pri-miRNA) to the precursor miRNA (pre-miRNA) by the nuclear Drosha, the second step includes processing of the pre-miRNA to the mature miRNA by the cytoplasmic Dicer. Using quantitative PCR (qPCR) assays developed in our lab to the pri-miRNA and the pri-/pre-miRNAs and commercially available assays to the mature miRNA, we were able to assay each of the miRNA species. This allowed us to determine if miRNA processing is regulated and at which step. The precursor and mature miRNA from 22 different human tissues, 37 human cancer cell lines and 16 pancreas and liver tissues/tumors was profiled using qPCR. Pearson correlation between the precursor and mature miRNA expression was closer to one for the tissues and pancreas tumors/tissues but was closer to zero for the cell lines and liver tumors/tissues, suggesting that processing of miRNA precursors is reduced in cancer cell lines and in liver cancer. Using Northern blotting, we show that many of these miRNAs (e.g., miR-31, miR-105 and miR-128a) are processed to the precursor, but in situ hybridization analysis demonstrates that these miRNA precursors are retained in the nucleus. Our results demonstrate that the miRNA precursors do not always predict the expression of the active, mature miRNA and that qPCR is an effective method to study post transcriptional regulation of miRNA. While microRNA expression has been shown to be deregulated in all cancers studied to date, a mechanism to describe the altered miRNA levels for most cancers is unknown. Post transcriptional regulation of miRNA processing appears to be a major means of regulating altered miRNA expression in human cancer.

IN080

TRADITIONAL AND FUNCTIONAL BIOMARKERS FOR MONITORING EXPOSED POPULATIONS FOR HEALTH RISK ASSESSMENT

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A variety of biomarkers have been used to monitor exposed populations to determine potential health hazards from their exposure to environmental toxic agents. Traditional biomarkers are categorized into Biomarkers of Exposure, Biological Effects and Health Risk. However, the majority of these biomarkers have been focused onto the identification of biological damage but not on functional deficits that are positioned along disease pathways, e.g. cancer. One of such pathways belongs to the extensive and complex DNA-repair machinery. The machinery thus becomes an enormous target for damage from exposure to environmental toxic agents and damage to any component of a repair pathway will interfere with the pathway-specific repair activities. Therefore, when cells from exposed populations are challenged with a DNA-damaging agent in vitro, the in vivo exposure-induced repair deficiency will be dramatically amplified and the deficiency will be detectable in a challenge assay as increased chromosome aberrations, micronuclei or un-repaired DNA strand breaks. The challenge assay has been used in different laboratories around the world to show DNA repair deficiency in a variety of exposed populations. These exposed populations include cigarette smokers; citizens exposed uranium mining and milling waste, heavy environmental pollutants and arsenic in drinking water; workers exposed to benzene, butadiene, styrene, mercury, lead, etc. The predicted health consequences of some of these studies have also been validated. Therefore, the challenge assay is a useful functional biomarker to compliment traditional biomarkers for population health risk assessment studies.

IN081

ENVIRONMENTAL AIR POLLUTION AND ASSESSMENT OF HEALTH RISK IN VARIOUS POPULATIONS

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In Asia, urban air pollution originates mainly from incomplete fossil fuel combustion resulting in emissions which contain carcinogenic compounds such as PAHs, benzene and 1,3-butadiene. Life style factors such as incense burning also contributes to the generation of these carcinogens. To assess the health risk of such exposure, personal monitoring of exposure to these carcinogenic compounds utilizing various biomarkers of internal doses and early effects have been conducted in school children, street vendors and temple workers. PAHs, benzene and 1,3-butadiene levels are high on the road and road sides in Bangkok. School children attending school in Bangkok are exposed to the aforementioned carcinogens at levels more than 6-fold, 2-fold and 4-fold higher respectively than those in rural areas. The health risks associated with such exposure have also significantly increased. In Bangkok children, PAH-DNA adducts were 4-fold higher, DNA strand breaks and 8-OHdG were significantly higher while DNA repair capacity was lower than those in rural children. Street venders who are working on the roadsides are also exposed to higher level of PAHs and benzene than the monks and nuns residing in the monasteries nearby. High level exposure to PAHs, benzene and 1,3-butadiene in the environment also occurs in areas where incense burning is practiced such as in temples or in the household. Personal monitoring in temple workers showed that urinary metabolites of these carcinogens were significantly higher those in the control workers. Increased DNA damage and reduced DNA repair capacity were also observed in temple workers. The results of these studies suggested an increased health risk of the development of cancer in various groups of population due to exposure to carcinogenic compounds in air pollution.

IN082

GENOTOXICITY OF AIR POLLUTANTS – IMPACT TO CHILDREN HEALTH

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The impact of air pollution to pregnancy outcome was related to increased exposure to carcinogenic polycyclic aromatic hydrocarbons (c-PAHs). In the Pregnancy Outcome Project, an increased risk of IUGR (intrauterine growth retardation) was established for mothers exposed to PM2.5 > 27 μg/m3 or c-PAHs > 15 ng/m3 (benzo[a]pyrene, B[a]P > 2.8 ng/m3) during the first month of gestation. This correlation proved to be strongly dose-response related. The relationship between the birth weight and genetic polymorphisms of metabolic genotypes were studied using DNA from placental samples from the cohort of 1 450 subjects. Birth weight was significantly decreased by smoking, ETS and genetic polymorphisms of CYP1A1*2A, CYP1A1*2C, GSTM1 null and c-PAHs. The same DNA samples were used to investigate association between 8-oxodG (8-oxodeoxyguanosine, a marker of oxidative damage to DNA) and Illumina custom-made 768 SNP panel with representatives of detoxification genes, DNA repair genes, genes mediating immune response and oxidative damage genes. The relationship between the selected 768 SNP panel and the respiratory morbidity in the cohort is further analyzed. Stratified random sample of 1 492 mother-infant pairs from the Pregnancy Outcome Project were recruited as a cohort for further study of respiratory morbidity. After 6 years the cohort created 1 007 children. The Rate ratio (RR) of the incidence of respiratory system diseases in both districts were evaluated. For children younger 2 years in the Teplice district was increased laryngitis and tracheitis (RR 2.07; CI 1.66-2.57), inflammation of the middle ear (RR 3.36; CI 2.50-4.50), pneumonia (RR 3.45; CI 1.92-6.22), at the age of 2-6 years this difference persists for laryngitis and tracheitis and pneumonia. Ambient c-PAHs and PM2.5 were associated with early-life susceptibility to bronchitis. New hot-spot was recently found in the Northern Moravia (Silesia) as the most polluted region in the Czech Republic by c-PAHs as (a concentration in the year 2007 was 8.8 ng B[a]P/m3). c-PAHs seem to be an important source of genotoxic and embryotoxic activities of organic mixtures associated with urban air particles.

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IN083

ARSENIC IN DRINKING WATER: GENETIC AND GENOMIC APPROACHES FOR IDENTIFY ARSENIC SUSCEPTIBILITY AND HEALTH EFFECTS

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In West Bengal, although about 25 million individuals are chronically exposed to arsenic through drinking water, yet, only 15 to 20% of them exhibit arsenic-induced skin lesions. This indicates that genetic variations might play an important role in arsenic-susceptibility. We have assessed the arsenic induced health effects and genetic damage in arsenic-exposed individuals with and with out skin lesions utilizing epidemiological and cytogenetic end points. To address this issue of arsenic susceptibility at genetic and genomic levels, comet assay and challenge assay were performed and single nucleotide polymorphism (SNPs) studies were carried for a number of genes that might be involved in the different pathways in arsenic metabolism and detoxification. Cytogenetic studies viz., chromosomal aberrations studies and micronucleus study show that individuals with skin lesion had higher genetic damage compared to exposed individuals without skin lesions, who in turn had higher genetic damage compared to unexposed controls. Results of DNA repair studies through Challenge and Comet assay shows that the individuals with arsenic induced skin lesions had suboptimal DNA repair capacity. From the GST's group the homozygous null gene frequency of GSTT1 and GSTM1 genes and SNPs of some other GST genes were also studied. SNPs of p53 gene, human purine nucleoside phosphorylase (PNP) and ERCC2 genes were also analyses. Distribution of homozygous GSTM1 null genotype was significantly higher in the no skin lesions group indicating a protective role of GSTM1 null in the no skin lesions individuals. On the other hand the individuals with p53 codon 72 Arg/Arg genotype is over represented in the skin lesions individuals indicating that this genotype is more susceptible to arsenic induced toxicity. Lys/Lys genotype in the ERCC2 polymorphism was almost five fold over represented in the arsenic induced hyperkeratosis skin lesions group when compared to no skin lesions group. Interestingly, Lys/Lys genotype individuals also showed to have significantly higher levels of chromosomal aberrations than those with non-risk genotypes. Thus the above results indicate that the sub-optimal DNA repair and the genetic variations are responsible for arsenic induced toxicity and carcinogenicity.

IN084

ENVIRONMENTAL HEALTH PRIORITIES AND CHALLENGES AROUND THE WORLD FOR THE NEXT DECADES

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Abstract not available at the time of publication.

IN085

ARISTOLOCHIC ACID NEPHROPATHY: AN ENVIRONMENTAL AND IATROGENIC DISEASE.

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Endemic (Balkan) nephropathy, characterized by an insidious onset, invariable progression to chronic renal failure and strong association with upper urothelial tract cancer (UUC), occurs exclusively in rural farming villages located near tributaries of the Danube River. Significant epidemiologic features of EN include a familial, but not inherited, pattern of disease and a mosaic distribution of cases in endemic villages. A variety of environmental agents, including mycotoxins and heavy metals, have been proposed as potential causative agents of EN. We hypothesize that chronic dietary exposure to aristolochic acid (AA) in genetically susceptible individuals, is uniquely responsible for EN and UUC. This hypothesis, based on clinical and pathological findings in patients with aristolochic acid nephropathy (AAN), is supported by the unequivocal

detection of 7-(deoxyadenosin-N6-yl)aristolactam DNA adducts in renal cortical and urothelial cancer tissues of patients with EN and/or UUC [PNAS, 104, 12129 (2007)]. Additionally, the spectrum of p53 mutations observed in UUC from residents of endemic villages in Croatia, Serbia, and Bosnia is dominated by A:T→T:A transversions, a mutational signature for exposure to AA. The mosaic distribution of EN suggests that one or several genes might account for the observed varying degree of susceptibility to EN. We have tested this hypothesis by conducting a nested, case-control study of EN in which we analyzed epidemiologic, biomarker and genetic polymorphism data from samples collected as part of a large cross-sectional health survey. Additionally, we identified genes involved in the cytotoxic response of mouse renal proximal tubules to AA, differentiating these changes from its genotoxic effects. Our molecular epidemiologic studies inform public health initiatives designed to eradicate EN and UUC in the several countries harboring this disease. They also serve as a warning of the profound nephrotoxicity and carcinogenicity of *Aristolochia* herbal remedies, used traditionally for medicinal purposes worldwide. Thus, from a global perspective, we conclude that AAN and its associated UUC currently persist, as in centuries past, as a generally unrecognized, but omnipresent iatrogenic disease (Adv Mol Tox. v3, 211 (2009).

IN086

HUMAN PREMATURE AGING PROTEINS PARTICIPATE IN DNA REPAIR

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The 5 human RecQ helicases protect the genome against genomic instability and participate in many DNA metabolic processes. Three of them are identified with specific human disorders: Werner syndrome (WRN), Bloom syndrome (BLM) and Rothmund Thomsons syndrome (RECQ4, RTS), characterized by clinical features of premature aging and cancer. The other two human RecQ helicases, RECQ1 and RECQ5, are as yet not associated with known disorders. The RecQ helicases appear to operate at stalled replication forks and to be involved in DNA repair. Studies from ours and other laboratories have suggested that WRN is involved in double- and single stranded DNA repair. BLM also appears to be involved with these DNA repair pathways, but much less is known about the role of RECQ4 and RECQ5. We are exploring the roles of RECQ4 and RECQ5 in double and single stranded DNA repair using biochemical and cellular approaches including confocal microscopy with laser beam. Cockaynes syndrome group B is also a humna premature aging deficiency, and this protein also participates in various DNA repair processes. Common for many of the premature aging proteins are functions in DNA double strand a DNA single strand repair. These observations support the general notion that aging is associated with deficiencies in DNA repair, a hypothesis that will be discussed.

IN087

ENVIRONMENTAL CAUSES OF TELOMERE DEFECTS

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Telomeres are regions of the genome that profoundly influence life span, disease and genomic integrity. Shortened telomeres are associated with numerous aging-related diseases, progeroid syndromes, and environmental factors such as cigarette smoking and oxidative stress. Werner syndrome is a segmental progeroid disorder that is characterized by telomere defects, and is caused by loss of the WRN helicase/exonuclease DNA repair protein. We are testing the hypothesis that DNA lesions that impede replication cause accelerated telomere loss, and that WRN preserves telomeres by facilitating recovery of stalled replication forks. Exposure of the environmental carcinogen chromium (VI) induces oxidative DNA damage and blocking lesions in G-rich sequences, such as telomeric TTAGGG repeats. Therefore, Cr(VI) exposure is an excellent model for investigating the consequences of environmental DNA damage on telomeric DNA replication. As evidence that Cr(VI) triggers stalled DNA replication, we observed cellular Cr(VI) exposure induced an accumulation of cells in S-phase that exhibited high levels of γH2AX foci, indicative of DNA breaks and stalled replication forks. We found that human cells deficient in WRN protein are hypersensitive to Cr(VI) toxicity, and exhibit a delayed reduction in γH2AX foci during recovery from Cr(VI) exposure. Cr(VI) induced WRN protein translocation from the nucleoli into nucleoplasmic foci in S-phase cells, and these foci colocalized with γH2AX foci indicating WRN responds to replication associated DNA damage. Currently we are testing whether Cr(VI) directly damages telomeres and find that Cr(VI) exposure induces the appearance of Telomere Dysfunction-Induced Foci (TIFs) and WRN mobilization to telomeres. We are further testing whether Cr(VI) induces sister telomere loss by analyzing metaphase chromosomes. Our data indicate that exposure to the environmental genotoxin Cr(VI) can induce telomere defects perhaps by interfering with telomere replication, and show a novel role for WRN protein in protecting against Cr(VI) toxicity.

IN088

EVIDENCE THAT DNA DAMAGE PLAYS A CAUSAL ROLE IN AGING AND AGE-RELATED DISEASE

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Background: Aging is defined as the progressive decline in functional reserve that occurs in all organisms over time. Aging causes a decrease in the capacity of tissues to maintain homeostasis and therefore impaired ability of an organism to recover from stress and an increased risk of morbidity and mortality. Aging is thought to be the consequence of stochastic damage to organelles and macromolecules with time. However, the cellular target that is life-limiting is not known, but may include mitochondria, telomeres, proteins, lipids or DNA. The aim of this study is to determine if DNA damage makes a significant contribution to the degenerative changes seen with aging.
Methods: To address this, we generated mice with increased DNA damage by genetically depleting DNA repair mechanisms. The mice were allowed to live their natural lifespan and the age at onset of symptoms associated with age-related diseases was recorded. Terminal animals were euthanized and histopathologic analysis of all major organ systems was conducted. The study was repeated while chronically treating the DNA repair-deficient mice with a radical scavenger to reduce endogenous oxidative stress, and thereby DNA damage.
Results: Mice expressing only 10% of the normal levels of ERCC1-XPF repair endonuclease have premature onset of numerous aging-related pathologies including impaired balance, peripheral neuropathy, muscle wasting, loss of vision and hearing, urinary incontinence, epidermal atrophy, bone marrow hypoplasia, decreased liver and kidney function, osteoporosis, loss of insulin-producing cells and intervertebral disc degeneration. The mice die prematurely at 7 months of age. Chronically treating the mice with a radical scavenger caused a significant delay in the onset of age-related symptoms and pathologies.
Conclusions: These data support the conclusion that endogenous DNA damage caused by oxidative stress promotes aging of most organ systems.

IN089

TRANSCRIPTION-BLOCKING DNA LESIONS: AT THE CROSSROAD OF AGING AND LONGEVITY

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There is abundant evidence that the gradual accumulation of stochastic damage to our genome is a driving force in aging. For instance, defects in the genome maintenance mechanisms can lead almost exclusively to

a variety of progeroid disorders suggesting a causative role of DNA damage in aging. However, in contrast to stochastic damage theories of ageing, longevity appears to be also regulated genetically. Single gene mutations in the GH/IGF1 hormonal pathway extend lifespan in worms, flies and mammals. Thus, stochastic accumulation of damage may drive functional decline with advancing age. However, the existence of genetic pathways that regulate longevity may set the pace on how rapidly damage builds up and function is lost. It remains unknown, however, to what extent stochastic events that drive genome instability are linked to genes that regulate longevity. Here we will present recent data indicating unexpected genome-wide gene expression associations between the progeroid mice carrying DNA repair defects and mutant dwarf and calorie-restricted animals that benefit from unusually long lifespan. We will show further evidence that the accumulation of unrepairable DNA lesions in the transcribed part of our genome elicits an age-related shift from growth to somatic preservation similar to that observed in natural aging but also in longevity mutants early in life. We propose that persistent transcription-blocking lesions act as instigators of somatotropic attenuation and enhanced stress resistance providing a link between stochastic damage events to genes that determine longevity.

IN090
CELLULAR SENESCENCE AS A DNA DAMAGE RESPONSE

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Cellular senescence is an irreversible state in which cells, although alive, are unable to further divide, despite favourable growth conditions. Historically, telomere attrition was the first observed cause of senescence. We and others have proposed that critically short telomeres are perceived by the cells as damaged DNA and that this triggers a DNA damage response (DDR) that enforces senescence. Our most recent data point to the presence of irreparable DNA damage as the discriminating event between transient checkpoint activation and irreversible senescence. We have also observed that mouse fibroblasts, that undergo premature cellular senescence because of suboptimal growth conditions, also activate a detectable DDR, suggesting that DNA damage is being generated under these conditions too. Perhaps surprisingly, also the expression of an activated oncogene induces cellular senescence. We have observed that this occurs as the consequence of the activation of a robust DDR following the generation of DNA damage. DDR impairment allows bypass of cellular senescence and favours cellular transformation. Senescence is preceded by a transient hyperproliferative phase and cells arrest with a partially re-replicated DNA. DDR is not activated in the absence of DNA replication. These and other observations have prompted us to propose that oncogene activation alters the normal DNA replication pattern and it is an intrinsically genotoxic event. Oncogene-induced senescence is also associated with a global heterochromatinization of nuclear DNA. Senescence-associated heterochromatic foci (SAHFs) are enriched in heterochromatin markers and they have been proposed to enforce cellular senescence by suppressing the expression of proliferative genes. Presently, DDR activation and heterochromatinization are considered the two main tumor suppressors mechanisms that control cellular senescence. We will discuss our most recent advancements on the interplay between DDR and chromatin modifications in the context of cellular senescence.

IN091
PERSONALISED AND POPULATION-BASED STRATEGIES FOR DIAGNOSIS OF DNA DAMAGE AND ITS PREVENTION VIA NUTRITIONAL AND LIFE-STYLE INTERVENTION.

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The evidence that increased genome and epigenome damage is the most fundamental risk factor for infertility, developmental defects, degenerative disease and accelerated ageing is increasing every year. There is therefore a need to develop an effective public health strategy aimed at the diagnosis and prevention of DNA damage both at the individual and population level. The impact of diet, life-style and environment on the genome depends on both inherited and acquired genetic characteristics and this is leading to an integration of new fields of research now known as Genome Health Nutrigenomics and Public Health Genomics. The term "Genome Health" is used because it is a positive term and easier for the public to comprehend the concept that harm to the genome can have serious health consequences and also because the term "DNA damage" may cause unnecessary excessive concerns. The presentation will provide an overview on current knowledge of the dietary and life-style factors that are associated with increased DNA damage both at the chromosomal level (e.g. micronucleus frequency) and the molecular level (e.g. telomere length) and the association of these pathologies with developmental and degenerative disease outcome. In addition I will outline the evolution of the Genome Health Clinic concept as a modern personalised and public health strategy for preventing diseases resulting from excessive genome damage due to inappropriate nutrition, life-style and environment. This approach is being supported by new initiatives to define Nutrition Reference Values for prevention of DNA damage.

IN092
NUTRITIONAL SYSTEMS BIOLOGY: FROM INTEGRATING MECHANISMS TO PREVENTION

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Our health status is the result of the combination of our genomic heritage and the cumulative environmental exposure. As our genome is stable, we just need to carefully quantify this and this is a technology challenge where rapid progress is being made. With the advent of the omics tools and concepts, nutrition science undergoes a dust-off and the transition (started by the functional food wave) from "optimizing our diet" (i.e. optimal amount of macro-and micronutrients in our diet) to "optimizing our health" (i.e. the right diet to optimize health and prevent disease) can be completed. It is essential to realize and embrace the key drivers: Nutrition science is part of the big biology revolution, and a massive integration of fundamental disciplines with nutritional science is essential. This integration involves not just technology implementation, but also a coordinated action on standardization, data warehousing and bioinformatics. A conceptual shift from "more" to "better", where very accurate quantification of food intake, nutrition related health status and health effects is essential. In following this path, we realize that the nutrition and health relationship is not targeted at disease pathologies or cancer progression, but much more towards the "overarching drivers" from which many nutrition related disease states originate, like metabolic stress, inflammatory stress, oxidative stress and even psychological stress. In optimal health, these systems are robust and resiliant, i.e. capable of absorbing environmental challenges. In fully understanding these highly balanced processes, we need a systems approach, where both the complexity and the individuality need to be taken into account. If this is properly achieved, we very likely will see a new wave of nutritional sciences where health optimization and disease prevention, maybe even at a personal level, can be achieved.

IN0093
BACTERIA-HOST INTERACTION IN CHRONIC DISEASE: INFLAMMATION MEETS METABOLISM

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The dramatic increase of chronically degenerative diseases in the industrialized world implies a complex interaction of host genetic predispositions and environmental factors. The gut acts as a highly selective barrier and communication organ between the luminal environment including food and bacterial components and the host responsible for the regulation of metabolic and immune functions. The peaceful and productive coexistence of the host with its intestinal microbiota is

tightly controlled at various levels and an accumulating body of evidence suggests, that the failure of this homeostasis is thought to contribute to the development of inflammation-driven metabolic pathologies. The gut acts hereby as a highly selective interface organ responsible for the regulation of immune-mediated and metabolically-driven processes. Specifically, cellular organelles like the endoplasmatic reticulum (ER) and mitochondria dramatically affect the inflammatory response to stress mechanisms. In the context of human IBD and animal models of chronic intestinal inflammation, we characterized ER- and mitochondrion-associated stress mechanisms at the epithelial and immune cell level. Following the hypothesis that nutritional factors may act as environmental triggers for the development of chronic diseases, dietary iron was identified as a risk factor for the pathogenesis of chronic intestinal inflammation.

IN094
PROTEOMIC BIOMARKERS OF VULNERABILITY TO CANCER IN THE ALIMENTARY TRACT

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Background: Carcinoma of the gastrointestinal tract develops from the mucosal epithelial cells, usually as the final stage in a prolonged sequence of morphological and functional changes. Clinical proteomics is widely used to characterize the various stages of tumour development. Here we describe the use of similar techniques to explore patterns of protein expression in the anatomically normal, but nevertheless vulnerable intestinal mucosa of individuals at high risk of disease. It is hoped that the information obtained by this approach can be used both to characterize subtle changes associated with the earliest stages of the disease process, and to explore the impact of nutrition and metabolic factors on the pre-cancerous mucosa. Methods: We have used 2-dimensional gel electrophoresis combined with mass spectrometry to define patterns of protein expression in oesophageal and colorectal mucosa, using protein extracts from colorectal biopsies obtained from patients with and without neoplastic disease. Results: The transition from morphologically normal mucosa to a precancerous lesion or tumour is marked by very significant changes in expression of proteins associated with both cytoskeletal structure and metabolic activity. However multivariate statistical analysis shows that protein expression in the normal mucosa also differs significantly amongst healthy subjects, polyp and cancer patients. A large proportion of the proteins showing evidence of differential expression in tumor tissue are cytoskeletal elements, and many of the same proteins are also over-expressed in apparently normal mucosa from polyp and cancer patients compared with healthy subjects. We are currently extending these studies to healthy individuals differing in their exposure to environmental and metabolic risk factors for colorectal cancer. Conclusions: These findings indicate that protein expression in the apparently normal colonic mucosal field is modified in individuals with neoplastic lesions at sites distant from the lesion. The recognition and characterization of these field-effects at the molecular level may provide protein biomarkers of susceptibility to cancer, and advance our understanding of the role of diet and other environmental factors in its causation.

IN095
HOW TO PROTECT INTEGRITY OF SCIENTIFIC RESEARCH

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There have been recently widely publicized fraud cases, and a fair number of scientists have agreed of themselves having been involved in various forms of scientific misconduct (Fanelli 2009). Publication misconduct is also "disturbingly frequent" (Wager et al. 2009). On basis of such revealed unethical behavior in science we have to accept that scientific enterprise, in addition to enthusiasm, collaboration and pursuit of truth includes also the negative sides of human nature: greed, jealousy, omnipotence, manipulation and selfishness. Humans are gregarious animals, and for that reason we are also prone to fashions, of which trends science is not free. We cannot step out of our humanity and thus we all are prone to these vices. However, especially such interdisciplinary fields, like environmental health-related research which is totally dependent on public trust, are very sensitive to the public image of science and any violation of scientific integrity may endanger the societal trust in research and scientists (Merlo et al. 2008). How to protect scientific integrity so that scientific research can provide reliable, impartial, and hopefully useful data? As important as a good peer review system is, it is self-evident, that peer-review cannot play a major role in detecting or preventing scientific misconduct, but is based on trust (Palma 2009, Malay 2009). Those who have really wanted have been easily able to publish fraudulent papers. Scientific integrity has to be inbuilt in the system and prevention of scientific misconduct and fraud is the common responsibility of the whole scientific community (Malay 2009). Education by example and prevention by giving a clear message to young scientists in research groups from the very beginning of their careers cannot be stressed too much (Vähäkangas 2004). Transparency at every level would be advisable, even in the world of increasing commercial interest in the results of research. To ensure impartial judgement, conflicts of interest are important to claim and already required in publications (Greco & Diniz 2008). It is also important to realize that research ethics boards play a significant role not just protecting those participating in research, but also promoting better science (e.g. McArthur 2009).

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IN096
OBTAINING, SHIPPING, BIOBANKING AND USING OF HUMAN SPECIMENS: LOGISTICAL AND ETHICAL CHALLENGES

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The advent of "-omics" technologies is generating a huge demand for high-quality human samples associated with comprehensive individual, epidemiological and pathological annotations. Population-based cohort studies are of particular interest since they provide access to samples (e.g. blood samples) collected ahead of clinically overt disease. Genome-Wide Association Studies have shown that it is often desirable to gather specimens from several large cohorts in order to achieve sufficient power. Developing strategies for interpolating cohort data and biobanks is a major challenge which requires international coordination (e.g. the cohort integration strategy developed by the P3G Observatory, http://www.P3G.org.). These opportunities place enormous demands on biobanking activities and raise new logistical and ethical questions in order to fulfill the promises of new biomarker technologies for better prevention and for personal medicine. The first and foremost question is: how big and how extensive should biobank be? New biobanks are being created at a high pace, often without consideration for realistic specimen usage. Many biobanks are developed as repositories of materials obtained in the course of medical procedures

and are not hypothesis-driven. They often lack appropriate controls and sufficient statistical power. At the same time, there is a lack of coordinated biobanking in the context of clinical trials, despite their remarkable potential for biomarker discovery, assessment and validation. The second question is: which "best practice" may ensure the optimal use of resources and preservation of individual molecular information? Most biobank practices are based on experience rather than evidence and there is a lack of adequate markers to monitor the quality of banked specimens. The third question, raised by the need to coordinate individual biobanks, is: what are the rules of access to biobanks and on which basis specimens may be exchanged between biobanks to achieve higher numbers when desirable? Answering these questions requires developing common standards, consensual ethical procedures, strict rules for protecting data, confidentiality and intellectual property, and international regulations for shipping and sending of research specimens across national borders. Inappropriate answers to these questions may have significant ethical consequences in wasting precious samples and resources, in breaching the confidence between study participants and researchers and in slowing down progress in public heath and personalized medicine.

IN097

CHILDREN AS RESEARCH SUBJECTS: TODAY'S RESEARCH FOR A BETTER FUTURE.

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As unequivocally stated by the German philosopher D. Bonhoeffer *"the test of the morality of a society is what it does for its children"*, environmental, pharmacological and clinical research in children must be solely aimed at protecting them from environmental hazards and curing the sick ones with efficient and safe medical treatments. To achieve these goals research in children is required to gather sound scientific evidences that often cannot be achieved simply and safely by extrapolating findings from adult populations. The integrity of children research must be guaranteed and age-related ethical concerns specifically addressed to protect a susceptible population. As for adults, pediatric research requires the careful development and testing of information sheets and informed consent forms, according to the level of the child's maturity and the country specific legislation. Very young children are not capable to fully understand even very simple research aspects, therefore the ethical principle of respecting their way of understanding and their own opinion needs to be taken in due consideration. For the little ones informed permission should be obtained from capable adults (mother, father, guardian) responsible for the child together with informed assent from children. Refusal by a child to participate should always be respected and taken on account. Both translational and environmental research is frequently seeking health effects or remission across a time window that may last decades during which children will became adults. Their willingness to participate (or opt out) should be reconsider across the study time window to fully respect their autonomy so to ensure the respect of the current ethical principles. Storing biological specimens collected at birth, when the child cannot give any kind of assent, is a practice that requires a careful evaluation from the ethics committees to protect subjects' from possible misconduct.

IN098

RECENT TRENDS AND CHALLENGES IN ENVIRONMENTAL HEALTH RESEARCH

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The concept of gene-environment interaction has gained wide acceptance in the scientific community and is central to the future of both genetic and environmental health research. These two fields, once separate and distinct, are now inextricable. Genetics, the study of individual genes, has expanded to include genomics and a host of new technological opportunities. Similarly, the definition of what constitutes the environment has evolved. Currently, and particularly as it relates to gene-environment interactions, the environment is considered to be anything outside the body that can affect an individual's health. This includes our air, water, soil, and climate, of course, but also takes into account elements such as the food, drink, and medicine we ingest, our behavioral choices such as consuming tobacco and alcohol, our exposure to infectious agents, our socioeconomic status, age or developmental status, stress, and even the structures and infrastructure around us (the so-called built environment). The questions of what causes disease and what can we do to prevent it, cure it, or minimize its impact on quality of life have been central to medicine from time immemorial. Today these questions propel the mission of environmental health research to understand and characterize gene-environment interactions. The vast majority of human disease arises when something is wrong in the relationship between our body and the environment. Although certain inherited disorders, such as Huntington's disease, cystic fibrosis, and Tay-Sachs disease, arise from mutation in a single gene, these are relatively rare, accounting for no more than 5 percent of human disease. Thus, the risk of such a disease for a person with a specific disease gene variant is relatively high, but the incidence of such monogenic diseases in the general population is low. However, many common human diseases appear to be polygenic, resulting from complex interactions of several genes. Such susceptibility-conferring genes increase disease risk only a few-fold, but because they occur so frequently in the human population, they can have a large effect on the incidence of a disease. Susceptibility genes alone are not sufficient to cause disease; they modify risk in combination with other genes and with exposure to environmental agents. Practical considerations regarding these points for the future of environmental health research will be discussed.

IN099

THE DNA DAMAGE PROBLEM IN THE CONTEXT OF CANCER, AGING AND LONGEVITY

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Nucleotide excision repair (NER) is one of the most versatile repair systems removing a wide range of helix-distorting lesions, mostly of exogenous (UV, bulky adducts), but also of endogenous (e.g. cyclopurines) origin. Two NER sub-pathways exist. Global genome NER operates genome-wide and in that way is important for preventing mutations. Transcription-coupled repair removes damage that obstructs transcription, counteracting cytotoxic effects of DNA injury. Photo(sun)sensitive inherited NER syndromes display a striking clinical heterogeneity: very strong (skin)cancer predisposition in xeroderma pigmentosum (XP) as well as dramatic neuro-developmental deficits such as in Cockayne syndrome (CS) and trichothiodystrophy (TTD) without any cancer susceptibility. Mutations in NER helicases XPB and XPD, subunits of the repair/transcription factor TFIIH, are associated with all three disorders or combinations. XPD^{TTD} mice, mimicking a XPD point mutation of a TTD patient, revealed that TTD is in fact a segmental premature aging syndrome, like CS, which appears to be protected from spontaneous cancer. $XPD^{XP/CS}$ mutant mice on the other hand are highly predisposed to cancer, but also display premature aging, demonstrating that both phenotypes can co-exist. Different single and double NER mutants exhibit multiple premature aging features, including osteoporosis, neuro-degeneration, early infertility and cessation of growth, liver and kidney aging, deafness, retinal photoreceptor loss, depletion of hematopoietic stem cells, etc. Life span is limited to 1,5 year for milder mutants to 3-5 weeks for dramatic double mutants. A striking correlation is found between severity of compromised repair and rate of onset and severity of the clinical aging manifestations providing strong experimental support for the DNA damage theory of aging. Conditional mutants in which dramatic

aging occurs only in e.g. the brain, display many signs of neurodegeneration whereas the remainder of the body appears normal, revealing organ-specific accelerated aging. We propose that endogenous oxidative lesions hamper transcription/replication and trigger cellular apoptosis-senescence and in the end (premature) aging. Microarray, functional and physiological studies have revealed that persisting DNA damage elicits a systemic downregulation of the IGF1 somatotrophic axis and upregulation of anti-oxidant defences, favouring maintenance and defences at the expense of growth and development, explaining the severe growth defect of repair mutants. Persisting DNA damage triggers this 'survival' response in a cell autonomous manner and implicates regulation by microRNAs. Caloric restriction and fasting trigger a similar 'survival' response, which maximizes anti-oxidant defence and -when constitutive-promotes longevity at least under laboratory conditions. These data link accumulation of DNA damage and the IGF1 control of life span and open perspectives for the promotion of healthy aging.

IN100

COMPLEX CELLULAR RESPONSES TO DNA DAMAGING AGENTS

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Alkylating agents are abundant in our cellular and external environment, and they are toxic, mutagenic, and carcinogenic. Because of their toxic properties, certain alkylating agents are also used for cancer chemotherapy. Therefore, it is important to elucidate the constellation of different biochemical and genetic pathways by which both normal and malignant cells respond to alkylation damage. Our ultimate goal is to develop ways of predicting how certain agents will affect these very different target cell populations. It is well known that a variety of DNA repair pathways and cell cycle checkpoint pathways protect cells against the toxic effects of alkylation damage. But now we have shown that a multitude of other unexpected pathways are also involved in cellular recovery, and our goal is to understand how these cellular pathways operate and interact with each other to determine the ultimate phenotypic response to such environmental assaults. We use a variety of cellular and animal model systems ranging from yeast to mice and humans. We have studied global genomic responses to alkylation damage in S. cerevisiae, in mouse and human cell lines, and in various tissues of exposed mice. The results demonstrate that global cellular responses to alkylation damage are far more complex than first thought and some of the unexpected pathways that protect against alkylation-induced toxicity will be presented and discussed.

IN101

NOVEL STRATEGIES IN THE PREVENTION OF MUTATION-RELATED DISEASES

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The 20th century was characterized by an "epidemiological revolution", which is well portrayed by the intricate crossover of mortality curves by the middle of that century. Infectious diseases were replaced by chronic degenerative diseases as main causes of death in the population. Although no generalization can be done, it is noteworthy that these diseases may share common risk factors as well as common protective factors. In addition, due to the considerable increase in life expectancy, different chronic diseases are likely to occur in the same individual. Certain mechanisms, such as damage to nuclear DNA and mtDNA, oxidative stress, chronic inflammation, signal transduction alterations and epigenetic changes may be involved in the pathogenesis of different diseases. Studies performed in our laboratory have shown that certain genomic and postgenomic changes that are usually investigated in cancer research may also been detected in other chronic diseases, such as atherosclerosis, degenerative heart diseases, chronic obstructive pulmonary diseases, neurological disorders, eye diseases, skin ageing, and alopecia. Similar alterations can be found in critical periods of life, such as birth and ageing. Together with other mechanisms, the nucleotidic alterations occurring at birth in the lung render the newborn particularly vulnerable to the action of environmental agents. In fact, we demonstrated that cigarette smoke, for which a suitable animal model has not been available so far, becomes a potent carcinogen in mice when exposure starts at birth and continues early in life. The most obvious approach to the primary prevention of mutation-related diseases is to minimize exposures to recognized risk factors. Growing importance is ascribed to a complementary strategy, called chemoprevention, which uses dietary and pharmacological agents to reinforce the host defense machinery through a variety of mechanisms. We investigated a number of chemopreventive agents by evaluating modulation of intermediate biomarkers and carcinogenicity. An optimal agent should not excessively alter the physiological patterns of gene expression and microRNA and proteome profiles, but at the same time it should be effective in inhibiting alterations induced by mutagens and carcinogens.

IN102

DNA DOUBLE STRAND BREAK REPAIR IN PARENTAL CHROMOSOMES OF MOUSE ZYGOTES

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DNA double strand breaks are of a special importance in the mammalian Radboud. They are instrumental for genetic recombination but also, in the male germline, for chromatin remodeling in elongating spermatids and for sperm chromatin remodeling in the cytoplasm of the zygote that originates after gamete fusion. Hence, when DNA repair in the male germline is shutting down at spermatid elongation, remnant double strand DNA breaks could be left for the oocyte to repair after sperm nucleus entry. Germline double strand breaks in the human are of special relevance knowing the high spontaneous reciprocal translocation frequency. In the mouse zygote, both non homologous enjoining (NHEJ) and homologous recombination (HR) are active in the repair of DNA double strand breaks. NHEJ plays a role in chromatin remodeling of paternal chromatin. Both HR and NHEJ are active during S-phase before first cleavage. An indication is found for the fact that both pathways communicate as to compensate for deficiency. Reciprocal translocations do arise at S-phase, also after single strand DNA damage, when HR is compromised. Overall, both before and during after S-phase, indications for a higher genotoxic sensitivity of the male genome by the presence of gammaH2AX and/or Rad51 foci, are found. Derijck et al., Human Molecular Genetics 2008, 17: 1922 – 37.

IN103

PATERNAL EXPOSURES AFFECT SPERM CHROMATIN AND DISTURB EPIGENETIC PROGRAMMING DURING EARLY EMBRYO DEVELOPMENT

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Over the past twenty years, a large body of evidence has accumulated establishing that paternal exposures to xenobiotics, including environmental contaminants and therapeutic or lifestyle drugs, can affect progeny outcome. However, the cellular and molecular actions of such agents on male germ cells and/or on early embryos are still poorly understood. Gross parameters, such as sperm numbers, are not adequate to characterize sperm quality or resolve the underlying mechanisms. Using several animal models and either a single drug (cyclophosphamide) or multiple drugs in the combined chemotherapeutic regimens used for the treatment of testis cancer or non-Hodgkin lymphoma, we have undertaken a systematic examination of the major sperm nuclear elements that are affected by in vivo exposures and the repercussions of these effects on the early embryo. We have used multiple sperm chromatin damage assays, including the comet assay, acridine orange, TUNEL, chromomycin A3

(CMA3) and monobromine bimane (MBBr) assays; we found that each provides differing, complementary information about chromatin quality. Furthermore, DNA damage induced by chemotherapeutic alkylating agents is germ cell phase specific, with the most extensive effects occurring during histone hyperacetylation and transition protein deposition, a key point of sperm chromatin remodelling. By investigating the proteome of the sperm nucleus, we found significant alterations in sperm basic proteins as well as the selective induction of numerous nuclear matrix proteins involved in cell defense and detoxification. Spermatozoa having extensive nuclear damage fertilize ova effectively. However, significant alterations of nuclear histone acetylation and DNA methylation of both paternal and maternal pronuclei ensue. Furthermore, phosphorylated histone H2AX was increased in a biphasic manner in the paternal genome, while poly(ADP-ribose) polymerase-1 was markedly elevated in both the paternal and maternal genomes in zygotes fertilized by drug-treated males. Therefore, paternal exposure to xenobiotics can alter critical sperm nuclear components, resulting in modifications of both pronuclei in the zygote, and may account for the adverse developmental outcomes that are observed after such treatments. Supported by CIHR.

IN104

EFFECTS OF TOBACCO SMOKE ON MALE GERM CELLS AND EARLY EMBRYONIC DEVELOPMENT

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Cigarette smoking in men has been associated with increased chromosomal abnormalities in sperm and with increased risks for spontaneous abortions, birth defects and neonatal death. Little is known, however, about the reproductive consequences of paternal exposure to second-hand (sidestream, SS) smoke. We used a rodent model and exposure to tobacco smoke and its components to determine whether paternal smoking: (a) affected the function and genetic integrity of sperm; (b) resulted in the induction of chromosomal aberrations after fertilization, and (c) delayed embryonic development. We found that male exposure to diepoxybutane, a tobacco smoke component, during the last two weeks of spermatogenesis resulted in the accumulation of sperm DNA lesions that were transmitted to the zygote where they were converted into chromosomal aberrations. These results indicated that the postmeiotic phase of spermatogenesis is a sensitive window for the generation of tobacco smoke-induced DNA lesions. Investigations of the effects of male exposure to mainstream (MS) or SS tobacco smoke during this critical window of sperm development showed that there was a differential sensitivity of male germ cells to MS and SS smoke. Specifically: (1) only SS smoke affected sperm motility; (2) only MS smoke induced DNA strand breaks in sperm; (3) both MS and SS smoke increased sperm chromatin structure abnormalities; and (4) MS smoke affected both fertilization and the rate of early embryonic development, while SS smoke affected fertilization only. Interestingly, for the majority of the endpoints investigated, there was little evidence for dose-related effects, as exposure to low doses of MS and SS were as effective as high doses. Furthermore, the detrimental effects on motility and sperm DNA integrity persisted for several weeks after the end of exposure, indicating that differentiating spermatogonia are also susceptible to tobacco smoke. However, no effects on stem cells were observed. These results show that MS and SS smoke have differential effects on the genetic integrity and function of sperm and provide further evidence that male exposure to second-hand smoke, as well as direct cigarette smoke, may diminish a couple's chance for a successful pregnancy and the birth of a healthy baby.

IN105

HERITABLE EFFECTS OF EXPOSURE TO COMBUSTION DERIVED PARTICLES

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Combustion-derived air pollutants are composed of complex mixtures of chemicals. These mixtures contain organic compounds and metals that are known to be capable of causing oxidative stress and DNA damage. Exposure to combustion by-products is ubiquitous in our environment. Developing an understanding of the risks posed to germline DNA following exposure to these agents is of critical importance for minimizing the potential for the transmission of genetic mutation and disease to offspring, and maximizing healthy births. A series of studies in mice has demonstrated that exposure to various sources of particulate air pollutants can result in the induction of DNA mutation in the germline that is transmitted to the offspring. Expanded simple tandem repeat (ESTR) loci have high spontaneous rates of mutation (1-10%) through gains and losses in repeat units. Significant increases in mutation frequency have been measured at ESTRs in laboratory mice following exposure to powerful mutagens such as ionizing radiation. In the field, exposure of male mice in situ to urban/industrial air particles results in a 1.5-2-fold increase in mutation frequency. This increase is accompanied by DNA strand breaks and global hypermethylation in sperm DNA. The lack of bulky DNA adducts in sperm suggests that metals and/or inflammatory response may be mechanistically linked to the observed effect. Exposure of male and female mice in utero to diesel exhaust particles causes a similar increase in both male and female germ cell mutation in F1 offspring, demonstrating that pre-meiotic gametes are responsive to particle exposure in both sexes. The increase is similar to what is observed in mice exposed to both mainstream and sidestream tobacco smoke. The data demonstrate that particles derived from combustion cause tandem repeat mutation in gametes, possibly mediated by epigenetic events. The mechanisms linking particle exposure and inherited mutation remain elusive but are the subject of intensive research.

IN106

ROLES OF THE FTO AND TREX1 ENZYMES IN REMOVAL OF DAMAGED OR DISPLACED DNA

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E. coli AlkB is an Fe(II) and 2-oxoglutarate dependent dioxygenase that reverts DNA base damage generated by alkylation or lipid peroxidation. Nine enzymes similar to AlkB are present in human cells; the one most recently discovered is FTO. In collaboration with L. Colleaux, S. O'Rahilly, and B. Sedgwick, an inherited FTO transition mutation has been investigated; it causes catalytic inactivation of FTO due to loss of ability to bind the 2-oxoglutarate cofactor, and in homozygotes is associated with serious developmental defects and early death. TREX1 is a DNA 3' exonuclease that acts preferentially on single-stranded DNA. Our group (Yun-Gui Yang, Peter Robins, Deborah Barnes and Tomas Lindahl) has shown that TREX1- negative murine and human cells exhibit chronic activation of the ATM-dependent checkpoint response. Such cells accumulate large amounts of a distinct and previously unrecognized by-product of lagging-strand replication, singlestranded DNA fragments of 62 nucleotides in length. The TREX1-negative cells grow slowly in culture, however, on prolonged propagation faster-growing variants can arise spontaneously with a largely suppressed phenotype.

IN107

INTEGRATING CELLULAR FUNCTION THROUGH A BASE EXCISION DNA REPAIR PROTEIN

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Many DNA base lesions are channelled into base excision repair (BER) pathways by DNA glycosylases; in addition, free-radical attack directly generates various oxidized abasic lesions. The 4'-oxidized and 1'-oxidized products are readily cleaved by the Ape1 AP endonuclease. However, the latter product, 2-deoxyribonolactone, can form an irreversible DNA-protein crosslink during attempted excision by the 5'-lyase activity of repair DNA polymerases (beta in the nucleus, gamma in mitochondria). We have shown that, in both cellular compartments,

this problem is averted by activating the “long-patch” BER pathway dependent on the flap endonucleases FEN1 (nuclear and mitochondrial) and Dna2 protein (mitochondrial). The switching mechanism that activates long-patch BER remains to be established. Repair via BER is an essential function: inactivating any of the central components causes embryonic lethality in mice, and (usually) apoptosis in mammalian cells in culture. Thus, Ape1 plays vital roles that we are seeking to understand in more detail. Careful examination of Ape1-depleted human cell lines (using RNAi methods) shows that significant mitotic defects are observed: multinucleated cells, micronuclei, chromosome aberrations, and chromosome missegregation. These effects appear to be associated with apparent cell cycle checkpoint defects and the disruption of the Chk2 pathway in Ape1-deficient cells. There is also disruption of the S-phase DNA damage checkpoint in Ape1-depleted cells. We are exploring whether these various effects all stem from the loss of the enzyme’s endonuclease role in BER, or some other function of the protein.

IN108

REGULATION OF BASE EXCISION REPAIR IN RESPONSE TO DNA DAMAGE

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DNA lesions occur due to the chemical instability of DNA or are induced by DNA damaging agents. The majority of non-bulky DNA lesions, including base damage, sites of base loss and single strand breaks, are repaired by proteins involved in the Base Excision Repair (BER)pathway. The level of endogenous DNA lesions depends on cellular metabolism and exogenous mutagens and although this level may vary, it is not clear how the BER response to the changing environment is controlled. We investigated the mechanism regulating BER capacity and found that BER enzyme levels are linked to and controlled by the level of DNA lesions. We demonstrate that stability of BER enzymes increases after formation of a repair complex on damaged DNA and that the proteins not involved in a repair complex are polyubiquitylated by the E3 ubiquitin ligase CHIP and subsequently rapidly degraded. Furthermore, the tumour suppressor ARF (p14ARF in humans and p19ARF in mice) relocates from the nucleoli to the nucleoplasm in response to DNA damage where it binds and inhibits the activity of the ubiquitin ligases MDM2 and ARF-Binding Protein-1 (ARF-BP1/Mule) that regulate cellular levels of p53 and Mcl-1, respectively, thus controlling DNA damage check-points and apoptosis. We now demonstrate a novel role for both ARF and Mule in the regulation of DNA repair by controlling the monoubiquitylation status of Pol β, the major base excision repair DNA polymerase. These data identify a novel mechanism controlling cellular levels of BER enzymes and the cellular response to DNA damage.

IN109

MUTYH AND DNA POLYMERASE λ COOPERATE IN A NOVEL LONG PATCH BASE EXCISION REPAIR OF 8-OXO GUANINE

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The potentially mutagenic A:8-oxo-G mispair synthesized by the replicative DNA polymerases (pols) δ and ε escapes proofreading activity. The mismatch is recognized by the MutY glycosylase homologue (MUTYH) that removes the A, leaving the lesion on the template strand. We have recently shown that the base excision repair enzyme DNA pol λ, together with the auxiliary proteins replication protein A (RP-A) and proliferating cell nuclear antigen (PCNA), has the unique ability among human DNA pols to efficiently incorporate a C opposite an 8-oxo-G, with error frequencies in the range of 10-3 (1). We identified the critical cellular components that specifically bind to DNA containing A:8-oxo-G mispairs and subsequently reconstituted a novel error-free pathway of 8-oxo-G. We showed specific binding of MUTYH, DNA pol λ, PCNA, FEN1 and DNA ligases I and III from human whole cell extracts to A:8-oxo-G DNA, but not to undamaged DNA. Based on this observation, we fully reconstituted a pathway for the repair of A:8-oxo-G mispairs. In a MUTYH and apurinic endonuclease 1 (APE1) initiated reaction, DNA pol λ in the presence of RP-A and PCNA incorporated dCTP opposite 8-oxo-G and added one nucleotide. The repair pathway was completed by FEN1 and DNA ligase I. These results identify a novel pathway, where a replication A:8-oxo-G mispair product is correctly repaired via MUTYH/DNA pol λ -dependent long patch base excision repair. Moreover, we identified DNA pol λ as an interaction partner of cyclin-dependent kinase 2 (Cdk2) that is central for the cell cycle G1/S transition and S phase progression. Four different phosphorylation sites were identified for DNA pol λ. Experiments with phosphorylation-defective mutants suggested that phosphorylation of the conserved T553 is critical for maintaining DNA pol λ stability, since it is targeted to the proteasomal degradation pathway via ubiquitination unless this residue can be phosphorylated. DNA pol λ is stabilized during cell cycle progression in late S and G2 phase, enableling DNA pol λ to properly conduct repair of 8-oxo-G damaged DNA before cells enter mitosis thus preventing G-C->T-A transversion mutations.

1. Maga, G., Villani, G., Crespan, E., Wimmer, U., Ferrari, E. Bertocci, B. and Hübscher,U.: Nature, 447, 606-609, 2007.

IN110

DNA REPAIR ENZYME NEIL1, METABOLIC SYNDROME AND CANCER

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Oxidatively induced DNA damage is known to play a role in disease processes such as cancer and aging. Mammalian cells possess elaborate repair mechanisms that repair DNA damage. Oxidatively induced DNA base lesions are mainly repaired by base-excision repair, which is initiated by a series of DNA glycosylases that include OGG1, NTH1, NEIL1 and others with broad or narrow substrate specificities. Human and mouse NEIL1 proteins have recently been isolated, characterized and extensively studied. These enzymes specifically remove purine-derived lesions 4,6-diamino-5-formamidopyrimidine (FapyAde) and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) from DNA that contains multiple oxidatively induced lesions. A minor activity for some pyrimidine-derived lesions has also been observed. However, NEIL1 possesses no significant activity for another major lesion, 8-hydroxyguanine. In the absence of exogenous oxidative stress, *neil1* knockout and heterozygotic mice develop severe obesity and subsequently dyslipidemia, fatty liver disease, hypertension and insulin resistance, collectively known in humans as the Metabolic syndrome. Inactivating mutations in *neil1* correlate with some diseases including cancer. Furthermore, knockdown in *neil1* significantly sensitizes cells to the killing effects of DNA-damaging agents. Polymorphic mutations in human *neil1* have been discovered. We characterized four known polymorphic variants of human NEIL1, S82C, G83D, D252N and C136R. While S82C, G83D, D252N retained near wild-type levels of nicking activity on abasic site-containing DNA, G83D did not catalyze the wild-type β-δ-elimination reaction, but exhibited β-elimination activity. Glycosylase activities of these proteins were measured using oligonucleotides with one single lesion and genomic DNA containing multiple lesions. S82C and D252N showed near wild-type enzyme specificity and kinetics, whereas G83D and C136R were devoid of glycosylase activity. Furthermore, *neil1*$^{-/-}$ mice accumulated significant levels of FapyAde and FapyGua in several organs and exhibited late onset of multiple types of cancer, indicating a role for these lesions in disease development. Extrapolation of these data suggests that individuals who are heterozygous for inactive variant *neil1* alleles may be at increased risk for diseases associated with the Metabolic syndrome and for carcinogenesis.

IN111

INTERACTION OF PROTEINS INVOLVED IN DNA REPAIR WITH AP SITE CONTAINING DNA

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One of the most abundant lesions in DNA is abasic (AP) sites arising spontaneously or as an intermediate in base excision repair (BER). AP sites are unstable and cytotoxic. Deoxyribose of AP site can form with amino groups of Lys residues a reversible covalent Schiff base intermediate, which can be stabilized by NaBH(4) treatments. This reversible intermediate can be involved in catalysis or, potentially, in temporal protection of AP sites and regulation of their processing. DNA duplexes with AP sites (AP DNA) were used to trap in cell extracts proteins reactive to AP sites. In HeLa cell extract a prevalent trap product with an apparent molecular mass of 95 kDa was observed. The cross linked protein was identified by MALDI-MS as the p80-subunit of Ku antigen (Ku). Ku antigen, a DNA binding component of DNA-dependent protein kinase (DNA-PK), participates in double-stranded break repair and is responsible for the resistance of cells to ionizing radiation. The specificity of Ku interaction with AP sites was proven by more efficient competition of DNA duplexes with an analogue of abasic site than non-AP DNA. Ku80 was cross-linked to AP DNAs with different efficiencies depending on the size and position of strand interruptions opposite to AP sites. Ku antigen was shown to inhibit AP site cleavage by apurinic/apyrimidinic endonuclease 1. We compared the results of dot-ELISA based on anti-Ku80 antibodies and the levels of Ku80 cross linking to AP DNA in the extracts derived from HeLa and several melanoma cell lines. The efficiency of Ku80 trapping varies considerably depending on the type of extract and correlates with the amount of Ku80 in the extracts. Thus, AP site containing DNA can be used as an efficient tool to test the content of Ku antigen in cell extracts. It was revealed that cofactor proteins of base excision repair(BER) system – PARP1, XRCC1 and HMGB1 – are also able to interact with AP sites via the same mechanism. This interaction was shown to modulate the efficiency of AP site hydrolysis by apurinic/apyrimidinic endonuclease 1. Therefore, it is likely that one of the functions of BER cofactor proteins consists in regulation of processing of AP sites in DNA repair. This work was supported by program of RAS 'Molecular and Cellular Biology'

IN112

INTRODUCTION TO SIMPOSIUM: CRITICAL ISSUES ON ENVIRONMENTAL GENOTOXICITY IN LATIN AMERICA

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Latin America (LA) is the region with the highest natural and cultural diversities on Earth. It is also one of the regions showing the highest levels of differences in development between countries and collectively make up the most urbanized region in the developing world with over three quarters of its populations living in often burgeoning cities such as, México city, São Paulo, Buenos Aires, Bogotá, Rio de Janeiro, Lima, Montevideo, Quito and Santiago. The region has more than 130 cities with over 500.000 people and more than 50 with over 1 million. The urban population grew from 69 percent to 77 percent of the region's total inhabitants between 1987 and 2005. The environmental problems in LA are nothing new: soil erosion, deforestation, water and air pollution. Deteriorating air quality is a major environmental problem in many LA urban centers. Exposure to air pollutants is higher around congested areas where informal and formal economic activities take place during the day. The most affected people are the most vulnerable: the children, the poor, the sick, the elderly. According to the WHO Global Burden of Disease Report (2002) the impact of outdoor air pollution in LA is 35,000 annual premature deaths and 276,000 lives lost per year. On the other hand poor farming practices, including the use of very toxic agrochemicals, have massively and negatively affected environment in many countries of our region. The World Health Organization (WHO) and the United Nations Environment Program estimate that pesticide poisoning injures between one and five million agricultural workers per year. At least 20,000 workers die from exposure every year, the majority in developing countries. Because of its great relevance, the symposium's main aim is to present studies on the mutagenic/genotoxic effects of environmental pollutants: Air pollution and the indiscriminate use of agrochemicals that contaminate environment are two of the most critical environmental issues in LA because of its impact on human health

IN113

DNA DAMAGE, OXIDATIVE BALANCE, AND EXPOSURE BIOMARKERS IN A RURAL POPULATION EXPOSED TO PESTICIDES.

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Pesticides have become indispensable in intensive agriculture to improve production, protect stored crops and control disease vectors. However, occupational exposure occurs during the preparation of mixtures, loading and/or washing equipment and spraying on crops. In addition, individuals are often exposed to different pesticides or pesticide mixtures, making it difficult to identify the effects of each one. In this frame, exposure and effect biomarkers can be used to detect alterations induced by pesticides in human tissues, occurring before clinical adverse health effects. Santa Fe province, in the Pampa region from Argentina, is one of the most productive and valuable territories. Favored by the climate and a dense/wide hydrographic network, the main crops are: soybean, wheat, maize, sorghum and sunflower, occupying the first place among the producing provinces, with more than 26% of the total national production. We report the first data obtained from a large evaluation of subjects occupationally exposed to pesticides (applicators and non-applicators) and a control group including oxidative balance, exposure biomarkers and DNA damage quantification. Cholinesterase (ChE) and Acetylcholinesterase (AChE) activities, Catalase (CAT), Lipid Peroxidation (by TBARS assay) and Damage Index Comet Assay (DICA) were studied. Our results showed an AChE inhibition in both groups in relation to controls ($P < 0.05$) with no significant modifications in ChE ($P > 0.05$). TBARS levels were higher in pesticide sprayers ($P < 0.05$) while CAT activity was reduced in both, applicators and non-applicators ($P < 0.05$). DICA was significantly increase in both direct and indirect exposed groups, compared to controls ($P < 0.001$). The influence of confounding factors in subjects occupationally exposed to pesticides was investigated in relation to all parameters. The results of this study show a relationship between pesticide exposure and an increase in genetic damage (DICA) as well as a decrease in AChE and CAT activities, suggesting that performing genotoxicity assays in parallel with the analysis of exposure biomarkers activity and oxidative balance in serial and routine monitoring of pesticide-exposed populations, would be important.

IN114

ATMOSPHERIC POLLUTION BY MUTAGENIC AGENTS IN AREAS OF INDUSTRIAL IMPACT: HUMAN BIOMONITORING

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Hazard waste environmental release plays a negative role on ecosystems and the life quality of population. Regarding the atmospheric compartment, chemical dispersions can modify ecosystems located far from original sources exposing populations to greater health risks. Also, some of these mixtures are likely to have genotoxic and carcinogenic agents. FEPAM, the environmental protection agency, RS/Brazil, has been implementing strategies to assess environmental genotoxins using genetic markers as early parameters to prevent contaminant effect risks. Researchers applied the Salmonella/microsome assay responses as markers to assess atmospheric compounds associated to wind distribution as indicators of chronic exposure. Comet assay in lymphocytes and micronucleus of oral mucosa cells were used as genetic markers for monitoring urban populations exposed to different types and levels of petrochemical industrial activities: Site1, 20 km from the source, in the main wind distribution; Site2 has the greatest inclination for the deposition of particulate matter from an oil refinery; Site3 is exposed to different contamination sources including fertilizers and petrochemicals, and Site4 is a reference area. In relation to monitoring, about 30-37 men (ages 18-40) were analyzed per site. The Comet assay was sensitive to DNA damage (tail intensity and moment) in subjects exposed directly to the particulate matter deposition, Site2. The data also showed higher levels of DNA damage (tail moment) in individuals from Site3. No differences in micronucleated cells frequencies were observed. About the airborne particulate matter, all sites tested positive for direct and indirect-acting mutagens. The diversity of PAHs and nitrocompounds like nitroarenes, nitro-PAHs and aromatic amines explain the different mutagenesis levels detected in organic extracts. Conclusion: The strategies applied led to selecting genetic markers as early indicators for assessing the release of hazard wastes into the ecosystems and preventing health risk effects on the population. The Comet assay proved to be sensitive to human environmental mutagenic compound exposition. Salmonella/microsome detected mutagenic compounds even in samples within legal air quality criterion. Support CNPq/CAPES.

IN115

POLYCYCLIC AROMATIC HYDROCARBON (PAH)-DNA ADDUCTS, CHROMOSOMAL ABERRATIONS, AND CYP1A, CYP1B1 AND GSTM1 RISK VARIANTS IN PERIPHERAL BLOOD LYMPHOCYTES FROM YOUNG ADULTS LIVING IN MEXICO CITY.

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According to the automated air quality monitoring system, the critical air pollutants in Mexico City are ozone, PAH and particulate matter < 10µm (PM_{10}) and <2.5 µm ($PM_{2.5}$). Particulate matter increases during the dry season (winter) and decreases during the rainy season (summer). PAHs are mainly derived from the incomplete combustion of organic materials and are also adsorbed in respirable $PM_{2.5}$ in urban air. Bioactivation of some PAHs produces reactive metabolites that bind to DNA resulting in mutagenesis and cancer. We compared levels of PAH-DNA adducts and chromosomal aberration frequencies using peripheral blood lymphocytes from non-smoking young adults (n=96) obtained during one winter and the following summer. PAH-DNA adducts were analyzed using a chemiluminescence immunoassay with antiserum elicited against DNA modified with r7,t8-dihydroxy-t-9,10-oxy-7,8,9,10-tetrahydro-benzo[*a*]pyrene (BPDE). Chromosomal aberration frequencies were determined in metaphases of cultured lymphocytes, and *Cytochrome P450* (*CYP)1A, 1B1* and *Glutathione-S-transferase M1 (GSTM1)* allelic variants were also evaluated. Smoking status was confirmed by urinary cotinine. The levels of DNA adducts determined in winter (10.69 ± 3.2 per 10^9 nucleotides) were slightly higher than those in summer (9.52±2.82 per 10^9 nucleotides) ($p<0.05$). The frequency of chromosomal aberrations was also significantly higher in winter than in summer, and the presence of at-risk alleles for *CYP1A, CYP1B1* and *GSTM1* was not associated with higher levels of PAH-DNA adducts. DNA samples from all individuals with the exception of those with variants *CYP1A1*4, GSTT1*0* or both , showed higher levels of adducts in winter. The study demonstrates that PAH-DNA adducts are modulated by level of external exposure, which, in Mexico City, varies by season. This work was supported by CONACYT grant 46341-M

IN116

USE OF AGROCHEMICALS IN ARGENTINA: GENOTOXIC AND CYTOTOXIC COMPARISONS BETWEEN PURE AND FORMULATED PRODUCTS

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To enhance the production in agriculture and horticulture, large amounts of agrochemicals are daily released into the environment, currently on croplands, pastures and gardening. It is known that agrochemicals not only affect target organisms but also have some side effects on non target organisms. Although these inconveniences, it is not possible for many countries, specially those emerging ones, to decrease the use of pesticides, mainly agrochemicals, without reducing crop yields. The evolution of the phytosanitary market in Argentina reveals that herbicides accounted for the largest portion of total use (69%), followed by insecticides (13%), and fungicides (11%). Studies demonstrated that occupational exposure to some pesticides as commercial formulations may be related to several neoplasias/diseases. In this presentation we evaluate comparatively the genotoxic and cytotoxic effects exerted in mammalian cells *in vitro* by several pure pesticides and their technical formulations commonly used in Argentina. Among them are listed the herbicides 2,4-D and 2,4-D DMA®, Dicamba and Banvel®, the fungicide Zineb and Azzurro®, the insecticides Carbofuran and Furadan®, Pirimicarb and Aficida®, and the endectocide Ivermectin and Ivomec®. The SCE, cell-cycle progression, chromosomal aberrations, comet assay, spindle disturbances, micronuclei, MAC, MTT and neutral red assays were used as end-points for geno and cytotoxicity in several *in vitro* cell systems. The results clearly demonstrated that the damage induced by the commercial formulations is in general greater than that produced by the pure pesticides, suggesting the presence of deleterious components in the excipients with a toxic additive effect over the pure agrochemicals. Accordingly, the results highlight that: 1) A complete knowledge of the toxic effect/s of the active ingredient is not enough in biomonitoring studies; 2) Pesticide/s toxic effect/s should be evaluated according to the commercial formulation available in market; 3) The deleterious effect/s of the excipient/s present within the commercial formulation should not be either discarded nor underestimated, and 4) A single bioassay is not enough to characterize the toxicity of a agrochemical under study.

IN117

NOVEL FUNCTION OF NUCLEOTIDE EXCISION REPAIR FACTOR AND ITS RELEVANCE TO XERODERMA PIGMENTOSUM AND COCKAYNE SYNDROME

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Nucleotide excision repair (NER) is a versatile DNA repair system that removes a wide range of DNA lesions including UV-damage. There are two subpathways in NER. One is transcription-coupled NER (TC-NER), which preferentially removes transcription-blocking DNA damage from the transcribed strand in active genes. Another is global genome NER (GG-NER), which removes lesions throughout the genome. The importance of NER has been suggested by the studies on human genetic disorders with NER deficiency such as xeroderma pigmentosum, Cockayne syndrome (CS), UV-sensitive syndrome (UVsS), and trichothiodystrophy (TTD). These patients show a variety of symptoms such as high incidence of skin cancer on sun-exposed skin, phys-

ical and mental retardation, neurological abnormalities and premature-aging. XP is classified into eight genetic complementation groups (XP-A ~ XP-G), and two in CS (CS-A and CS-B). CS-A, CS-B and UVsS are specifically deficient in TC-NER, while XP-C and XP-E are deficient in GG-NER but proficient in TC-NER. However, a variety of symptoms in XP, CS and TTD cannot be explained by NER-deficiency alone. Mutations in XPA, which is required for both TC-NER and GG-NER, never cause CS, while, in rare cases, mutations in XPB, XPD, and XPG cause features of CS combined with XP (XP-B/CS, XP-D/CS, and XP-G/CS). XPB and XPD are subunits of TFIIH, which is a multifunctional complex involved in basal transcription, transactivation, the cell cycle, and NER. We showed that XPG forms a stable complex with TFIIH and functions in maintaining the architecture of TFIIH, underlining the contribution of XPG to transcription. Moreover, we demonstrated that the XPG mutations found in XP-G/CS patients disturb the interaction of both CAK and XPD with the core TFIIH, resulting in defective transactivation of nuclear receptors. These results indicate that the features of CS in XP-G/CS are at least partly due to abnormal transcriptional activity. In this symposium, novel functions of CSB and XPD, and their relevance to CS features will be discussed.

IN118

CHROMOSOMAL INSTABILITY IN CANCER PATHOGENESIS AND TREATMENT

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Instability of chromosome structure or number is a hallmark of common epithelial malignancies. Accurate prosecution of the cellular response to double-strand DNA breaks (DSBs) is essential for the suppression of chromosomal structural anomalies in dividing cells. Phosphorylation by PIK kinases of the variant histone, H2AX, which recruits the machinery for DSB repair, is the earliest known marker of DNA breakage. A new signalling pathway, proximal to H2AX modification, promotes chromatin changes that trigger the DNA damage response (1). Germline mutations in the breast cancer susceptibility gene, BRCA2 give rise to numerical and structural chromosomal aberrations (reviewed in 2). One major function of BRCA2 is in the control of the RAD51 recombinase during the reactions that lead to DSB repair by homologous DNA recombination. Varshavsky's N-end rule has been used to create a thermosensitive form of RAD51 in vertebrate cells, enabling dissection of the cell cycle co-ordination of DNA replication with homologous recombination (3). The role played by the RAD51-binding regions of BRCA2 in the control of homologous recombination has been dissected using biochemistry (eg., 4) and somatic cell genetics (5), revealing new functions for the BRCA2 tumour suppressor.

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IN119

ROLE OF TOPOISOMERASE I IN GENOMIC STABILITY

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DNA topoisomerase I (Top1) is an essential gene in mice and flies. Top1 levels are tightly regulated both in replicating and post-mitotic cells. Top1 is also the target of anticancer drugs with the camptothecin derivatives, topotecan and irinotecan being used in standard treatment for ovarian, lung and colon cancers.[1] To uncover the basic cellular functions of Top1 we generated cell lines with low expression levels of Top1[2] and used Top1 inhibitors to interfere with Top1 functions. We will report our finding showing genomic alterations and replication-associated DNA damage measured by endogenous phosphorylation of histone H2AX[3] and single molecule analyses of DNA replication fibers[4,5] in Top1-deficient cells and in cells treated with camptothecins. Together these findings demonstrate a key role for Top1 in DNA replication.

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IN120

OXIDATIVE STRESS-INDUCED TUMORIGENESIS IN THE SMALL INTESTINES OF VARIOUS TYPES OF DNA REPAIR-DEFICIENT MICE

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Oxygen radicals are produced through normal cellular metabolism, and the formation of such radicals is further enhanced by radiation and by various chemicals. Oxygen radicals attack DNA and its precursor nucleotides, and consequently bases with various modifications are introduced into the DNA of normally growing cells. One such modified base, 8-oxo-7, 8-dihydroguanine (8-oxoG) is highly mutagenic because of its ambiguous pairing property. Three enzymes, MTH1, OGG1, and MUTYH, play important roles in avoiding the 8-oxoG-related mutagenesis in mammalian cells. We have established an experimental system for oxidative DNA damage-induced mutagenesis and tumorigenesis in the gastrointestinal tracts of mice. Oral administration of an oxidizing reagent, potassium bromate ($KBrO_3$), effectively induced G:C to T:A transversions and epithelial tumors in the small intestines of *Mutyh*-deficient mice, implying the significance of Mutyh in the suppression of mutagenesis and tumorigenesis induced by oxidative stress. To elucidate the roles of other DNA repair genes in the suppression of oxidative DNA damage-induced tumorigenesis, we performed $KBrO_3$-induced tumorigenesis experiments using *Ogg1*-, *Mth1*-, *Msh2*- and *Xpa*-deficient mice. We observed an enhanced tumor-formation in the small intestines of *Msh2*-deficient mice, as compared with the wild type and heterozygous mice. No such enhancement was observed in *Xpa*-deficient mice. These results indicate that mismatch repair, but not nucleotide excision repair, is involved in the suppression of oxidative stress-induced intestinal tumorigenesis in mice. The number of tumors was marginally increased in *Ogg1*- and *Mth1*-deficient mice, in comparison to the wild-type mice, suggesting that in contrast to Mutyh, Ogg1 and Mth1 may play a limited role in the suppression of intestinal tumorigenesis caused by oxidative stress. Our data indicate that among the repair factors examined, only Mutyh and Msh2 play a significant role in the suppression of $KBrO_3$-induced intestinal tumorigenesis in mice. These findings are well corre-

lated with the fact that among many DNA repair factors, only MUTYH and mismatch repair factors are, so far, identified to be associated with hereditary colorectal cancers in humans.

IN121
ROLE OF MICROENVIRONMENT ON TUMOR PROGRESSION: ENDOTHELIUM, ANGIOGENESIS AND INFLAMMATION.

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Tumor angiogenesis is increasingly recognized as a crucial process for cancer progression as well as an important target of therapy. The initially transformed cell in an avascular state can only form tumors of relatively small size and rapidly reaches a state of equilibrium between cell growth and cell death mainly due to the lack of oxygen and nutrients. Tumor cells that acquire growth autonomy through the induction of vessel growth into the tumor in a crucial phase in tumor development termed the “angiogenic switch”, through the release of pro-angiogenic factors such as vascular endothelial cell growth factor, VEGF, or basic fibroblast growth factor, bFGF, have a selective growth advantage. Intriguingly, many of the biological processes involved in angiogenesis, such as cell migration and invasion, expression of matrix metalloproteases (MMPs) and control of their inhibitors (TIMPs) are also required for metastasis, and the newly formed tumor vessels are also a major route of metastatic cell dissemination. Further, tumor inflammation also promotes angiogenesis and tissue remodeling. These considerations have led to the development of anti-angiogenic drugs. Clinical trials have shown that angiogenesis inhibition is a promising, but also an elusive, target; only those strategies directed against a specific angiogenic factor, VEGF, have shown positive responses. The disruption of VEGF signaling is active on incompletely formed vessels, causing localized endothelial cell death. An alternative, valuable approach should contemporarily targeting the endothelium and inflammatory cells at the beginning of the pathologic process, and should be continued chronically. We identified “Angioprevention”, the chemoprevention of angiogenesis, as one possible concrete strategy. Several molecules share the ability to inhibit inflammatory and angiogenic processes, many of them are already approved for clinic use, or dietary components. We have shown that several flavonoids, antioxidants and retinoids act on the tumor micro-environment to inhibit enrollment and activation of endothelial cells and innate immune cells, thus linking inflammation and vascularization to tumor onset and progression and providing a key target for angiogenesis prevention.

IN122
ANTIMUTAGENIC STRATEGIES APPLIED TO CHEMOPREVENTIVE DRUG DEVELOPMENT

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The prevention of cancer is one of the most important public health and medical practices of the 21st century. Much progress has been made in this emerging field, but a significant amount or work remains before accepted practice and widespread use become commonplace. The process of carcinogenesis typically requires 20-40 years to reach the endpoint called invasive cancer. It follows multiple, diverse and complex pathways in a stochastic process of clonal evolution. Chemoprevention research has demonstrated that these pathways are amenable to inhibition, reversal or retardation at various stages. We must therefore identify the key pathways in the evolution of cancer that must be blocked to prevent this carcinogenesis process. Current mechanistic approaches include administering agents which block the activation of carcinogens, promote the detoxification of carcinogens (Phase II enzyme inducers), enhance glutathione, block the production of reactive oxygen species, inhibit carcinogen uptake, promote repair processes, and block cell cycle progression. Antimutagens become chemopreventive agents since oncogenes can be activated and/or tumor suppressor genes may become mutated or even deleted, rendering them inactive. Receptors for growth factors can become over or constitutively expressed, under expressed or mutated, angiogenesis factors can be expressed, telomerase becomes activated, and many other defects occur in cellular machinery which lead to evolution of the cancer phenotype. Many of these early precancerous lesions favor cell division over quiescence and protect cells against apoptosis or senescence when growth signals are present. Many of these antimutagenic agents are now in chemoprevention clinical trials in humans. To conquer this diverse disease, we must attack multiple key pathways at once. Furthermore, each organ-specific cancer type may require a custom combination of prevention strategies to be successful.

IN123
CLINICAL STRATEGIES FOR DEVELOPING ANTIMUTAGENIC CHEMOPREVENTIVE DRUGS

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Carcinogenesis at the cellular and tissue levels is characterized by accelerating mutagenesis and proliferation, and chemopreventive drug development strategies involving modulation of these processes by targeting key molecular targets and pathways are increasingly important. Advances in genomics, proteomics and functional and molecular imaging allow better definition of carcinogenesis mechanisms, more precise early cancer detection and cancer risk estimates, and quantitative histopathological evaluation of precancerous tissues. All these technologies contribute to discovery and characterization of chemopreventive agents and evaluation and validation of carcinogenesis biomarkers as surrogate endpoints for cancer incidence. These tools can therefore provide evaluation of individual and population-based cancer risks allowing selection of cohorts benefitting from chemoprevention and suitable for evaluating chemopreventive strategies. Many classes of antimutagenic and antiproliferative agents have already shown chemopreventive activity in animal models of carcinogenesis and in clinical trials to reduce precancer—for example, agents that inhibit the inflammatory process (cyclooxygenase-2 selective inhibitors and other nonsteroidal antiinflammatories in colon, lipoxygenase inhibitors in lung; antioxidant tea polyphenols in prostate) and epigenetic modulators (histone deacetylase inhibitors in breast). Three challenges that must be met for successful development of these and other cancer preventive drugs in the setting of precancer are identifying populations at high risk for developing cancer; defining endpoints for clinical studies that demonstrate chemopreventive efficacy and clinical benefit to populations studied; and demonstrating safety of long term administration. Net clinical benefit of chemopreventive interventions is determined by the change in significant efficacy event rates (e.g., reduction in precancer or cancer) compared to the change in significant toxicity event rates (e.g., increased cardiovascular events). Proving chronic safety in chemopreventive settings is more challenging than proving efficacy. Chronic safety is very important, since these drugs may be prescribed to large populations at relatively low absolute risk of developing cancer.

IN124
TARGETING KEAP1-NRF2 SIGNALING WITH DRUGS

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Oltipraz, a drug originally developed for the chemotherapy of schistosomiasis, is an effective inducer of enzymes that detoxify carcinogens (e.g., glutathione S-transferases and UDP-glucuronosyltransferases) and is a potent anticarcinogen in animals. A common cis-acting sequence, the Antioxidant Response Element (ARE), is found in the promoter regions

of these protective genes. The transcription factor Nrf2 regulates inducible and/or basal expression of genes by the ARE. An actin-binding protein, Keap1, sequesters Nrf2 in the cytoplasm and facilitates its degradation through ubiquitination. Keap1 is a sulfhydryl-rich protein, and several cysteine residues mediate the Keap1-inducer interaction. Treatment with oltipraz or other inducers such as sulforaphane alters the interaction between Keap1 and Nrf2, allowing Nrf2 to translocate to the nucleus. Highlighting the importance of this signaling pathway, Nrf2-deficient mice are considerably more sensitive to carcinogenesis than wild-type mice, perhaps reflecting a lower constitutive expression of carcinogen detoxication enzymes. Moreover, the cancer chemopreventive efficacies of oltipraz and sulforaphane are completely lost in the knockout mice. Genomic, proteomic and biochemical analysis indicate that Nrf2 regulates the expression of carcinogen detoxication and antioxidative genes, as well as those affecting glutathione homeostasis, NADPH generation, solute transporters, and proteasome function. Monitoring for induction of Nrf2-regulated genes led to the original identification of oltipraz as a potential chemopreventive agent and, more recently, the recognition that some triterpenoids are exceptionally potent inhibitors of aflatoxin carcinogenesis in vivo. As proof of principle for the merit of targeting Nrf2, administration of oltipraz in a placebo-controlled, randomized, double-blind clinical trial also conducted in Qidong resulted in a 2.6-fold increase in the excretion of aflatoxin-mercapturic acid, a detoxication product of the reactive, DNA-damaging intermediate of aflatoxin . Follow-up clinical trials are evaluating newer generations of Nrf2 activators. Supported by NIH Grants ES06052, CA39416, & CA94076.

IN125

CHEMOPREVENTION OF CIGARETTE SMOKE GENOTOXICITY AND CARCINOGENICITY

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The most obvious way to prevent lung cancer and other smoke-related diseases is either to refrain from smoking or to quit smoking or not to live in smoke-contaminated environments. A complementary strategy is chemoprevention, which uses dietary and pharmacological agents capable of protecting addicted active smokers, ex-smokers, and passive smokers. The biological effects of cigarette smoke (CS) as a complex mixture, either mainstream (MCS) or sidestream (SCS) or environmental (ECS), have been poorly explored. During the last two decades we showed that MCS and ECS induce a broad variety of alterations of intermediate biomarkers in animal models, including adducts to nuclear DNA and mtDNA, oxidatively generated DNA damage, proliferation, apoptosis, alterations of oncogenes and tumor suppressor genes, multigene expression, microRNA and proteome profiles as well as cytogenetic damage in the respiratory tract, bone marrow and peripheral blood. CS-altered end-points were variously modulated by chemopreventive agents of natural or pharmacological origin, such as N-acetyl-L-cysteine (NAC), 1,2-dithiole-3-thione, oltipraz, 5,6-benzoflavone, phenethyl isothiocyanate (PEITC), indole-3-carbinol, sulindac, and budesonide. Combinations of agents were also assayed. Unfortunately, until recently a suitable animal model for evaluating CS carcinogenicity was not available. We demonstrated that ECS and especially MCS become potent carcinogens when exposure of mice starts at birth. The carcinogenic response induced by MCS is characterized by very short latency times, high incidence and multiplicity of benign lung tumors, early occurrence of malignant lung tumors, and lesions in other organs. This mouse model was successfully used to demonstrate the ability of NAC, PEITC, and budesonide to prevent smoke-induced lung cancer, according to protocols mimicking the situation either in current smokers or in ex-smokers. Moreover, NAC was successful to prevent lung cancer induced by MCS after birth when the chemopreventive agent was administered during the prenatal life. Therefore, it is now possible to investigate in vivo not only alterations of intermediate biomarkers but also the carcinogenicity of CS and their modulation by chemopreventive agents.

IN126

LIKE FATHER LIKE SON: TRANSGENERATIONAL GENOMIC INSTABILITY IN MAMMALS

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Mutation induction in the directly exposed cells is currently regarded as the main component of the genetic risk of ionizing radiation and chemical mutagens. However, recent data on the delayed effects of exposure to ionizing radiation represent a new challenge to the existing paradigm. The results of numerous *in vitro* studies show that ionizing radiation can not only induce mutations in the directly exposed cells, but can also lead to delayed effects, with new mutations arising many cell divisions after the initial irradiation damage. Apart from the studies on mutation rates in somatic cells, considerable progress has been made in the analysis of radiation-induced instability in the mammalian germline, where the effects of radiation exposure were investigated among the offspring of irradiated parents. Our results show that mutation rates at tandem repeat DNA loci and protein-coding genes are substantially elevated in the germline and somatic tissues of non-exposed offspring of irradiated male mice. According to our data, this remarkable transgenerational destabilization can be attributed to the presence of a subset of endogenous DNA lesions. We have recently shown that paternal treatment by the alkylating agent ethylnitrosourea also results in the transgenerational effects, thus implying that this phenomenon is not initiated by a specific sub-set of DNA lesions and is most probably triggered by a stress-like response to a generalized DNA damage. Our data imply that instability detected in the non-exposed offspring is caused by some DNA-dependent signal transmitted from the irradiated father and implicate an epigenetic mechanism for the transgenerational instability. The potential implication of these results for the estimates of genetic risks for humans will be discussed.

IN127

THE MECHANISM AND CLINICAL UTILITY OF SOMATIC MITOCHONDRIAL MUTAGENESIS IN CANCER

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Several studies have revealed a substantial number of clonally expanded (homoplasmic) somatic mitochondrial DNA (mtDNA) mutations in a variety of human cancers. It has been debated whether these mutations are involved in early or late carcinogenesis, or merely accumulate by chance. We address this question by studying mutations in mtDNA isolated from nine patient-matched sets of normal, adenoma, and carcinoma colorectal tissues. These tissues allow us to establish a mutational timeline during colorectal cancer progression. First, we identified homoplasmic mutations in adenoma and carcinoma tissues by sequencing the entire mitochondrial genome from each sample, and found at least one mutation in 33% of adenomas and 56% of carcinomas. We hypothesized that the abundance of these mutations was driven by an elevated rate of mutagenesis in mtDNA, i.e., a mitochondrial mutator phenotype. We investigated this hypothesis by adapting the Random Mutation Capture (RMC) assay to assess the frequency of random mutations in the 12S rRNA and COXI regions of the mitochondrial genome in all patient-matched colorectal tissue samples. The results of this study revealed no statistically significant difference in random mutation frequency between mtDNA isolated from normal and adenoma tissues at either the 12S rRNA ($p=0.39$) or the COXI sites ($p=0.07$). Mitochondrial DNA isolated from carcinoma tissues, however, exhibited a greater than two-fold reduction in random point mutations compared to mtDNA isolated from normal colonic tissue at both the 12S rRNA ($p=0.01$) and COXI sites ($p=0.05$). The increase in clonally expanded mtDNA mutations we observed in carcinoma, when contrast-

ed with a decreased random mutation frequency, suggests their selection during carcinogenesis. Irrespective of the precise mechanisms of homoplasmic mtDNA mutation accumulation in cancer pathogenesis, mutational homoplasmy in cancer provides a more specific biomarker of disease, than any other yet described. As such, we have developed new technologies that exploit mtDNA mutations to monitor tumor progression, therapeutic response and cancer recurrence.

IN128

CROSS-SPECIES, ENDOGENOUS MUTATION ASSAY BASED ON THE *PIG-A* GENE

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This laboratory has developed a flow cytometric technique for monitoring gene mutation at the *pig-a* locus. The *pig-a* gene product is responsible for a critical step in the formation of glycosylphosphatidylinositol (GPI) anchors that are used to target certain proteins to the cell surface. Preliminary experiments were directed at optimizing mouse and rat blood staining strategies whereby lack of a specific GPI-anchored surface protein served as a phenotypic marker of *pig-a* mutation. The kinetics of the *pig-a* response to known mutagens was characterized using *N*-ethyl *N*-nitrosourea (ENU), 7,12-dimethyl-1,2-benz[*a*]anthracene (DMBA), 4-nitroquinoline-1-oxide, benzo(a)pyrene, and *N*-methyl-*N*-nitrosourea (MNU). For all studies, treatment of Wistar Han rats or CD-1 mice occurred on three consecutive days via oral gavage (i.e., Days 1-3), with blood samples collected on Days -1 (rat only), 4, 15, 30, 45 and 90. Mutant phenotype erythrocytes and reticulocytes were measured following staining with SYTO 13 in combination with either anti-CD59-PE (for rats) or anti-CD24-PE (mice). Mutant phenotype cells were not evident on Day 4, but significant increases were observed on Day 15 for each chemical. The persistence of the responses were markedly different for these chemicals, which presumably relates to the degree to which mutation is occurring in long-lived hematopoietic stem cells versus cells with limited self-renewal capacity. These data support flow cytometry-based assessment of *pig-a* gene function as a cross-species indicator of mutation.

IN129

MUTATION NOT CANCER: VALIDATION AND UTILITY OF TRANSGENIC GENE MUTATION ASSAYS

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The Organization for Economic Cooperation and Development (OECD) has recently approved the development of a Test Guideline on Transgenic Rodent (TGR) Gene Mutation assays following the acceptance of a Detailed Review Paper on this endpoint. The process of OECD Test Guideline development will require consideration of the extent to which these assays have been "validated" according to specific criteria. One basic issue is the selection of the most relevant endpoint against which validation should be made. The predictivity of genotoxicity tests for carcinogenicity is an important consideration in the acceptance and interpretation of such tests for regulatory use since mutagenicity is a primary event in the etiology of most cancers. Despite the close association between these two endpoints, it is an imperfect association, which is evident for all tests for genotoxicity, including TGR assays. There is a small, but distinct, proportion of non-carcinogens that are genotoxic, presumably because mutagenicity per se was insufficient for the development of tumours in such cases. Furthermore, there are carcinogens that are non-genotoxic, due to mechanisms that do not involve genotoxicity as a primary event. Carcinogenicity, therefore, may not be the most appropriate endpoint against which to validate a new assay such as the transgenic rodent gene mutation assay, since there is an expectation of non-concordance among test results. The most biologically relevant endpoint for use in validation of the predictivity TGR assays would be another, well-established in vivo gene mutation assay that is not limited to a single tissue. Since such an assay does not exist, we have used sequence data from the DNA isolated from mutant phenotypes (i.e. plaques or colonies) to estimate the Positive Predictive Value (PPV) of these presumptive mutant genotypes. The PPV is the proportion of mutant phenotypes that are confirmed as mutant genotypes. We have reviewed the data from over 140 studies in which a total of 32,751 mutant phenotypes were sequenced yielding 31,659 mutant genotypes and a PPV of 0.967, validating the effectiveness of TGR assays for detecting gene mutations, and facilitating the establishment of an OECD Test Guideline.

IN130

OVEREXPRESSION OF SOME DNA REPAIR PATHWAYS ARE ASSOCIATED WITH METASTASIS RISK IN MELANOMA PATIENTS

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Melanoma is the most life-threatening human neoplasm of the skin and shows a worldwide dramatic increase in incidence and mortality. The underlying molecular events leading to metastasis have not been clearly elucidated.

Using a collection of 83 frozen human primary cutaneous melanomas we determined their genome-wide gene expression profiles. Thanks to a new multiple random validation strategy we identify a signature of 254 genes allowing us to predict with a high probability distant metastasis free-survival as well as overall survival at 4 years. Clusterization of these genes into functional groups identified distinctive trends in gene expression throughout tumor progression.

Because melanoma development is partly linked to ultraviolet exposure, we analyzed the role of genes involved in DNA replication, DNA repair and recombination in primary melanomas that are going to metastasize. We used a newly-developed bioinformatic tool allowing us to analyze the differential gene expression by looking at whole biological pathways rather than individual genes. Among the most significant pathways associated with progression to metastasis, we found the DNA replication (p=10-14) and the DNA repair pathways (p=10-16). We concentrated our analysis on DNA repair in a large sense and found that 47 genes of this category, among a list of 234 DNA repair genes, are associated with metastatic progression and poor prognosis. Most of the genes involved in the regulation of replication origin firings are overexpressed in primary melanomas that will lead to metastasis as well as numerous genes involved in the maintenance of genetic stability, such as homologous recombination and the pathways leading to recovery of replication fork stalling, due to spontaneous blockage or induced DNA lesions such as double-stranded breaks or crosslink's. It looks if genome stability is necessary for a primary tumoral cell to invade and succeed to inducing distant metastasis. Moreover, this overexpression of repair genes explains nicely the extraordinary resistance of metastatic melanoma to chemo- and radiotherapy. New tailored therapies should be developed by inhibiting specifically these DNA repair processes to better cure melanoma metastasis.

IN131

DNA DAMAGE AND DNA DAMAGE RESPONSES AFTER THIOPURINE/UVA TREATMENT

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Background The immunosuppressant azathioprine (Aza) is prescribed to prevent graft rejection in organ transplant patients. The incidence of non-melanoma skin cancer is extremely high in these patients and the duration of immunosuppression and sunlight exposure are both acknowledged risk factors. The DNA of Aza-treated patients contains measurable 6-thioguanine (6-TG) which, unlike canonical DNA bases, absorbs the ultraviolet A (UVA) radiation that comprises 95% of the ultraviolet in incident sunlight. The skin of patients taking Aza is selectively UVA sensitive, consistent with the formation of DNA photoproducts. UVA photoactivation of 6-TG generates reactive oxygen species (ROS) and UVA irradiation of cultured cells containing DNA 6-TG produces DNA lesions that block replication and transcription. The DNA 6-TG/UVA combination is synergistically cytotoxic and mutagenic. Aims To determine the products of the interaction between DNA 6-TG and UVA and their impact on DNA repair and DNA damage responses. Results Photochemical lesions include damaged DNA bases, DNA strand breakage, oxidized DNA-associated proteins, and DNA-protein crosslinks. Potentially lethal photochemical damage caused by DNA 6-TG is not removed by nucleotide excision repair (NER); neither NER-deficient nor transcription-coupled NER-defective cells are hypersensitive to 6-TG/UVA. The interaction between DNA 6-TG and low dose UVA induces both single- and double-strand (DSB) DNA breaks. The DNA of S phase cells - and particularly DNA at replication forks - is exceptionally sensitive to breakage and cells defective in the repair of DSB by homologous recombination are hypersensitive to killing by this treatment. Photochemical activation of 6-TG provokes the ATM- and ATR-mediated DNA damage responses and both the Chk2 and Chk1 proteins are rapidly activated together with the G2/M cell cycle checkpoint. Higher levels of damage cause a proteasome-dependent degradation of Chk1 and abrogation of the checkpoint. Conclusion The DNA 6-TG that is present in the skin cells of patients undergoing long-term Aza therapy can interact with low doses of UVA to generate persistent DNA damage. These findings have implications for the development of skin cancer in this patient group.

IN132

GENOTOXIC STRESS RESPONSE: MECHANISMS AND RELEVANCE TO CANCER

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Recent work in the field of DNA damage recognition, signaling and repair identified multiple protein modifications that operate in concert with the 'classical' phosphorylation/dephosphoryla- tion network governed by the ATM/ATR-regulated DNA damage response (DDR). This lecture will summarize our recently published and unpublished data documenting the biological and pathophysiological role of the emerging ubiquitylation/deubiq- uitylation cascade, including the RNF8, RNF168, HERC2/UBC13 and BRCA1 ubiquitin ligases, USP7 and other deubiquitylation enzymes and additional components, in DNA damage signaling and repair in human cells. The data will include results from pan-genomic RNAi-based screens for novel DDR components, live-cell imaging of human cells to analyze the spatiotempral orchestration of the key DDR pathways, and mechanistic insights into the cooperation between phosphorylation, ubiquitylation and protein-protein inter- actions in response to DNA double strand breaks. Finally, recent results extending our concept of DDR as a tumorigenesis barrier in early human cancer development, and exploitation of DDR defects in tumors as predictive markers to guide individualized chemotherapy, will be presented.

IN133

PROCESSING OF DNA ADDUCTS INTO DOUBLE STRAND BREAKS.

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DNA double strand breaks (DBSs) are highly toxic lesions which can trigger apoptosis and mitotic cell death. DSBs arise as a result of physiological, pathological, pharmacological or environmental exposure. Our current knowledge of the biological response to DSBs rely principally on experiments with ionizing radiation. However, radiation-induced DSBs are primary lesions with two blunt ends, whereas most other types of DSBs are secondary lesions formed within the context of macromolecular complexes, which may conceivably change the nature of the DSB. We here describe the processing of 3 anticancer agents originally derived from natural sources: ecteinascidin 743 (yondelis) a marine product, S23906, a plant alkaloid, and irofulven, a fungal product. All three compounds form bulky monofunctional adducts which can be converted into DSBs following collision with the replication fork and/or the transcription machinery. Interestingly, interaction with DNA repair proteins may either lead to the removal of the lesions or convert them into different type of lesions with enhanced toxicity. Characterization of the processing of these natural-derived agents should permit the identification of biomarkers for clinical response prediction and provide us with unique probes to dissect the biological response to DNA damage.

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IN134

IMPLICATION OF THE NUCLEOTIDE EXCISION REPAIR MACHINERY ON THE RESPONSE TO DOXORUBICIN TREATMENT IN HUMAN FIBROBLASTS

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Doxorubicin (DOX), a member of the anthracycline group, is a widely used drug in cancer therapy. The mechanisms of DOX action include topoisomerase II-poisoning, free radicals release, DNA adducts and interstrand cross-link (ICL) formation. DNA repair capacity is important not only for cell survival but also influences the response to chemotherapy, being the major contributor to drug resistance in cells. Therefore, we aimed to test the role of nucleotide excision repair (NER) in the response to DNA lesions and sensitivity of cells treated with DOX. Human fibroblast cell lines carrying different mutations in NER machinery, either in global genome repair (GGR-NER) – XPC, or both GGR and transcription coupled repair (TCR-NER) – XP-D, TTD and XP/CS, were employed. They were treated with different DOX concentrations and times and were further analyzed for cell survival, apoptosis levels and cell cycle arrest. The formation of double-strand breaks (DSB) and their repair kinetics, measured by the generation of gama-H2AX nuclear foci, were also investigated. Our results show that XP-D, TTD and XP/CS cells are extremely sensitive to DOX and present elevated levels of apoptosis, while XPC cells are weakly sensitive to DOX and present a cell cycle profile similar to wild type cells. The NER-deficient cell lines do not show different patterns of DSBs formation as assayed by phosphorylated H2AX foci formation. The Topoisomerase II alpha knock-down with siRNA leads to increased survival in both MRC5 and XP-D cells, while XP-D cells still remained significantly more sensitive to the treatment by DOX. This indicates that DNA lesions other than breaks induced by TopoII blockage (i.e. DNA-adducts induced by DOX) may be responsible for the increased cell death in XPD-mutated cells.

IN135
INTOGEN: A NOVEL FRAMEWORK FOR INTEGRATION AND DATA-MINING OF MULTIDIMENSIONAL ONCOGENOMIC DATA

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The availability of data from a growing number of oncogenomic studies provides an unprecedented opportunity to understand tumor development from a genomic perspective. However, new integrative methodologies are necessary in order to take full advantage of these valuable data. IntOGen is a novel framework that addresses this by collecting, organizing, analyzing and integrating genome-wide experiments that study several forms of alterations in numerous cancer types. IntOGen explores the data at different levels, from individual experiments to combinations of experiments that analyze the same tumour type, and from individual genes to biological modules, pathways or gene sets. IntOGen is designed to be an updatable, flexible, extensible and efficient system for integrative oncogenomics analysis and visualization. The system consists of three main components: 1) Data, including oncogenomics experiments and biological modules. 2) Statistical methods for analysis and integration of the data. A specifically designed statistical framework has been implemented with the objective of identifying driver genes and modules significantly altered in different tumour types. 3) Visualization methods to explore the results in intuitive and efficient ways. We have developed two complementary visualization systems, i) A publicly accessible web system (www.intogen.org) which allows an easy and efficient access to IntOGen results and ii) A standalone Java application, GiTools, which permits a more flexible and sophisticated navigation of IntOGen data and results. IntOGen is a unique and high valuable resource for the research community studying cancer. We expect that it will be a highly useful addition to the field and will assist researchers by allowing exploration of altered genes and modules in many tumour types.

IN136
THE COMPARATIVE TOXICOGENOMICS DATABASE: A DISCOVERY TOOL FOR IDENTIFYING CHEMICAL-GENE-DISEASE NETWORKS

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The Comparative Toxicogenomics Database (CTD; http://ctd.mdibl.org) is a curated database that promotes understanding about the effects of environmental chemicals on human health. This is a critical area of research because the etiology of many chronic diseases involves interactions between environmental factors and genes and proteins that modulate important physiological processes. CTD integrates manually curated data from peer-reviewed published literature with diverse public data sets to provide a centralized, freely available resource for exploring cross-species chemical-gene and protein interactions and chemical- and gene-disease relationships. Over 167,000 interactions between 4,700 chemicals and 15,900 genes have been curated from 289 species, and over 8,100 gene-disease and 5,000 chemical-disease relationships have been curated. By integrating these data, 555,000 gene-disease relationships and 153,000 chemical-disease relationships can be inferred. CTD also integrates external data sets like Gene Ontology annotations and KEGG pathways, which greatly expands query and analysis options in CTD. Several unique features were recently implemented to enhance data access and analysis, including: a) data download options; b) batch query options; c) a VennViewer tool that allows users to compare associated data sets for chemicals, genes/proteins or diseases; and d) GeneComps and ChemComps, which are statistical metrics used to identify comparable genes or chemicals, respectively, based on similar toxicogenomic profiles. CTD's integrative approach provides researchers with important connections between chemicals, genes/proteins and diseases that may not otherwise be apparent, and provides the basis for developing novel hypotheses about the mechanisms underlying the etiology of environmental diseases. This presentation will demonstrate the current scope and functionality of the database using a CTD-curated data set.

IN137
METABOLIC PROFILING AS A TOOL IN BIOMARKER RESEARCH AND SYSTEMS BIOLOGY

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Metabolic biomarkers have much potential in biomedical and toxicological research. They can be measured non-invasively via imaging or body fluid profiling, which is better for the welfare of both patients and animals and facilitates longitudinal studies and translation of results between models and man. Metabolites are also defined chemical entities without genetic variation or post-translational modifications, which also helps to translate analytical methodologies directly from the laboratory to clinical and population studies. A substantial body of research has shown that metabolic profiles can report sensitively and specifically on a number of pathological states, both in terms of clinical disease processes and laboratory studies of genetic manipulation or chemical exposure. Certain conditions, such as Type II diabetes or cancer have defined metabolic phenotypes that are already exploited in diagnosis and therapy. Importantly, metabolic biomarkers have been shown to be predictive of the way that individual people or animals metabolise and respond to chemicals. In this lecture I will review some of this evidence, and go on to present data from our own laboratory to show that metabolic profiling (metabonomics/metabolomics) is a crucial element of systems biology, enhancing the information ("pathway") recovery from other "-omics" datasets.

IN138
BLOOD TRANSCRIPTOMICS AND EXPOSURE BIOMARKERS IN A POPULATION-BASED COHORT – THE NOWAC POSTGENOME STUDY

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Environmental exposures, used in the broadest sense of lifestyle, infections, radiation, chemicals and occupation, are a major cause of human cancer. Surrogate analysis is not a new concept in exposure biomarker research, but the development of -omic technologies has broadened both the range of tissues that can be examined and the number of targets that can be analyzed in a single experiment. Thus, there is growing evidence that use of peripheral blood cells for transcriptome analysis is valuable to assess environmental- or disease-associated gene signatures. To date, these studies investigating various blood cell subsets are characterized by relatively small number of subjects not representative of the general population, and a lack of consideration of potential confounders. During this talk, I will focus on altered gene expression in blood by lifestyle factors and exposure to perfluorinated compounds measured in plasma of a representative sample set of postmenopausal women in the NOWAC postgenome study. We have identified and deciphered blood gene expression signals associated to lifestyle (body mass index, fasting, smoking), and exposure to some perfluorinated compounds (perfluorooctane sulphonate and perfluorooctanoate). Perturbed biological pathways were more or less numerous according to specific exposure, some interconnected, and potentially associated with pattern of other behaviors. Overall, these data indicate that environmental exposures at low level in human population can elicit changes in blood gene expression, and also provide valuable information pertinent to the design of studies where pre-cancerous or cancer itself might be the outcome.

IN139
EXPERIMENTAL TESTS AND MODELING APPROACHES: GETTING THE BEST FROM BOTH

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The in vivo mutagenicity studies, shortly followed by carcinogenicity, are posing high demand for test-related recourses: therefore, the development and extensive use of estimation techniques such as (Q)SARs, read-across and grouping of chemicals, might have a huge saving potential for these endpoints. In particular, the Structure-Activity Relationships paradigm provides a wide range of tools that have different degrees of uncertainty and apply to different scopes. On one side, there are coarse-grain approaches such as the Structural Alerts (SA). Beside being a repository of the science on chemical biological interactions, the SAs have a crucial role in risk assessment, for: a) description of sets of chemicals; b) preliminary hazard characterization; c) formation of categories; d) generation of subsets of congeneric chemicals to be analyzed subsequently with Quantitative Structure-Activity Relationships (QSAR) methods; e) priority setting. On the other side, there are fine-tuned QSARs for congeneric classes of chemicals. A range of good quality, local QSARs for mutagenicity and carcinogenicity have been assessed in our laboratory, and challenged for their predictivity in respect to real external test sets. The QSARs for potency generated predictions 30 to 70 % correct, whereas the QSARs for discriminating between active and inactive chemicals were 70 to 100 % correct in their external predictions. A crucial issue is that of the uncertainty of the modeling approaches. More properly, their uncertainty should be compared with that of the competing experimental tests. For example, the ability of SAs to predict rodent carcinogenicity is of the same order of the Ames test (around 65% accuracy). Equally illuminating is the fact that the external predictivity of good local QSARs (70 to 100 % accuracy) is of the same order of the reported inter-laboratory variability of the Ames test (85%). Thus, uncertainties are proper to both modeling and experimental systems. The crucial issue is that of exploiting and combining –at their best- both methods.

IN140

NEW CHEMICAL/BIOLOGICAL PROFILING AND INFORMATICS APPROACHES FOR EXPLORING MUTAGENICITY & CARCINOGENICITY: UPDATES OF EPA TOXCAST™ AND TOX21 PROGRAMS

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EPA's National Center for Computational Toxicology is building capabilities to support a new paradigm for toxicity screening and prediction through harnessing of legacy toxicity data, creation of data linkages, and generation of new in vitro screening data. In association with EPA's ToxCast™, ToxRef DB, and ACToR projects, the DSSTox project provides cheminformatics support and is improving public access to structure-annotated chemical toxicity information to facilitate modeling, data-mining and read-across approaches. Phase I of EPA's ToxCast™ research project is building on three rich data tiers: 309 unique, structurally diverse chemicals (predominantly pesticides), activity and concentration response data from approximately 500 in vitro (cell-based and cell-free) high-throughput screening (HTS) assays, and extensive in vivo rodent bioassay data extracted from EPA pesticide registration records (entered in EPA's ToxRefDB). Contained within these data tiers are chemicals with mutagenic and non-mutagenic mechanisms of carcinogenicity, target-specific bioassay data for multiple rodent species pertaining to tumorigenicity, and HTS assay results potentially relevant to, and informative of mutagenic and carcinogenic mechanisms in rodents and humans. Highlights of the first ToxCast™ Data Analysis Summit will be presented, along with some preliminary analysis of genetox HTS assays. A future course for broadening the chemical test space, HTS assay coverage, and reference genotoxicity studies contained within ToxRefDB will be described, in the context of both the ToxCast™ programs and the expanded multi-laboratory Tox21 research projects. These efforts, combined with progress in expanding public genotoxicity databases and integrating structure-activity relationship (SAR) approaches, point to exciting prospects for computational toxicology impacting the field of mutagenesis and carcinogenesis. *This work was reviewed by EPA and approved for publication, but does not necessarily reflect EPA policy, nor does mention of trade names constitute endorsement.*

IN141

A KNOWLEDGE-BASE APPROACH TO IDENTIFY SIGNATURES FOR BIOLOGICAL AND CHEMICAL PAIRS IN THE RISK ASSESSMENT PROCESS

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Developing computational toxicology methods to assist the risk assessment process has recently gained much attention both in regulatory agencies and industries. The FDA Center for Food Safety and Applied Nutrition's Office of Food Additive Safety (CFSAN OFAS) applies TTC (Threshold of Toxicological Concern) approach to address the carcinogenicity and uses (Q)SAR methods as part of the evaluation process of new food contact materials, food additives, and their impurities and breakdown products. The structural classes defined in the TTC can be further stratified across various toxicity endpoints. In addition, they can become a major component of the knowledge-base when transformed to chemical signatures by association with biological assays from high throughput screening (HTS) experiments that are anchored to in vivo phenotypic effects. The challenges of this new paradigm are being addressed within research projects such as, for example, the US EPA ToxCastTM and Critical Path Initiative Projects at US FDA. The Food Additives KnowledgeBase will be a part of FDA's larger effort to link understanding of chemotypes to phenotypes and eventually to genotypes. Our methodology to reduce the inherent noise in HTS assays includes the introduction of an interpretation layer of chemical classes between compound, HTS assays and in vivo data. The chemical classification methods for such compound profiling range from purely unsupervised structural classifiers to supervised rules, e.g., structural alerts or the new phenotype-dependent TTC categories. Integrating the biological similarities not only helps find more biologically similar analogs, but is also critical to improved understanding of molecular mechanism, which subsequently helps extract biologically meaningful structural rules and mode-of-action driven models. The Food Additives KnowledgeBase project initiated in US FDA CFSAN therefore will address these issues and demonstrate how this paradigm can bridge the gap between toxicologists and chemists involved in regulatory science.

IN142

THE USE OF (Q)SAR IN FOOD SAFETY ASSESSMENT

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In the dietary risk assessment of pesticides, considering that the consumer is exposed not only to the active substance as applied, but also to a wide range of chemical compounds as a result of metabolic and degradation processes, the assessment of food safety should include metabolites and degradates of toxicological relevance. However, only the toxicological properties of the active substance and their mammalian metabolites are directly investigated through the range of toxicological studies required by Directive 91/414/EEC and in contrast, very limited information about the toxicological properties of metabolites and degradates is available in the majority of cases. Moreover the requests by the authorities for further toxicological studies are restricted as far as possible to minimise the use of animals in toxicological testing. Assessment methods and alternative scientific tools, not involving animal testing, therefore need to be developed and used to optimize the consistency and robustness of the evaluation the toxicological profile of metabolites and degradates of pesticides. For this reason, the European Commission's Joint Research Centre (JRC) and the European Food Safety Authority (EFSA) are working on a collaborative to evaluate the possible contribution of Quantitative Structure-Activity Relationship (QSAR) analysis in the evaluation of pesticide metabolites toxicity for dietary risk assessment. The EFSA-sponsored project has the eventual purpose to develop an opinion and a guidance document on the establishment of the residue definition for risk assess-

ment in food commodities. This presentation will provide an overview of the project and will present preliminary results, including an evaluation of the potential applicability of QSARs for mutagenicity and carcinogenicity in dietary risk assessment.

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IN143

THE RATE OF SOMATIC MUTATIONS AND HUMAN CANCER

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Given the multiplicity of environmental factors and the large number of tumor types, it is not surprising that estimates of what prorportion of tumors can be attributed to environmental causes vary a great deal (from 40 to 80%). As for inherited factors predisposing to cancer, when only high penetrance susceptibility genes are considered, figures of 5-20% are quoted for different types of tumors; but if we include low penetrance genes the figures may be much larger. In any case, and whatever the cause(s) of any individual tumor, its development requires one or more discrete genetic events, i.e. somatic mutations, stochastic events which occur at random. Somatic mutations take place throughout life, and most of them are innocuous: only when a certain set of genes is mutated does an individual cell becomes a cancer cell. It follows that the rate of somatic mutation (μ) probably plays a major role in determining the risk of developing cancer. In principle, inherited factors may affect μ, for instance by affecting DNA repair; and environmental mutagens will, by definition, increase μ: thus, we have a rationale and a measurable output for the role of these factors. Recently, by using as reporter the X-linked PIG-A gene (which, when inactivated, gives to the cell a distinctive flow cytometry phenotype), we have shown that μ is increased in patients with inherited cancer-prone syndromes such as Fanconi anemia and ataxia-telangiectasia; and we are investigating the genetic determinants of the variability of μ in the general population.The model of cancer as a clonal disease in which successive mutations confer to mutant cells varying degrees of Darwinian selective advantage was first explored over 30 years ago (see J Cairns, Nature 255: 197,1975). Since that time, much evidence has accumulated to support this model: particularly with the identification of over 400 genes for which somatic mutations have a causal role in oncogenesis, and with the identification of the individual pathways that control cell growth, apoptosis, and response to stress, to which most of these genes belong. It will be important now to explore to what extent μ itself mediates our risk of cancer, both in terms of baseline and in terms of exposure to environmental mutagens; and to consider whether we can find ways to deliberately reduce μ itself, as a new approach to cancer prevention.

IN144

CAUSES AND MECHANISMS OF COLON CANCER DEVELOPMENT, AND STRATEGIES FOR ITS PREVENTION

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Epidemiologically, a high fat intake and obesity are reported to be associated with increased risks of several types of cancers including colon cancer. However, the underlying mechanisms of how the high fat intake and obesity are associated with colon carcinogenesis remain to be elucidated. Thus, the search for colon cancer-causing and preventive substances is an important and urgent task. More than 20 mutagenic and carcinogenic heterocyclic amines (HCAs) are known to be formed in meat and fish under ordinary cooking conditions. Humans are exposed to HCAs in daily life. Among these HCAs, PhIP and IQ induced colon tumors in rats, with alterations in β-catenin and Apc genes in the tumors. Colon cancer by PhIP was significantly promoted by high-fat diet in animal studies. Several epidemiological studies have provided evidence of positive associations between the high consumption of well-done red meat and the risk of colon cancer, suggesting that HCAs are involved in the development of human colon cancer. We recently noted that an age-dependent hyperlipidemic state exists in Apc-deficient mice in which mRNA levels of lipoprotein lipase (LPL) are down-regulated in the liver and small intestine. The levels of serum triglycerides (TG) in Apc-deficient mice were approximately 10-fold higher than those in wild-type mice. Moreover, steatosis in the liver and accumulation of lipid in the polyps were clearly observed. Adipocytokines such as TNFα and plasminogen activator inhibitor-1 (Pai-1) were remarkably over-expressed and adiponectin was down-regulated in the liver of Min mice. LPL induction by PPAR agonists and a LPL selective inducer resulted in the concomitant suppression of hyperlipidemia and intestinal polyp formation. A genetic study in Apc-deficient mice with increasing LPL mRNA levels also clearly indicated that hyperlipidemia contributes to intestinal carcinogenesis. Using Pai-1 inhibitors and adiponectin-deficient Min Mice, adipocytokine's contribution to the polyp formation was demonstrated. Taken together, our results indicate that LPL and adipocytokines play an important role in intestinal carcinogenesis. Based on these results, effective approaches to colon cancer prevention will be discussed.

IN145

AN INTEGRATED VIEW OF INDUCED MUTAGENESIS IN E. COLI

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When a replicative DNA polymerase encounters a chemically altered base that it is unable to copy, a process called Translesion Synthesis (TLS) takes place during which the replicative polymerase is transiently replaced by a specialized DNA polymerase. It is during the process of TLS that point mutations are induced. TLS not only involve replicative and translesion DNA polymerases but also accessory factors such as the general replication processivity factor, the so-called β-clamp. In E. coli, besides the β-clamp, RecA plays a fundamental role in TLS as an essential co-factor of Pol V the major bypass polymerase in this organism. We will show that under physiologically relevant conditions, PolV activity requires both the β-clamp and a RecA filament in cis. Both co-factors endow Pol V with the functional stability that is required for achieving a productive biological outcome. An essential role of the cis-RecA filament is to stretch the template DNA in order to allow smooth elongation of the nascent strand by PolV. In this talk we will summarize the insights into TLS gained over the last 25 years by studying a frameshift mutation hot spot, the Nar I site. This site was initially discovered by serendipity when establishing a forward mutation spectrum induced by a hepatocarcinogen, N-2-acetylaminofluorene (AAF). Indeed, this carcinogen covalently binds to DNA forming adducts with guanine residues. When bound to G* in the Nar I site, 5'-GGCG*CC-, AAF induces the loss of the G*pC dinucleotide at a frequency that is ≈ 10exp7 fold higher than the spontaneous frequency. In vivo studies showed that the Nar I mutation hot spot is neither restricted to the Nar I sequence itself, nor to the carcinogen AAF. Genetic analysis initially revealed that the Nar I frameshift pathway is SOS dependent but umuDC (i.e. Pol V) independent. More recently, DNA Pol II was identified as the enzyme responsible of this frameshift pathway. Concurrently the AAF adduct in the Nar I site can be bypassed in an error-free way by Pol V. The Nar I site offers a unique opportunity to study the interplay between two TLS pathways. Lessons gained from the reconstitution of the two pathways will be presented and discussed.

IN146

INTERPLAY OF DNA REPAIR, DNA POLYMERASES AND TRANSCRIPTION IN THE PROCESS OF SPONTANEOUS MUTAGENESIS IN YEAST

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Spontaneous mutagenesis is a complex phenomenon, both deleterious for genome integrity but also necessary for evolution. Origin of spontaneous mutation is diverse including replication of miscoding endogenous DNA damages and errors of DNA polymerases. Spontaneous

mutation may represent limits of the fidelity of DNA repair and replication mechanisms. Transcriptional activity also impacts spontaneous mutagenesis: the TAM process (Transcription Associated Mutagenesis). Here, we discuss processes that impact spontaneous mutagenesis in a model eukaryote, the yeast Saccharomyces cerevisiae. In a first part, we discuss the role of 8-oxoGuanine, an abundant and mutagenic oxidative DNA lesion. We present evidence for cooperation between the Ogg1 DNA N-glycosylase, Rad18-Rad6-dependent monoubiquitylation of PCNA at K164, the damage-tolerant DNA polymerase eta and the mismatch repair system (MMR) to prevent 8-oxoG-induced mutagenesis. We also discuss the impact of transcription on spontaneous mutation rate. Under high transcription condition, spontaneous mutation rate at the CAN1 reporter gene is about 30-fold higher than that observed at low transcription. We show that high transcription results in enhanced base pair substitutions (BPS) and 2 nucleotides deletions. Our results point to DNA polymerase zeta and Topoisomerase I are important players in the TAM process.

IN147

OXIDATIVE STRESS, DNA ALKYLATION AND MUTAGENESIS

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Reactive oxygen and nitrogen species can generate oxidative stress in cells. These species directly cause damage to DNA, and the resultant DNA lesions can be the sites of replication errors during DNA synthesis. These reactive species also can cause damage to membrane lipids, and the products of lipid damage can themselves damage DNA, forming organic compound-DNA adducts, such as 1,N6-ethenoadenine. Cancer and other genetic diseases can result from the accumulation of these genetic changes over time. This presentation will describe the genoprotective role of DNA repair proteins from the alpha-ketoglutarate dioxygenase category. These enzymes usually remove oxidative or alkylative damage to DNA by a direct reversal mechanism. In addition, this class of enzymes helps cells counter the toxic effects of DNA adducts made by bis-chloroethylnitrosourea, a commonly used anticancer drug.

IN148

MUTAGENIC OR ACCURATE OUTCOME OF TRANSLESION DNA SYNTHESIS IS DETERMINED BY SPECIFIC TWO-POLYMERASE MECHANISMS IN MAMMALIAN CELLS

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Translesion DNA synthesis (TLS), also termed error-prone DNA repair, is a cellular DNA damage tolerance pathway that manages stalled replication forks and replication gaps caused by DNA lesions. The key components in TLS are specialized low-fidelity DNA polymerases, which have a remarkable ability to replicate across DNA lesions, with the concomitant formation of mutations. Mammalian cells contain 5 dedicated TLS DNA polymerases, and 5-10 additional polymerases that may participate in this process. The significance of TLS is illustrated by the disease xeroderma pigmentosum variant (XPV), which is characterized by high predisposition to sunlight-induced skin cancer caused by germ-line mutations in the POLH gene encoding the TLS DNA polymerase eta. TLS must be tightly regulated in order to avoid an escalation in mutations rates. This regulation is primarily at the posttranslational level, and involves monoubiquitination of PCNA, as well as the action of p53, and p21 via its interaction with PCNA (Avkin et al., Mol. Cell 22, 407-413, 2006). Quantitative Analysis of TLS in cultured mammalian cells in which the expression of specific TLS polymerases was either knocked-out or knocked-down revealed that specific two-polymerase mechanisms determine mutagenic or accurate outcome (Shachar et al., EMBO J. 28, 383-393, 2009). The activity of the mechanisms that back up TLS across UV light-induced cyclobutane pyrimidine dimers in cells from XPV patients will be described. These mutagenic mechanisms are likely to play a critical role in the high cancer predisposition of XPV patients.

IN149

EPISTASIS ANALYSIS OF THE DNA DAMAGE RESPONSE

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Chromosomes are continuously threatened by exposure to intracellular (e.g. free radicals) and environmental agents (e.g. ionizing radiation) that induce DNA damage. The DNA Damage Response (DDR) protects chromosomes from the mutagenic effects of these agents, and as such prevents genome instability and carcinogenesis. The DDR is an evolutionary conserved signaling network that orchestrates various biological processes, including chromatin remodeling, cell cycle progression, DNA repair and DNA replication, in response to genetic insult. However, how the interplay between proteins involved in these diverse processes regulates the DDR remains unclear. To address this issue we investigated the interaction between DDR factors by performing a systematic genetic analysis, called EMAP (Epistatic MiniArray Profiling), under DNA damage-inducing conditions in the model organism *S. cerevisiae* (budding yeast). Our novel DDR EMAP provides new insights into how interactions between DDR factors, particularly those which involve chromatin remodeling complexes, orchestrate the DDR.

IN150

TRANSLATIONAL RESPONSES TO DNA DAMAGE

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Transcriptional and post-translational signals are known mechanisms which promote efficient responses to DNA alkylation damage. Using high throughput screens of *E. coli* and *Saccharomyces cerevisiae* gene deletion libraries we have identified a number of translation associated proteins as modulating the toxicity of the DNA alkylating agent MMS. We postulate that specific translational programs optimize the DNA damage response, and in support we have identified *Saccharomyces cerevisiae* tRNA methyltransferase 9 (Trm9) as an enzyme that prevents cell death via translational enhancement of DNA damage response proteins. Trm9 methylates the uridine wobble base of $tRNA^{ARG(\underline{U}CU)}$ and $tRNA^{GLU(\underline{U}UC)}$. We used computational and molecular approaches to predict that Trm9 enhances the translation of some transcripts over-represented with specific arginine and glutamic acid codons. We found that translation elongation factor 3 (*YEF3*) and the ribonucleotide reductase (*RNR1* and *RNR3*) large subunits are over-represented with specific arginine and glutamic acid codons, and demonstrated that Trm9 significantly enhances Yef3, Rnr1, and Rnr3 protein levels. In addition, we identified 425 genes, which included *YEF3*, *RNR1*, and *RNR3*, with a unique codon usage pattern linked to Trm9. We propose that Trm9-specific tRNA modifications enhance codon-specific translation elongation and promote increased levels of key damage response proteins.

IN151

GLOBAL ANALYSIS OF SIGNALING NETWORKS BY HIGH-RESOLUTION MASS SPECTROMETRY-BASED QUANTITATIVE PHOSPHOPROTEOMICS

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Phosphorylation is a reversible covalent modification that is tightly controlled by the action of protein kinases and phosphatases, and it plays a universal role in regulating essentially all signaling pathways in the eukaryotic cell, for example the insulin signaling network, cell-cycle progression and the DNA damage response. Protein phosphorylation events are dynamic, spatial and temporal in their distribution, and often present an activating or deactivating switch to control protein activity. Deregulation of signaling networks is a hallmark of cancer and other diseases, and protein kinases are therefore prominent drug targets. In molecular biology, phosphorylation events are usually probed in a targeted manner by using phospho-specific antibodies or radioactive phosphate labeling. In contrast, advances in proteomics, including phosphopeptide enrichment methods, high-accuracy mass spectrometry, and associated bioinformatic tools now make it feasible to obtain an unbiased view of phosphoproteomes at a ´systems-level´. Combining all these methodologies with stable isotope labeling of amino acids in cell culture (SILAC) that allows for accurate quantitation, we obtained a global view of dynamic regulation of phosphorylation in mammalian cells as a function of a growth factor (epidermal growth factor; EGF) stimulus in a time-resolved manner [Olsen et al, Cell 2006]. In this study, we were able to determine phosphopeptides with very high accuracy and obtained kinetics of 6600 *in-vivo* phosphorylation sites. This data set provided the first and fascinating view into temporal regulation of the phosphoproteome and we have now used this technology platform to study other phosphorylation networks in detail. Applications range from fundamental studies of signal transduction pathways to cross talk in cell signaling and to drug development in cancer and other diseases. We believe that the ability of MS-based proteomics to quantitatively 'read out' the phosphoproteome will revolutionize the cell signaling field.

IN152

TOXICOGENOMICS OF (NON)-GENOTOXIC CARCINOGENS - TOWARDS ALTERNATIVES FOR EXPERIMENTAL ANIMALS.

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Genotoxic effects of chemicals are the primary cause of genomic instability followed by the onset of carcinogenesis. Alternatively, carcinogenesis is initiated by a variety of non-genotoxic carcinogens with diverse mechanisms of action. Our goal is to establish novel predictive cell-based models to classify (non)-genotoxic carcinogens. Since tissue stem cells are the primary site where chemical-induced mutation may initiate tumor formation, we use normal mouse embryonic stem (mES) cells as a model. We carefully characterized the timing and mechanism of the DDR in mES cells using cisplatin as a model. Next, we further determined the transcriptional program of a panel of direct acting DNA-damaging agents, indirect DNA-damaging agents and non-genotoxic carcinogens. Bioinformatics analysis allowed the identification of a group of around fourty different candidate predictive genes. The promoter regions of these genes have been cloned into fluorescent protein DsRed-based reporter constructs and several reporter mES cell lines have been generated that now allow the selective quantitative evaluation of the DNA damage response. Our work demonstrates the feasibility to generate novel mechanism-based alternative in vitro methods to predict carcinogen hazard. Future prospects of unraveling the signaling programs underlying the responses to carcinogens will be discussed.

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IN153

CHIP-SEQ APPROACH TO STUDY THE CELLULAR RESPONSE TO DAMAGE INDUCED TRANSCRIPTION INTERFERENCE

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One of the major cytotoxic events in organisms is the interference of DNA damage with transcription. In particular, the persistent encounter of the elongating RNA polymerase II (RNAPIIo) with DNA lesions has severe consequences for the cell as this event can provide a strong signal for cell death. On the other hand, damage-stalled RNAPIIo will initially trigger a cascade of protective cellular responses to DNA damage i.e. signalling, cell cycle arrest, chromatin remodelling and repair. The main pathway for the removal of transcription blocking DNA lesions and restoration of damage-inhibited transcription is the transcription coupled nucleotide excision repair (TC-NER). Defects in TC-NER are manifested in patients that suffer from Cockayne syndrome (CS) and CS associated rare human disorders such as the combined Xeroderma Pigmentosum (XP)/CS and cerebro-oculo-facio-skeletal syndrome type 1 (COFS1), all characterised by neurological abnormalities, growth retardation and premature ageing. To unravel the molecular events that underlie the cellular responses to DNA damage-induced transcription interference and to gain insight into the complex genotype-phenotype relationship manifested in CS associated genetic disorders we have used normal and patient derived cell lines and advanced proteomic and genome wide technologies. Whereas activities so far were limited to the analysis of single model genes or gene clusters, utilising the Illumina/Solexa second generation technology of direct genome wide sequencing of chromatin immunoprecipitated (ChIP-seq) DNA has enabled the global scale mapping of TC-NER factors and RNAPIIo occupancy in response to UV irradiation and other agents that block transcription. Using this approach, profound differences in the binding profile of CS factors to their respective genomic target sites in response to damage-induced transcription blockage have been identified. These data suggest an as yet unanticipated regulatory function of CSB in the expression of genes involved in cell cycle arrest and inhibition of apoptosis underscoring the power of this technology to provide valuable information on the regulation of the interplay between DNA repair, damage signalling and gene expression.

IN154

FANCONI ANEMIA: OMIC APPROACHES AND THERAPEUTIC APPLICATIONS

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Fanconi anemia (FA) is a rare genetic disease characterized by chromosome fragility, congenital malformations, progressive pancytopenia and cancer susceptibility. 13 FA genes have been identified and their products interact with many other DNA damage response proteins in an intricate pathway1-2. The genetic characterization of FA patients is critical for future therapeutic applications including gene therapy and regenerative medicine. In this context we were able to generate disease-free hematopoietic progenitors from skin cells of FA patients by combining gene therapy and induced-pluripotent stem (iPS) cell technology3. Interestingly, the FA pathway is essential for the maintenance and proliferation of iPS. In order to identify novel molecular partners of the FA/BRCA pathway in the repair of stalled-replication forks, 34 unique proteins of the FA signalling network (including all 13 FA proteins, BRCA1, BLM, ATM, ATR, NBS1, CHEK2, XPF, EMSY, RAD51, RPA1, etc…) were selected for an interactome mapping. The corresponding genes were subdivided into 137 baits for a massive yeast-2-hybrid (Y2H) screen. Of the baits showing interactions, we defined 28 high-confidence interactions, of which two were previously known. The 26 newly identified interacting proteins are mainly involved in DNA damage response, signal transduction, transcription,

cell cycle, cell proliferation, chromosome segregation and DNA recombination. Data on the role of these new interacting proteins in the DNA cross-link repair and in breast cancer susceptibility will be presented. Finally, we applied differential proteomics by DIGE (or difference in gel electrophoresis) with cells deficient in FANCA or FANCC and the spontaneously reverted isogenic counterparts (FANCA-R and FANCC-R). 49 proteins were up/down regulated in FANCA, 36 in FANCC, and 43 in FANCA versus FANCC and, of these, 47 proteins were unequivocally identified by mass spectrometry#. The pathways recurrently involved are inflammatory response, cell growth, transcription, mitochondrial function, apoptosis and redox status.

1Surrallés et al (2004). Genes and Development 18: 1359-1370.
2Bogliolo et al (2007). The EMBO J 26:1340-1351
3Raya et al (2009) Nature (31st May aop)
#Patent pending

IN155

FANCM CONNECTS THE TWO GENOME INSTABILITY DISORDERS BLOOM'S SYNDROM AND FANCONI ANEMIA

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Fanconi Anemia (FA) is a genetic disorder characterised phenotypically by bone marrow failure, cancer predisposition and cellular sensitivity to agents that induce DNA interstrand cross-links (ICLs). Mutations in one of 13 different genes can cause FA, and 8 of these gene products form the Fanconi core complex. The core complex is required to mono-ubiquitylate FANCD2 after ICL damage. The only DNA binding component of the core complex is the 230kDa protein FANCM, thought to be the "anchor" of the complex at sites of ICL damage. We show that FANCM also "anchors" another DNA repair complex: the Bloom's complex, after similar types of damage. BLM of the Bloom's complex is mutated in an FA-related disorder, Bloom's Syndrome. We used immunoprecipitation of Flag-tagged fragments or deletion mutants of FANCM and identified the region of FANCM essential for interaction with the FA and Bloom's complexes respectively. Distinct motifs in FANCM mediate the interactions, showing that the protein provides a link between the complexes in cells. This is a critical interaction as we show that in FANCM-depleted cells the FA complex and the BLM complex assemble correctly, but fail to come together at sites of DNA damage. We demonstrate that FANCM knockdown caused increased sensitivity to DNA damaging agents and decreased activation of the FA and Bloom's pathways. We have generated mutants that bind one complex but not the other and measured their ability to rescue the phenotype of FANCM knockdown using a novel complementation system. FANCM appears to be a central co-ordinator of ICL repair. In a heterodimer with FAAP24, the FANCM C-terminus recognises fork DNA, the key substrate of the FA pathway. Through direct binding of the FANCM N-terminus to HCLK2, it also activates the ATR dependent cell cycle checkpoint. The mapping of two novel domains has defined two further protein:protein interactions essential for FANCM function. With deletion and point mutations that independently ablate specific protein interactions within conserved domains we have devised separation of function within the FANCM protein. This separation of function provides vital clues to the activity of the FA and Bloom's syndrome pathways.

IN156

GENETIC PATHWAYS REQUIRED FOR TEMPLATE-SWITCH MEDIATED DAMAGE BYPASS REPLICATION

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Gaps occur during replication and their filling is required for cell viability and replication completion. Homologous recombination (HR) and post-replication repair (PRR) genes are required for damage-bypass of the lesions and promote the formation of X-shaped sister chromatid junctions (SCJs) at damaged replication forks. It has been suggested that these catenated molecules represent template switch intermediates and are resolved by the RecQ helicase Sgs1/BLM in cooperation with topoisomerase III (Top3/Top3α). Here we examine the contribution of different replication factors to the initiation and the DNA synthesis step of template switch-mediated repair. We analyzed the contribution of the polymerase alpha-primase, nucleases such as Exo1 and Rad27, proteins mediating HR or stabilizing the Rad51 nucleofilament such as Rad51, Rad55, Rad57, Rad52 and RPA, translesion synthesis polymerases (polymerase eta and zeta), lagging and leading strand polymerases responsible for the bulk DNA synthesis (polymerase delta and epsilon) and accessory replication factors such as RFC and PCNA. The results will be presented and discussed.

IN157

ACTIVATION OF THE CELLULAR DNA DAMAGE RESPONSE IN THE ABSENCE OF DNA LESIONS

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The cellular DNA damage response (DDR) is initiated by the rapid recruitment of repair factors to the site of DNA damage to form a multiprotein repair complex. How the repair complex senses damaged DNA and then activates the DDR is not well understood. Here we demonstrate that prolonged binding of DNA repair factors to chromatin can elicit DDR in an ATM- and DNAPK- dependent fashion in the absence of DNA damage. Targeting of single repair factors to chromatin revealed a hierarchy of protein interactions within the repair complex and suggests amplification of the damage signal. We conclude that activation of DDR does not require DNA damage and stable association of repair factors with chromatin is likely a critical step in triggering, amplifying and maintaining the DDR signal.

IN158

ATM AND TGF PATHWAY SIGNALING FOLLOWING X-RAY AND HEAVY IONS EXPOSURE

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A goal at NASA is to develop event-based systems biology models of space radiation risks that will replace the current dose-based empirical models. Complex and varied biochemical signaling processes transmit the initial DNA and oxidative damage from space radiation into cellular and tissue responses. Mis-repaired damage or aberrant signals can lead to genomic instability, persistent oxidative stress or inflammation, which contribute to cancer risk. Protective signaling through adaptive responses or cell repopulation is also possible. We are developing a computational simulation approach to galactic cosmic ray (GCR) effects that is based on biological events rather than average quantities such as dose, fluence, or dose equivalent. Conventional space radiation risk assessment employs average quantities, and assumes linearity and additivity of responses over the complete range of GCR charge and energies. To investigate possible deviations from these assumptions, we studied several biological response pathway models of varying induction and relaxation times including the ATM, TGFβ-Smad, and WNT signaling pathways. We then considered small volumes of interacting cells and the time-dependent biophysical events that the GCR would produce within these tissue volumes to estimate how GCR event rates mapped to biological signaling induction and relaxation times. We considered several hypotheses related to signaling and cancer risk, and then performed simulations for conditions where aberrant or adaptive signaling would occur on long-duration space mission. Our results do not support the conventional assumptions of dose, linearity and additivity. A discussion on how event-based systems biology models, which focus on biological signaling as the mechanism to propagate damage or adaptation, can be further developed space radiation risk projections is given.

IN159

CYTOGENETIC EFFECTS OF HEAVY IONS

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Chromosomal aberrations are generally considered as an excellent biomarker of exposure and risk. They are very much used indeed in radiation biodosimetry. The recent multi-color painting techniques allow a much better resolution and characterization of the radiation-induced damage at DNA level.
Several in vitro studies on chromosomal aberrations induced by heavy ions have demonstrated that high-LET particles produce a much higher fraction of complex-type exchanges, but the extension of these observations to the in vivo case seems to be problematic. We will present here new data on the cytogenetic effects of energetic heavy ions, obtained at the SIS synchrotron at GSI (Germany) using human peripheral blood lymphocytes exposed in vitro or in vivo during radiotherapy for prostate cancer.

IN160

DNA DAMAGE AND REPAIR FROM SPACE RADIATION

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While ionizing radiation in the terrestrial environment is largely composed by sparsely ionizing radiation (such as X- or gamma-rays and electrons), with the exception of low energy alpha-particles, mainly deriving from the radioactive decay of radon and radon progeny, space radiation is characterized by the presence of high energy protons and high charge and energy (HZE) particles. An improved knowledge of the biological effects of densely ionizing HZE particles is required to evaluate the radiation health risk during long term space travels with a reasonably low level of uncertainty, and to develop appropriate countermeasures. Although less abundant than protons, HZE particles are more effective in damaging biological systems. It is thought that this is due to the production of spatially correlated and/or clustered DNA damage, in particular double strand breaks (DSB) or DSB associated with other lesions within a localized DNA region. This kind of complex damage, difficult to repair accurately, is rarely produced by sparsely ionizing radiation or by endogenous processes. Various approaches have been exploited to characterize DNA breakage and to study DSB repair in human cells irradiated with charged particles. DNA fragmentation studies have shown that the yield, the spatial correlation and the rejoining kinetics of DSB are influenced by radiation quality and related to the differences in biological effectiveness for cellular effects among different radiation types. Immunofluorescence techniques, based on antibodies against proteins involved in DNA damage response, made it possible to visualize how the different patterns of energy distribution for various charged particles are reflected by the different distributions of DSB in the cell nucleus, to analyze the morphology of the fluorescent foci as a function of radiation quality, and to study the mechanisms of DBS induction and processing in single cells at doses as low as those released by one particle traversal and in situations where only few cell are hit in the overall cell population. Quantitative evaluation of DNA damage in un-hit cells as a consequence of signals released by cells traversed by one or few tracks is a further crucial point in space radiation protection.

IN161

POPULATION ACTION AS A MODIFIER OF RADIATION-INDUCED CARCINOGENESIS

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Background: Carcinogenesis risk estimation is complicated by a number of factors, among these being the lack of a common platform to integrate and analyze the available data, and the inherently systems-biologic nature of the problem. Carcinogenesis must be treated as a systems biology problem because it is a multi-scale phenomenon, influenced critically by determinants not only at the molecular level, but at the cell and tissue-levels as well. A comprehensive carcinogenesis paradigm must therefore take into account multi-scale effects. Methods: A Two-Stage Logistic model we developed considers the molecular-level events of initiation and promotion, while incorporating in a rudimentary way the larger-scale growth-limiting role of cell-cell interactions. Likewise, to account for cell-level carcinogenesis progression as influenced by inter-tissue signaling at the next higher scale, a dynamic carrying capacity construct has been studied that both explains and quantifies the growth-limiting effect of induced vasculature on tumor growth. Recent data has suggested this mechanism to important for whether nascent tumors ever rise to become a disease threat. Results: Intercellular interactions, even after the fact of cancer cell creation, are seen to play a major and sometimes decisive role in whether that cancer will even become epidemiologically significant. Intercellular interactions are therefore a cancer risk determinant that must be accounted for in any prediction of clinical occurrence. We report on a number of results showing how proton and Fe radiations alter cancer outcomes at this level, and propose a mechanistic rationale for what is taking place. Following the initiation-promotion-progression paradigm, we have begun to quantitatively link the successive scales of influence on risk. Conclusion: The current mutational paradigm for cancer alone is inadequate. Specific, population-level mechanisms must be introduced as additional steps in the model pathway to cancer disease to reconcile mechanism with epidemiologic data.
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IN162

THE EUROPEAN AND INTERNATIONAL AGENDA OF PUBLIC HEALTH GENOMICS

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The task of Public Health Genomics (PHG) has become a challenge for all healthcare systems having major implications for future research and policy strategies. The various stakeholders in public health play a key role in translating the implications of genomics such as deriving from systems biology and integrative genomics including epigenomics or genome-environmental interactions. Recent advances in systems biology indicate that specific cellular functions are infrequently carried out by single genes, but rather by groups of cellular components. This network-based research is already starting to change nosology. Seemingly dissimilar diseases and health outcomes are being lumped together. What were thought to be single diseases are being split into separate ailments. The approach offers a novel method for human disease classification. It defines disease expression on the basis of its molecular and environmental elements in a holistic way. This knowledge will not only enable clinical interventions but also health promotion messages and disease prevention programmes to be targeted at susceptible individuals as well as subgroups of the population (personalized healthcare). So far there has been no systematic integration of genome-based knowledge and technologies into public health research, policy and practice. Thus, the public health agenda demands a vision of translational research that reaches beyond the research horizon to arrive at application and public health impact of these innovations. Both, the EU funded Public Health Genomics European Network (PHGEN) (www.phgen.eu) as well as the Genome-based Research and Population Health International Network (GRaPHint) (www.graphint.org) aim to fulfil this task. While PHGEN is involving all European Member States, EFTA-EEA and Applicant Countries and is developing European best practice guidelines for quality assurance, provision and use of genome-based knowledge and technologies, GRaPHint has a push-pull function between governmental bodies such as CDC or PHAC and academia on the international level.

IN163

PUBLIC HEALTH AND GENOMIC EPIDEMIOLOGY

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Continuous advances in genotyping technologies and the inclusion of DNA collection in observational studies have resulted in an increasing number of genetic association studies. The ability to perform genome-wide analyses has provided in the recent years tantalizing new clues to disease causation and therapeutic targets. As a result of that, companies have sprung up to use these new technologies to provide information to individuals about predicted health and disease, and about behavioural traits. Nevertheless, the evidence surrounding the benefits and harms of these genomic tests is frequently weak, with their predictive value usually small and an unclear clinical utility. Public Health Genomics contributes to this discussion by focussing on the use of genome-based information for epidemiological research, surveillance systems, health policy development, individual health information management and effective health services. As a premature introduction of technologies into healthcare settings could potentially overwhelm the health system financially, legally and ethically. The main challenges to the successful translation of new research findings about genotype-phenotype associations into clinical practice are presented, highlighting the need for genomic medicine to become more evidence-based. The next decade will provide the opportunity to establish infrastructures and educate health providers to enable the genome-based technologies to be translated into evidence-based guidelines and policies.

IN164

ASSESSING GENE-ENVIRONMENT INTERACTIONS IN CANCER

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The Human Genome Epidemiology Network has developed interim guidelines for the evaluation of the rapidly expanding cumulative f evidence on the role of genetic variants in human disease. A comparable effort for a comprehensive assessment and evaluation of environmental causes of cancer has been done by the IARC Monographs program. The importance of gene-environment interactions in cancer etiology is becoming more apparent as knowledge on causes and mechanisms of human cancer evolve. However, no theoretical framework has been elaborated on to assess the evidence of such type of interactions, which is derived mainly from observational studies. There is the need to develop guidelines on how to assess the cumulative evidence on the role of gene-environment interactions in cancer causation, and to explore the performance of such guidelines in selected examples of gene-environment interaction in human cancer. This will help to determine how knowledge on genetic variants can help in the identification of human carcinogens and vice-versa, how evaluations of environmental carcinogens contribute to the identification of cancer-causing genetic variants.

IN165

TRANSLATIONAL RESEARCH IN GENOMICS

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Genome-wide association studies are rapidly unraveling genetic susceptibility variants that are implicated in the etiology of common multifactorial diseases such as coronary heart disease, type 2 diabetes and non-familial forms of breast cancer. Expectations about the future impact of these discoveries on preventive and clinical health care practice are high. Future use of genetic tests is foreseen for the prediction of disease susceptibility, targeting pharmacotherapy and tailoring lifestyle and health behavior recommendations. Fueled by the enormous progress in gene discovery, many researchers are already investigating the prediction of common diseases based on *genetic profiling*, the simultaneous testing of multiple susceptibility variants, and an increasing number of companies already offer personalized lifestyle health recommendations and nutritional supplements based on clients' genetic profiles. Despite the current euphoria, the predictive value of genetic profiling is still limited for most disorders, with only some promising exceptions. This presentation will present the framework for the translational research that is needed, and review recent studies in this area.

IN166

PRACTICAL IMPLEMENTATION OF PUBLIC HEALTH GENOMICS: THE CASE OF GENAR INSTITUTE

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Epidemiological and demographic transition has brought populations to an extended life expectancy in 21st century. The diseases of this century are complex diseases, which stem from mainly the complex interaction of human genome with life style and environmental factors. These diseases are common, chronic and costly. Cardiovascular and cerebrovascular diseases, cancers, diabetes and osteoporosis are among major chronic and complex diseases, which account for approximately two third of all deaths in the world. Currently, the best known prevention for complex diseases is adopting a healthy lifestyle. However, this is not achieved in many places of the world. Effective intervention models including lifestyle changes for prevention of these diseases is urgently needed. Genome based information has a very important potential to improve human health, quality of life and performance, and extend life span. At this point, a new concept called 'Public Health Genomics' emerges, which focuses on translating the genomic discoveries into individual and public health interventions. Utilizing public health genomics and personalized medicine approach, GENAR Institute for Public Health and Genomics Research (Turkey) has been working on a preventive health care model to combat with the complex diseases. It is based on application of public health genomics tools and concepts in individual level, in order to stratify individuals according to risk groups, prevent diseases and detect them early. It is an integrative preventive model utilizes individuals' clinical information, detailed lifestyle analysis, biochemical markers and the genetic make-up in order to prevent, early detect and treat complex diseases in a targeted way. Based on the results of the aforementioned components, an optimum lifestyle plan is drawn, including personal menu plans and exchange lists, exercise plans, smoking cessation recommendations based on the individual causes of smoking, and medical follow up plan. The mission of this model is changing the behaviour of individuals. It creates awareness by informing individuals about their current lifestyle and genetic predispositions. Further, it causes an attitude change by creating a vulnerability perception. Finally, behavior change is achieved with the follow-up programme and the trainings. Public health genomics and personalized health care will play major role in combating the chronic-complex disease burden of the aging populations of 21st century. In parallel to exponentially increasing knowledge gained through research, health care systems need to foresee these upcoming developments and prepare for the transition. The approach of GENAR is an example to translation of genome-based knowledge into preventive health care.

IN167

THE FUNCTIONS OF AID AND OTHER DNA DEAMINASES IN IMMUNITY

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The immune system is unique in mammals in its use of active genom-

ic mutation for a programmed physiological purpose. Within the adaptive immune system, the functionally rearranged immunoglobulin genes in activated B lymphocytes are targeted for deamination at cytosine residues by activation-induced deamination (AID). Deamination within the immunoglobulin V gene triggers antibody gene diversification by somatic hypermutation and gene conversion. Deamination in the vicinity of the immunoglobulin switch (S) regions triggers the shift from the expression of IgM to that of one of the downstream isotypes (IgG/A/E). Programmed DNA deamination is also used within the innate immune system where deamination of retroviral replication intermediates by members of the APOBEC3 family (which show sequence homology to AID) is associated with pathways of retroviral restriction. The action of AID needs to be targeted: to the IgV for somatic mutation and to the relevant S regions for appropriate class-switching. Furthermore, off-target action of AID can lead to genomic instability and predispose to cancer. Results will be presented on the need for an interaction with CTNNBL1 for AID targeting and on the possible role of the carboxy-terminal region of AID in immunoglobulin class-switching. Data will also be presented suggesting that the specific activity of AID may have been limited by the risk of cancer-associated off-target mutation. Using high-throughput assays to select for AID mutants with improved activity, we have found that many of the up-mutations map close to the active site. Some of these variants are more potent not just in vitro but also in respect of their ability to induce immunoglobulin somatic mutation and class-switching in vivo. However, the AID up-mutants are also more potent in the induction of cancer-associated chromosomal translocations suggesting that the specific activity of AID can be increased to improve antibody maturation but at the risk of increasing predisposition to cancer. Interestingly, many of the up-mutations in AID bring the sequence of AID closer to that of APOBEC3s which predominantly function in the cytosol and whose evolution might therefore not have been limited by an associated risk of genomic instability.

IN168

INTENTIONAL MUTAGENESIS IN B CELLS: PCNA-UBIQUITYLATION CONTROLS SOMATIC HYPERMUTATION

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BACKGROUND: Proliferating cell nuclear antigen (PCNA) encircles DNA as a ring shaped homotrimer and by tethering DNA polymerases to their template PCNA serves as a critical replication factor. In contrast to high-fidelity DNA polymerases the activation of low-fidelity translesion synthesis (TLS) DNA polymerases can require damage inducible monoubiquitination (Ub) of PCNA at lysine residue 164 (PCNA-Ub). TLS polymerases tolerate DNA damage and replicate directly across DNA lesions. The lack of proofread activity renders TLS highly mutagenic. Interestingly, B cells take advantage of mutagenic TLS to introduce somatic mutations in immunoglobulin genes to generate high affinity antibodies. AIM: Determine the role of PCNAK164 modifications in DNA damage tolerance, cell cycle control, and somatic hypermutation of Immunoglobulin genes. METHODS: PCNAK164R knock-in mice and cell lines thereof were generated following standard procedures. RESULTS: Primary pre B cells from PCNAK164R mutant mice are highly sensitive to DNA damage. PCNAK164R delays but does not abolish the progression of replication forks in bypassing UV-C induced lesions in vivo. Interestingly, UV-C treatment of PCNAK164R mutant cells delays the synthesis (S) phase and the gap (G) phases of the cell cycle. Consistent with these observations, the recruitment of Polη into nuclear repair foci strongly depends on PCNA-Ub. Given the critical role of PCNA-Ub in activating TLS we analyzed the mutation spectrum of somatically mutated Ig genes in B cells from PCNA K164R knock-in mice. A ten-fold reduction in A/T mutations is associated with a compensatory increase in G/C mutations- a phenotype similar to Polη and mismatch repair deficient B cells. The G/C mutator(s) do not depend on PCNA-Ub. CONCLUSIONS: Our data indicate a dual physiological role for PCNA-Ub dependent TLS by Polη: Anti-mutagenic TLS to survive UV-C-induced DNA lesions and mutagenic TLS at template A/T of AID-induced lesions to generate high affinity Ig variants. During SHM Polη is recruited by PCNA-Ub to establish the vast majority of mutations at template A/T. PCNA-Ub controls cell cycle progression and damage tolerance throughout the interphase of the cell cycle, i.e. during DNA replication and long patch DNA repair synthesis.

IN169

DIVERSITY-GENERATING RETROELEMENTS

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Host-parasite interactions are often driven by mechanisms that promote genetic variability. In the course of our studies on bacterial pathogenesis, we discovered a group of temperate bacteriophages that generate diversity in a gene that specifies tropism for receptor molecules on host *Bordetella* species, which cause respiratory infections in humans and other mammals. This microevolutionary adaptation is produced by a novel "diversity-generating retroelement" (DGR) that combines the basic retroelement life cycle of transcription, reverse transcription and integration with site-directed, adenine-specific mutagenesis. Central to this process is a reverse transcriptase-mediated exchange between two repeats, one serving as an donor template (TR) and the other as a recipient of variable sequence information (VR). Recent work has focused on the genetic basis of diversity-generation. The directionality of information transfer is determined by the initiation of mutagenic homing (IMH) sequence present at the 3' end of VR. We have demonstrated that DGR function occurs through a TR-containing RNA intermediate by a unique target-primed reverse transcription mechanism that precisely regenerates target sequences. This non-proliferative, "copy and replace" mechanism enables repeated rounds of protein diversification and optimization of ligand-receptor interactions. The potential utility of DGRs is illustrated by the identification of over 40 related elements in bacterial, phage, and plasmid genomes. DGRs are present in human pathogens (*Treponema*, *Legionella spp.*), human commensals (*Bacteroides*, *Bifidobacterium spp.*), green sulfur bacteria (*Chlorobium*, *Prosthecochloris spp.*), cyanobacteria (*Trichodesmium*, *Nostoc spp.*), magnetotactic bacteria (*Magnetospirillum spp.*), and many other diverse species. DGRs comprise a new family of retroelements with the potential to confer powerful selective advantages to their host genomes. In addition to shedding light on DGR function, our results suggest novel approaches for DGR-based protein engineering.

IN170

ERROR-PRONE REPAIR PATHWAYS MOBILIZING TLS DNA POLYMERASES IN IMMUNOGLOBULIN GENE HYPERMUTATION

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Hypermutation of immunoglobulin (Ig) genes is initiated by the enzyme AID (activation-induced cytidine deaminase) that deaminates cytidines into uracils at the Ig loci. Most mutations observed at these loci are however produced by the processing of these lesions, generating further mutations at the site of the initial deaminated base or at distance from it, a process mobilizing mutagenic polymerases specialized in the by-bass of damaged bases. Our studies are focused on the role of DNA polymerase eta in this process, and on the specific pathways (error-prone repair vs. translesion DNA synthesis) ensuring its specific recruitment.

IN171

EDITING DEAMINASES: STORY OF A MULTI-TALENTED DOMAIN

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The AID/APOBECs are a family of cytidine deaminases acting on (deoxy)nucleotides in the context of nucleic acids. While sharing similar enzymatic properties, the AID/APOBECs are players in a diverse set of pathways: AID and the APOBEC3s are DNA mutators acting respectively in the antigen-driven antibody diversification processes and in an innate pathway of defense against retroviruses; APOBEC1 edits the mRNA encoding for the Apolipoprotein B, involved in lipid transport. But their peculiar activity comes at a steep price: several lines of evidence link the AID/APOBECs to the promotion of cancer. All AID/APOBECs bear a characteristic Zinc-coordination motif, which forms the core of the catalytic site. Here I use a phylogenetic approach coupled to an analysis of the available structural information to place the AID/APOBEC proteins in the context of other zinc-dependent deaminases. The ancestral AID/APOBECs have originated at the beginning of the vertebrate radiation from the tRNA adenosine34 deaminases (TadA/ADAT2), a branch of the Zinc-dependent deaminase superfamily. Other members have arisen in the mammals from duplication of the AID locus, and present a history of complex gene duplications and positive selection. Given the paucity of available data on the recognition and binding of the AID/APOBECs to their substrate, the similarities of the TadA/ADAT2 deaminases to the AID/APOBECs can provide a useful structural paradigm to understand the specificities of the mode of action of the AID/APOBECs.

IN172

REGULATION OF DNA POLYMERASE ETA IN HUMAN CELLS

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An important pathway by which cells are able to tolerate unrepaired DNA damage during replication is translesion synthesis (TLS). In this process DNA is synthesized past the damaged bases by specialized DNA polymerases, most of which belong to the Y-family. These polymerases have an open structure which allows them to accommodate damaged DNA bases in their active sites. Deficiency in one of these polymerases, DNA polymerase (pol) eta, is responsible for the variant form of the highly skin cancer-prone disorder xeroderma pigmentosum. Regulation and control of pol eta is mediated by several important motifs close to the C-terminal third of the protein. These motifs are required for their correct localisation in replication factories and for protein-protein interactions. The sliding clamp accessory protein PCNA plays a crucial role in regulating TLS. When the replication fork is blocked, PCNA becomes ubiquitinated. This increases the affinity of pol eta for PCNA, because it contains both PCNA-binding and ubiquitin-binding motifs. Localisation of pol eta in replication factories and interaction with PCNA are complex and highly dynamic processes.

IN173

DNA POLYMERASE KAPPA-DEFECTIVE MICE ARE SPONTANEOUS MUTATORS

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DNA polymerase kappa (polχ) is one of multiple DNA polymerases involved in translesion DNA synthesis (TLS) in eukaryotes. Polk-deficient mice have no observable phenotypes except for a slightly reduced life span. Polk-/- mice were bred with BigBlue mice carrying a reporter gene (the cII gene of bacteriophage λ), which allows the measurement of mutation frequencies in any tissue of interest. We have observed enhanced spontaneous mutagenesis in the kidney, liver and lung (but not in the spleen) of Polk-/- mice. The majority of mutations arose from GC->AT transitions, suggesting that either G or C is a target for spontaneous mutations. Consistent with recent studies by others (1), tissues from mice defective for polη (for which the "cognate" substrate for TLS is presumably the cyclobutane pyrimidines dimer) are not spontaneous mutators. Cells from Polk-/- mice are hypersensitive to killing by benzo[a]pyrene, which like many planar polycyclic chemicals binds primarily to the N2 position of G in DNA. Additionally, Polχ accurately bypasses ring compounds that form N2adducts in G in vitro. We postulate that some type(s) of naturally occurring planar ring compound(s) bound to G in DNA is the "cognate" substrate for accurate TLS by polχ in vivo. Cholesterol or cholesterol derivatives, such as various steroids, are prime candidates for such spontaneous DNA damage.

(1). R. A. Busuttil, et al., Mutation frequencies and spectra in DNA polymerase η-deficient mice. Cancer Res. 68: 2081 (2008)

IN174

HUMAN DNA POLYMERASE NU (POLN), A UNIQUE A-FAMILY DNA POLYMERASE WHICH CAN BYPASS DNA DAMAGE

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The human genome encodes two nuclear A-family DNA polymerases related to *E. coli* DNA polymerase I, DNA polymerase θ (POLQ) and DNA polymerase ν (POLN). POLQ possesses both a helicase-like and a DNA polymerase domain while POLN encodes only a DNA polymerase domain. POLQ homologs are present in the genomes of most animals and plants whereas POLN homologs appear more recently in evolutionary time, in the deuterostome lineage of animals. It is important to determine the cellular functions of these enzymes, which have unusual fidelity and DNA lesion bypass properties. *POLQ* is expressed in many tissues, cell lines, and cancer cells. Mice with disruption of the gene have elevated spontaneous and radiation-induced frequencies of micronuclei, and bone marrow stromal cell lines show increased sensitivity to γ-irradiation. POLQ may aid in cellular tolerance of DNA damage that can lead to double-strand breaks. Biochemically, POLQ is a low fidelity enzyme and can catalyze efficient DNA synthesis opposite an apurinic site or a thymine glycol (Tg), which both present strong blocks for synthesis by replicative DNA polymerases. In contrast, we find that *POLN* is expressed in testis of mammals and the zebrafish but is generally weakly or not expressed in other tissues. POLN has several unique properties, including strong strand displacement activity. It is a low-fidelity enzyme favoring incorporation of T almost as often as C for template G, resulting in frequent GC to AT transitions. POLN can catalyze accurate translesion synthesis past a 5*S*-Tg, which is a major DNA lesion generated by reactive oxygen species. Unlike POLQ, POLN cannot bypass an apurinic site. To explore the basis for these features of POLN, we altered residues in the DNA polymerase domain. In Motif 4, a positively charged Lys (K) is present at residue 679, a conserved position in the POLN group. This residue is an uncharged Ala (A) in *E. coli* pol I or Thr (T) in *Taq* pol I and is one of the most important for controlling fidelity of the procaryotic enzymes. The K679A or K679T POLN mutants are active but poorly incorporate T opposite template G and do not bypass 5*S*-Tg efficiently. This residue is critical for the unique fidelity and bypass properties of POLN.

IN175

REPAIR AND TOLERANCE MECHANISMS OF DNA-PROTEIN CROSSLINK DAMAGE

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Various chemical agents such as aldehydes and heavy metal ions and physical agents such as ionizing radiation and ultraviolet light induce DNA-pro-

tein crosslinks (DPCs). DPCs are unique among DNA lesions in that they are extremely bulky as compared to conventional bulky DNA lesions such as pyrimidine photodimers and the base adducts of aromatic compounds. Covalently trapped proteins would impede the progression of replication and transcription machineries, posing an enormous threat to cells. However, it has not been fully elucidated how cells alleviate the genotoxic effect of DPCs. Recently we have shown that in bacterial cells nucleotide excision repair (NER) and homologous recombination (HR) contribute to the repair/tolerance of DPCs, but differentially. NER removes DPCs with crosslinked proteins (CLPs) smaller than 12-14 kDa, whereas oversized DPCs are processed exclusively by RecBCD-dependent HR. These results rigorously define the size limit of bulky DNA lesions amenable to bacterial NER, and provide insight into how NER and HR collaboratively alleviate the genotoxic effect of DPCs in bacterial cells. We further asked whether NER and HR were similarly involved in the repair and tolerance of DPCs in mammalian cells. Analysis of the incision activity with cell extracts revealed that the upper size limit of CLPs amenable to mammalian NER was around 8 kDa, a value notably smaller than that for bacterial NER. Chromosomal DPCs induced by formaldehyde were released at comparable rates in NER-proficient and NER-deficient cells. CLPs in chromosomal DNA were not polyubiquitinated. These results together indicate that NER and NER coupled with the proteasomal degradation of CLPs are not involved in the repair of DPCs. Cells deficient in HR were hypersensitive to DPC-inducing agents and accumulated RAD51 and gamma-H2AX nuclear foci following treatment with DPC-inducing agents, demonstrating the pivotal role of HR in mitigating the toxic effects of DPCs. The present results show the differential involvement of NER in the repair of DPCs in bacterial and mammalian cells, and highlight the versatile and conserved role of HR in tolerance to DPCs among species.

IN176

NEW FUNCTIONAL ROLES OF THE HUMAN DNA POLYMERASES ETA AND KAPPA DURING GENOMIC DNA REPLICATION

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DNA polymerases eta (pol η) and kappa (pol k) are members of the Y-family of DNA polymerases that are thought to function primarily in trans-lesion synthesis (TLS) past different types of exogenous DNA damage. In order to explore additional functional roles and importance of these DNA polymerases in human cells, we used sh/siRNA to selectively deplete Pol eta or Pol kappa from cells and then determine how the loss of these polymerases affected cell cycle progression, cell proliferation and DNA replication dynamics in unperturbed cells or following the addition of replication inhibitors. Pol η-depleted cells demonstrated two consistent abnormalities that may be mechanistically linked: altered replication factory dynamics and elevated instability of a common fragile site. Of importance, these abnormalities were demonstrated in unperturbed replicating cells and in several different cell types, including primary fibroblasts, and appeared to depend on Pol η catalytic activity as demonstrated by a combination of depletion and complementation experiments. Moreover, these effects are specific to Pol η since none of these cellular or karyotypic defects were observed in cells depleted for Pol i, the closest relative of Pol η.
Pol k depletion experiments allowed us to uncover a novel Pol k function in the replication stress response. DNA replication stress triggers the activation of the intra-S checkpoint signalling cascade resulting in the ATR-mediated phosphorylation of Chk1 protein kinase, thus preventing genomic instability. We found that Pol k regulates Chk1 phosphorylation after UV damage and under conditions of nucleotide starvation. We showed that Pol k interacts with Claspin, an adaptor protein that promotes Chk1 phosphorylation, and is required for loading Claspin on chromatin under replication stress. This novel checkpoint function of Pol k is required for cell proliferation, for normal replication fork progression and for the maintenance of genomic stability in unstressed human cells. These data extend therefore the functions of Pol η and Pol k to normal DNA replication in cycling cells, and indicate that a functional interplay between TLS and replication checkpoint signalling is important to maintain genomic stability.

IN177

GENE-ENVIRONMENT INTERACTIONS AS COMMON DETERMINANTS OF NONCANCER-DEGENERATIVE DISEASES

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Genomic and postgenomic changes resulting from gene-environment interactions are extensively investigated in cancer research. Similar alterations, affecting genome, transcriptome, mirnome and/or proteome end-points, have been detected in a variety of other chronic degenerative diseases, such as atherosclerosis, degenerative heart diseases, chronic obstructive pulmonary diseases, neurological disorders, eye diseases, and skin ageing. No generalization can be made due to the myriad of diverse clinical entities classified as chronic degenerative diseases. Moreover, the detection of molecular changes does not automatically imply their causal role. Nevertheless, common mechanisms, such as DNA damage, epigenetic alterations, oxidative stress, and chronic inflammation, in addition to genetic predisposition, are often involved in noncancer diseases. Typically, mitochondrial DNA is a common target of pathogenic factors involved in a variety of noncancer degenerative diseases. Genomic and postgenomic changes do also occur during critical periods of life, including the prenatal life, the perinatal period, and ageing. In addition, stem-derived cells are more susceptible to molecular damage than more differentiated cells. All these data are relevant in the perspective of preventive medicine. In fact, there is evidence that the genomic and postgenomic alterations occurring not only in several pathological conditions but also in paraphysiological situations that affect critical periods of life can be modulated by means of dietary and pharmacological agents.

IN178

GENETIC INFLUENCES ON SMOKING BEHAVIOR AND PREVENTION OF CHRONIC DEGENERATIVE DISEASES

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Tobacco smoking remains the major preventable cause of premature morbidity and mortality throughout the world. Research on tobacco use and smoking cessation has contributed to reductions in smoking prevalence. However there are still more than 1.2 billion smokers world-wide, resulting in 3-4 million deaths per year. Currently, 35% of all deaths can be attributed to cardiovascular diseases, making it the most common cause of death from smoking. On the other hand, by 2015 about one-third of the smoking-related deaths will probably be caused by cancers, closely followed by cardiovascular diseases and chronic respiratory diseases (both ~30%). Because smoking is a modifiable risk factor, a large part of related deaths and diseases could be prevented. Smoking is a complex behavior and both genetic and environmental factors are believed to be involved. Recent studies have found significant influences of heredity on several aspects of smoking behavior (e.g. initiation and maintenance, number of cigarettes smoked, and cessation). More recently significant genetic influences on more clinically relevant phenotypes, such as indicators of nicotine dependence and withdrawal, have been found as well. The search for specific genetic effects on smoking behavior has focused on genotyping of two broad classes of genes: (1) genes that may predispose to addictive behavior via their effects on key neurotransmitter pathways (especially dopamine) and (2) genes that are involved in the metabolism of nicotine (determining its half-life). Even with help of pharmacological and behavioral therapies more than 70% of smokers motivated to stop smoking start smoking again within 12 months after their quit attempt. We recently analyzed multiple genetic variants of smoking-behavior related genes in clinical trials of different types of smoking cessation therapy in order to define an algorithm that can be used to predict in advance which therapy could be used best. Personalized treatment based on a person's genetic profile is expected to result in a more efficient use of anti-smoking therapies, less frustration by smokers, more effort by health care providers in stimulating cessation attempts,

increased quit rates and ultimately, in reduced toxic exposures and deaths from smoking.

IN179

THE IMPACT OF GENETIC AND ENVIRONMENTAL FACTORS IN NEURODEGENERATION: THE EMERGING ROLE OF EPIGENETICS

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Neurodegenerative diseases are a heterogeneous group of pathologies of the nervous system which includes complex multifactorial diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD) and Amyotrophic lateral sclerosis (ALS). Familial forms represent a minority of the cases (ranging from 5 to 10% of the total), whereas the vast majority of AD, PD and ALS occurs as sporadic forms, likely resulting from the contribution of complex interactions between genetic and environmental factors superimposed on slow, sustained neuronal dysfunction due to aging. Several causative genes for the familial forms have been discovered in recent years, they are inherited as Mendelian traits and their discovery has led to a better comprehension of the molecular pathways responsible for the selective neuronal degeneration which is specific for each of these disorders. Moreover several environmental factors, including pesticides, metals, head injuries, lifestyles and dietary habits have been associated with increased disease risk or even with protection. Hundreds of genetic variants have been investigated as possible risk factors for the sporadic forms, but results are often conflicting, not repeated or inconclusive. It is now clear that various environmental and dietary components and lifestyles can modulate both genome and epigenome, this last by altering genetic expression and potentially modifying the risk and/or severity of a variety of disease conditions including neurodegenerative ones. The possible role of epigenetic modifications of human genes has been the focus of recent studies aimed at a better understanding of both the origin and the possible treatment of several neurodegenerative diseases. Dietary modification can indeed have a profound effect on DNA methylation and genomic imprinting, moreover among dietary factors, antioxidant compounds seem to exert a neuroprotective role throughout epigenetic mechanisms. Many of the processes with a key role in the neurodegeneration such as the formation of senile plaques, the accumulation of ROS, the cleavage of APP by neuroscretases, can be now analysed in light of the new epigenetic knowledge, to facilitate the implementation of future disease prevention strategies.

IN180

POLYMORPHISMS OF CYTOCHROME P450 1A1, CIGARETTE SMOKING AND RISK OF CORONARY ARTERY DISEASE

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Background/Aims: The relationship between cigarette smoking and coronary artery disease (CAD) has been previously demonstrated with strong epidemiological evidence. Cytochrome P450 1A1 (CYP1A1) is one of the key enzymes that metabolize cigarette smoking derived toxin and may be relevant to smoking-induced atherogenesis. This case-control study was designed to examine whether CYP1A1 polymorphisms, including *CYP1A1*2A* (T6235C) and *CYP 1A1*2C* (A4889G), play a role in susceptibility to smoking-related CAD. Methods: We recruited participants who had undergone coronary angiography form a hospital in Taiwan. Cases (n = 481) were those with any coronary arteries with 50% or more luminal obstructions. Normal subjects (n = 228) served as controls. Information about socidemographic factors and smoking status were obtained by a self-administered questionnaire. Genotypes of *CYP1A1*2A* and *CYP1A1*2C* polymorphisms were determined by polymerase chain reaction or in combination with restriction fragment length polymorphism methods. Logistic regressions were used to calculate the odds ratios (ORs) and 95% confidence intervals (CIs). Results: We found that the *CYP1A1*2C* G allele was more prevalent in controls (p = 0.03) and it exerted a dose effect on the protective risk of CAD (p for trend = 0.016). After adjusting for potential confounders, the *CYP1A1*2C* G/G genotype compared to the A/A genotype was significantly associated with a decreased risk of CAD (OR = 0.32, 95% CI = 0.15-0.70). This protective effect was pronounced among never cigarette smokers, but the interaction between genotype and smoking status was not statistically significant. Never cigarette smokers who carrying the *CYP1A1*2C* G/G genotype have 77% decrease risk of CAD than those carrying the A/A genotype (OR = 0.23, 95% CI = 0.08-0.71). However, there was no significant association between *CYP1A1*2A* polymorphism and CAD risk. Conclusions: Our findings suggest that the *CYP1A1*2C* G/G genotype may reduce the risk of CAD in the Taiwanese population, especially among never smokers.

Keywords: Cytochrome P450 1A1; Polymorphisms; Coronary artery disease; Cigarette smoking

IN181

USE OF THE COMET ASSAY FOR THE DETECTION OF DNA PROTECTIVE CONSTITUENT IN THE HUMAN DIET

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The COMET assays is based on the determination of DNA migration in an electric field and is increasingly used in human intervention trials for the detection of DNA protective dietary constituents in lymphocytes. It enables the detection of prevention of single and double strand breaks, measurements of endogenous formation oxidised bases by use of lesion specific enzymes, and also the investigation of alterations of the cells sensitivity towards damage caused by reactive oxygen species and DNA reactive dietary carcinogens (such as PHAs, mycotoxins, HAAs, acrylamide, nitrosamines and heavy metals). Recently also protocols were developed which enable to monitor alteration of the DNA repair capacity (BER and NER). At present the results of 85 studies have been published, and in about half of them the protective effects were found. Examples for chemoprotective foods which we studied in the last years in human trials comprise Brussels sprouts, spinach, the spice sumach, coffee and wheat sprouts. All of the them reduce the formation of oxidised DNA bases and/or protect against DNA damage induced by selected carcinogens. The effects could be attributed to alterations of enzymes involved in the detoxification of ROS (e.g. SOD by coffee and Brussels spouts) and to alterations of enzymes involved in the activation/detoxification of carinogens (GST induction by coffee, SULT inhibition by sprouts). Gallic acid which we identified as the active principle in sumach was found to be a "super antioxidant" which is 30-50 times more effective than vitamins C and E (in human SCGE trials) and we showed in follow up experiments that it protects also rodents against oxidative DNA damage in a variety of inner organs and prevents the formation of ROS (radiation) induced precursors of liver tumours in rats (GSTp+ foci). Also with coffee protective effects were seen in the latter model. This approach (i.e. the combination of human trials with animal experiments aimed at relating the results seen humans to the prevention of diseases) appears to be a promising strategy for the identification of food constituents which cause beneficial heath effects.

IN182

TRANSIENT GENERATION OF REACTIVE OXYGEN SPECIES AS AN IMPORTANT SIGNALLING MECHANISM IN CANCER CHEMOPREVENTION

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Excessive production of reactive oxygen species (ROS) has been associated with oxidative stress, cellular damage and carcinogenesis. Many natural plant polyphenols are capable of scavenging ROS and have antioxidant functions. These have been related to cancer preventive potential. Xanthohumol (XN) from hops is a broad-spectrum cancer chemopreventive agent, acting by distinct mechanisms including radical-scavenging, anti-estrogenic, anti-proliferative, anti-angiogenic, and apoptosis-inducing potential (Gerhauser *et al*., Mol. Cancer Ther. 2002). To better define the role of ROS in cancer preventive potential of XN, we established test systems based on sensitive fluorescent markers to detect and quantify ROS formation in cell culture and in isolated mitochondria. We used 2',7'-dichlorodihydrofluorescein diacetate (H_2DCF-DA) to monitor ROS in benign prostate cancer cells (BPH-1). We detected an immediate XN-mediated dose- and time-dependent increase in fluorescence, indicative of ROS production, with a maximum at 12.5 µM concentration. ROS detection was not due to artificial H_2O_2 generation, as described previously for selected polyphenols. Rather, after treatment with XN up to 25 µM, we monitored a dose-dependent intracellular increase of superoxide anion radicals (O_2^{-*}) by dihydroethidium oxidation. Fluorescence microscopy images of BPH-1 cells stained with MitoSOX Red specific for mitochondrial O_2^{-*} suggested a mitochodrial origin of O_2^{-*} formation. This was confirmed by XN-mediated induction of O_2^{-*} in isolated mitochondria. Furthermore, in rho-zero BPH-1 cells harboring non-functional mitochondria, XN treatment did not induce O_2^{-*} formation. Detection of O_2^{-*} in BPH-1 cells was significantly reduced by co-treatment with ascorbic acid, N-acetyl cysteine and the superoxide dismutase mimetic MnTMPyP. Also, co-treatment of BPH-1 cells with MnTMPyP significantly prevented XN-mediated anti-proliferative activity and induction of apoptosis, monitored by flow cytometry and poly(ADP-ribose)polymerase cleavage. These data suggest that apoptosis induction by XN is a functional consequence of mitochondrial O_2^{-*} production. In conclusion, transient generation of ROS in addition to ROS scavenging may be an important determinant for cancer preventive potential of natural products.

IN183
IN SEARCH OF A MECHANISM: FOOD POLYPHENOLS FROM ANTIOXIDANTS TO MODULATORS OF GENE EXPRESSION

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Food is an important determinant of cancer. A high intake of fruit and vegetables has been linked to a decreased incidence of human tumours. Since most plant materials contain polyphenols, the possibility was suggested that plant polyphenols might have a cancer-preventive activity. Polyphenols from tea and grapes have shown cancer-inhibiting activity in experimental animal models; however, the mechanism through which food polyphenols modulate cancer risk were unknown or speculative. We carried out experiments in rodents to study the effect of plant materials containing polyphenols on the gene regulation of the colon mucosa and liver, which come into contact with polyphenols or their metabolites (mutant strains of Arabidopsis Thaliana, varieties of apples and strawberries containing various amounts of phenolic acids and complex polyphenols, polyphenol-rich and depleted olive oil). No major changes in DNA oxidative damage were found in the organs studied, except in animals treated with flavonol-rich Arabidopsis seeds. Gene expression profiles were investigated in liver and colon mucosa samples using rat oligonucleotide arrays, (Rat Genome Oligo Set Version 1.1™ , Operon Technologies, CA, USA) and composed of 5,677 oligonucleotides (70mers), each representing one gene. We compared the gene expression profile of animals fed high polyphenol diets vs reference pools obtained by RNA extracts from rodents fed the same diets devoid or with low levels of polyphenols. GenMAPP and MAPPFinder analysis revealed effects of polyphenols on genes regulating cell growth, transport, regulation of transcription, metabolism, apoptosis, inflammatory and defense responses. The combination of gene-regulatory effects were variable with different polyphenol-containing foods. Some of the foods containing high phenolic acids (Marie Ménard apples) had a marked anti-inflammatory effect. We propose that the change in gene regulation induced by polyphenol-containing foods, associated with variation of intestinal flora, might explain their cancer- modulating activity in vivo. Some of the anti-inflammatory phenolic acids present in foods may have cancer-preventive properties on their own.

IN184
NON-ANTIOXIDANT EFFECTS OF PHYTOCHEMICALS: DNA REPAIR

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It is clear from many human supplementation trials in humans that pure antioxidant compounds do not protect against cancer (or other serious chronic diseases). However, the evidence still indicates a protective role for fruits and vegetables. How else might phytochemicals be acting? One possible role is in modulating DNA repair activity. The comet assay can be used in different ways to measure DNA repair. If cells are treated with a specific agent to induce DNA damage, and incubated, the removal of the damage can be followed using a lesion-specific endonuclease; for example, base excision repair (BER) of 8-oxoguanine is monitored using formamidopyrimidine DNA glycosylase. Another approach is more biochemical; a subcellular or nuclear extract is incubated with a substrate, consisting of agarose-embedded nucleoids from cells treated with a specific DNA-damaging agent, such as a photosensitiser plus light to induce 8-oxoguanine. The rate of accumulation of incisions in the DNA (the first step in repair) reflects the extract's repair capacity. We previously reported that daily consumption of kiwifruit led to an enhancement of BER in lymphocyte extracts, measured on a substrate containing 8-oxoguanine. Similar findings emerge from a recent intervention study with whole fruits and vegetables. We recently modified the assay to measure nucleotide excision repair (NER), and found that a phytochemical-rich diet, rather than enhancing repair, significantly decreased the NER rate. In parallel, we are carrying out experiments in which cells are preincubated with a phytochemical, and then monitored for effects on repair. β-Cryptoxanthin, for example, accelerates strand break rejoining, and removal of 8-oxoguanine, in HeLa or Caco2 cells; it also enhances the BER capacity of cell extracts. There are wide inter-individual variations in BER and NER activities in humans. The next challenge is to find out how much of the variation is genetic in origin, and how much is due to environment, including nutrition.

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IN185
CELLULAR ADAPTIVE SURVIVAL RESPONSE TO OXIDATIVE, NITROSATIVE AND INFLAMMATORY STRESSES: ROLES OF REDOX-SENSITIVE TRANSCRIPTION FACTORS

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In an almost every moment, living organisms are subjected to diverse types of stress both external and internal sources. While excessive stress leads to necrotic or apoptotic death, moderate amounts of noxious stimuli may render the cells adaptive or tolerant to ongoing or subsequent insults. Such adaptive survival response normally accompanies *de novo* synthesis of proteins through activation of distinct stress-responsive signaling. One of the key signaling molecules involved in cellular adaptation or tolerance to a wide array of noxious stmuli is nuclear factor-kappa B (NF-χB). Our previous studies have revealed that NF-χB plays a pivotal role in Bcl-2-mediated resistance to oxidative PC12 cell death through augmentation of cellular antioxidant

capacity. Induction of phase-2 detoxifying or antioxidant genes also represents an important cellular defence in response to oxidative and electrophilic insults. Nuclear transcription factor erythroid 2p45 (NF-E2)-related factor 2 (Nrf2) plays a crucial role in regulating cytoprotective gene induction. Nrf2 is sequestered in the cytoplasm as an inactive complex with the inhibitory protein Keap1. Upon activation, Nrf2 binds to antioxidant responsive element (ARE) or electrophile responsive element (EpRE) sites, leading to the coordinated up-regulation of down-stream target genes that boost cellular antioxidant potential. Many dietary phytonutrients can induce ARE-driven upregulation of antioxidant/phase-2 detoxifying enzymes or other cytoprotective proteins, thereby fortifying cellular defence against oxidative, nitrosative insult. Recent studies highlight the protective role of Nrf2 against inflammatory cell and tissue damage. Cysteine thiols present in various transcription factors and their regulators function as redox sensors in fine-tuning of transcriptional regulation of many genes essential for maintaining cellular homeostasis. Thus, oxidation or covalent modification of thiol groups present in the above redox-sensitive transcription factors and their regulating molecules can provide a unique strategy for molecular target-based chemoprevention and cytoprotection.

IN186
INVOLVEMENT OF MUTATOR DNA POLYMERASES IN CARCINOGENESIS

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In order to account for the large number of mutations in human tumors, we proposed that cancers exhibit a mutator phenotype. The basic concept is that normal mutation rates are insufficient to account for the large numbers of mutations in tumors, and as such cancer cells have an increased mutation rate during the course of tumorigenesis. Mutations in the primary enzymes involved in base-selection, DNA polymerases, provide an attractive mechanism for a progressive increase in mutation frequency throughout the genome during each round of cell division. So far evidence for the prominence of mutator DNA polymerases in human tumors has been limited to Pol-beta and to overexpression of certain translesion DNA polymerases. DNA polymerase-δ (Pol-δ) is responsible for lagging strand DNA synthesis during replication, and functions in various DNA repair pathways. Based on homology to mutations in other polymerases that alter the fidelity of DNA synthesis, we mutated position L606 in the catalytic domain of human Pol-δ. Wild type human Pol-δ is highly accurate, catalyzing base-substitution or frameshift errors at rates less than 5 X 10-6 nucleotides polymerized. Inactivating the proof-reading exonuclease or introducing substitutions at L606 increases mis-incorporation. L606G Pol-δ exhibits an increased rateof single-base substitutions. In contrast, L606K is accurate in base-selection but is defective in copying past site-specific nucleotide modifications. These differences in the in vitro phenotype were reinforced by analysis of yeast harboring the homologous substitutions. In vivo, the G-substitution yields single-base substitutions and the K-substitution results in random deletions. Moreover, in mouse embryonic fibroblasts, established from heterozygous mice, the frequency of chromosomal aberrations was 17-fold and 38-fold greater in cells harboring the G and K substitutions, respectively. Mice heterozygously expressing the K-mutation, but not the G-mutation, exhibited decreased survival in concert with enhanced tumor progression. These studies indicate that a heterozygous deletion mutator in Pol-δ can promote genetic instability and accelerate tumor progression.

IN187
THE EFFICIENCY OF DNA MISMATCH REPAIR IN SACCHAROMYCES CERIVISIAE

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We are investigating whether the efficiency with which the mismatch repair machinery corrects replication errors varies in yeast depending on which replicative polymerase generates the mismatch, the identity of the mismatch, and/or the DNA strand or the local sequence context in which the mismatch resides. To identify the polymerase involved, we use haploid yeast strains harboring pol1 (Pol alpha), pol 2 (Pol epsilon), and pol3 (Pol delta) mutant alleles that retain robust replicative capacity but have elevated spontaneous mutation rates at the URA3 locus that are further increased upon inactivation of Msh2-dependent DNA mismatch repair. Sequence analysis of ura3 mutants previously revealed strand-specific and site-specific differences in mutation rates in patterns implying that Pol epsilon is the primary leading strand polymerase, while Pol alpha and Pol delta primarily replicate the lagging strand. Here we compare ura3 mutational spectra in wild type versus msh2Δ derivatives of these mutator strains. The results reveal that Msh2-dependent mismatch repair efficiently corrects most, but not all, replication errors made by all three polymerases. On average, MMR efficieny is highest for single base indel mismatches and slightly lower for single base-base mismatches, with transition mismatches repaired slightly more efficiently than transversion mismatches. The efficiency of repairing the same mismatch at different locations can vary widely. Consequently, errors at the least efficiently corrected sites are observed even in MMR-proficient strains, i.e., these sites are at greater than average risk for replicational mutagenesis.

IN188
PATHWAYS SUPPRESSING SPONTANEOUS MUTATION AND CANCER IN MICE

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Organisms require faithful DNA replication to avoid deleterious mutations. This is achieved through a network of conserved pathways that repair DNA damage and correct replication errors. In yeast, replicative leading- and lagging-strand DNA polymerases (Pols epsilon and delta, respectively) have intrinsic proofreading exonucleases that cooperate with each other and mismatch repair to restrict spontaneous mutation to less than one per genome per cell division. The relationship of these pathways in mammals and their functions in vivo are unknown. Here we show that mouse Pol epsilon and delta proofreading suppress discrete mutator and cancer phenotypes. We found that inactivation of Pol epsilon proofreading elevates base-substitution mutations and accelerates a unique spectrum of spontaneous cancers. The types of tumors that develop are entirely different from those triggered by loss of Pol delta proofreading. Intercrosses of Pol epsilon-, Pol delta- and mismatch repair-mutant mice show that Pol epsilon and delta proofreading act in parallel pathways to prevent spontaneous mutation and cancer and to ensure normal embryogenesis. These findings uncouple Pol epsilon and delta functions in vivo and reveal tissue-specific requirements for DNA replication fidelity.

IN189
GENOMIC INSTABILITY AND CANCER IN MOUSE DNA REPLICATION MUTANTS

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Genomic instability (GIN) and uncontrolled cell growth are hallmarks of cancer cells. Although proper regulation and execution of DNA replication is required to prevent these outcomes, most studies on genetic causes of GIN have focused on DNA damage response (DDR) and cell cycle checkpoint genes rather than the DNA replication machinery. Previously, we reported the first evidence that a defect in the core DNA replication machinery can cause cancer. Using a forward genetic screen for mutations causing GIN in mice, we isolated a hypomorphic allele (*Chaos3*) of the essential gene *Mcm4,* which encodes a

subunit of the MCM2-7 DNA replication-licensing complex and replicative helicase. Nearly all C3H-*Mcm4*$^{Chaos3/Chaos3}$ nulliparous females get mammary adenocarcinomas. The mutation causes a ~30% reduction in all MCM2-7 members, attenuates the ATR checkpoint response, and elevates chromosome breaks in replication-stressed cells. To better understand the events leading to GIN and tumorigenesis, we have explored the biochemical defects of the *Chaos3* mutation in yeast, and investigated the potential role of MCM reductions. Budding yeast containing the orthologous amino acid change exhibit recombination-dependent, elevated chromosome aberrations linked to replication fork defects. We have generated mice containing mutations of all the other *Mcm* genes, and crossed them in various combinations and to DNA repair-defective backgrounds. These studies indicate that 1) the levels of MCM proteins are coordinately regulated; 2) rather small reductions in MCMs can cause synthetic lethality and/or cancer; and 3) different types of cancers can arise in different genetic backgrounds and with different mutant combinations. Tumor genome resequencing is being conducted to explore the nature of secondary mutations that we hypothesize to be the "drivers" of cancer in these replication-defective mice. Overall, these studies will provide a comprehensive assessment of the importance of properly tuned replication licensing in maintaining the integrity of the mammalian genome and in cancer prevention.

IN190

CHALLENGES IN THE DESIGN AND STATISTICAL ANALYSIS OF POPULATION STUDIES WITH HIGH-THROUGHPUT ASSAYS

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The dramatic evolution of high throughput assays has greatly changed the scenario of human population studies. Molecular epidemiologists experience a period of great uncertainty, due to the inadequate knowledge regarding the most appropriate study design to be used with new techniques, and particularly due to the lack of statistical models specifically developed for the analysis of massive databases originated from high-throughput assays. This presentation will discuss major limitations of traditional study designs when biomarkers generated by new technologies are involved. More space will be given to the use of innovative methods for statistical data analysis. In particular the pro's coming from the use of a game theory algorithm will be discussed together with some details concerning the evolution of methods based on measures of centrality. The rapid evolution of high-throughput techniques has generated a puzzled situation, with methods now technically reliable and increasingly cheap that still need validation for preventive, diagnostic, or therapeutic purposes. On the other hand, the evidence accumulated on classic biomarkers should not be dismissed, especially since many of them are extensively used to study genotoxic exposures, susceptibility or to predict cancer incidence. An approached based on the mixed evaluation of data from high- and low-throughput assays seems to be the most efficient approach. The association between micronuclei frequency and the gene expression pattern of a selected number of candidate genes in a population exposed to air pollution will be discussed as an example.

IN191

CLUES TO CANCER AETIOLOGY AND CARCINOGENIC MECHANISMS DERIVED FROM DNA ADDUCTS

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DNA adducts detected in human tissues indicate carcinogen exposure and may be also be indicative of carcinogenic risk. Accessible surrogate tissues, such as blood cells, provide the means to investigate occupational or environmental exposure in healthy individuals. Such exposure, e.g. to polycyclic aromatic hydrocarbons, has been demonstrated in several industries and in defined populations by the detection of higher levels of adducts. Adducts detected in many tissues of smokers are at higher levels than in non-smokers, although the magnitude of the elevation does not predict the magnitude of the risk and the exact nature of the adducts detected remains obscure in some cases. The detection of identified DNA adducts in target tissues, e.g. in urothelial tissues of patients with Balkan endemic nephropathy, and in oesophageal tissue of Chinese populations at high risk of oesophageal cancer has provided important insight into environmental cancer causation. The influence of genetic polymorphisms on DNA adduct levels may also shed light on factors affecting cancer susceptibility. Recently we found that smoking-related DNA adducts were higher in the lungs of lung cancer patients with the TERT-CLPTM1L lung cancer susceptibility variant on chromosome 5p15. Current controversies on which DNA adduct measurements may shed light are whether women are at greater risk than men of lung cancer from smoking, and whether smoking causes breast cancer. DNA adduct measurements provide a useful means of exploring mechanisms of carcinogenesis in in-vitro and in-vivo systems. Animal experiments show that the occurrence of DNA adduct formation in a tissue does not always mean that it is a target organ for carcinogenesis. The dependence of DNA adduct formation by benzo[a]pyrene in human cells on p53 reveals a hitherto unknown link between p53 expression and metabolism by cytochrome P450. Experiments with synchronised cells indicate that activation of benzo[a]pyrene is cell-cycle dependent. While cytochrome P450 enzymes are involved in the metabolic activation of benzo[a]pyrene in vitro, recent results with genetically modified mice suggest that their role may primarily be one of detoxification in vivo.

IN192

THE USE OF DNA ADDUCTS IN RISK ASSESSMENT

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Mange chemical carcinogens are metabolized into metabolites that react with DNA to form covalently bound DNA adducts. The chemical structure of these adducts have in many cases been elucidated. Early studies indicated that the ability to bind to was linked to the carcinogenic potency, the so-called carcinogen binding index. It was also observed that the biological activity was associated with the level of specific adducts, rather than total adduct levels. The association between specific adducts and biological endpoint relevant for the carcinogenic process in target cells indicate that DNA- adducts are relevant in the mode of action (MOA) concept for cancer risk assessment. One of the ongoing questions in cancer risk assessment is the question about threshold. The use of DNA adducts suggest that a threshold may exists, as initial damage will be repaired efficiently at low dose exposure. However DNA adducts cannot be used in isolation in the risk assessment process but must be used in an integrated fashion with other information, i.e., dosimetry, toxicity, tumor incidence. Recent epidemiological studies have indicated that the adduct level is an indicator of cancer risk, that reflects both exposure and genetic susceptibility. The usefulness of carcinogen-DNA adducts, as an indicator of risk at low exposure situations, will depend on the technological development of sensitive and specific analytical procedures.

IN193

GENOMIC ALTERATIONS AS EARLY INDICATORS OF ADVERSE EFFECTS OF EXPOSURES

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To respond to potential adverse exposures properly, health care providers need accurate indicators of exposure levels and early indicators that precede the development of overt injury or disease. This is par-

ticularly important in the case of acetaminophen (APAP) intoxication, the leading cause of liver failure in the USA. We hypothesized that gene expression patterns derived from blood cells would provide useful early indicators of acute exposure levels. To test this hypothesis, we used a blood gene expression data set from rats exposed to APAP to train classifiers in two prediction algorithms and to extract patterns for prediction using a profiling algorithm. Prediction accuracy was tested on a blinded, independent rat blood test data set and ranged from 88.9 to 95.8 %. Genomic markers outperformed predictions based on traditional clinical parameters. Analysis of human blood samples revealed separation of APAP-intoxicated patients from control individuals based on blood expression levels of human orthologs of the rat discriminatory genes. These results support the hypothesis that gene expression data from peripheral blood cells can provide valuable information about exposure levels, well before liver damage is detected by classical parameters. To directly test this hypothesis, a clinical study has been initiated to investigate the gene expression changes in blood from healthy volunteers exposed to therapeutic doses of acetaminophen in a well controlled, clinical environment. Our preliminary results support the potential use of genomic markers in the blood as surrogates for clinical markers of potential acute liver damage.

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IN194

MASS SPECTRAL DETECTION OF DNA ADDUCTS PRODUCED BY EXPOSURES TO CARCINOGENS

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Human DNA is continuously being damaged by exposure to endogenous and exogenous electrophilic molecules and reactive oxygen species. The extent of background damage in DNA reaches more than 1 modification/million nucleotides. Accurate assessment of these damage products, many of which may be used as specific markers of the biologically effective dose of genotoxins, is very valuable prior to approaches for risk determination. Many highly specific and sensitive analytical procedures for determining individual DNA damage products (covalently bound adducts from genotoxic compounds and oxidative DNA damage products) are already available. These include 32P-postlabelling, HPLC-fluorescence, HPLC-electrochemical detection, immunoassay based procedures, modified Comet assays and mass spectrometry (MS) normally coupled to a liquid chromatograph (LC). MS techniques are playing an increasing role in these determinations owing to their chemical specificity. Two main approaches have been used: the determination of modified 2'-deoxynucleosides (separated from enzymically digested DNA, or present in urine), or of adducted purine bases (derived from thermal degradation of DNA or repair). The former group of compounds show a common fragmentation in MS/MS of neutral loss of 116u, allowing high throughput, selective and sensitive detection, which may be further improved by the use of on-column switching in LC-MS/MS. This has allowed our recent development of analytical methods for N2-ethyl-2'-deoxyguanosine (a monitor of acetaldehyde exposure), various polycyclic aromatic hydrocarbon adducts, and 8-oxo-2'-deoxyadenosine (a marker of oxidative DNA damage). Screening systems ('adductomics') also seem possible by monitoring this MH+ - [MH-116]+ fragmentation. Accelerator mass spectrometry, which has the advantage of much increased sensitivity, may be used for the detection of 14C- or 3H-labelled adducts following administration of labelled carcinogen. Using this technique we have differentiated between adduct formation at the N7-position of guanine from exogenous and endogenous ethylene oxide, and showed the effect of endogenous production on dose-response relationships for exogenous adduct formation.

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IN195

THE NUCLEOTIDE EXCISION REPAIR: TFIIH AND CO

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Abstract not available at the time of publication

IN196

INSIGHTS INTO GENOTYPE-PHENOTYPE RELATIONSHIPS IN THE REPAIR/TRANSCRIPTION SYNDROME TRICHOTHIODYSTROPHY

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Trichothiodystrophy (TTD) is a rare autosomal recessive multisystem disorder characterized by hair anomalies, nail dysplasia, ichthyosis, physical and mental retardation, proneness to infections, signs of premature aging and, in many cases, photosensitivity but no cancer. Sun-sensitive TTD cases are mutated in one of three genes (XPB, XPD and GTF2H5/TTDA) that encode distinct subunits of TFIIH, a multi-protein complex essential for RNA polymerase II transcription and nucleotide excision repair (NER), the main pathway removing DNA lesions induced by ultraviolet (UV) light. TTD cells are unable to repair UV-induced DNA damage and are characterised by reduced amounts of TFIIH. Several lines of evidence support the hypothesis that TFIIH may become limiting in TTD terminally differentiated tissues, thus being insufficient to provide adequate transcriptional activity of highly expressed genes. Furthermore, emerging in vivo and in vitro data indicate that mutations responsible for TTD interfere with the role of TFIIH in basal transcription and in gene expression regulation. By gene expression profiling, we have identified about one hundred genes differentially expressed in primary skin fibroblasts of TTD patients mutated in XPD. The majority of these genes are down-regulated, in agreement with the notion that the pathological phenotype of TTD mainly reflects subtle defects in transcription. The altered expression of some of these genes was paralleled by quantitative alterations of the corresponding product. By focusing on proteins involved in skin formation and maintenance, we have obtained the first evidence of alterations in components of the extracellular matrix (ECM), the structure that forms the bulk of the dermis and plays fundamental roles in normal tissue homeostasis, cell adhesion and cell migration. As well as identifying new markers for TTD diagnosis, these findings contribute to understand the aetiology of those symptoms that TTD shares with other disorders caused by mutations in ECM genes. Biochemical and molecular studies are elucidating how TTD-specific mutations in the XPD subunit of TFIIH interfere with the normal expression of genes/pathways relevant for ECM.

IN197

TRANSCRIPTION STALLING AND CELLULAR RESPONSES

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Transcription coupled nucleotide excision repair (TC-NER) is a critical survival pathway that protects against acute toxic and long-term effects (cancer) of UV light or chemicals. Deficiency in TCR is a hallmark of the rare human disorder Cockayne syndrome (CS) associated with severe clinical symptoms such as dwarfism, mental retardation and photosensitivity. Two complementation groups (A and B) have been identified. The CSB protein displays ATPase, DNA binding activity and nucleosome remodelling activity, whereas the CSA protein is part of a E3 ubiquitin ligase (E3-ub ligase) complex. CSB fulfils a key role as coupling factor to attract nucleotide excision repair (NER) proteins and the CSA- E3-ubiquitin ligase complex to the stalled RNA polymerase. CSA is dispensable for attraction of NER preincision proteins to lesion-stalled RNAPIIo, yet in cooperation with CSB is required to recruit NER postincision factors. Both CS proteins have distinct functions in recruitment of chromatin remodelers and TFIIS. The emerging

picture of TCR is complex: repair of transcription blocking lesions requires the NER factors, chromatin remodelers and at least two essential assembly factors i.e. the CSA and B proteins. Together, these and yet unidentified proteins will accomplish not only efficient repair but also contribute to DNA damage signalling events.

IN198

INTERSECTING DNA REPAIR PATHWAYS AND COORDINATION WITH TRANSCRIPTION

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Human XPG protein is a structure-specific endonuclease whose enzymatic activity is strictly required in nucleotide excision repair (NER) to make the 3' incision in removal of bulky lesions. Defects in this function result in the cancer-prone disease xeroderma pigmentosum (XP). However, XPG appears to be highly pleiotropic, and it also has critical non-enzymatic functions. Mutations in XPG that inactivate these functions result in the severe postnatal developmental disorder Cockayne syndrome (CS), and mice and humans with knockout or severe truncations of XPG die in very early life. We are investigating the molecular basis for this postnatal requirement for XPG. Suggesting a role in initial steps of transcription-coupled repair (TCR), XPG interacts with stalled RNA polymerase II (RNAPII), the TCR proteins CSB and TFIIH, and transcription-sized bubbles in DNA. In addition, XPG directly and functionally interacts with multiple proteins throughout the pathway for base excision repair (BER) of oxidative DNA damage. Supporting the importance of XPG in this pathway, XP-G/CS cells lacking XPG protein are hypersensitive to ionizing radiation and to hydrogen peroxide treatment. Extracts of these cells are both deficient in incision at oxidative lesions and defective in Long Patch-BER, consistent with the demonstrated stimulation by XPG of glycosylase activity and of DNA Ligase I activity, respectively. Of significance for a possible mechanistic connection between BER of oxidative DNA damage and transcription, XPG exists in a large cellular complex that includes the NEIL2 glycosylase, RNAPII, and other TCR proteins. However, whether the multiple roles of XPG in BER are functionally connected to its role in early steps of TCR remains to be definitively determined. The existence of two large domains in XPG that appear to be unstructured and flexible and that mediate all of its many known protein-protein interactions is consistent with the emerging concept that conformational changes in intrinsically disordered regions of scaffold proteins allow reversible interactions that determine partnerships, control pathway progression, and regulate pathway intersections. Intriguingly, these unstructured domains of XPG have been implicated in CS by patient mutations and mouse models.

IN199

DIETARY FACTORS, MUTATION AND CANCER

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Compelling evidence supports an accumulation of somatic mutations during carcinogenesis, leading to the activation of oncogenes and/or inactivation of tumour suppressor genes. Genome-wide association studies have identified genes associated with familial cancers, but genes and mutations involved in sporadic events are less well characterised. Many dietary components are mutagenic, including natural dietary components, mutagens generated during cooking and processing of food, or through contamination. Association studies, supported by molecular epidemiology, provide evidence that certain dietary mutagens, including aflatoxin B1, aristolochic acid and benzo[a]pyrene, are causal in some human cancers. Similar studies have correlated the level of oxidative DNA damage, DNA adducts and clastogenesis with cancer risk. Micronutrient deficiencies lead to comparable effects on mutagenesis to adding dietary carcinogens. The balance between mutagens and antimutagens in the diet remains critical, and may require molecular epidemiological approaches to interpret.

IN200

BASE EXCISION REPAIR, OXIDATIVE STRESS AND CANCER

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Oxidative stress is involved in the pathology of human cancers. Functional assays performed in blood leukocytes of cancer patients and matched controls show that specific BER pathways are decreased in cancer patients, and may be risk factors. These include 8-oxoguanine (8-oxoG) repair in lung cancer patients, as well as repair of lipid peroxidation (LPO) derived exocyclic adducts in patients developing lung and colon cancers. However, during carcinogenic process in target tissues repair mechanisms may be regulated differentially. BER activity for 8-oxoG, ethenoA and ethenoC is enhanced in colon cancers in comparison to unaffected surrounding tissues. In contrast in lung tumors, repair of ethenoadducts is similar to that in unaffected lung tissue, but 8-oxoG repair is decreased. Regulation of BER proteins activity may be related to oxidative stress. Transcription of several DNA glycosylases and AP-endonuclease is activated by reactive oxygen species. In rodent model this is accompanied by the appearance of preneoplastic colon lesions, aberrant crypt foci. Enzymatic activities of repair proteins may, however, be abolished by oxidative stress. One of the major lipid peroxidation products, 4-hydroxynonenal (HNE) inhibits in vitro, methylpurine- and thymine DNA glycosylases (ANPG and TDG), but not APE-1 and OGG1. Moreover, incision of oligonucleotides with ethenoA and ethenoC by cell extracts is decreased when cells are pretreated with HNE. HNE also sensitizes cells to oxidizing and alkylating agents. Oxidative stress-driven modulation of BER enzymes activities may be, then, an important factor determining the risk of cancer.

IN201

DIETARY INTAKE OF ARISTOLOCHIC ACID AS A RISK FACTOR FOR BALKAN ENDEMIC NEPHROPATHY-ASSOCIATED UROTHELIAL CANCER

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Balkan endemic nephropathy (BEN) is a chronic renal interstitial fibrosis with slow progression to end-stage renal failure associated with a high risk of urothelial cancer found in certain rural areas of Bosnia, Bulgaria, Croatia, Romania and Serbia. Substantiated by the investigations on aristolochic acid nephropathy (AAN) the proposal has been put forward that the primary cause of BEN is exposure to food crops contaminated with seeds of Aristolochia spp, which contain high levels of aristolochic acid (AA). On both clinical and morphological grounds, AAN is very similar to BEN. Recently, tumour DNA samples from patients with BEN were found to harbour principally A to T mutations in the TP53 tumour suppressor gene (Grollman et al., Proc Natl Acad Sci USA 2007; 104:12129-34). A to T tranversions are typical mutations observed after AA exposure in experimental animal models and are consistent with AA-DNA adduct formation primarily at adenine residues. Using a novel mutation assay that has been designed to induce and select mutations in human TP53 sequences in vitro by exposure of cultured cells to a mutagen, we found A to T mutations were elicited by AA at sites in TP53 rarely mutated in human cancers in general, but which were observed in the BEN patients. This concordance of specific mutations in patient tumours and AA-exposed cultures strongly support the argument that AA has a direct role in the aetiology of BEN-associated cancer.

IN202

THE ROLE OF GENETIC AND NON-GENETIC MECHANISMS IN FURAN RISK

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After the discovery of the formation of acrylamide during heat processing of food, more recent studies have also indicated the presence of the simple organic molecule furan in a wide variety of heat-treated foods. Furan is a potent hepatotoxicant and liver carcinogen in rodents, but existing data on furan toxicity do not provide a suitable basis for risk assessment of human exposures to furan with food. To improve risk assessment, a 6th FP-STREP project (FURAN-RA) was initiated to assess the role of genotoxic and non-genotoxic mechanisms and their dose-response relationships in furan carcinogenesis. In rat liver, a dose-dependent increase in the 14C-content of DNA was observed following treatment of male F344 rats with 14C-furan at a known carcinogenic dose (2 mg/kg bw) and at a dose close to estimated human exposures (0.1 mg/kg bw). Importantly, 14C-label was not associated with normal nucleosides, suggesting covalent binding of furan to DNA. Repeated administration of furan in the same dose-range resulted in increased hepatocyte proliferation along the edge of the left and caudate liver lobes accompanied by very thin foci of non-specific inflammation, indicating that short-term furan administration may induce proliferative changes in rat liver in the absence of appreciable cytotoxicity. Increased mitotic activity was associated with reversible change in the expression of cell cycle and apoptosis-related genes independent of DNA methylation, but surprisingly – and in contrast to higher doses - no significant alterations in DNA damage related genes were found. Collectively, our data suggest that both genotoxic and non-genotoxic events contribute to furan carcinogenicity and that chronic administration of furan at doses close to human exposure may promote tumor formation in rat liver.

This work was supported by FP6 of the European Union (SSPE-CT-2006-44393) and DFG (MA 3323/3-1).

IN203

REPORTS FROM THE 5TH INTERNATIONAL WORKSHOP ON GENOTOXICITY TESTING

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Just a few days before this ICEM the 5th in the series of IWGT workshops will have taken place in Basel. As usual, some challenging topics will have been discussed and recommendations, based on data-driven consensus, will (hopefully) have been reached. A report on one of the topics “Improvement of In Vivo Genotoxicity Assessment – The link to Standard Toxicity Testing” has already been given by the chair, Andreas Rothfuss, in the session on New Developments in Regulatory Genetic Toxicology earlier in this ICEM. Reports from the other working group leaders will be given here, namely: A re-evaluation of the top concentration for testing non-toxic substances in mammalian cells *in vitro* – David Kirkland (Covance, UK). Top concentration and cytotoxicity issues in mammalian cell chromosomal aberration/micronucleus and mutation assays – Sheila Galloway (Merck, USA) and Martha Moore (NCTR, USA). A re-appraisal of the recommendations for photogenotoxicity testing– Peter Kasper (BfArM, Germany) Recommendations for choice of better test systems to improve the predictivity of *in vitro* mammalian cell tests – Stefan Pfuhler (Procter & Gamble, Switzerland) The collection and use of historical control data in the interpretation of *in vitro* positive results. – Makoto Hayashi (BSRC, Japan) Suitable follow-up risk assessment testing for *in vivo* positive results – Veronique Thybaud (Sanofi-Aventis, France)

IN204

THE MOLECULAR BASIS OF LIFE’S ROBUSTNESS

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The key approach of classical molecular genetics and molecular biology, i.e., the phenotypic change caused by gene inactivation, taught us plenty about the functions of genes/proteins in the wild type organism. However, to learn how a standard species could become more resilient, we turned to the study of robust organisms, e.g., bacterium *Deinococcus radiodurans* and the aquatic animals Bdelliod Rotifers (collaboration with Prof. M. Meselson, Harvard University), both extremely resistant to desiccation and radiations. So far we have learned the following:
(1) DNA is equally susceptible to double-strand breakage (DSB) in robust and sensitive species. What matters for survival is the efficacy of the DNA repair system[1].
(2) *D. radiodurans* did not acquire any recognizable new DNA repair activities[2], instead:
(3) Both *D. radiodurans* and the rotifer *Adineta vaga* show a remarkable intracellular protection system against the oxidative damage (carbonylation) to their proteome (both constitutive and radiaiton-induced), as compared with their standard counterpart species: the bacterium *E. coli* and the nematode *C. elegans*[3]. In all four species death correlates with the sharp increase in protein oxidation at lethal doses of radiation.
(4) The protective agent is a small molecule that acts also as a protector of proteins from sensitive speces.
(5) When normalized against killing fraction, all four species show the same quantitative correlation with the oxidation of their proteome[4] caused either gamma or UV irradiation.
(6) Protein carbonylation appears as the cause of cellular degeneracy and death, a feature that seems to apply also to human aging.
(7) We propose that “intrinsic aging” – the fundamental chemistry of aging, age-associated diseases, and death – is the progressive oxidation of the cellular proteome leading to its increasing fatal functional degeneracy by the failing cellular interactome and catalysome.

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IN205

MUTATIONS IN MICRORNA PRECURSORS IN HEMATOPOIETIC MALIGNANCIES

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We have examined different leukemias for mutations in microRNA genes. We have found a number of germ lines and somatic mutations of microRNA precursors. Such alterations affect the processing of the microRNAs, resulting in lower levels of mature microRNA expression. The role of these alterations in cancer predisposition will be discussed.

ABSTRACTS

Forum abstracts

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FOR01

ANTIMUTAGENESIS AND CHEMOPREVENTION IN A U-SHAPED WORLD

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The perception that is pervasive among the public is that, when it comes to anti-cancer nutrients or anti-cancer drugs, more is better. A growing body of scientific evidence, however, suggests that the dose response between DNA damage and the dietary intake of cancer-fighting nutrients, such as selenium, zinc, and beta-carotene, is in fact U-shaped. Therefore, more of these "good things" may not necessarily be a good thing. Ironically, health-conscious men and women may be at highest risk for the ill-effects of over supplementation because they are already consuming high quality diets rich in vitamins and minerals. This forum emphasizes the need for a new approach to cancer prevention that is personalized. This forum will explore mechanisms related to U-shaped dose responses and will show how non-linear dose-responses have important implications for public health.

FOR02

DEFINING THE OPTIMAL DOSE OF SELENIUM FOR PROSTATE CANCER RISK REDUCTION

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Our work in dogs has revealed an intriguing U-shaped dose response between selenium (Se) status and prostatic DNA damage that remarkably parallels the relationship between dietary Se and prostate cancer risk in men, suggesting that more Se is not necessarily better. Recently, we extended this work to test the hypothesis that the Se dose that minimizes prostatic DNA damage upregulates apoptosis in prostatic epithelial cells. In a randomized feeding trial, 62 elderly beagle dogs (equivalent to 65 year-old men) received nutritionally adequate or supranutritional levels of Se for 7 months. Prostatic DNA damage (alkaline Comet assay), apoptosis (TUNEL staining of formalin-fixed prostate tissues) and Se status (neutron activation analysis of toenails) were measured. The extent of apoptosis was compared between dogs with low Se status (<.67 ppm in toenails, the level above which selenoenzymes like GPX are maximally expressed), moderate Se status (.67-.92 ppm), and high Se status (>.92 ppm, which exceeds one s.d. above mean Se level in U.S. men). Dogs with moderate Se status were 84% less likely to have high prostatic DNA damage than dogs in the low Se group (OR, 95% CI = 0.16, 0.04-0.63); prostatic damage in the low and high Se groups was not significantly different. Foci of intense apoptosis ("hot spots" with >30 apoptotic cells/200X field) were seen 4.1X (95% CI, 1.1-15.3) more often in the moderate Se group than in the low Se group; there was no significant difference between the frequency of apoptosis in the high and low Se groups. These findings are leading to a new line of thinking about how selenium may reduce cancer risk. Mid-range Se status (.67-.92 ppm toenails) favors homeostatic housecleaning – an upregulated apoptosis that preferentially purges damaged cells. Our observations converge with the null results from oversupplementing men in SELECT to set a new research priority: to test whether men with low, suboptimal Se can achieve cancer risk reduction through daily Se supplementation. Clearly, not all men will benefit from additional Se. Recognizing the U-shaped dose-response offers fresh insight into clinical trial design by categorizing individuals as Se-responsive or Se-refractory based upon the likelihood of reducing their cancer risk by Se supplementation.

FOR03

MUTAGENIC MODE OF ACTION FOR CARCINOGENS: HOW HIGH IS THE BURDEN OF PROOF?

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In 2005 U.S. EPA issued its final Guidelines for Carcinogen Risk Assessment (Cancer Guidelines) and Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens (Supplemental Guidance). Among other topics, the Cancer Guidelines provides guidance on evaluating data to determine mode of action (MOA) of biologically active chemicals. The 2005 Cancer Guidelines specify a two step extrapolation process: modeling data in the observed range and choosing a point of departure; followed by linear or non-linear extrapolation. MOA information is used in the choice of linearity or non-linearity at low dose. The Supplemental Guidance evaluates evidence that early life exposure may result in increased cancer risk by comparison to later life exposure, and recommends that for carcinogens that induce cancer through a mutagenic MOA, age dependent adjustment factors be used to increase slope factors calculated from standard cancer bioassays or epidemiologic data. Based on the recommendations from these two documents, it is likely that increased use of MOA analyses will have a major impact on future risk management decisions. This calls for new thinking concerning assumptions implicit in cancer MOA evaluation. It can be argued that there are currently three potential conclusions from the MOA analyses for carcinogenicity: (1) sufficient evidence to support a MOA, and mutation is NOT the key event; (2) sufficient evidence that mutation IS the key event; and (3) insufficient evidence to determine the MOA. Before the publication of the Supplemental Guidance, the focus of the cancer MOA evaluation was on weighing the evidence that the MOA did not include mutation as the key event and on assessing the weight of the evidence (WOE) for other MOAs. When mutation could not be excluded, the risk assessment practice was to default to a linear extrapolation model (generally the linearized multistage procedure). With the publication of the new Supplemental Guidance, it becomes necessary to consider the WOE for actually establishing that mutation IS a key event for carcinogenicity, rather than an ancillary observation. This new requirement modifies the strategy, focus and the burden of proof for the MOA assessment. This forum will explore the implications of the new requirements for MOA assessment with a focus on evaluating mutagenicity as the MOA for cancer and will provide a lively discussion as to just how much information should be required before establishing a chemical as a "mutagenic carcinogen".

FOR04

RATIONALE OF GENOTOXICITY TESTING OF NANOMATERIALS

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The Forum discussion will address the following questions.

1) Are the tests recommended by the OECD appropriate and sufficient to screen for the genotoxic effects of NM? How can we interpret the positive results of the studies?
Are nanomaterials primary or secondary genotoxic? What is the best methodological approach to evaluate this? Do we have to propose other tests?

2) What is the biological relevance of NM found systemically such as in the cardiovascular system or the brain?

3) What should be the minimal set of parameters that we have to report in a study? Includes stability of aggregates and agglomerates in biological environment. Do they disintegrate? Are agglomerates more toxic than a single particle of the same size?

4) Which kind of NM could be used as reference material? What should be the main characteristics of a reference NM? Is it possible to have one reference NM for all the tests or should we define different representative NM in function of the endpoints studied?

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FOR05
RATIONALE OF GENOTOXICITY TESTING OF NANOMATERIALS: REGULATORY REQUIREMENTS AND APPROPRIATENESS OF AVAILABLE TEST SYSTEMS

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The development of a risk management system for nanoscale particle-types requires a base set of hazard data. Assessing risk is a function of hazard and exposure data. When proposing a strategy for conducting nanoparticle research, mechanistic studies on "representative" nanoparticles should be considered. Alternatively, with regard to nanoparticle-types in commerce, an environmental, health and safety (EHS) framework may include a minimum base set of toxicity studies. These could include the following criteria: substantial particle characterization, pulmonary toxicity studies, acute dermal toxicity and sensitization studies, acute oral and ocular toxicity studies, along with screening type genotoxicity, and aquatic toxicity studies. We have previously reported the toxicity results of a base set of hazard tests on a set of newly developed, well-characterized, ultrafine TiO2 (uf-TiO2) particle-types. In vivo pulmonary and oral toxicity studies in rats demonstrated low hazards. Acute dermal irritation studies in rabbits and local lymph node assay results in mice indicated that uf-TiO2 was not a skin irritant or dermal sensitizer. Genotoxicity tests demonstrated that uf-TiO2 was negative in both the Bacterial Reverse Mutation Test and in an In Vitro Mammalian Chromosome Aberration Test with Chinese hamster ovary cells. To summarize the findings, the results of most of the studies demonstrated low hazard potential in mammals or aquatic species following acute exposures to the ultrafine TiO2 particle-types tested in this program. More recently, we have conducted short-term inhalation studies in rats with aerosols of amorphous silica nanoparticles. In addition to assessments of pulmonary hazards, we also conducted a flow cytometric-based micronucleus assay following 1 or 3-day inhalation exposures. The results of the flow cytometric-based micronucleus assay did not indicate any increases in the number of micronuclei in immature erythrocyte cell populations. In conclusion, the results obtained from these two sets of studies support the use of already established in vitro and in vivo methodologies for evaluating the biological and genotoxic potential of nanoparticles for hazard and risk assessment purposes.

FOR06
POSSIBLE GENOTOXIC MECHANISMS: CRITERIA FOR IMPROVED TEST STRATEGIES

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Particle-induced genotoxic effects arise by two mechanisms – direct and indirect mechanisms. Direct mechanisms involve interactions between the particles and the target cell that lead directly to genotoxic change including oxidative adducts formation and mechanical interference with the mechanisms of segregation of chromosomes during anaphase and telophase. Indirect mechanisms involve the induction of inflammation by the particles and the involvement of the inflammatory cells in leading to genotoxic effects. The mechanisms are not necessarily mutually exclusive. Many *in vitro* studies have shown that nanoparticles (NP) have the potential to cause genotoxic and a number studies where the same material (TiO_2, carbon black) in the form of NP were genotoxic but not in the form of larger particles. This is very similar to the SA dose-dependent effects experienced with NP in pro-inflammatory effects in the rat lungs and highlights the likely role of surface area and surface reactivity. This highlights the need to employ surface area dose and plausible dosimetry in the selection of doses of NP; the determination of plausible dose is complicated by hotspots in deposition. Nel has described a hierarchical particle –induced oxidative stress model where different degree of oxidative stress can cause transcription of phase II antioxidant enzymes (Tier 1), MAPK and NF-κB activation leading to inflammation (Tier 2) and finally perturbation of the mitochondria resulting in apoptosis or necrosis at the highest levels (Tier 3). High doses of some NP types in *in vitro* genotoxicity assays very likely induce high levels of oxidative stress consistent with Tier 3, whilst the lower doses encountered *in vivo* might be more likely to induce inflammation. Reference to Paracelsus' dictum suggests that we should be careful of effects found only at high doses that may not be relevant for human exposures and the interpretation of *in vitro* genotoxicity assays must be carefully related to dosimetry. There need to be inclusion of adequate control particles that allow us to contextualise the outcome of studies with unknown NP to those found with conventional particles of known carcinogenicity in order that risk assessment can be done. A number of NP are in common use and have been examined in long term animal studies but found to be negative for carcinogenic effects, apart from overload –induced effects and such particles should be the starting point for selection of negative controls. The relevance of combustion-derived NP like diesel soot, containing metals and organics, to manufactured NP is questionable. In the long run a structure:activity model of NP genotoxicity needs to be constructed that allows prediction on the basis of physicochmemical characteristics and for this to be attained we need good chracterisation and to this end some minimal criteria for particle characterisation accompanying data on genotoxicity have been suggested.

FOR07
ECOGENOTOXICOLOGY: PAST SUCCESSES AND FUTURE NEEDS

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Genotoxic compounds including carcinogens, are the components of complex environmental matrices, including surface water, aquatic sediment, soil, and urban air that can have adverse health effects on humans and on indigenous biota. A wide number of common marine pollutants are studied only for acute effects, and their long-term adverse environmental hazard is not known. The impact of pollutant mixtures frequently may be manifest only after relevant important effects on ecosystems and on human health have occurred. The application of short-term bioassays, *in vitro* or *in situ*, allows for the assessment of mutagenic hazards and/or for the identification of the sources and fate of the contaminants without *a priori* information about the identity or physico-chemical properties of the contaminants. *In vitro* tests that measured different genetic endpoints were used to evaluate urban air particulates and water or soil samples to characterize the toxicological features of environmental complex mixtures and to assess human health risk. Inconsistencies between studies have been often observed because of the use of different extraction procedures and methodologies. Aquatic organisms such as teleosts and bivalves were used as bioindicators to monitor surface water contaminated with genotoxic chemicals. Earthworms have been proposed as sentinel species for detecting genotoxicity in soil. No effective animal assay for in situ monitoring of air pollution has been identified, however a number of plant bioassays are used for this purpose. A limited number of well-established biomarkers were applied in different sentinel species in environmental programs, but a large number of non-standardized parameters are evaluated in risk assessment studies. The aim of this forum is to discuss the role of genotoxicity tests in environmental monitoring, the reliability and reproducibility of tests, the choice and validation of specific bioassays in the field, and the expression and interpretation of the results. The potential role of genomic and proteomic approaches to identify new biomarkers and to develop new bioassays in environmental genotoxicology will be also discussed.

FOR08

EVALUATING THE MUTAGENICITY OF AIR, WATER, AND SOIL BY THE *SALMONELLA* ASSAY: WHERE ARE WE AFTER 40 YEARS?

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The *Salmonella* (Ames) assay is nearly 40 years old, and prior to its introduction in 1970, little was known regarding the mutagenicity of our environment. However, after its introduction, the Ames assay was the first to reveal the mutagenicity of air (1977), river water (1977), drinking water (1978), and soil (1982). Since then, most studies on the mutagenicity of these environmental matrixes have used the Ames assay, resulting in thousands of publications. These studies have indicated that essentially all air, drinking water, and soil samples are mutagenic, as well as most river water samples. PAHs account for <5% of the mutagenic activity of air but correlate highly with the mutagenic activity of soil in TA98 +S9. Concentrations of chemicals monitored routinely in air do not correlate with air mutagenicity because few of the monitored compounds are mutagenic. The predominant mutagens in air are nitroaromatics, aromatic amines, and ketones. The smallest particles are the most mutagenic, and organic extracts of air and water induce primarily G to T base substitutions in TA100. Few air sheds around the world have been tested for mutagenicity. Sewage, industrial chemicals, and petrochemicals account for most of the mutagenicity of rivers, whereas disinfection by-products cause the mutagenicity of drinking water. Although much has been learned from these studies, the Ames assay needs to be incorporated into a systematic, routine analysis of air, drinking water, river water, and soil to complement current chemical analyses. [Abstract does not reflect the policies or views of the US EPA.]

FOR09

ECOGENOTOXICITY APPLIED TO ENVIRONMENTAL QUALITY CONTROL

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FEPAM, the environmental protection agency of the State of Rio Grande do Sul, Brazil, is developing programs for ecogenotoxicology assessment applied to environmental quality control to subsidize environmental legislation. These programs comprehend interdisciplinary studies which combine the efforts of specialists from different institutions to assess the impact of pollutants on air, water and soil and, thus, estimate possible acute, chronic or genotoxic damage occurrences in high-contaminated risk urban and industrial sites. The presence of toxic and genotoxic substances are primarily investigated from the contaminated sources and their action is verified at different environmental compartments. In regard to such ecogenotoxicity monitoring, our multi-disciplinary research group applies different strategies to it using biological approaches such as acute and chronic ecotoxicity essays, mutagenicity measured by *Salmonella*/microssoma assay in different compartiments, cytogenetic endpoints *in vitro* applied to water samples, genetic biomarks in plants, aquatic biota or human biomonitoring - all followed by chemical analyses to establish cause-effect relationships and different pollutant dispersion models. Also, as a consequence of contamination, studies have been carried out on fish malformation, human teratogenesis and cancer incidence. One of our goals has been achieved through the studies conducted: the *Salmonella*/microssoma essay, along with other acute and chronic ecotoxicological essays, have been included in an environmental legislation which defines and enforces criteria and patterns as to the emission of industrial liquid effluents discharged in the superficial waters of the state of Rio Grande do Sul made effective in November 24, 2006 (RESOLUÇÃO CONSEMA N º 129/2006, que “Dispõe sobre a definição de Critérios e Padrões de Emissão para Toxicidade de Efluentes Líquidos lançados em águas superficiais do Estado do Rio Grande do Sul”). Support: PADCT/FINEP/ FAPERGS/ CNPq.

FOR10

INDUSTRIAL SOLID WASTE LEACHATES INDUCED GENOTOXICITY: MODELS AND ASSAYS

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Industrial processes generate waste that are dumped and desired to be free from causing toxicity to the environment. However, a number of studies showed that these wastes either dumped in engineered sites or in an unplanned manner may cause leaching which subsequently can reach both ground and surface water for their percolation into different strata of the food chain thereby causing toxicity to flora and fauna. Hence, genotoxicity studies are aimed to assess the DNA damaging potential of these leachates for safety purposes. A number of sentinel species are being used to assess the real life genotoxic potential of the wastes with different DNA damage measuring endpoints. Data so far generated reveal that a number of species and a number of assays to be performed. Our laboratory has used a genetically well defined model organism, Drosophila and a widely accepted non-biased assay, Comet assay to determine the genotoxic potential of wastes generated from three industries situated at the vicinity of the city of Lucknow, India. Our data showed that of these, metal based industrial waste leachates have the maximum genotoxic potential to this organism. Subsequent examination of two different tissues viz. gut and brain of the exposed organism showed a comparable assessment of genotoxicity caused by the leachates. Studies from the other laboratory from this institute with plant (Allium cepa chromosome aberrations assay) and mammalian genotoxicity assays also suggest a comparable genotoxic potential of these solid waste leachates as observed in Drosophila. In addition, cellular toxicity of these leachates in Drosophila was assessed by stress gene expression and oxidative stress markers. While sentinel species remain at the forefront of assessing genotoxicity of wastes, genetically defined model like Drosophila based assay provides useful information on assessing genotoxicity of complex chemical mixture.

FOR11

AN APPROACH TO VALIDATE GENOTOXICITY BIOMARKERS IN ENVIRONMENTAL ANIMALS: THE EXAMPLE OF THE MICRONUCLEUS TEST.

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Micronucleus (MN) test, due to its simplicity, seems to be one of the most promising techniques to identify genomic alterations in environmental biomonitoring studies. Micronuclei frequency provides an index of accumulated genetic damage during the lifespan of the cells and it is an index of integrated response to the complex mixture of contaminants monitored or not that contributes to the toxic load. This test has the advantage that it can be applied in interphase to any proliferating cell population regardless of its karyotype. MN assay, originally developed with mammalian species, is today widely applied in fish and other aquatic organisms, including sea urchin, mussels, oysters, crabs and worms, in wild and transplanted animals. Hemocytes and gill cells in bivalves, erythrocytes in fish are the most frequently targets used for MN evaluation. Nuclear anomalies, chromatin buds, invaginations, vacuoles are also considered as biomarkers of genetic events. The validation process of the test involves the evaluation of the kinetics of cytogenetic effects in sentinel species and the identification of the potential sources of variability. Sensitivity and specificity of the assay, in revealing the exposure to the main classes of genotoxic pollutants, have to be determined in experimental studies under controlled conditions. Interspecies and interindividual variability in spontaneous MN frequency, related to differences in metabolic competency and DNA repair mechanisms as well as in the MN expression, has been observed.

Two main sources of variability impairing the measurement of the MN frequency in environmental animals have been identified. The first one is inherent to the laboratory method and sample collection, that could easily be controlled by the standardization of the protocol and quality assurance. The lack of uniform criteria of scoring for MN and other nuclear abnormalities is the main critical factor preventing the comparison of the results from different labs. Other sources of variability include biotic factors such as age and reproductive status and environmental factors associated to seasonal changes and nutritional status. Multiple samplings during the year allow to overcome environmental and seasonal effects.

FOR12

APPLICATIONS OF BIOMARKERS FOR THE MONITORING OF AQUATIC ENVIRONMENT: CHALLEHENGES AND NEW TRENDS

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Determination of sub-lethal biological responses following exposure to environmental contaminants contributes to assess exposure, estimate internal and biological effective doses to elucidate underlying basis of diseases, identify susceptible groups and to take preventive action to avoid detrimental outcomes. Despite the fact that in common with humans, similar biological responses could be observed in natural biota, until recently relatively little importance has been given to applications of biomarkers in environmental risk assessment (ERA). With growing interest for the adoption of an ecosystem-based approach for the management of aquatic environment, however, new legislations are emphasizing the need for biological effects of contaminants as the criteria for ERA. Given the complexity and dynamism of the aquatic environment, there is however need to adopt a more pragmatic approach to environmental monitoring with emphasis on the elucidation of cause-effect coupling for potential environmental degradation. It is also emerging that pollutants could simultaneously manifest their toxicity in varieties of way. This expression of toxic response could be cell, tissue and species specific depending on routes of exposure, life stages and ecological niche in addition to physico-chemical and natural variations. It is therefore essential that an integrated approach for the determination of ecosystem health is implemented in which biomarkers provide complementary information and an added value while obtaining information through bioassays, chemical and ecological measures. It is also important to benefit from the rapid technical and methodological developments taking place in human health arena for the use of biomarkers in the wild species. We have attempted to develop and implement a range of genotoxicological and biological responses to (a) determine the relative sensitivity of key species to a range of common contaminants and (b) link and compliment observed biomarker responses to bioavailability, body burden and the site chemistry. In ecotoxicology, such a rationale could offer the opportunity to adopt preventive approach to hazard and risk assessment for environmental sustainability and to simultaneously protect human health.

FOR13

RENAISSANCE MEDICINE BETWEEN TYPIFICATION AND DIRECT OBSERVATION

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Renaissance medical theories and practices, which basically consisted of a mixture of natural philosophy, physiology, herbal medicine, metaphysics, astrology and magic, were at a crossroads between the corpus of doctrines inherited from ancient and medieval authors Galen, Hyppocrates and Avicenna, first and foremost, whose works were already at the core of past tradition but were newly rendered into Latin, discussed and sometimes even harshly criticized and the development of "experimental method" though the effects of "scientific revolution" were more readily perceived in other disciplinary fields like physics and astronomy. After delineating, in a brief overview, the main cornerstones of Renaissance medicine and presenting the structure of the curriculum of physicians in early modern universities, the presentation will focus on two different but interrelated aspects concerning the attitude of doctors towards patients and diseases: on one hand, the use of general classifications of human types - the theory of four humours and temperaments, complexions, resemblances between men and animals (physiognomy), astral influences - and, on the other hand, the importance of the direct observation of the sick person, of the visible manifestations of the illness, of matter discharged from the body. The relationship between these two elements lies in the fact that medical treatises recommended a global examination of patients and of their body features also under normal conditions, since the interpretation of symptoms and the choice of a remedy could not neglect the peculiar physical characteristics of the patient. Another intersection between general rules and taxonomy and specific examples is represented by the importance of "time": the determination of the beginning of a disease and the individuation of the moments when it was more or less acute were of crucial importance, and appropriate timing in the administration of medicines was essential to recovery. In this latter case, physicians often resorted to astrological doctrines such as the idea of the "Lords of the hours", according to which a specific planet "superintended" specific days, hours and parts of the body: something between traditional Oriental medicine and circadian rhythms.

FOR14

ART, SCIENCE AND NATURE IN THE RENAISSANCE FROM BOTTICELLI TO LEONARDO

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In Florence during the Humanism a Renaissance of the arts and sciences took place, having as protagonists great artists like Sandro Botticelli, Piero della Francesca and Leonardo. They developed their work on ideas of the neo-platonic philosophical movement flourishing at the court of Lorenzo dei Medici, according to which art had to celebrate humans and their capacity to study nature and to experiment new methods of scientific research. In fact, precisely in Florence the first public Library of the modern age was established with the patronage of Cosimo dei Medici. The Biblioteca di San Marco gathered Italian translations of many scientific and philosophic texts: the Greek Euclide for Mathematics, Aristoteles and Plato for philosophy; the Latin Lucretius and Plinius for natural and physical sciences and Ptolemy for cosmology. The Medici sponsored costly expeditions to collect rare plants, like the voyage of Benincasa to Crete, favoring naturalistic collections and natural sciences, especially botany, through the foundation of botanical gardens and the attempt to plant classification. These efforts were coupled with a very high artistic quality of scientific illustrations, from the extraordinary designs by Leonardo of plants, flowers, animals and fossils to the watercolors of Jacopo Ligozzi. The interest of the Florentines for anatomy, mathematics and geometry was exceptionally high and inspired the new studies on perspective of the architect Leon Battista Alberti and of the painter Piero della Francesca. The supreme synthesis of the Renaissance was accomplished by Leonardo in all fields of human learning, using an approach that we can now define as "scientific". In his innumerous Codici and Trattati, he reported and theorized about his experimental observations in botany, bird flight, anatomy, hydraulics, mechanics, engineering and optics. In his famous Uomo Vitruviano, created using science and geometry, he put humans at the center of the Universe, in equilibrium with the micro and micocosmos, becoming the symbol of universal harmony, a primary goal of Renaissance humans. Numerous examples of the art of this period in Florence will be shown during the conference.

FOR15

WORKSHOP ON CYTOTOXICITY MEASURES IN THE *IN VITRO* MICRONUCLEUS TEST

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The draft OECD guideline for the in vitro micronucleus (MN) test has been almost 10 years in development. A final version was very nearly adopted in 2007, but issues relating to the recommended measures of cytotoxicity were still outstanding and unresolved. It was therefore necessary to conduct a collaborative trial across several European laboratories, and different cell types. The objective was to test 11 different genotoxic chemicals with different modes of action and different profiles of activity, and to compare the more well known measures of cytotoxicity (namely replication index in the presence of cytochalasin B or relative cell count in the absence of cytochalasin B) with the newer recommendations for relative increase in cell count or relative population doubling. In addition, 3 further chemicals, which could not from the literature be clearly expected to induce MN, were evaluated by 2 US laboratories. In this workshop the results of this joint EU/US/OECD trial will be presented.

FOR16
RISK ASSESSMENT OF GENOTOXIC TRACE SUBSTANCES IN FOOD

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The deliberate addition of genotoxic substances to food is generally considered not acceptable by regulatory authorities, whatever the dose level. Yet, trace amounts of genotoxic compounds may occur in everyday diet as natural food components, as by-products from food processing, or as environmental contaminants. The assessment and management of the health risks posed by genotoxic trace substances in diet, as well as by the numberless *unknowns* with undefined identity and toxicity, are challenging tasks which call for both good science and pragmatism. In this Forum, mechanistic aspects of low doses effects, including practical thresholds, and examples of regulatory options for trace genotoxins and /or *unknowns* in food will be shortly reviewed and discussed, also with reference to the application of the Threshold of Toxicological Concern approach.

FOR17
THE MARGIN OF EXPOSURE APPROACH TO SUBSTANCES IN FOOD THAT ARE GENOTOXIC AND CARCINOGENIC

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Substances that are genotoxic *in vivo* are not permitted for deliberate use in food production, whether as food additives, pesticides, veterinary medicines or in materials that come into contact with food. However an appreciable number of known or suspected genotoxic carcinogens occur inadvertently in food, resulting from natural occurrence, environmental contamination, generation during cooking and processing, or as impurities in permitted food chemicals. There are also incidents of adulteration of food with genotoxic substances, including unauthorised dyes and veterinary medicine residues. Long-standing UK and EU advice has been that, unless there is evidence to the contrary, a non-threshold approach should be adopted for risk assessment of substances that are genotoxic and carcinogenic, i.e. that there is some risk, albeit small, even at extremely low levels of exposure. However the mathematical models that attempt to provide a best estimate of cancer risk by extrapolation below experimental data points are not scientifically justified, and therefore the advice has generally been that exposure should be kept as low as reasonably practicable (ALARP). The ALARP approach has been criticised as not informative for prioritisation of risk management action because it does not take into account carcinogenic potency or exposure. The Joint Expert Committee on Food Additives of the Food and Agriculture Organisation and World Health Organisation (JECFA) and the European Food Safety Authority (EFSA) have both proposed a margin of exposure approach to inform on possible magnitude of health concerns at different levels of intake. The margin of exposure is defined as a reference point on the dose-response curve (e.g. a benchmark dose lower confidences limit derived from a rodent carcinogenicity study) divided by the estimated human intake. A small margin of exposure indicates a higher concern than a very large margin of exposure. Whilst the margin of exposure cannot be directly equated to risk, it supports prioritisation of substances for further research or for possible regulatory action. If the MOE is very large, it can also be used to underpin on communication of a low level of human health concern.

FOR18
POSSIBLE MECHANISMS UNDERLYING PRACTICAL THRESHOLDS FOR GENOTOXIC CARCINOGENS

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Toxicological studies with rodents including genotoxicology are usually conducted at high doses based on the maximum tolerable dose for the animals. However, the doses used for the assays are sometime more than 1,000 times higher than the doses at which humans are actually exposed in daily life. Therefore, a question rises whether the genotoxicity and/or carcinogenicity at the high doses can be linearly extrapolated at the low doses. In regulatory sciences, it is supposed that genotoxic compounds can induce mutations and/or chromosome aberrations even at single molecules and that there are no thresholds for genotoxic carcinogens. Thus, no ADI (acceptable daily intake) can be established for food additives, pesticides and veterinary medicines when the compounds are genotoxic carcinogens. The assumption, i.e., linear non-threshold (LNT) model, seems counterintuitive, however, because all organisms including humans possess defense mechanisms such as detoxication metabolism, antioxidants, DNA repair, error-free translesion DNA synthesis, apoptosis and so on, which may suppress the genoxocity and carcinogenicity of chemicals and constitute practical thresholds at low doses. In addition, it is not clear how to define the genotoxicity. There are a number of genotoxicity assays, e.g., Ames Salmonella assay, mouse lymphoma gene mutation assay and in vivo micronucleus assay, and chemicals usually exhibit different results (genotoxic or non-genotoxic) depending on the assay employed. In this forum, I will discuss (1) possible mechanism underlying the practical thresholds for genotoxic and carcinogenic compounds and (2) in vivo transgenic mutation assay, i.e., *gpt* delta transgenic rats, for the suitability to identify genotoxicity at target organs of carcinogenicity.

FOR19
US FDA SAFETY ASSESSMENT OF GENOTOXIC FOOD CONTACT SUBSTANCES

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The Office of Food Additive Safety at the US FDA Center for Food Safety and Applied Nutrition is charged with regulating industry to ensure that food contact substances are safe. The food contact substance (FCS) includes polymers (plastic packaging materials), pigments and antioxidants used in polymers, can coating, slimicides and biocides
(antimicrobial agents), and sealants for lids and caps. Introduction of genotoxic food contact substances or their components into food may occur from their contact with food, and our approaches for the safety assessment of such components are described in the Guidance to Industry: Toxicology Recommendations. The safety assessment of such substances includes the assessment of all the components that may migrate to food as a result of the use. An overview will be presented on the assessment of genotoxic components with a dietary concentration at or below 0.5 parts per billion (ppb) or 1.5 µg/person/day assuming an intake of 3 kg of food/person/day. At these low dietary levels, additional (new) genotoxicity studies are not recommended, but it is necessary to review all available information on genotoxicity studies, carcinogenicity studies as well as structural similarity to known mutagens or carcinogens. For risk assessment, a genotoxic component is regarded as a potential carcinogen and cancer risk is estimated based on linear non-threshold risk extrapolation model and QSAR (Quantitative

Structural Activity Relationship) analysis. Risk assessment is only applicable if the so-called Delaney anti-cancer clause in U.S. law is not triggered. For dietary concentrations at or below 0.05 ppb (0.15 µg/p/d), even a genotoxic component can be regarded as safe if it presents only a negligible risk at this dietary exposure. Our definition of the term "safe" is a "reasonable certainty in the minds of competent scientists that a substance is not harmful under the intended condition of use".

FOR20
PREPARING THE NEXT GENERATION OF SCIENTISTS THROUGH EDUCATION AND RESEARCH

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At the opening of *Tale of Two Cities* Dickens wrote "*It is the worst of times. It is the best of times."* These words have probably rung true often in human history since first written, but perhaps ring truer than ever now as applied to science, and science education, in the world. The worst of times always provide opportunity, and that is what is there for the next generation of scientists. Young scientists of today are the foundation upon which the careers of those future scientists will be constructed. In her book *We are the Ones We Have Been Waiting For* Alice Walker notes that *" because we live in an age in which we are able to see and understand our own predicament with so much greater awareness than our ancestors with such a capacity for insight, knowledge, and empathy we are uniquely prepared to create positive change within ourselves and our world."* This forum will allow to discuss the how indeed 'we are the ones' to change the face of how we do science, teach science, and serve society with our science.

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ABSTRACTS

Abstracts of posters and selected oral presentations

"The abstracts are divided according to the following six tracks:
Mutational and epigenetic mechanisms
DNA damage responses
Environmneetal mutagenesis
Mutagenesis and health effects
Prevention of mutation-related diseases
Risk assessment
The abstracts are allocated in each track according
to the indication of the authors, when available.
Text of abstracts is reproduced as it was submitted by the authors"

Mutational and epigenetic mechanisms

ME001
DEXRAZOXANE ERADICATES THE GENOMIC DAMAGE INDUCED BY ETOPOSIDE VIA THE APOPTOTIC MODE

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Etoposide is a highly successful anticancer drug that has been in clinical use for nearly 30 years. However, full therapeutic efficacy of this drug is limited due to the development of various side effects in the host as the development of acute myeloid leukemia due to its genomic damage in normal cells. Dexrazoxane was originally developed as an anti-tumour agent. However, it now is clinically used to reduce doxorubicin-induced cardiotoxicity. The protection of mouse bone marrow from etoposide-induced genomic damage by dexrazoxane was established in our laboratory by a battery of short-term cytogenetic tests. One of the possible mechanisms of their anti-mutagenic action could be an increase in apoptotic elimination of heavily-damaged cells. Therefore, a series of three related but separated methods were applied for the detection of apoptosis in genotoxically-damaged bone marrow cells in animal treated with etoposide and/or dexrazoxane. First, the exposure of phosphatidylserine on the surface of early apoptotic cells caused by the test chemicals was assessed using the annexin V assay. Second, the counting of nuclei with a sub-diploid DNA content was quantified after staining with propidium iodide. Lastly, the activity of caspase-3 in signalling transduction was investigated by the caspase-3/CPP32 assay. Data presented here indicate that etoposide induce death in marrow cells with morphological and biochemical characteristics typical of apoptosis. Dexrazoxane did not significantly change the frequency of apoptotic cells in animals not post-treated with etoposide, whereas it markedly and significantly enhanced the degree of apoptosis of etoposide-treated animals. Dexrazoxane enhanced the percentage of both early and late apoptotic cells and fragmentation of nuclear DNA induced by etoposide as detected by the exposure of phosphatidylserine on the surface of apoptotic cells and the appearance of nuclei with sub-diploid DNA content, respectively. Moreover, caspase-3 activation, an event that has been shown to play a critical role in apoptosis signalling transduction, becomes evident in animals pre-treated with dexrazoxane. The results suggest that the enhancement of apoptosis by dexrazoxane can explain a major part of its anti-genotoxic activity.

ME002
avß3 INTEGRIN MEDIATED DRUG RESISTANCE IN HUMAN TONGUE SQUAMOUS CELL CARCINOMA

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Integrin-mediated drug resistance is based on the adherence of cells to extracellular matrix proteins through integrins. In human laryngeal carcinoma cells a mechanism of multidrug resistance mediated by avß3 integrin have been described. In the present study we investigated whether similar mechanism exists in tongue squamous carcinoma cells (Cal27) which express a small amount of avß3 integrins, and Cal27-derived stable transfectants with increased expression of avß3 integrin. The cell clones were produced by stable transfection of Cal27 cells with a plasmid expressing the ß3 subunit. In one stable transfectant the expression of avß3 was increased but the expression of avß5 remained the same in comparison to parental cell line. The other stably transfected cell line showed an increase in avß3 expression and a moderate decrease in avß5 expression, due to the competition of ß3 for available av in the cell. The sensitivity of Cal27 and Cal27-derived avß3 integrin expressing clones to anti cancer drugs was determined using MTT assay. Our results showed that both Cal27-derived avß3 integrin expressing cell lines were resistant to cisplatin, doxorubicin and mitomycine C. This thesis shows that increased avß3 integrin expression can protect cells from various citostatics. Thus measuring the expression of avß3 integrin in head and neck cancer cells could be an important indicator of tumor resistance to anti-cancer drugs.

ME003
COMPARISON OF REPAIR ACTIVITIES OF PROKARYOTIC AND EUKARYOTIC NUCLEOTIDE EXCISION REPAIR SYSTEMS FOR DNA-PROTEIN CROSSLINKS

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DNA-protein crosslinks (DPCs) are unusually large DNA lesions and are produced by a number environmental mutagens and chemotherapeutic agents. Proteins irreversibly trapped on DNA strands will hamper DNA transactions such as replication and transcription and exert an adverse effect on cells. In both prokaryotic and eukaryotic cells, bulky DNA lesions are generally repaired by nucleotide excision repair (NER). In NER, the damage recognition protein binds to the lesion and recruits the nuclease that incises the 5' and 3' sides of the lesion. The DNA fragment containing the lesion is released by helicase and the resulting gap is filled by polymerase. Finally the nick is sealed by ligase. Unlike conventional bulky lesions, it is possible that steric hindrance imposed by trapped proteins inhibit the binding of a damage recognition protein or the assembly of NER proteins at the DPC site. Keeping this in mind, we assessed the incision activity of prokaryotic and eukaryotic NER systems for DPCs in vitro. To prepare DNA substrates containing DPCs (DPC-DNA), oxanine was site-specifically incorporated into oligonucleotides and crosslinked to proteins. With the UvrABC nuclease, the incision efficiency initially increased and then decreased with increasing the size of crosslinked proteins (CLPs). The upper size limit of CLPs incised by UvrABC was around 12–14 kDa. The efficiency of DNA-UvrB complex formation was dependent on the CLP size, indicating that the damage recognition step by UvrA2B was key to the excision of DPCs by UvrABC. DPC-DNA was also incubated with HeLa cell free extracts (CFEs). The incision activity was dependent on the CLP size. The upper size limit of CLPs incised by HeLa CFEs was around 8-10 kDa, which was notably smaller than that for prokaryotic UvrABC. E. coli and human cells were treated with formaldehyde, a typical DPC inducing agent, and the removal of chromosome DPCs was analyzed. Consistent with the in vitro data for UvrABC, chromosome DPCs with CLPs smaller than 11 kDa were removed in wild type E. coli but not in the uvrA mutant. Conversely, the rates of removal of chromosome DPCs were similar in HeLa and NER-deificient XPA cells, suggesting that NER was not involved in the repair of DPCs in human cells.

ME004
MECHANISM OF DNA REPAIR BY FPG FROM E. coli

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Reactive oxygen species appear in the cell as metabolism byproducts and may be generated by environmental factors such as ionizing radiation. They react with DNA to produce a variety of genotoxic lesions that are considered as possible causative agents in aging and a number of diseases. In particular, 7,8-dihydro-8-oxoguanine is one of the most common products of base oxidation in DNA. It can pair with C, forming a Watson–Crick-type oxoG:C pair, but also easily forms a premutagenic Hoogsteen pair oxoG:A. In the latter case, replication of an oxoG:A mispair produces a T:A pair thus leading to a G:T transversion.

Although the mechanism of Fpg action and structures of several complexes in the reaction pathway are known, it is still not clear how Fpg can robustly select sparse substrate lesions among the vast excess of normal DNA or non-substrate lesions. One possibility is that the conformational transitions during substrate binding occur sequentially, with at least some steps structurally optimized for the selection of correct substrates. This model is supported by our previous stopped-flow studies of Fpg catalytic cycle that used tryptophan and 2-aminopurine as fluorescence reporters [1-3]. This pre-steady-state kinetic analysis of Fpg acting on various DNA substrates and ligands reveals several sequential conformational changes that precede the chemical steps in the reaction pathway. The most likely sequence of structural rearrangements leading to preferential recognition of oxoG:C by Fpg has been thus described [3]. In this study, we have used a micro-volume rapid quench instrument and mass spectrometry to examine the reactions catalyzed by Fpg. According to these mass spectrometry data combined with the kinetic scheme derived from the quench-flow analysis, hydrolysis of the Schiff base intermediate involving Pro-1 of the enzyme and the 4-oxo-2-pentenal residue can be rate-limiting under the steady-state conditions of the reaction. Support from RFBR (07-04-00191, 08-04-00596), SB RAS (2009-48) and Russian Ministry of Education and Science (MK-987.2008.4) is acknowledged.

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ME005
GENOTOXICITY OF DOPAMINE: ROLE OF THE DOPAMINE TRANSPORTER AND OF MONOAMINE OXIDASE

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We have shown that the neurotransmitter dopamine causes DNA damage in the low micromolar concentration range in the rat neuronal cell line PC12. The role of the dopamine transporter (DAT) in the genotoxicity of dopamine was investigated using the two DAT-inhibitors nomifensin and GBR12909. Both of them decreased the dopamine induced DNA damage. Real time PCR mRNA expression results revealed that treatment of PC12 cells with dopamine resulted in upregulation of DAT mRNA. This provides an explanation for the observed reduction of the genotoxicity of dopamine by cotreatment with the D2-antagonist sulpiride, which might have prevented the upregulation of DAT upon dopamine-treatment. To investigate the role of DAT in more depth, we used the dog kidney MDCK cell line which is believed not to express DAT and MDCK-DAT cells which have been transfected with human DAT gene. In MDCK cells, dopamine was not able to increase the number of micronuclei, whereas MDCK-DAT cells showed elevated micronucleus formation after treatment with dopamine. Once transported inside the cells, dopamine is either metabolized by the enzyme monoamine oxidase (MAO), other oxidizing enzymes or undergoes autoxidation. To distinguish which of these ways is more important in the genotoxicity of dopamine, we inhibited MAO with the specific inhibitor trans-2-phenylcyclopropylamine hydrochloride (PCPA), which reduced the number of micronuclei induced by dopamine. In conclusion, dopamine exerted genotoxicity in vitro in our cell models upon transport into the cells and oxidization by MAO.

ME006
GENE EXPRESSION PROFILE OF 1,2-DIMETHYLHYDRAZINE-INDUCED COLON CANCERS IN F344 RATS BY MICROARRAY ANALYSIS

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Background: Genome wide gene expression analysis may identify genes and molecular pathways relevant for colon cancer. Aim: To study gene expression profile in colon cancers induced in rats by 1,2-dimethylhydrazine (DMH), a widely used model of colon carcinogenesis, mimicking the various steps of colon carcinogenesis as observed in humans. Methods: DMH-induced F344 rats were sacrificed after 32 weeks to harvest colon tumours. Eight carcinomas and their paired normal mucosa (NM) were hybridized on Agilent Whole Rat Genome arrays containing 41,000 genes and transcripts. After spot quality control and normalisation, differential analysis was performed (t-moderated test with Benjamini-Hochberg correction). The functional analysis was performed with GenMAPP/MAPP Finder software. Results: 27,329 probes passed the quality control step, 55.8% of which were differentially expressed in tumours compared to NM; 256 (0.9%) and 566 (2%) with a fold change $\geq$ 2 and $\leq$-2, respectively. Defcr4, Slc30a2, Lum, Igfbp5, Nos2, Mmp7, S100A8 and S100A9 were among the most up-regulated genes, while Slc26a3, Mptx, Retlna and Muc2 were among the most down-regulated. RT-PCR on selected genes confirmed microarray results. GenMAPP analysis showed that pathways linked to ribosomal proteins, translational factors, mRNA processing, proteasome degradation, cell cycle, nucleotide metabolism and glycolysis were up-regulated. TNF-alpha/NF-Kb, TGF□ and hepatocyte growth factor signalling pathways were also up-regulated as well as the prostaglandin synthesis and matrix metalloproteinases. Krebs cycle, electron transport chain, and the fatty acid beta oxidation were down-regulated. Conclusion: Tumours exhibit intense protein metabolism, increased proliferation, an energy metabolism shifted toward glycolysis and increased inflammation. Given the relevance of this model for human pathology, the complete analysis of the differentially expressed genes in carcinomas as well as in earlier steps of the carcinogenesis process may be useful for cross-species analyses and for the identification of conserved and functionally relevant genes in the carcinogenesis process.

ME007
HIGH FREQUENCY OF GENOMIC DELETIONS INDUCED BY ME-LEX, A SEQUENCE SELECTIVE N3-ADENINE METHYLATING AGENT, AT THE HPRT LOCUS IN CHINESE HAMSTER OVARY CELLS

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Background. Many antineoplastic agents currently used in cancer therapy generate a wide panel of DNA lesions with different mutagenic and/or toxic potential. One serious complication associated with the use of alkylating chemotherapeutic agents is the induction of pre-mutagenic lesions that could give rise to therapy–related secondary tumors. Me-lex is a methylating agent that preferentially generate N3-methyladenine (3-MeA) mainly in A-T rich regions. Unlike other methylating agents (i.e., MMS, MNU) that induce highly mutagenic adducts, such as 7-MeG and 6-MeG, Me-lex induces more than 95 % of 3-MeA, a DNA lesion that is highly cytotoxic and poorly mutagenic in different yeast strains. Aim of this work is to study the toxicity and mutagenicity of Me-lex in eukaryotic cell lines by using the HPRT as target gene. Results. Me-lex treatment was cytotoxic but poorly mutagenic, resulting only in a 3-fold induction above background in the Hprt mutation frequency. The molecular nature of 43 Hprt mutations induced by Me-lex was determined by sequence analysis of the Hprt cDNA and genomic analysis of the gene locus. Base pair substitutions represented about 25% of Me-lex induced mutations. The mutation spectrum revealed a high percentage of genomic deletions comprising single/multiple exon(s) and even the loss of the complete locus (51%). The difference between the spontaneous and Me-lex induced CHO spectra was statistically significant ($p<0.012$), indicating that the sites where mutations occurred were Me-lex specific. To study the role of DNA repair on Me-lex mutagenicity, Hprt deficient mutants were also isolated from the EM-C11 cell line, a CHO-9 derivative cell line defec-

tive in the ligation step of the BER pathway (xrcc1-). The definition of the Hprt mutation spectrum induced by Me-lex in these cells is under investigation and will be discussed. Conclusions. Based upon our results we hypothesize that a large proportion of mutations induced by Me-lex may result from the processing of 3-MeA within A/T rich sequences in non-coding regions of the Hprt gene. The processing of these lesions by DNA polymerases could result in recombination and genomic deletions, thus representing a severe threat for genome integrity.

ME008

MUTAGENICITY OF ME-LEX, A SEQUENCE SELECTIVE N3-ADENINE METHYLATING AGENT IS A MULTI TRANSLESION POLYMERASES AFFAIR

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Background. A number of exogenous and endogenous methylating agents form N3-methyladenine (3-mA) in DNA. Bacteria, yeast and mammals have evolved and maintained specific DNA repair enzymes that are selective for removal of 3-mA from their genomes, a strong indication of the deleterious effect of 3-mA. Using Me-lex, an agent that efficiently and selectively generates 3-mA in A-T rich regions of double-stranded DNA due to its minor groove binding conferred by the lexitropsin dipeptide, we showed that the lesion itself is cytotoxic in *E. coli*, *S. cerevisiae* and in mammalian cells. In particular, we showed in yeast that the toxicity and mutagenicity of Me-lex, is dependent on the nature of the DNA repair background. The fixation of lesions into mutations depends on the activity of TLS DNA polymerases that can bypass specific DNA lesions that stall replication forks. In *S. cerevisiae*, there are three known TLS polymerases: Polη, Rev1 and Polζ. We recently demonstrated that Polζ and Rev1 contribute to alleviate the lethal effects of Me-lex by error prone bypass. Aim and experimental approach. In order to determine the role of Polη in the biological fate of Me-lex induced lesions, the RAD30 (Polη) gene was deleted in the yIG397 parental yeast strain and in its rev3 (Polζ) derivative and the strains were transformed with plasmid DNA damaged *in vitro* by Me-lex. Results. Our results indicate that Polη is involved in the processing of Me-lex induced lesion(s) and can reduce their toxic effect with a limited and dose-dependent impact on mutagenicity. Me-lex induced mutation spectra in WT and rad30 strains were not significantly different. However, we found mutational events suggestive of error free (significant increase of AT>GC in rad30 vs RAD30) and error prone (significant decrease of AT>TA at a mutation hotspot in rad30 vs RAD30) bypass dependent on Polη. This dual effect could explain why a significant increase in induced mutation frequency in the absence of Polη was observed only at the highest concentration of Me-lex used.
Conclusion. Altogether, our results suggest that mutagenicity of Me-lex lesions is a multi-DNA polymerases process that is most effective when all three TLS polymerases are present.

ME009

DNA METHYLATION AT THE C-5 POSITION OF CYTOSINE BY CHEMICAL REACTIONS: A POSSIBLE ROLE FOR EPIGENETIC CHANGE DURING CARCINOGENESIS BY ENVIRONMENTAL AGENTS

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During carcinogenesis, methylation of the C-5 position of cytosines in the promoter region of tumor suppressor genes is often observed. Enzymatic DNA methylation is a widely accepted mechanism for this phenomenon. However, the exact mechanisms of hypermethylation, particularly in relation to environmental factors during carcinogenesis, are not clear. It is interesting to propose a free radical mechanism for 5-methyldeoxycytidine (5mdC) production, because the C-5 position of cytosine is an active site for free radical reactions. When deoxycytidine (dC) and cumene hydroperoxide (CuOOH), a tumor promoter and a methyl radical producer, were reacted in the presence of ferrous ion at pH 7.4, the formation of 5mdC was clearly identified by HPLC equipped with a photodiodo array UV detector. When a radical scavenger, TEMPO or POBN, was added to the reaction mixture, the 5mdC formation was inhibited. From these results, it can be concluded that this reaction proceeds via a free radical mechanism, probably via a methyl radical. The same reaction also proceeded with t-butyl hydroperoxide. After a double-stranded homopolymer, poly(dG) • poly(dC) or an alternating copolymer, poly(dG-dC) • poly(dG-dC), was reacted with CuOOH in the presence of ferrous ion, the formation of 5mdC was examined by an immuno-dot blot analysis. The formation of 5mdC was clearly detected in both DNA polymers after the treatment. The formation of 5mdC from dC or in the DNA polymers was also confirmed by LC/MS/MS analysis. This is the first report of chemical DNA methylation at cytosine C-5 by environmental tumor promoters. We propose that this reaction is one of the important mechanisms of de novo DNA methylation during carcinogenesis, because methyl radicals are produced by the biotransformation of various endogenous and exogenous compounds.

ME010

POLYMORPHISMS IN THE MTHFR GENE: SUSCEPTIBILITY TO COLORECTAL CANCER AND ROLE IN THE RESPONSE TO THERAPY

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Background: Sporadic colorectal cancer (CRC) is a multifactorial disease resulting from the combined effects of numerous genetic, environmental and behavioral risk factors. Genetic association studies have suggested that low-penetrance alleles of an extremely large variety of genes are involved in susceptibility to CRC in Caucasian populations. The Czech Republic in the last decade has reported one of the highest incidences of CRC in the world. Aims/Methods: The objective of the present study was to evaluate whether methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C polymorphisms are associated with an increased risk of sporadic colorectal cancer in a population from the Czech Republic. We also evaluated the possibility that these polymorphisms may be a prognostic and predictive marker for CRC patients treated with 5-fluorouracil-based adjuvant chemotherapy. The analysis has been done on 666 colorectal cancer patients, 614 controls with negative colonoscopy and 767 healthy blood donors from the Czech Republic. Results and Conclusions: Preliminary results have shown a decresead risk of CRC in individuals carrying MTHFR 677TT genotype (odds ratio, OR: 0.60; 95% confidence interval, CI: 0.37-0.87; p = 0.009) when compared with controls with negative colonoscopy. Similar results have been found when CRC patients were compared with the second control group (OR: 0.62; 95% CI: 0.41-0.93; p = 0.02). These results confirmed previous studies on the same topic and in different populations. At the moment the effect of the MTHFR haplotype is under investigations. We evaluated also the possibility that these polymorphisms may be a prognostic marker in CRC patients treated with 5-fluorouracil-based adjuvant chemotherapy. For this purpose we analysed the effect of MTHFR polymorphisms in a subgroup of 290 CRC patients treated with 5-FU. The analysis of the obtained results is currently ongoing. This study was supported by the grants: Czech Science Foundation, no.: GACR 310/07/1430 and GACR 305/09/P194.

ME011
ANALYSIS OF SOMATIC MUTATIONS SPECTRA IN MITOCHONDRIAL DNA OF WISTAR RATS
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Mutation frequencies vary significantly along nucleotide sequences sometimes resulting in the formation of so called hotspots. The most variable part of human and mouse mitochondrial genome is a noncoding region which is about 900 base pair between the tRNA genes for proline and phenylalanine, and including D-loop and control region. To present day it is a great interest to investigate the accumulation of somatic mutations in the D-loop for rat's mtDNA. The work was performed on 3 and 12 month old male rats of Wistar strain from the Laboratory of Animal Breeding (ICG SB RAS, Novosibirsk) and The Animals Breeding Center (Branch of Institute of Bioorganic Chemistry RAS, Pushchino). Total liver DNA was isolated from frozen tissues stored at -70°C. PCR of investigated fragment was performed using a high-fidelity DNA polymerase HF2 (Clontech, USA). Amplified fragment was cloned into the T-vector and then transformed to *E.coli* strain *XL1-Blue MRF'*. Individual clones containing the vector with insertions were taken by white-blue selection. Plasmid DNA was isolated and the presence of insertions and mutation in them was confirmed by sequencing according to Sanger. In mutations spectra of base substitutions in rats of both ages were a prevalence of transitions – about 95% of all mutations. The most part of base substitutions (about 75%) was observed in the AT pairs. The mutation frequency in investigation region of mtDNA for 3 and 12 month Wistar rats was $5,3*10^{-4}$ and $3,5*10^{-3}$ respectively. Also was observed the increasing of mutation frequency in the regulatory regions ETAS and in highly conserved sequences CSB1, 2 and 3 with age. The dislocation mutagenesis model was analyzed for the reconstructed mutation spectrum using a Monte-Carlo procedure (the KUNKEL program). Analysis of the 3-month-old Wistar (ICG SB RAS) spectrum revealed that many base substitutions (50%) are consistent with the dislocation model. No statistically significant support for the dislocation model was found in the 12-month-old Wistar spectrum. These results suggested that there are differences in mechanisms of mutagenesis in the two studied ages of rats.

ME012
DINAMIC CHANGES OF CAJAL BODIES AND HISTONE LOCUS BODIES DURING MITOSIS.
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Cell nucleus contains many specialized organelles, including Cajal Bodies (CBs), marked by the presence of coilin and involved in various functions including snRNA biogenesis. We and others demonstrated the existence of a new group of organelles named: Histone locus bodies (HLB), characterized by at least two main proteins: FLASH and NPAT. HLBs associate with CBs in a cell cycle dependent manner (3).While their function is still in part elusive, we have shown that they are involved in replication-dependent histone gene transcription. Phosphorylation of a HLB component p220/NPAT by cyclinE/Cdk2 is required to activate histone transcription, exit from G1 and progression through S phase. Also another HLB component: FLASH is essential to this function and its down regulation results in structural alterations of HLBs and CBs, reduction of replication-dependent histone gene transcription, and block of cells in the S phase (1, 2). In the last few years the characterization and classification of the different nuclear bodies has progressed, but there is still much to investigate. Moreover the destiny of these highly dynamic structures, at the beginning of mitosis, when the nucleus and nucleoli disappear, is not known. We therefore decided to investigate the presence and localization of different proteins characterizing CBs and HLBs during mitosis. HT1080 and Saos2, human cell lines, already used in our previous studies (3), were grown on coverslips, fixed in 4% paraformamide in PBS and subjected to Immunofluorescence with antibodies against FLASH, NPAT, coilin, SMN and α or β tubulin and secondary fluorescent antibodies. We examined cells in the different phases of mitosis. We observed that HLBs disappear during prophase to reappear only at the end of mitosis. On the contrary CBs labelled by antibodies directed against coilin remain visible during the whole cell division and are not associated with chromosomes. This is a further confirmation of the existence of these two different bodies and suggests that their components might play different roles during mitosis.
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ME013
EPIGENETIC CHANGES AND MICRONUCLEI RELATED TO ANTI-B[A]PDE-DNA ADDUCT LEVELS IN PAH-EXPOSED INDIVIDUALS.
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From some years chromosomal instability and/or epigenetic changes by exposure to genotoxins has been an issue in the literature, but the clear demonstration that such alterations are caused by one or more specific agents in people has been demanding In this study the effect of carcinogenic polyaromatic hydrocarbons(PAHs) exposure on DNA methylation (percentage of methylated cytosines(%mC)) and telomere shortening in Polish male non-smoking coke-oven workers and matched controls was investigated. Methylation of gene-specific promoters (*p53*, *p16*, *HIC1* and *IL-6*) and of *Alu* and *LINE-1* repetitive elements, as surrogate measures of global methylation, were quantified by pyrosequencing and telomere length by real-time quantitative PCR in peripheral blood lymphocytes (PBLs). We also evaluated if PAH-induced DNA methylation and telomere changes were in turn associated with micronuclei (MN) in PBLs, an indicator of chromosomal instability. Workers were heavily exposed to PAHs, as their urinary 1-pyrenol concentrations exceeded the biological exposure index (BEI) in 79% workers vs. 0% controls ($P<0.001$). Workers, besides to exhibit higher levels of genetic alterations in PBLs,i.e., *anti*-B[*a*]PDE-DNA adduct ($p<0.0001$) and MN ($p<0.0001$), presented significantly higher epigenetic changes, such as telomere shortening and, global and gene-specific promoters methylation (mainly *p53*), compared to controls ($p<0.05$ and $P\leq0.001$). Global, *IL-6* and *p53* methylation states were significantly correlated to PAH-exposure (urinary 1-pyrenol and *anti*-B[*a*]PDE-DNA adduct levels, $p<0.01$). Telomere shortening were significantly correlated with raise in working years ($P=0.039$), *anti*-B[*a*]PDE-DNA adduct ($P=0.0415$) and *p53* hypomethylation ($P=0.0053$). In a linear multivariate regression analysis *p53* hypo-methylation, was the only significant determinant in increasing MN ($P<0.01$), and not methylation of other genes. Moreover MN levels raised with the increase in telomere shortening ($p<0.001$). DNA methylation changes (mainly *p53* hypomethylation), telomere shortening and MN in circulating PBLs, related to *anti*-B[*a*]PDE-DNA adduct, are some characteristics acquired by PBLs of subjects exposed to PAHs suggesting that these events could be determined to identify subjects at high cancer risk.

ME014

DI-(2-ETHYLHEXYL)-PHTHALATE EFFECTS ON RETINAL VASCULARIZATION IN NEWBORN RATS.

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Di-(2-ethylhexyl)-phthalate (DEHP), released from PVC medical devices, is toxic for several tissues and species. In pre-term babies, a correlation was found between endotracheal intubation with PVC tubes and incidence of chronic diseases and DEHP treatment of pregnant and lactating rats was found to impair the lung maturation of their pups. In the present research DEHP was orally administered to rats since day 15 of pregnancy. Retinal vessels of 7 and 14 day-old pups were examined in fluorangiographies, in flat-mounted retinas labelled with the endothelial marker isolectin B4 and in eye-cup sections incubated with biotinylated IB4. In angiographies taken from DEHP-treated 7 day-old animals the differences with age-matched controls mainly consisted in non-perfused segments of radial arterioles and less regular distribution of superficial and deep capillary nets. At 14 days, the alterations affecting the superficial vascular plexus, generally heavier than in younger animals and qualitatively different among the individual specimens, mainly consisted in strongly dilated and branched radial venules, not uniformly perfused radial arterioles and persistence of periarteriolar capillary-free regions. In few specimens, FITC leakages and non-vascularised areas were observed, while immunolocalization of α-SMA failed to reveal alterations in vessel mural cells. In eye sections from DEHP-treated 14 day-old rats the mean number of vessels belonging to the superficial plexus was significantly decreased with respect to controls. On the basis of these observations, we suggest that indirect assumption of DEHP by foetal and neonatal rats causes structural alterations (probably with a flow reduction) in their retinal radial vessels; modifications of other vessels include vascular shunts in the retinal mid periphery, but not include increased permeability or pre-retinal tufts. The high responsiveness to DEHP shown, in the present research, by the immature rat retina is in agreement with that referred for neonatal liver, kidney and lung and strongly supports the idea that DEHP, released by PVC medical devices utilised for intensive care of preterm babies, can influence the maturation of several of their organs.

ME015

EFFECTS OF NATURAL POLYPHENOLS ON PHYSIOPATHOLOGY OF RETINAL ANGIOGENESIS IN NEWBORN RATS.

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Angiogenesis is a key step in tumour growth, invasion, and metastasis, besides than in numerous diseases; its modulation constitutes an important therapeutic strategy for human health. Several polyphenols, produced by different plants as reaction against biotic and abiotic stress, are effective in inhibiting endothelial proliferation and migration. Our aim is to study pro- and/or anti-angiogenic properties of different compounds (camptothecin and derived compounds, resveratrol, quercetin, curcumin, ecc.), in cultured endothelial cells (e.g. HUVEC), which proliferation, migration and tube formation activities we are now evaluating, and in vivo in physiological conditions. Rat retinal angiogenesis was chosen as a useful system for analyzing events involved in the construction of organized vascular architecture. In rodents, in fact, at birth retinal vessels starts to develop from the optic disc, forming a plexus that rapidly evolves into large and small vessels and grows over the retinal surface to form a two-dimensional structure. In the mean time, retinal vessels sprout into deeper layers, finally leading to a three-layered system and vessels of superficial layer establish a hierarchical architecture consisting of arteries, veins, and capillaries; the vascular network can be examined as a whole in flat-mount preparations obtained from FICT-dextran-injected animals. Utilising these specimens, we described the effects of a camptothecin semisynthetic derivative (the topoisomerase I inhibitor Topotecan), peribulbary injected to 7 day-old rats. In fluorangiographies from treated animals many radial vessels showed both non-perfused and abnormally dilated segments; persistence of capillary-free periarteriolar regions, vessel mislocations with respect to retinal layers and spots of extravascular FITC were also detected.

ME016

SIGNIFICANCE OF DNA ADDUCTOME ANALYSIS IN IN VITRO MICRONUCLEUS TEST.

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In vitro micronucleus (MN) test is widely used as a screening assay for genotoxicity, but it often produces false positive results for chemical carcinogenicity. To consider the significance of positive results in *in vitro* MN test, it is very important to analyze the DNA-damaging capability of the test chemicals. In this study, the usefulness of DNA adductome analysis (Matsuda et al, 2006) for assessing the DNA-damaging capability of chemicals was evaluated. CHL/IU cells were treated with various classes of chemicals, A; carcinogens known to produce bulky DNA adduct (Benzo[*a*]pyrene, 4-Nitroquinoline-1-oxide etc.), B; carcinogens known to produce small DNA adduct (Ethyl methanesulfonate, *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine etc.), C; aneugens (Colchicine etc.), D; non-carcinogens (Caffeine etc.) for 6 hours with or without metabolic activation followed by a 20-hour recovery period, and the incidences of micronucleated cells were measured. Concurrently, DNA was extracted from the cells at the termination of the treatment period at doses producing positive results on MN test. The extracted DNA was enzymatically hydrolyzed to deoxyribonucleoside by the micrococcal nuclease/bovine spleen phosphodiesterase or P1 nuclease method, and subsequently applied to the LC/ESI-MS/MS analysis. The strategy was designated to detect the neutral loss of deoxyribose from positively ionized deoxynucleoside adducts by monitoring the DNA samples transmitting their $[M+H]^+ > [M+H-116]^+$ transitions. The transitions were monitored over the *m/z* range of 250-702. All test compounds gave positive results on MN test. Many DNA adducts were detected in cells treated with carcinogens, and some of those *m/z* corresponded to those of known adducts. There was no DNA adduct detected in cells treated with non-carcinogens or aneugens, indicating that these compounds produced micronuclei through mechanisms other than DNA adduct formation. These results indicate that this DNA adductome approach would be useful to identify the DNA reactivity of *in vitro* micronucleus test-positive compounds, and to facilitate understanding of their mechanism of action.

ME017

NITRATIVE AND OXIDATIVE DNA DAMAGE BY K-RAS MUTATION IN MICE.

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Activating mutations of Ras family genes have been found in human cancers, and K-ras mutations in codon 12 and 13 are frequently observed especially in lung cancer. It is reported that the activation of the K-ras oncogene leads to lung cancer in mouse model. Therefore K-ras mutations have been thought important for lung carcinogenesis. On the other hand, it is considered that carcinogenesis consists from multi-step process with mutations in more than 10 genes. In this study, we analyzed the formation of DNA damage after oncogenic activation of K-ras in mouse model. We prepared transgenic mice with oncogenic K-ras, conditionally expressed by Cre/LoxP system. The mutated K-ras has a point mutation in codon 12 with change of amino acid glycine to

valine. For conditional activation of K-ras, the adenovirus expression vector of Cre recombinase was infected to airway of the transgenic mouse. Adenocarcinomas were developed in the lung of the transgenic mice infected of Cre-adenovirus. To investigate the role of DNA damage in carcinogenesis initiated by K-ras mutation, we performed immunohistochemical analysis of nitrative and oxidative DNA damage in lung tissues of mice. A double immunofluorescence labeling study demonstrated that 8-nitroguanine and 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) was apparently formed in lung adenocarcinoma, whereas little or no immunoreactivity was observed in normal lung. The accumulation of 8-nitroguanine was co-localized with expression of inducible nitric oxide synthase (iNOS) and of oncogenic K-ras. These results suggest that oncogenic K-ras activation causes additional DNA damage via iNOS induction. It is reported that 8-nitroguanine and 8-oxodG can cause mutations. Our recent report revealed that the critical role of translesion DNA synthesis (TLS) polymerases in cellular tolerance to NO-induced DNA damage and suggested that the contrubution of these error-prone polymerases to accumulation of single base substitutions. It may be concluded that the accumulation of mutagenic 8-nitroguanine and 8-oxodG contribute to lung carcinogenesis initiated by oncogenic activation of K-ras.

ME018

4-HYDROXYESTRADIOL INDUCES ANCHORAGE-INDEPENDENT GROWTH OF HUMAN MAMMARY EPITHELIAL CELLS THROUGH ACTIVATION OF IκB KINASE-NF-κB SIGNALING: ROLE OF REACTIVE OXYGEN SPECIES.

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Multiple lines of evidence support that excessive production of estrogen is implicated in pathophysiology of human breast cancer. Estrogen is converted primarily by cytochrome P450 1B1 to 4-hydroxyestradiol (4-OHE_2), a putative carcinogenic metabolite of estrogen. This catechol estrogen metabolite is oxidized further to produce a reactive quinone via semiquinone. Redox-cycling between 4-OHE_2 and its quinoid generates reactive oxygen species (ROS). In the present study, we found that 4-OHE_2-induced ROS production contributed to enhanced proliferation and neoplastic transformation of human mammary epithelial (MCF-10A) cells as evidenced by elevated levels of proligerating cell nuclear antigen and anchorage-independent growth on soft agar, respectively. ROS overproduced by 4-OHE_2 increased the activation of NF-χB through induction of IχB kinase (IKK)-α and IKKβ activities. The pharmacologic inhibition of the IKK activities with Bay 11-7082 abrogated 4-OHE_2-induced anchorage-independent growth. In addition, 4-OHE_2 (20 μM) induced increased expression of heme oxygenase-1 (HO-1), an important stress-responsive antioxidant enzyme involved in heme degradation, by activating antioxidant response element (ARE)-mediated Nrf2 signaling. By utilizing human HO-1 promoter reporter plasmids, we demonstrated that 4-OHE_2 transcriptionally activated the upstream ARE-rich enhancer region of human HO-1 promoter. The direct binding of Nrf2 to HO-1-specific ARE sequence was verified by the chromatin immunoprecipitation (ChIP) assay. Induction of HO-1 by 4-OHE_2 was attenuated by siRNA knock down of Nrf2 gene. 4-OHE_2-induced HO-1 expression was also abrogated by the antioxidant *N*-acetyl-$_L$-cysteine and Trolox. In conclusion, ROS concomitantly overproduced during redox cycling of 4-OHE_2 activates IKK-NF-χB signaling which stimulates neoplastic transformation of MCF-10A cells. In contrast, the Nrf2-drived upregulation of HO-1 expression may provide MCF-10A cells with adaptive survival response against 4-OHE_2-induced oxidative stress and cell transformation.

ME019

NOVEL BIOCHEMICAL PATHWAYS FOR 5-FLUOROURACIL IN MANAGING EXPERIMENTAL HEPATOCELLULAR CARCINOMA IN RATS.

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Background : Five fluorouracil (5-FU) is extensively used in the treatment of hepatocellular carcinoma (HCC). 5-FU and its metabolites inhibit DNA synthesis by inhibition of thymidylate synthetase. A little is known about additional pathways for 5-FU in managing HCC.
Aim of the study: To study possible biochemical pathways that can be added to 5-FU mechanisms of action. Design : Four groups of rats were recruited as control (given saline), tichloroacetic acid,TCA (0.5 g/kg/d for 5 days, orally), 5-FU (75 mg/kg, IP, once weekly for 3 W) and TCA plus 5-FU (24 hours apart of TCA last dose). Methods: Biochemical (serum alpha fetoprotein, liver tissue contents of total glycosaminoglycan (TGAGs), collagen (as hydroxyproline (HP), total sialic acid (TSA), free glucosamine (FGA), proteolytic enzyme activity (pepsin and free cathepsin-D) and histological examination of liver tissue. Results: Histological changes as, central vein congestion, irregular-shaped, substantially enlarged, vesiculated and binucleated hepatocytes were seen. The nuclei were pleomorphic, hyperchromatic and vacuolated cytoplasm encircling the nucleus with masses of acidophilic material. 5-FU corrected these changes, except some necrotic and cytotoxic effects of 5-FU. AFP was significantly elevated in TCA- but reversed in 5-FU groups. Increased proteolytic activity by TCA was reversed by 5-FU, which restored TGAG to normal, but both TCA and 5-FU depleted collagen . TCA significantly elevated FGA but depressed TSA, this action was reversed by 5-FU treatment. Conclusion : Proteolytic activity , expressed as up-regulated pepsin and free cathepsin-D is increased in HCC. This is accompanied by ECM macromolecular disturbance , decreased TGAGs, collagen and TSA, with marked increase in FGA liver tissue content. Elevated FGA with depressed TSA content of liver tissue may be attributed to a cancer hampered N-acetylation of FGA into SA. 5-FU markedly depressed hepatic proteolysis, re-activated N-acetylation of FGA into SA and elevated TGAGs without stopping tissue fibrosis.This study explores additional pathways for the mechanism of action of 5-FU, through conservation of ECM composition in situ, inhibiting invasion and metastasis in addition to its DNA disturbing pathway.

ME020

DNA METHYLTRANSFERASES, GLOBAL DNA METHYLATION AND MICRONUCLEI IN MOUSE EMBRYONIC FIBROBLASTS EXPOSED TO TCDD AND CADMIUM.

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There is limited understanding of the significance of epigenetic mechanisms, such as DNA methylation, in biological and toxic effects of exposures to environmental agents. The aim of this study was to evaluate the effects of a dioxin (2,3,7,8-tetrachlorodibenzo-p-dioxin, TCDD) and a heavy metal (cadmium, Cd) on epigenetic modifications and genomic instability. Mouse C3H/10T½ embryonic fibroblasts were exposed to 1, 10 and 100 nM of TCDD or 0.5 and 1 μM Cd. A flow cytometric assay was used to analyze micronucleus (MN) frequency. The mRNA expression of DNA methyltransferases (DNMT1, DNMT2, DNMT3a and DNMT3b) was determined by quantitative RT-PCR and global DNA methylation was quantified using the Methylamp Global DNA Methylation Quantification Kit by Epigentek. Measurements of delayed effects several days after the end of exposure were included in all assays to address persistent changes and induction of genomic instability. No significant differences were found in MN frequency between fibroblasts exposed to TCDD for 24 h and controls at 2 or 8 days after

starting the exposure. When the cells were exposed to TCDD for 48 h an elevated MN frequency was found at 8 days (but not at 2 days). Cadmium induced elevated MN frequency 2 days after the beginning of 24 or 48 h exposures but no effect could be confirmed at day 8. Cadmium also dose-dependently decreased the mRNA expression of the de novo methyltransferases DNMT3a and DNMT3b, as well as DNMT2, after 3 or 6 day exposures, but after an additional incubation for 2 or 4 weeks without exposure, this down regulation was not detectable anymore. TCDD (100 nM) had only slight effects on DNMTs. In contrast, our preliminary results from global methylation measurements revealed that the DNA methylation profiles after 3 day exposures to TCDD or Cd persisted after 2 week incubation without exposure. TCDD as well as 0.5 µM Cd induced hypermethylation whereas 1 µM Cd hypomethylation. According to our preliminary results, TCDD induced DNA hypermethylation that was not transmitted through changes in DNMTs. This result, as well as the confusing dose-response between Cd and global methylation requires further studies. The results from the MN assay suggest that a 48 h exposure to TCDD induced genomic instability.

ME021

HYPERMETHYLATION OF TUMOUR SUPPRESSOR GENES IN LUNG TUMOURS FROM NEVER-SMOKERS WITH AND WITHOUT SECOND-HAND TOBACCO SMOKE EXPOSURE.

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Smoking is the main cause of lung cancer, but a fraction of lung cancer cases occurs in patients with no history of smoking. Epidemiological studies identify second-hand tobacco smoke (SHS), a widespread contaminant of indoor air, as a significant cause of lung cancer in never-smokers. To provide further insight into mechanisms by which SHS participates in the development of lung cancer we analysed the frequency of gene promoter hypermethylation in lung tumours from never-smokers with (n = 34) or without (n = 9) documented exposure to SHS in comparison to tumours from smokers (n = 35). Promoter hypermethylation in five tumour suppressor genes (TSGs), namely p16, RARb, RASSF1A, DAPK and MGMT, was analysed by means of methylation-specific PCR. The overall frequencies of promoter hypermethylation were 41% for RARb, 26% for DAPK, 25% for RASSF1A, 15% for p16, and 12% for MGMT gene. DAPK hypermethylation was more prevalent in tumours from female patients (32% vs 6% in males; $p<0.05$), while RARb hypermethylation occurred more frequently in adenocarcinomas than in other histologies (53% vs 24%; $p<0.05$). No major differences in the frequencies of hypermethylation were detected between tumours from smokers and never-smokers. However, the p16 gene hypermethylation was detected in lung tumours from smokers (20%) and from the SHS-exposed never-smokers (15%), but not in tumours from non-exposed never-smokers (0%). Overall, the profile of epigenetic alterations, particularly hypermethylation of the p16, RARb and DAPK genes, was similar in tumours from the SHS-exposed cases and smokers. In addition, mean methylation index was identical (MI = 0.25) in tumours from smokers and SHS-exposed never-smokers. In conclusion, the results of our study support the role of SHS exposure in inducing lung tumourigenesis in never-smokers, and for the first time show an association between well-documented SHS exposure and gene promoter hypermethylation in lung tumours from never-smokers.
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ME022

VIDENCES OF AN ANTITUBULIN ACTIVITY OF Xanthium strumarium L. EXTRACTS IN CHO CELLS.

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Chemotherapy with cytostatic agents is one of the most widely employed methodologies in the fight against cancer. This approach includes the employment of compounds that interfere with the cellular division. In this sense, the application of techniques that allow to evaluate the capacity of interfere and/or modify the mitotic cell course, constitutes a valuable practice in the search of new therapeutically active molecules. Plants are an important natural source of compounds that, potentially, can drive to development of new cytostatic drugs. It has recently been highlighted that complex mixtures present in vegetal extracts can have an even greater therapeutic power than that of the isolated active principle(s) they contain, due to possible positive interaction with cofactors present in the mixture. *Ex vivo* assays demonstrated that the vegetable species *Xanthium strumarium* L. possesses an effective anti-tubulin activity. The aim of the present work is to study the effect of a hydroalcoholic extract obtained from *X. strumarium* on CHO cells proliferation, evaluating parameters related to cell cycle progression and mitotic division. Experimentally, CHO cells cultures were treated with different extract concentrations and the following parameters were analyzed: growth of the cellular population, cell cycle progression, mitotic progression and mitotic spindle morphology. We observed a dose-dependent inhibition of cell growth and a dose-dependent decrease of anatelophases, abnormal mitotic spindle assembly and significant blockade of cellular cycle in G_2-M phase. These experimental evidences are consistent with the *in vitro* data on *X. strumarium* extract anti-tubulin capacity and reinforce the valuation of this vegetable species as a potential source for the development of new cytostatic drugs.

ME023

EFFECTS OF ARSENITE ON DNA STRAND BREAKS, EXPRESSION OF XRCC1 AND GLOBAL DNA METHYLATION IN HUMAN LYMPHOBLAST CELL LINE

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Arsenic is a ubiquitous environmental pollutant and a known human carcinogen. A number of studies have reported that possible mechanisms of arsenic carcinogenesis include both genotoxic and epigenetic mechanisms. DNA methylation is an important factor of epigenetic control of gene expression. In terms of human biomonitoring, circulating blood cells such as lymphocytes are easily accessible and have been suggested as surrogate samples to reflect pathological changes and an alternative to tissue samples for molecular profiling of human diseases. Therefore, the study aimed to investigate the effects of arsenic on DNA damage and DNA methylation in human lymphoblasts.
The human lymphoblasts (RPMI 1788) were cultured and treated with sodium arsenite. Dose-response and time-course study of arsenite on DNA strand breaks were investigated in lymphoblasts at concentrations 0-100 µM for 2, 4, 8, 12 and 24 hours of treatment. DNA strand breaks analyzed by Comet assay showed that arsenite significantly increased DNA strand breaks at all concentrations ($P<0.05$) at 2 hours and peak at 4 hours then decreased; the maximum level of DNA strand breaks was observed at 50 µM of arsenite treatment. XRCC1 expression which is responsible for DNA strand break repair was determined by real-time RT-PCR. Conversely, mRNA expression of XRCC1 was

decreased in 50 μM arsenite-treated lymphoblasts in which a maximum reduction was observed at 4-8 hours after treatment. In addition, global DNA methylation in Long Interspersed Nuclear Elements1 (LINE-1) was also determined by Combined Bisulfite Restriction Assay (COBRA). When compared to untreated lymphoblasts, global DNA hypomethylation was observed in the arsenite-treated lymphoblasts with a reduction of 6.45%, 16.32% ($P<0.05$), 37.45% ($P<0.01$) and 33.84% ($P<0.01$), following 4 hours of treatment at 10, 20, 50 and 100 μM, respectively. The results clearly showed that arsenite caused DNA strand breaks, reduced XRCC1 expression and global DNA hypomethylation in human lymphoblasts. The profile of arsenite-induced DNA strand breaks and global hypomethylation may indicate that DNA damage could in part be associated with aberrant DNA methylation; however the mechanistic link between these two mechanisms is of interest to be investigated further.

ME024

DICHLORVOS, AN ORGANOPHOSPHATE PESTICIDE, IMPAIRS MITOTIC SPINDLE ASSEMBLY AND CAUSES ANEUPLOIDY.

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There is an increasing concern for the possible impact of environmental factors on genomic stability in human populations. We studied the aneuploidy-inducing capacity of pesticides of significant human exposure in cultured human cells and demonstrated that dichlorvos, an organophosphate insecticide classified as possible carcinogen, significantly induced CREST-positive micronuclei in binucleated lymphoblastoid cells (Mattiuzzo et al., 2006). To identify the mechanisms involved in the aneuploidy-inducing ability of this chemical, we investigated the influence of the drug on mitotic progression and spindle assembly. In both lymphoblasts and Hela cells Dichlorvos greatly increased mitotic index and inhibited the metaphase/anaphase transition: this resulted in a mitotic arrest at promethaphase with hyper-condensed chromosomes. Analysis of mitotic spindles and chromosome congression by immunofluorescence staining with anti- alpha tubulin and anti- Ser10 phospho H3 antibodies showed that the chemical was able to perturb spindle dynamics and chromosome behaviour. Spindle microtubules in treated cells were not organized in parallel fibres but collapsed in tubulin aggregates or formed distorted fibres that did not interact with the kinetochores. At higher doses Ser10 phospho H3-positive picnotic nuclei were associated to monopolar spindles, showing two close gamma tubulin signals. These results demonstrate that Dichlorvos mimics spindle poison effects, inducing mitotic arrest and altering the structure and the function of the mitotic apparatus in cultured human cells. In vivo analysis of mitotic progression showed that a large proportion of dichlorvos-treated cells did not progress beyond prometaphase. The few cells that completed mitosis, did so without chromatid separation producing polyploid or multinucleated cells. Finally, B tubulin in dichlorvos treated cells was heavily phoshorylated at a residue that is specific for tubulin not incorporated into microtubules (Fourest-Lieuvin et al., 2006), suggesting that the pesticide affects microtubule dynamics during mitosis. These effects of the chemical may be relevant for its potential carcinogenic activity.

ME25

THE ASSOCIATION OF METHYLATION PATTERN AND PRENATAL POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) EXPOSURE

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Background:PAHs are a group of carcinogenic environmental pollutants. Prenatal Exposure to PAHs can alter the pattern of DNA methylation, resulting in adverse biological effects. We sought to explore if prenatal airborne PAH exposure is associated with methylation pattern change in cord blood. Methods:In a cohort study of 730 non-smoking women in New York City, we measured PAH exposure during pregnancy using personal air monitors. DNA was extracted from cord blood. The Illumina Infinium® assay was used to measure 27,578 CpG loci across 14,495 genes on a subset of 24 Dominican women, 12 with prenatal PAH exposure in the highest and lowest tertiles of exposure, respectively. Results:The array data was processed by Illumina BeadStudio for background normalization. The inter-quartile range (IQR) of the methylation levels for each of the CpG loci was obtained, and an IQR filter with cutoff point at the median IQR was applied. Spearman's correlation coefficient between the methylation levels and PAH exposure was calculated, and 99 most highly correlated CpG loci were selected; A4GALT ACCN3 ADORA1 AMID ANXA4 APBA3 ASPH ATP5I BMP8A C10orf33 C19orf30 C1orf164 C2 C21orf84 CAPN12 CD9 CDH24 CDH5 CEACAM4 CHRNA2 CHRNG CR2 CRTAM CRYGD CTNND1 CYP2W1 CYP7B1 ETNK2 FBXL12 FBXO27 FLJ20186 FLJ20699 FLJ21106 FLJ27505 GBGT1 GGTLA1 GLB1L GNAS GPR85 GSTO2 GZMB HOXA2 HSPA12B HTRA4 IGF2 INPP5E INT1 JDP2 KCNK3 KCNQ1 KIAA0372 KNS2 KRT25A LILRB3 LOC92345 LRIG3 MEG3 MGC31967 NAP1L5 NCOA6 NDST1 NGFR NYX OFCC1 PCDHB3 PCDHB6 PGCP PHB2 PKP4 POU5F1 PPAPDC3 PTGES PTPRC RAB37 RHOBTB3 ROR2 SCGB3A1 SCMH1 SFRP1 SLAMF1 SOCS1 STK3 SULT1C2 TCN1 TINAGL1 TMEM42 TMEM59 TRA2A TSC1 UBE4A WDR52 WFDC13 WWP1 ZNF512and ZNF662. The heatmaps produced by the hierarchical clustering algorithms showed that the methylation pattern of the females within the highest prenatal PAH exposure are distinctly different than the pattern of those in the lowest tertile of exposure. Conclusion:Our exploratory results indicate that prenatal PAH exposure is associated with the methylation pattern of a subset of genes measured in cord blood. This suggests that DNA methylation status can be influenced by environmental exposures in early life. Further analyses will explore pathways of exposure-related methylation differences.

ME026

STRUCTURAL ANALYSIS OF THE EPIGENETIC CONTROLLER, HUMAN DNA METHYLTRANSFERASE I

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DNA methylation, one of the epigenetic mechanisms, plays an important role in numerous biological functions in cells, from inhibition of cleavage by endonucleases to inhibition of transcription factor binding. The DNA methyltransferase I (DNMT1) protein represents a major DNA methyltransferase activity in human cells and is therefore a prominent target for environmental epigenetic control and experimental cancer therapies. However, there are only few available structural information and inhibitors as well as their high toxicity and low specificity have so far precluded their broad use in chemotherapy. Therefore, cloning and bacterial expression of several functional domains of DNMT1 were carried out for the structure determination by X-ray crystallography and NMR technique. One of the functional domain, DBD (the non-catalytic amino terminus of DNMT 1) binds a new protein, DMAP 1 (for DNMT 1 associated protein), and can mediate transcriptional repression. Bioinformatics, CD (circular dichroism) and NMR (nuclear magnetic resonance) spectroscopic approaches indicate this domain adopt the all helical topology. In addition, DBD showed DNA binding activity, and the responsible sequence was narrowed to about 80 amino acid residues involving the proliferating cell nuclear antigen (PCNA) binding motif. The DNA binding activity did not distinguish between DNA non-methylated and methylated states, but preferred to bind to the minor groove of AT-rich sequence. Furthermore, DNA binding activity of DBD may contributes to the localization of DNMT1 to AT-rich sequence such as satellite, Line 1, and the promoter of tissue-specific silent genes. The structural analysis of DBD of human DNMT1 could provide biological explanation for how the domain interacts with other regulation proteins onto the particular sites in genes expression, and give the structural view for specific inhibitor development.

ME027

COMPARATIVE STUDY OF RADIATION-INDUCED GENOMIC INSTABILITY MANIFESTATIONS IN THE PROGENIES OF DIRECTLY IRRADIATED AND BYSTANDER CELLS

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Radiation-induced genomic instability (RIGI) is defined as a persistent increased rate in the level of *de novo* formed alterations in the progeny of irradiated cells. RIGI is observed in the progeny of directly irradiated cells as well as in the progeny of non-irradiated cells communicated with irradiated cells (bystander effect). However the question concerning the differences in RIGI-induction and perpetuation in directly irradiated and bystander cell populations is still open. The aim of present study was to compare the RIGI manifestations in the progeny of totally irradiated cells vs. the progeny of cells mixture containing 90% of non-irradiated cells and 10% of irradiated cells. Chinese Hamster Ovary cells (CHO K1 cell line) were used in this study. There was a significant increasing in the DNA breaks level, apoptotic cells percentage and intracellular ROS content in 9-32 cell generations after total acute irradiation of cells at a dose of 1 Gy (γ-radiation, ^{60}Co) as an evidence of RIGI induction. The increased ROS content in the progeny of irradiated cells testifies in favor assumption of important role of mitochondrial dysfunction in perpetuating of RIGI. The ROS overproduction leads to elimination of cells that are sensitive to the oxidative stress. Earlier, we have demonstrated a selective pressure of RIGI leading to the radioresistant cell clones forming. The results of the experiments on cells mixture (co-culture containing 10% of cells irradiated at a dose of 1 Gy and 90% of non-irradiated cells) showed a statistically significant increase in the ROS content in 9(11) cell generations as well as in all tested end-points (DNA breaks, apoptosis and ROS) from 12(14) cell generations. It is necessary to note that the effects manifestation was not differ from the genomic instability expression in generations from totally irradiated cells. These studies show that the secondary bystander effect via signals secreted by minor population of genome unstable cells has been involved in the forming of genomic instability. This work was supported by RFBR grant (# 07-04-01009-a)

ME028

THE USE OF RESTRICTION ANALYSIS FOR THE IDENTIFICATION OF THE METABOLIC STAGE OF ANTHRACYCLINES' REDUCTION BY CYTOCHROME P450 REDUCTASE THAT GENERATES DNA BINDING INTERMEDIATES

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Previous studies demonstrated that metabolic activation by NADPH cytochrome P450 reductase (P450red) increased cytotoxicity of doxorubicin towards cultured tumour cells. Moreover, with the use of [14C]-labelled doxorubicin, we demonstrated that this metabolic pathway generated intermediates capable of covalent DNA binding in cellular and cell-free systems. These observations suggested that one-electron reduction might play an essential role in antitumour activity of anthracyclines. In this study, we applied the restriction analysis method to find out the individual metabolite(s) responsible for DNA modification for three clinically used anthracyclines: doxorubicin, daunorubicin and epirubicin. The method employs 695 bp DNA amplicon that includes two restriction sites; one containing only GC and the other only AT base pairs, recognized respectively by restriction enzymes MspI and Tru1I. The covalent modification of the restriction site(s) abolishes their recognition inhibiting cleavage by the enzymes and simultaneously pointing at the kind of base pairs involved in DNA adduct formation. Since the metabolic activation by P450red is associated with 5 sequential changes of anthracycline UV-VIS spectrum, we were able to monitor the reaction course and to collect separately consecutive intermediates formed upon one-electron reduction of drugs studied. The collected samples were mixed with the amplicon, allowed to react with DNA for 2 or 24 hr and then unbound anthracyclines were removed by solvent extraction. The aqueous fraction containing the amplicon was submitted to restriction analysis. The experiments confirmed that all three anthracyclines studied are able to bind to DNA after P450red activation. Only intermediates formed at the 3rd stage of reduction, corresponding to conversion of leuko form to hydroquinone, inhibited cleavage by MspI. The cleavage by Tru1I was not affected. This suggests that only GC base pairs are substrates for modification by anthracyclines. Doxorubicin and daunorubicin derivatives abolished completely DNA cleavage by MspI as soon as after 2 hr of incubation with the amplicon, while less toxic epirubicin needed 24 hr for the reaction with DNA to be accomplished.

ME029

STUDY ON DNA METHYLATION PROFILE OF ALKBH1-9 GENES PROMOTERS IN TEN HEALTHY HUMN TISSUES

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BACKGROUND: Bacterial dioxygenase AlkB protein oxidatively demethylates 1meA and 3meC in DNA resulting the recovery of the natural A and C bases. Among the human homologues ALKBH1-9 there are two proteins: ALKBH3 (PCA, prostate cancer antigen) and ALKBH9 (FTO, fat mass- and obesity-associated) which are known to affect the human health. The level of mRNA expression of human AlkB homologues is different in various tissues what seems that expression might be under epigenetic control. DNA methylation is a widely studied epigenetic mechanism that affects cell function and genome stability by altering gene expression and refers to the covalent addition of a methyl group, catalyzed by DNA methyltransferase (DNMT), results 5meC in a CpG islands. This leads to down-regulation of gene expression. METHODS: Modifying DNA using sodium bisulphite to convert unmethylated cytosines to uracils and subsequently detect methylated cytosines using methylation specific PCR (MSP) technique is used for study of DNA methylation profile of ALKBH1-9 genes promoters in ten healthy human tissues (brain, heart, lung, liver, spleen, small intestine, prostate, testis, ovary, skeletal muscle). RESULTS: The model of bisulphite treated DNA by substituting all cytosines which are not in CpG context into tymines was generated, and 3 pairs of primers to each gene promoter were designed. Moreover, the methylation profile of ALKBH3 promoter in various tissues was established and seams to be similar in ones. Study undergoes and more results will be presented soon. CONCLUSIONS: Our study will increase the current knowledge of genome wide DNA methylation profile.

ME030

EFECTIVENESS OF BUDESONIDE TO REVERSE THE HIGH RATE OF ENDOREDUPLICATION IN THE REPAIR DEFICIENT CHO MUTANT EM9

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We have previously reported a dose-dependent increase in the yield of endoreduplication after treatment with 5-azaC in the AA8 cell line that correlated with the level of DNA hypomethylation. In order to better understanding the nature of these effects, we have extended these studies to the CHO mutant cell line EM9. a repair deficient mutant that shows elevated indices of spontaneous endoreduplication as compared to its parental cell line AA8, while previous work has shown no apparent difference in topo II activity between both cell lines. First aim of this study was to analyse global genome methylation in AA8 and EM9 cells in order to see if any differences could exist between both parental and mutant cell lines. Our

data seem to be indicative of reduced methylcytosine content in EM9 global genome DNA as compared to AA8. To further corroborate a direct relationship between the methylcytosine content in global genomic DNA and the rate of endoreduplication, we did a series of experiments with the aim to reverse the hypomethylation in the EM9 cell mutant. For that purpose, we have taken advantage of the ability of budesonide, a known methylating agent, to produce remethylation of the CpG sites in DNA. Actively growing EM9 cells were cultured for 24 h in the presence of a wide range of different concentrations of budesonide and global genome methylation was evaluated with HpaII and MspI restriction enzymes, followed of DNA electrophoresis and densitometric analysis of the cutting pattern. Data appear to indicate that at least in part it is possible to reduce the high frequency of endoreduplication observed in EM9 cells (approximately a reduction of 50%) by means of the chemical modification of the methylation status of their DNA. Previously we had proposed that hypomethylation of DNA induced by 5-azaC leads to reduced chromatid decatenation that ends up in endoreduplication, most likely due to a failure in topo II function. A plausible explanation for the results presented would be that treatment with budesonide restore at least partially in EM9 the physiological methylation patterns (i.e. that found in the parental cell line AA8). This might be a pre-requisite for optimal topoisomerase II function including the observed decatenation activity.

ME031

MODULATION OF GENE EXPRESSION NETWORKS BY 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD) DURING VERTEBRATE DEVELOPMENT

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Normal vertebrate jaw development can be disrupted by exposure to 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)—a potent inducer of the aryl hydrocarbon receptor (Ahr) transcription factor required for transducing the toxic effects of TCDD. We used zebrafish (*Danio rerio*) embryos to investigate their transcriptional response to TCDD with the goal of uncovering novel, jaw-specific genes affected by exposure. Our results uncovered a novel target of TCDD-induced transcriptional upregulation belonging to the evolutionarily conserved family of forkhead box transcription factors. This Fox gene is highly conserved in mammals, including humans and mice, and was the most significantly upregulated gene (20-fold) in a set of novel TCDD targets of induction. We compared the timing of induction to *Cyp1A1* as a function of TCDD exposure and observed no significant difference, suggesting that this Fox gene—like *Cyp1A1*—is a direct target of Ahr-mediated transcriptional activation. This result was consistent with the presence of multiple Ahr binding sites within the gene's promoter region. Quantitative real-time polymerase chain reaction analysis demonstrated that TCDD induction was strongest at 24 and 48 hours post-fertilization (hpf). *In situ* hybridization analysis at 48 hpf unambiguously demonstrated that this gene is specifically expressed in the jaw primordium. We hypothesized that upregulation of this gene plays a critical role in TCDD-mediated jaw abnormalities. Morpholino knockdown of this gene followed by exposure to TCDD resulted in fry that appeared to be identical to uninjected, unexposed fry. These results identify a novel target of TCDD-mediated Ahr induction that also functions as a transcription factor critical for normal craniofacial development.

ME032

EVIDENCES OF AN ANTITUBULIN ACTIVITY OF Xanthium strumarium L. EXTRACTS IN CHO CELLS.

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Chemotherapy with cytostatic agents is one of the most widely employed methodologies in the fight against cancer. This approach includes the employment of compounds that interfere with the cellular division. In this sense, the application of techniques that allow to evaluate the capacity of interfere and/or modify the mitotic cell course, constitutes a valuable practice in the search of new therapeutically active molecules. Plants are an important natural source of compounds that, potentially, can drive to development of new cytostatic drugs. It has recently been highlighted that complex mixtures present in vegetal extracts can have an even greater therapeutic power than that of the isolated active principle(s) they contain, due to possible positive interaction with cofactors present in the mixture. *Ex vivo* assays demonstrated that the vegetable species *Xanthium strumarium* L. possesses an effective antitubulin activity. The aim of the present work is to study the effect of a hydroalcoholic extract obtained from *X. strumarium* on CHO cells proliferation, evaluating parameters related to cell cycle progression and mitotic division. Experimentally, CHO cells cultures were treated with different extract concentrations and the following parameters were analyzed: growth of the cellular population, cell cycle progression, mitotic progression and mitotic spindle morphology. We observed a dose-dependent inhibition of cell growth and a dose-dependent decrease of anatelophases, abnormal mitotic spindle assembly and significant blockade of cellular cycle in G_2-M phase. These experimental evidences are consistent with the *in vitro* data on *X. strumarium* extract anti-tubulin capacity and reinforce the valuation of this vegetable species as a potential source for the development of new cytostatic drugs.

DNA damage responses

DD001

SEALER 26 AND ZINC OXIDE EXHIBIT DIFFERENTIAL RESPONSE RELATED TO GENOTOXICITY USING TWO CELL LINEAGES IN VITRO

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The purpose of this study was to evaluate whether Sealer 26 and zinc oxide powder induce genetic damage in vitro. Genotoxicity was assessed by single cell gel electrophoresis (comet assay). Chinese hamster ovary (CHO) or murine fibroblast cells were exposed to increasing powder concentrations ranging from 10-1000 µg/mL and liquid from Sealer 26 in increasing concentrations ranging from 0.1 to 10%. All treatments were performed for 1 hour at 37oC. Sealer 26 liquid promoted DNA breakage in CHO cells at the highest concentration tested (10%) and at all concentrations tested in murine fibroblasts. Similarly, zinc oxide powder induced DNA damage at the highest concentration tested when compared to negative controls for both cells types evaluated. However, the powder from Sealer 26 presented no detectable effect on genotoxicity at all the concentrations tested. In summary, the results indicated that Sealer 26 and zinc oxide induce genetic damage in vitro, with more pronounced effect obtained for Sealer 26.

DD002

SLEEP LOSS INDUCES DIFFERENTIAL RESPONSE RELATED TO GENOTOXICITY IN MULTIPLE ORGANS OF THREE DIFFERENT MICE STRAINS

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The present purpose was to determine the genetic damage induced by paradoxical sleep deprivation (PSD) in three different male mice strain in peripheral blood, heart, kidney and liver tissues by the single cell gel (comet) assay. Swiss, C57BL/6j and Hairless mice were submitted to PSD by the platform technique for 72 hours and DNA damage was evaluated. Significant statistically differences ($p<0.05$) for DNA damaging were found in blood cells of Swiss mice strain when compared to negative controls. By contrast, no significant statistically differences ($p>0.05$) were noticed to C57BL/j or Hairless (HRS/j) mice strains as depicted by the tail moment data. Regarding the liver, extensive genotoxic effects were noticed in the Swiss strain only. Hairless (HRS/j) and C57BL/j mice strains did not show any signs of genotoxocity for this organ. The same lack of effect was noted in kidney or heart cells, i.e., no statistically significant differences ($p>0.05$) were detected between experimental group and the negative controls for all strains evaluated. In conclusion, our results reveal that sleep deprivation exerted genetic damage in the form of DNA breakage in blood and liver cells of Swiss mice strain only. This kind of approach should be considered when testing noxious activitives on genetic apparatus induced by sleep deprivation in mice since the Swiss strain is more suitable for this purpose.

DD003

A MRN/TIP60 COMPLEX INVOLVED IN DNA DOUBLE STRAND BREAKS REPAIR

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Chromatin modifications and chromatin modifying enzymes are believed to play a major role in the process of DNA repair. The Tip60 histone acetyl transferase is physically recruited to DNA double strand breaks (DSB) where it mediates histone acetylation. Here, we show that Tip60 expression is required for DNA DSB repair through Homologous Recombination. Tip60 is involved in the formation of Rad50 foci following ionizing radiations, suggesting that Tip60 expression is necessary for recruitment of the DNA damage sensor MRN (Mre11, Rad50, Nbs1) complex to DNA DSB. Moreover, Tip60 physically interacts with MRN proteins in a complex which is distinct from the classical Tip60 complex. Finally the Tip60/MRN complex is rapidly mobilized to chromatin following DNA DSB induction, suggesting that the effects of Tip60 on DNA repair are mediated within this complex. Taken together, our results provide important new insights into the role and mechanism of action of Tip60 in the process of DNA DSB repair.

DD004

THE HUMAN HEPATOMA HEPARG CELLS – RECENT ADVANCES IN KNOWLEDGE OF MECHANISMS OF MUTAGENICITY AND CARCINOGENICITY

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The HepaRG hepatocyte-like cells express various liver specific functions in vitro after differentiation in the presence of DMSO. Various phase I and phase II metabolism enzymes are express in these cells at comparable levels to that found in human hepatocytes and higher levels than any other available hepatocytes in vitro models. Therefore, HepaRG cells represent a promising in vitro model in genetic toxicology that should be more predictive for hazard assessment. Recently, we showed the attractivity of this model to detect human procancerogens by the comet and the micronucleus assays, especially B[a]P, Aflatoxin-B1 and PhIP. First, in order to improve the sensitivity of the comet assay to detect oxidative DNA damage and alkylated bases, we performed the modified comet assay with FormamidoPyrimidine DNA-Glycosylase (FpG) protein. We found that FpG strongly enhanced Cyclophosphamide-, B[a]P-, Acrylamide- and Glycidamide-induced DNA damage in HepaRG cells after 24-h treatment. Whether the comet assay in its standard version at pH>13, only detects a small fraction of DNA damage induced by cyclophosphamide and glycidamide, the response obtained in the comet assay in the presence of FpG was observed at lower doses and was strongly enhanced at each dose. Secondly, combined exposure to heterocyclic aromatic amines (HAAs) in differentiated HepaRG cells was investigated in the comet assay. We tested different co-exposition scenarios with PhIP, IQ and MeIQx in HepaRG cells. Whereas only PhIP induced comet formation when incubated alone, we showed that IQ and MeIQx inhibited PhIP induced-DNA strand breaks in HepaRG cells. This work was supported by an ANR contract number 06SEST17

DD005

URINARY 8-HYDROXY-2'-DEOXYGUANOSINE, A METABOLITE OF OXIDIZED DNA, IS NOT ELEVATED IN INFANTS FED WITH MELAMINE-CONTAMINATED POWDERED FORMULA

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Oxidative DNA damage is involved in the process of carcinogenesis.

There is no evidence in humans for the carcinogenicity of melamine. In this study, urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) was measured to determine if melamine-contaminated formula feeding leads to oxidative damage to DNA in infants. A cross-sectional study was carried out measuring urinary 8-OHdG in four groups of infants according to the type of feeding: Infants of observation group 1 and 2 receiving 90% and 50% to 90% of their intake as melamine-contaminated powdered formula, respectively; infants of reference group 1 and 2 receiving respectively 90% and 50% to 90% of their intake as imported milk powder formula, which has been proven not to have melamine contamination. The mean spot urinary 8-OHdG levels (micromoles per mole of creatinine) for the observation groups 1 and 2 and the reference groups 1 and 2 were: 2.3±1.02, 1.7±0.76, 1.9 ±0.85 and 2.1±1.10 (mean ± standard deviation), respectively. No significant difference in mean urinary 8-OHdG excretions was observed among the four groups. In addition, no correlation between mean urinary 8-OHdG excretions and percentage of melamine-contaminated powdered formula intake was observed. Our data suggest that melamine does not lead to oxidative damage of DNA in infants.

DD006

MICRORNAs IN HUMAN IRRADIATED LYMPHOCYTES: TIME AFTER IRRADIATION AND MICROGRAVITY INCUBATION MODULATE MICRORNA EXPRESSION

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Cell response to ionising radiation (IR) depends both on genetic and physiological features of the biological systems, and on environmental conditions occurring during DNA repair. In this work we analysed the profile of microRNAs of peripheral blood lymphocytes (PBLs), a class of short single strand RNAs, whose expression inhibits target protein translation and regulates a large variety of cellular processes. We identified the expression changes of miRNAs during repair time (4 and 24h), at two doses of ionising radiation, like early bio-indicator of radiation exposure, and to correlate them with their target levels. In addition, we investigated if MMG during repair time can modify miRNA profiles, to provide important information about the risks of human in space. Microgravity is a condition of weightlessness experienced by astronauts in space, which can be only simulated on Earth. Our previous results provided evidences that incubation in modelled microgravity (MMG) during repair time affects cell survival and mutant frequency and delays the rejoining of IR-induced DSBs, increasing the genotoxic effects of ionising radiation. Our experiments were carried out on human PBLs, obtained from healthy donors, irradiated with γ-rays at the doses of 0.2-2Gy and incubated in normal gravity and in parallel MMG. MiRNA and gene expression profiles were assessed at 4 and 24 h after irradiation by using Agilent Microarray platforms. As reported in previous works, we confirmed that DNA damages induce up-regulation of miR-34a, which is directly transactivated by p53, and increase its activity promoting apoptosis. We found others miRNA whose expression patterns were affected by radiation: cluster of miR-513, miR-630, miR-188-5p, that are up-regulated after 24h of repair time with a higher de-regulation after 2Gy of γ-rays. Moreover, we found some miRNAs specifically deregulated by MMG incubation during 24h of repair time, with the share of MMG on radiation effect that is opposite in low respect high dose of γ-rays. On the whole, our results suggest that miRNA expression patterns are function of radiation dose and repair-time, and the effect of radiation/MMG on miRNA expression varied according to radiation levels, individual-radiosensivity and post-irradiation time point.

DD007

OXIDATIVE DNA DAMAGE AND CELL DEATH IN PERIPHERAL LYMPHOCYTES FROM PATIENTS SUBMITTED TO SURGERY UNDER PROPOFOL OR ISOFLURANE ANESTHESIA

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To investigate genotoxicity of anesthetics widely used in human surgery has drawn considerable attention because of their possible secondary effects. Nothing is known about the oxidative potential of propofol (PF) on DNA, and there are controversial results regarding the in vivo isoflurane (ISF) genotoxicity. On the other hand, surgery is often associated with a temporary perioperative immunological alteration, and some anesthetics seem to contribute to a transient lymphocytopenia after surgery. PF is an intravenous anesthetic used for induction and maintenance of anesthesia, while ISF is used for maintenance of inhalation anesthesia. Since both compounds are very used in anesthesia, this study aimed to evaluate their possible genotoxic effect (DNA strand breaks and purine and pyrimidine oxidation) as depicted by the comet assay, and whether anesthesia with these anesthetics could induce apoptosis or necrosis in T lymphocytes, by using flow cytometry. The experimental design included 24 patients classified as physical status ASA I, submitted to otorhinolaryngologic surgery, and under at least 120 minutes anesthesia. Blood samples were collected at three moments: before anesthesia, at 120 minutes of surgery, and at the following day of surgery. Tail intensity, as evaluated by the software Comet Assay II, was considered to estimate DNA damage; lymphocytes were phenotyped as CD4+ and CD8+ and also stained with annexin-V and 7-AAD to detect dead cells. Results demonstrated no statistically significant difference in the levels of DNA damage and of oxidized bases among the three moments, for both anesthetics. Anesthesia with PF or ISF also did not increase the percentage of early or late apoptosis, and necrosis in both subpopulations of T lymphocytes. In conclusion, our data indicated that general anesthesia maintained with PF or ISF did not induce DNA damage (DNA strand breaks and purine and pyrimidine oxidation) in lymphocytes from ASA I patients submitted to elective surgery. Furthermore, none of the anesthetic procedures led to ex vivo death of CD4+ or CD8+ T lymphocytes after surgery and at the postoperative first day.

DD008

APOPTOSIS, CELL CYCLE PROGRESSION AND GENE EXPRESSION IN URINARY BLADDER TUMOR CELLS SUBMITTED TO IN VITRO TREATMENTS WITH CISPLATIN AND GEMCITABINE

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Currently, the combination of cisplatin and gemcitabine is considered a standard chemotherapeutical protocol for bladder cancer. Nevertheless, the mechanism by which these drugs act on tumor cells is not completely understood. The present study was designed to evaluate cellular and clonogenic survival, apoptotic index, cell cycle and gene expression in urinary bladder transitional carcinoma (TCC) cell lines, under different treatment protocols with cisplatin and gemcitabine. Three cell lines, with different TP53 status, were used: RT4, with wild type TP53, and established from a low-grade papillary tumor; 5637 and T24 lines, with mutated TP53, and established from high-grade tumors. Results showed that: 1) treatment with cisplatin (1μM) induced apoptosis only in the wild type TP53 cells (RT4); 2) RT4 was also more sensitive to apoptosis and presented lower percentage of clonogenic survival when treated with gemcitabine (1.56μM); 3) the combined treatment (cisplatin + gemcitabine) induced higher apoptosis rates in the wild type TP53 cells; 4) G1 cell-cycle arrest was observed in the three cell lines after treatment with gemcitabine or with combination of both; 5) significant cell death (other than apoptosis) was detected in mutant TP53 cell lines after treatments with cisplatin or gemcitabine; 6) lower percentage of survival occurred when the antineoplastic compounds were given in combinations, independently on TP53 status; 7) the combination of lower concentrations of cisplatin and gemcitabine had the same

cytological effect than those obtained with high concentrations of each compound; 8) more significant alterations on gene expression were observed after combined treatment; 9) mainly genes related to cell proliferation inhibition were affected. In conclusion, it was demonstrated that treatment with combination of cisplatin and gemcitabine is effective, despite of tumor-associated TP53 mutations or grade of tumor. Besides apoptosis, other cell death mechanisms occurred in mutated TP53 cells treated with these drugs. Since high concentrations of cisplatin is known to be toxic to the whole organism, the synergism between low concentrations of cisplatin and gemcitabine is a good alternative for chemotherapy of TCC. Supported by FAPESP and CNPq.

DD009

THE ROLE OF ASN 211 RESIDUE IN A CATALYTIC MECHANISM OF HUMAN AP ENDONUCLEASE 1

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Apurinic/apyrimidinic (AP) sites belong to the most common DNA lesions arising under the action of UV and ionizing radiation, alkylating agents and oxygen radicals. In human cells AP endonuclease 1 (Ape1) recognizes AP sites and makes a single nick in the phosphodiester backbone 5' to the AP site. In our previous study (Pshenichnikova et al., 2007) we have investigated the conformational dynamics of wild type (wt) Ape1 protein. The obtained data suggests the multi-stage character of recognition of damaged sites in DNA and their incision, and can explain the origin of the high specificity of the Ape1 enzyme. It was suggested earlier (Rothwell et al., 1996) that Asn-211 residue of the Ape1 active site is essential for AP sites binding and DNA cleavage as well. The goal of present research was to ascertain a role of Asn-211 in the catalytic mechanism of Ape1. We have employed two Ape1 mutants where Asn-211 residue was substituted for alanine or aspartate. The conformational dynamics of N211A and N211D Ape1 mutants was investigated by stopped-flow technique combined with fluorescence detection. Conformational transitions in the protein molecule were followed by changes in the fluorescence intensity of its Trp residues. In addition, we have used the fluorescent analogue of cytosine, pyrrolocytosine (pyC), introduced into DNA sequence in order to observe a conformational dynamics of DNA during enzymatic cleavage. The 12-bp DNA duplexes 5'CTCTCXCCTTCC3'•3'GAGAGYGGAAGG5' (where X is G residue, natural AP site or its tetrahydrofuran analogue; Y is C or pyC residues) were used as specific and non-specific substrates for the Ape1 mutants. The quantitative analysis of fluorescent data obtained in single-turnover conditions has shown that the rate of reaction catalyzed by mutant Ape1 proteins is decreased significantly in comparison with wtApe1. Based on these findings we have shown that Asn-211 residue is not essential for DNA substrates recognition and binding but highly required for effective catalysis.

DD010

KINETIC MECHANISMS OF HUMAN AP ENDONUCLEASE (APE1) AND ITS MUTANT APE1K98A IN NUCLEOTIDE INCISION REPAIR PATHWAY (NIR)

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APE1 is involved in the alternative NIR pathway that bypasses the glycosylase step. In avoiding formation of a potentially toxic AP-site (AP) intermediate, NIR may have certain advantages over BER. We analyzed the conformational dynamics and the kinetic mechanism of APE1 action on 5,6-dihydro-2'-deoxyuridine (DHU) containing substrate in NIR process. For this purpose the changes of fluorescence intensity of 2-aminopurine, located in two different positions of the substrate, were detected. We have shown, that APE1 had at least three centers for DHU-substrate binding, ~100% of APE1 molecules possessed a binding activity. Using [32P]-labelled DHU-substrate we have shown, that only ~15% of APE1 had a catalytic activity towards this substrate. Probably, APE1 formed three types of complexes with DHU-substrate, and only one of them was catalytically active.

We have studied the influence of Lys-98 substitution for Ala (APE1K98A) on kinetic parameters of APE1 action in NIR. As for APE1 only ~15% of APE1K98A possessed the catalytic activity towards the DHU-substrate. The cleavage of DHU-substrate by both enzymes was described by the identical kinetic schemes. The enzyme-substrate complex formations were followed by fast substrate cleavage in the catalytically active complex. The rapid isomerisation of the protein-nucleic complex occurred and followed by the slower conformational change resulting in the formation of stable enzyme-product complex. Further the limiting process of product release from this stable complex took place. It progressed very slowly, although we didn't detect the Schiff-bases formation. The equilibrium dissociation followed the limiting stage. Rate constant of DHU-substrate catalytic cleavage by APE1 in NIR conditions was comparable with rate constants of tetrahydrofuran and AP containing substrates cleavage in BER conditions (Timofeyeva et al. JBSD 26, 2009). These data showed NIR to be the biologically relevant process. The catalytic cleavage of DHU-substrate by APE1K98A occurred ~200 fold slower in comparison with APE1 demonstrating the critical role of Lys-98 in the catalysis in NIR.

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DD011

MODELING STUDY OF EFFECT OF AMINOACID MUTATION AND COFACTOR STRUCTURE ON THE β-ELIMINATION STAGE OF CATALITIC CYCLE OF HUMAN 8-OXOGUANINE-DNA GLYCOSYLASE ENZYME OF DNA REPAIR SYSTEM

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The effect of oxidative damages to DNA include miscoding and disregulation of gene expression and may lead to cancer and aging. Base excision repair of damaged bases is initiated by DNA glycosylases, enzymes that recognize lesions and excise the damaged base. The human 8-oxoguanine-DNA glycosylase hOgg1 plays a prominent role and excise the 8-oxoguanine (8-oxoG), a major damaged purine, from DNA. Defects in hOgg1 have been associated with human cancer and enhanced mutagenesis. Recently it was show that recognition and excision of damaged bases by DNA glycosylases is accompanied by several conformational rearrangements that bring the excised damaged base into enzyme catalytic site, i) initial encounter with DNA and forming of enzyme/DNA complex with inversion of the damaged base, ii) insertion of several enzyme residues into DNA, iii) enzyme isomerization to the catalytically competent form, and finally, iv) reaction of β-elimination to break the damaged DNA strand. At the last stage, the 8-oxoG base bound to the active site of hOgg1, acts as a cofactor of the β-elimination reaction . It was shown that the rate of β-elimination reaction increased at least 10-folds by 8-BrG, an 8-oxoG analog [1,2] . In this work we report results of computer simulations of 8-oxoG and 8-BrG in the active site of enzyme to understand the nature of the difference between the two compounds. Docking, analysis of interactions of cofactors and calculations of binding rate are performed for wild type hOgg1 and three hOgg1 mutants of amino acids in a spatial proximity to the 8-oxoG binding pocket. A computational analysis reveals details of coupling between cofactor nature and structure of hOgg1 mutants at the β-elimination reaction stage. Acknowledgement. This work was supported by RFBR grants # 09-04-00136

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DD012

CHANGES IN EXPRESSION OF GPX AND SOD IN MICE TREATED WITH CHRONIC DOSES OF CYCLOPHOSPHAMIDE

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Cyclophosphamide (CP) is an alkylating agent used for the treatment of malignant and non-malignant disorders. Despite its wide spectrum of clinical uses, CP also possesses wide spectrum of cytotoxicity to normal cells not only in patients but also in pharmacists and nurses occupationally exposed to CP during its production or distribution. Limited information has been reported on the effect of CP on the expression of genes specially those coding for antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPX). Changes in levels of expression of these two antioxidant genes, SOD and GPX were investegated in liver and brain cells of mice injected intrapritoneally with cyclophosphamide (4 mg/kg) for 5, 10, and 15 consecutive days using reverse transcriptase polymerase chain reaction (RT- PCR) technology. Results indicated that GPX expression has been declined in liver cells whereas; changes in SOD gene expression were comparable to those of the control. In brain cells, a dramatic depression in the levels of expression of SOD and GPX occurred after chronic administration of CP for five consecutive days. Further exposure to CP for 10 and 15 days, up-regulated the expression of GPX and down-regulated SOD expression compared to the control. Analysis of SOD/GPX expression ratios recorded a significant increase in the SOD/GPX expression ratios in liver cells after 15 days of treatment with CP. A significant decrease in SOD/GPX expression ratios were also recorded in brain cells after ten and 15 days of treatment. Changes in the SOD/GPX expression ratios suggested that the liver is more injured by chronic treatment with CP and that an adaptive response has developed in the brain cells by upregulating GPX gene expression.

DD013

COMET ASSAY STUDY OF HUMAN LEUKOCYTES EXPOSED TO OKADAIC ACID

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Okadaic acid (OA), a toxin produced by several dinoflagellate species and responsible for frequent food poisonings associated to shellfish consumption, is a specific inhibitor of serine/threonine protein phosphatases 1 (PP1) and 2A (PP2A) and a tumour promoter in two-stage carcinogenesis experiments. Although several studies have documented this role of phosphatase inhibition by OA on cell transformation, the molecular mechanistic basis for the effects induced by OA is not completely understood and the number of controversial data on OA genotoxicity is increasing in the literature. In the present study, the possible genotoxic effects of OA on human leukocytes were examined. Cells were treated with a range of OA concentrations (5, 10, 20, 50, 100 and 1000nM) in presence and absence of S9 fraction with the purpose of determining if this compound acts directly or it needs metabolic activation. Standard comet assay was used to evaluate DNA strand breaks, and combination with hOGG1 enzyme incubation enabled the specific recognition of oxidative damage. Data obtained showed increases in DNA damage, both strand breaks and oxidative, in all OA treatments in absence of S9-fraction, significant only for the highest concentrations. Nevertheless, no differences were found with regard to the control for any OA concentration in leukocytes cultured with S9-fraction, neither strand breaks nor oxidative damage. These results suggest that OA can act as a direct genotoxicity inducer in human leukocytes at the concentrations tested. Research supported by Xunta de Galicia (INCITE08PXIB106155PR).

DD014

DIFERRENTIAL DNA REPAIR RESPONSE OF CANCER CELLS TO CHEMOTHERAPEUTIC DRUGS STUDIED USING A MULTIPLEX IN VITRO ASSAY - PREDICTIVE VALUE?

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Aim: There is strong indication that the sensitivity of tumour cells to chemotherapeutic drugs is partly related to their reduced ability to repair drug-induced damaged DNA. On the other hand cancer cells can become resistant to drugs by acquiring DNA repair capacity. Investigating DNA repair is still a challenge due to a lack of appropriate tool that would take into account the complexity of the interplay between the different repair pathways. In this study we showed that the multiplex excision/synthesis repair assay we have developed allowed gaining insights into the complex response of several tumor cells treated by a panel of genotoxic drugs. Methods: 8 cancer cell lines were treated by 5 anticancer drugs (20% cytotoxiciy at 72h) and their repair capacity toward several lesions was investigated using a quantitative in vitro assay on biochip using cell extracts. We first had to set up the method that would allow interpretation of the data. Two parameters were defined: (1) global DNA repair capacity which is total repair activity that can be measured (2) DNA repair profile which is the contribution to total repair of the different repair pathways (toward photoproducts, cisplatin adducts, oxidized bases, AP sites). We then calculated for each cell line/drug pair, the ratio treated/non-treated for each parameter. A range of significance was established that was applied to each ratio. We could then express the effects of the drugs on DNA repair (global capacity and profile) according to 3 terms: stimulating, no effect, inhibitory. Results: at basal state each cell line exhibited a typical DNA repair profile. Effect of the drugs on DNA repair was not necessarily correlated with the corresponding cytotoxicity. Roughly, two categories of responses were observed: (1) global effect of drugs on repair capacity with no modification of the repair profile, (2) specific drug effect on certain repair pathways. Repair phenotypes were compared to available data on known mutations in cell regulatory and repair genes. Conclusion: we hope identifying mutations in driver genes that direct the response to therapy. Applied to cancer patients this strategy could help identifying predictive markers of individual response to chemotherapy.

DD015

IS THERE A WAY TO PREDICT THE CLINICAL OUTCOME OF DNA STRAND BREAKS GENERATING DRUGS? THE CASE OF CALICHEAMICIN.

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Gemtuzumab Ozogamicin (GO) is a chemotherapeutic agent conjugated with a cytotoxic antibiotic, calicheamicin. The antibody portion of GO binds to the CD33 antigen, an adhesion protein found on leukemic blasts. Binding of the anti-CD33 antibody portion of GO with the CD33 antigen results in the formation of a complex that is internalized. The calicheamicin derivative is then translocated into the nucleus and binds to the minor groove of DNA. This results in DNA double strand breaks and in cell death. A variable clinical response to GO has been observed in spite of the presence of CD33 on leukemic cells and this seems not only related to the presence of membrane proteins involved in drugs efflux (P-gp/MDR-1). The amount of GO-related DNA damage was evaluated by different methodologies in freshly isolated blasts from AML patients to identify the possible mechanisms of drug resistance and to correlate the in vitro individual response with the clinical outcome. For this purpose patients with relapsed leukemia were included in the study. The effect of GO was evaluated also in HL60 cells

(CD33+ acute promyelocytic leukemia). The evaluation of genotoxic effect in cells exposed to GO was obtained by microscopic visualization of DNA damage by Comet Assay. DNA strand breaks were detected by immocytochemical evaluation of the expression of γ histone AX (H2AX). The identification of apoptotic cells was performed by annexin/PI FACS technique. GO treated HL-60 cells showed high cito- and geno-toxic effects. Generally, GO induced nuclear damage and cellular death in a time dependent manner on patients CD33+ blasts. In vivo drug resistance and low level of DNA damage were associated with high nuclear damage level, as detected by Comet test and by H2AX expression. The presence, in some cases, of high levels of early DNA damage not related to consistent in vitro apoptotic death but showing a poor clinical outcome, suggests that DNA repair mechanisms or alternative death pathways activation could be connected with GO resistant phenotypes. Better understanding of these systems may provide a useful way to increase CD33+ blast sensitivity against GO and to find efficient therapy combination.

DD016

ANIMAL MODLE FOR AGE-RELATED GENOTOXICITY OF DIETHYSTILBESTROL

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Background: Environmental xenoestrogens pose a significant health risk for all living organisms. There is growing evidence on different susceptibility to xenoestrogens in developing and adult organism, but little is known about their genotoxicity in pre-pubertal mammals. Aim: In this study we developed an animal model to show gender and age specific genotoxcity of a synthetic oestrogen diethylstilbestrol (DES). Material and methods: Genotoxicity of DES was tested on the reticulocytes of pre-pubertal and adult mice using the in vivo micronucleus (MN) assay. Diethylstilbestrol had been administered intraperitonealy (0.05 µg/kg ,0.5 µg/kg and 5 µg/kg) for three days. Animals were sampled 48 h,72 h, 96 h, and 2 weeks after exposure. Results: After the dose of 0.05 µg/kg of DES pre-pubertal mice showed a significant increase in MN frequency (P<0.001) while in adults it remained at reference values (5,3 MN/1000 versus 1,0 MN/1000 reticulocytes). At doses of 0.5 µg/kg and 5 µg/kg, MN frequency significantly increased in both age groups. In pre-pubertal male animals, the MN frequency remained above the reference values even two weeks after exposure. Conclusion: Our animal model for pre-pubertal genotoxicity assessment using in the vivo MN assay proved to be sensitive enough to distinguish age and sex differences in genome damage caused by DES. Diethylstilbestrol showed to be more genotoxic in prepuberal animals especially in males.

DD017

INTERLABORATORY COMPARISON OF NUCLEAR BUDS, NUCLEOPLASMIC BRIDGES AND MICRONUCLEI SCORING FOR POTENTIAL FOOD AND ENVIRONEMNTAL MUTAGENS;benzo(a)pyrene , 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine and 2-amino-3-methyl-imidazo[4,5-f]-quinoline

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BACKGROUND: Continous increase in cancer incidence reported worldwild is providing evidence that only small proportion of cancerogenesis can be attributale to genetic traits. Particulary critical period is intrauterine development where maternal exposure to food and/or environmental genotoxicants might contribute to health risk in the unborn child and programed onset of diseases occuring later in life. Within the NewGeneris framework programme, biomarker technology is used to evaluate, early effects of, and individual susceptibility to, a selection of food and/or environmental genotoxicants. The in vitro micronucleus assay, a well-validated method, is being used for the screening of polycyclic aromatic hydrocarbons and heterocyclic amines. METHODS: Blood samples were collected from 60 healthy adult male and female donors ranging from 20 to 35 years old. Peripheral blood lymphocytes were isolated from whole blood, and three selected chemicals: Benzo[a]pyrene (BaP), 2-amino-1-methyl-6-phenylimidazol[4,5-b]pyridine (PhIP) and 2-amino-3-methylimidazol[4,5-f]quinoline IQ were tested by in vitro micronucleus assay with S9 for several doses (1%, 10% and 100%). Micronucleus assay was performed and slides were scored according to the criteria defined by Fenech and Morley 1985 and Fenech et al. 2003. The slides were manually scored at the University of Bradford and the Institute for Medical Research and Occupational Health in Zagreb. RESULTS: Dose- dependent increase in MN frequency in binucleates (BiMN) was observed for BaP and IQ, but the differences were not statistically significant. There is only significant increase of BiMN at concentration of 1% for PhIP chemical. BaP, PhIP and IQ did not generate MN in mononucleated lymphocytes, nuclear buds and CBPI. CONCLUSION: Interlaboratory collaborative study was performed in order to reduce heterogeneity and give more objective results of scoring and therefore increasing accuracy of results. At our experimental conditions BaP and IQ we were not able to detect their genotoxicity. As S9 fraction was used for all tested compounds, the difference in detected genotoxic effect may be influenced by potency of S9 to activate all compounds with the same efficiency.

DD018

MICRONUCLEI FREQUENCY AND GENE EXPRESSION IN CHILDREN EXPOSED TO AIR POLLUTION

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The Teplice area in the Czech Republic is a mining district whit high levels of air pollution including airborne carcinogens. This environmental threat may impact human health [1]. Gene expression analysis and micronuclei (MN) frequency was measured in lymphocytes of children living in this area and compared with a similar group of children living in the much less polluted area of Prachtice [2]. The raw and transformed data files were downloaded from http://www.ncbi.nlm.nih.gov/GEO [2]. Aim of this study has been to investigate the association between the frequency of MN in peripheral blood lymphocytes and a list of 20 candidate genes which were reported in the literature to be associated with chromosome malsegregation (BUB1, Cyclin2E, hCDC4, Face-1, TP53, WAF1, BAX, TPX2, PRC1, CDC2, FOXM1, KIF20A, AURKB, NEK2, H2AFX, CDC20, ZWINT, CCNB1, CCNB2 and AURKA). The role of major covariates such as area, gender, and age, as effect modifiers of this association was evaluated. Only 16 of 20 genes were found into microarrays database. First of all, univariate statistical parameters were evaluated, linking gene expressions to MN frequency (Pearson correlation). After this, the association between chromosome malsegregation and gene expression, taking into account the influence of the area, gender, and age, was investigated according to a binomial negative regression model. The extent of linear correlation between MN frequency and the expression level of the 16 gene investigated was quite low, never reaching the threshold of statistical significance. The differential expression analysis did not show statistically significant difference for any of the covariates included in the model. The binomial negative regression

model confirmed the association between MN frequency and region and age (p-value < 0.01). The Likelihood ratio tests showed a statistically significant interaction between the expression of TP53 gene and gender (p-value < 0.02).

1. van Leeuwen,D.M. et al. (2006) Genome-wide differential gene expression in children exposed to air pollution in the Czech Republic.Mutat. Res., 600, 12–22.
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DD019

ALDOSTERONE ACTIVATES NF- B IN KIDNEY CELLS THROUGH NADPH OXIDASE-MEDIATED OXIDANT PRODUCTION.

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An inappropriate increase of the mineralocorticoid aldosterone (ALD) can be induced by a stimulated renin-angiotensin system. Epidemiological exploring the connection between hypertension and cancer found higher cancer mortality and an increased risk to develop kidney cancer in hypertensive individuals. This work investigated if high concentrations of ALD could activate the transcription factor NF-κB, an oncogenic signal when overstimulated. The incubation of LLC-PK1 and MDCK kidney cells in the presence of ALD caused a dose (5-100 nM)- and time (0-4 h)-dependent activation of NF-κB as evaluated by EMSA. In LLC-PK1 and MDCK cells the maximum nuclear NF-κB-DNA binding occurred at 100 nM ALD after 30 and 120 min, respectively. ALD-induced NF-κB activation was inhibited by the antioxidants lipoic acid (LA) and N-acetylcysteine (NAC), and by the superoxide scavenger tempol. Significantly, cell oxidant levels, measured with the probe 5-(and-6)-carboxy-2'7'-dichlorodihydrofluorescein diacetate (DHDCFDA), increased in both kidney cell lines upon treatment with 100 nM ALD. This increase was prevented by LA, NAC, and tempol. The mechanisms involved in ALD-mediated oxidant increase and NF-κB activation were investigated. Both, the increase in cell oxidant levels and NF-κB activation were inhibited by the NADPH oxidase (NADPHox) inhibitors apocynin (Apo) and diphenyleneiodonium chloride (DPI), and by the protein kinase C (PKC) inhibitor Ro 320432. The results indicate that high levels of ALD can trigger an increased oxidant production in kidney cells, through the PKC-mediated activation of NADPHox activating NF-κB. These events could in part underly the genotoxic actions of ALD.

DD020

MULTIPLE FACTORS FEATURING HYPERSENSITIVITY OF P53 DEFICIENT CELLS TO TOPOTECAN

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Topotecan (TPT), a camptothecin derivative, belongs to the group of topoisomerase I (topoI) inhibitors and is a relevant anticancer drug. Previously we showed that the frequency of TPT-triggered apoptosis depends on the p53 status, i.e. we observed that p53 or one of its target proteins support the degradation of topoI in the so-called topoI cleavable complex, thus making p53 proficient cells more resistant to TPT. We dissected the apoptotic pathway in great detail and demonstrate that p53 deficient (p53-/-) MEFs undergo abundant apoptosis (annexin V fraction) in response to TPT whereas p53 proficient (wt and apaf-1-/-) MEFs are slightly sensitive and fully resistant to TPT, respectively. In TPT-treated p53-/- cells the H2A histone variant, H2AX (a hallmark of stalled replication forks and SSB/DSB formation), was strongly phosphorylated. Both Bax stabilization in p53 proficient and Bcl-xL downmodulation in p53 deficient cells led to cytochrome c release and with exception of apaf-1-/- cells to caspase-9/-3 activation. Since none of the cell lines displayed caspase-8 activity, we infer that cells undergo apoptosis by activating the mitochondrial pathway with caspase-9 crucially involved, as substantiated by experiments with caspase inhibitors and dominant-negative mutated constructs of caspase-9/-3. In p53-/- cells caspase-2 was significantly processed, cleaving Bid. In wt and apaf-1-/- cells neither caspase-2 was active nor Bid was cleaved. Cotreatment of cells with caspase-3 inhibitor and TPT abolished caspase-2 activation and Bid cleavage, implying that caspase-2 is processed downstream of the apoptosome by caspase-3. Although caspase-9 and -3 were similarly processed in wt and p53-/- cells, only in p53-/- cells caspase-3 was significantly active. This was due to proteasomal degradation of the anti-apoptotic proteins XIAP and survivin in p53-/- cells. In accordance, in wt cells TPT-induced apoptosis was enhanced by the knockdown of XIAP and survivin. Overall, the data show that p53 deficient cells are hypersensitive to TPT because of lack of degradation of topoI, accumulation of DNA double-strand breaks, down-regulation of XIAP and survivin, and amplification of the intrinsic pathway via caspase-2-mediated Bid cleavage.

DD21

ERCC1-XPF IS A DETERMINANT FOR CROSSLINK REPAIR DEFICIENCY AND CISPLATIN HYPERSENSITIVITY IN TESTIS TUMOR CELLS

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Metastatic testicular germ cell tumors (TGCT) are cured in over 75 % of patients using cisplatin-based combination therapy. In contrast, almost all other solid cancers in adults are incurable once they have spread beyond the primary site. The reasons underlying the cisplatin sensitivity of TGCT are not yet known. Cell lines derived from TGCTs retain the cisplatin hypersensitivity in vitro, therefore providing a good model system for investigating the factors controlling cisplatin sensitivity. We previously showed that testis tumor cells have a reduced capacity to remove cisplatin-induced DNA platination suggesting that repair deficiency might be a factor for the observed cisplatin sensitivity. In further studies we found that the nucleotide excision repair (NER) factor ERCC1-XPF is reduced in testis-tumor cell lines indicating a possible role of ERCC1-XPF for repair deficiency and cisplatin sensitivity of testis tumor cells. Cisplatin induces both intrastrand adducts (IAs) and interstrand crosslinks (ICLs). To investigate repair of IAs we used the method of DNA slot blotting. Repair of ICLs was investigated using the Comet assay. We found that repair of IAs is similar in the testis tumor cell lines 833K and SuSa compared to the cisplatin resistant bladder cancer cell line MGH-U1. Repair of ICLs, however, was significantly reduced in the testis tumor cells compared to the bladder cancer cell line. To analyse the causal role of ERCC1-XPF for repair deficiency and cisplatin sensitivity in testis tumor cells we overexpressed ERCC1-XPF in the testis tumor cell line 833K using a mammalian expression vector. Overexpression of ERCC1-XPF increased ICL repair and made the cells more resistant to cisplatin induced apoptosis. In proof of principle experiments we demonstrated that downregulation of ERCC1-XPF sensitized MGH-U1 bladder cancer cells to cisplatin. These findings suggest that ERCC1-XPF levels modulate sensitivity to cisplatin and identify ERCC1-XPF as a target for sensitizing cells to cisplatin and related drugs that cause ICLs.

DD022

DNA POLYMERASES BETA AND LAMBDA AS A POTENTIAL PARTICIPANTS OF TLS DURING GENOMIC DNA REPLICATION ON THE LEADING AND LAGGING STRANDS

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The main strategy used by pro- and eukaryotic cells for replication of damaged DNA is translesion synthesis (TLS). Here, we investigated TLS activity of the human X-family DNA pols beta and lambda on DNA duplexes containing different lesions resulted from the oxidative

stress – abasic site, oxoguanine and thymine glycol. We determined kinetic parameters of native and photoreactive dNTP incorporation opposite different DNA damages by DNA pols activity in the presence of Mg(II) or Mn(II) ions. Additionally, we investigated the influence of hPCNA and hRPA on TLS-reaction. Further, to discriminate the surrounding proteins that could potentially act during TLS in the cell we applied the photoaffinity labelling approach for modification of Bovine Testis (BT) and HeLa (RC) extract proteins. We found a limited number of modification products among the general pool of proteins. It was confirmed: (i) by Western blotting that the RC 75-80 kDa crosslinking product is the covalent adduct of DNA to pol lambda; (ii) by immunoprecipitation with human antibodies that the BT 105 kDa crosslinking product is PARP1. On the basis of experimental results, DNA pols beta and lambda can be proposed as a “good” candidates for participation in TLS process across AP-site, 8-oxoG and Tg during genome DNA replication on the leading and lagging strands. Moreover, DNA pols beta and lambda can be a components of TLS machine not only during the first stage of the process (i.e. incorporation of dNMP opposite damage) but on the stage of processing of uncomplementary 3’-end of primer. Both DNA pols are very attractive for construction and application of photoreactive DNA structures for studying TLS process in eukaryotic cellular/nuclear extracts. This work was supported by a grant from the Russian Foundation for Basic Research (No. 09-04-00899-a), and program of Russian Academy of Science “Molecular and cellular biology”.

DD023

DEFECTIVE REPAIR OF 5-HYDROXY-2’DEOXYCYTIDINE IN COCKAYNE SYNDROME CELLS AND ITS COMPLEMENTATION BY E.COLI FORMAMIDOPYRIMIDINE DNA GLYCOSYLASE AND ENDONUCLEASE III

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Repair of the oxidized purine 8-oxo-7,8-dihydroguanine (8-oxoGua) is inefficient in cells belonging to both complementation groups A and B of Cockayne syndrome (CS), a developmental and neurological disorder characterized by defective transcription coupled-repair. We show here that both CS-A and CS-B cells are further defective in repair of 5-hydroxy-2’-deoxycytidine (5-OHdC), an oxidized pyrimidine with cytotoxic and mutagenic properties. The defect in repair of oxidatively damaged DNA in Cockayne syndrome cells thus extends to an oxidized pyrimidine, indicating a general flaw in repair of oxidized lesions in this syndrome. The defect could not be reproduced in in vitro repair experiments on naked oligonucleotides substrates, suggesting a role of both CS-A and CSB proteins in chromatin remodeling during 5-OHdC repair. Expression of E. coli formamidopyrimidine DNA glycosylase (FPG) or endonuclease III (NTH) complemented the 5-OHdC repair deficiency. Hence, expression of one single enzyme, FPG from E. coli, stably corrects the delayed removal of both oxidized purines and pyrimidines in CS cells and represents a possible gene therapy tool.

DD024

DEFECTIVE REPAIR OF 5-HYDROXY-2’DEOXYCYTIDINE IN COCKAYNE SYNDROME CELLS AND ITS COMPLEMENTATION BY E.COLI FORMAMIDOPYRIMIDINE DNA GLYCOSYLASE AND ENDONUCLEASE III

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Repair of the oxidized purine 8-oxo-7,8-dihydroguanine (8-oxoGua) is inefficient in cells belonging to both complementation groups A and B of Cockayne syndrome (CS), a developmental and neurological disorder characterized by defective transcription coupled-repair. We show here that both CS-A and CS-B cells are further defective in repair of 5-hydroxy-2’-deoxycytidine (5-OHdC), an oxidized pyrimidine with cytotoxic and mutagenic properties. The defect in repair of oxidatively damaged DNA in Cockayne syndrome cells thus extends to an oxidized pyrimidine, indicating a general flaw in repair of oxidized lesions in this syndrome. The defect could not be reproduced in in vitro repair experiments on naked oligonucleotides substrates, suggesting a role of both CS-A and CSB proteins in chromatin remodeling during 5-OHdC repair. Expression of E. coli formamidopyrimidine DNA glycosylase (FPG) or endonuclease III (NTH) complemented the 5-OHdC repair deficiency. Hence, expression of one single enzyme, FPG from E. coli, stably corrects the delayed removal of both oxidized purines and pyrimidines in CS cells and represents a possible gene therapy tool.

DD025

LESION RECOGNITION AND CATALYSIS IN BER PATHWAY INVOLVE MULTIPLE CONFORMATIONAL CHANGES IN ENZYMES AND DNA

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Base-excision repair (BER) pathway is one of the main ways to repair damages of individual DNA bases. The key enzymes in BER are DNA glycosylases, which recognize a variety of modified or mismatched bases and catalyze cleavage of the N-glycosidic bond to release the inappropriate base from the deoxyribose ring. Many glycosylases also catalyze a beta-elimination (or lyase) reaction to effect strand scission after the base removal. Subsequent action of apurinic-apyrimidinic (AP) endonucleases and 3’-phosphodiesterases remove the remaining sugar fragment to produce a single-nucleotide gap with the proper 3’-OH and 5’-phosphate termini, a substrate for DNA polymerases. After the DNA polymerase adds the correct nucleotide, DNA ligase completes the BER process. Bacterial Fpg and eukaryotic OGG1 are two proteins that share no sequence homology nor are they structurally similar. In spite of this, they both are able to remove 8-oxoguanine, an abundant pre-mutagenic oxidized nucleobase, from DNA. Recently we have investigated the conformational transitions in several DNA repair enzymes, including DNA glycosylases (E. coli Fpg and Nei, human OGG1) and AP endonucleases (human APE1), and in their DNA substrates by stopped-flow detection of tryptophan and 2-aminopurine fluorescence as well as using FRET labels in DNA. DNA substrates contained damaged bases or abasic sites of different natures. In all cases, multiple transient changes in fluorescence intensities of enzymes and DNA substrates were observed, indicating sequential conformational changes in both macromolecules during the catalytic cycle. Detailed kinetic schemes were derived that describe the mechanisms for substrate recognition and cleavage. A comparison of the fluorescence traces for wild-type Fpg and its mutant forms F110W and F110A suggests that the search for damaged bases in DNA proceeds through intercalation of Phe-110 residue into the DNA helix. This step could initiate the eversion of the damaged deoxynucleoside into the catalytic center of enzyme. The research was supported by grants from RFBR (No 08-04-12211, 07-04-00191), SB RAS (No 28, 48), Russian Ministry of Education and Science (652.2008.4).

DD026

DNA FRAGMENTATION IN BACTERIA ASSESSED IN SITU

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BACKGROUND AND AIMS: Chromosomal DNA fragmentation, resulting from massive DNA double-strand breaks, is a hallmark of cell death. Despite its importance from both a basic research and a clinical point of view, DNA fragmentation has not been assessed in the microbiology laboratory. This may be due to the lack of a simple and rapid evaluation procedure. Here we present an adaptation of a diffusion-based assay, developed as a kit, which allows for simple, fast, reproducible and accurate discrimination of bacteria with fragmented DNA. METHODS: Intact microorganisms were processed using de Micro-Halomax® kit. In this way, cells were embedded in an agarose microgel on a slide and incubated in a lysis buffer at 37ºC for 5 minutes to partially remove the cell walls, membranes, and proteins. After washing, the slide was dehydrated and then stained with a DNA fluorochrome, SYBR Gold. RESULTS: Cells without DNA fragmentation show nucleoids with DNA loops spreading from a central core, which corresponds to the residual bacterium, with a compact, microgranular surface extended peripherally to many branches. Identifying cells with fragmented DNA have a very big halo of DNA spots radiating from the residual central core. These results have been seen in several gram-negative and gram-positive bacteria. Detection of DNA fragmentation was confirmed by fluoroquinolone treatment and by DNA breakage detection-fluorescence in situ hybridization (DBD-FISH). Analysis of spontaneous DNA fragmentation in Proteus mirabilis cultures, and analysis of antibiotic or reactive oxygen species induced damage in Escherichia coli demonstrated the great potential of this procedure to determine chromosomal DNA fragmentation in microorganisms. CONCLUSIONS: Our assay may be useful for the evaluation of DNA damage and repair as well as cell death, either spontaneous or induced by exogenous stimuli, including antimicrobial agents or environmental conditions. Its simplicity, short assay time (50 min), and efficacy makes this technique useful for the routine determination of DNA fragmentation and intracellular variation. Applications may be extensive for both basic and clinical research.

DD027

ASSESSMENT IN SITU OF DNA DOUBLE-STRAND BREAKS INDUCED BY CIPROFLOXACIN ON CHROMOSOMAL DNA FROM Escherichia coli

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Background/Aims: Fluoroquinolones are extensively used antibiotics that induce DNA double-strand breaks (DSB) by trapping DNA gyrase and topoisomerase IV on DNA. In bacteria, this effect is usually evaluated using biochemical or molecular procedures but not at the single-cell level. We have assessed ciprofloxacin (CIP) induced chromosomal DNA breakage in single-cell Escherichia coli by a procedure of direct visualization of DNA fragments that diffuse from the nucleoid. Method: We have recently developed a kit (Micro-Halomax® Kit) that allows the simple and rapid assessment of the presence of fragmented DNA at the single cell level in microorganisms. Cells immersed in a microgel on a slide are lysed, stained, and visualized with a fluorescence microscope. The nucleoids with fragmented DNA are discriminated clearly by the peripheral halo of diffused DNA fragments. The greater the fragmentation, the greater the number of DNA spots and the greater the circular surface area of diffusion. Results: When the E. coli strain TG1 was exposed to increasing doses of CIP for 40 min, DNA damage started to be detectable with the minimum inhibitory concentration (MIC) dose. As the dose increased, the DNA affectation was clearly visualized immediately after processing, its degree progressively increasing when rising the antibiotic incubation time. The affectation level was much higher when bacteria come from liquid LB broth and, CIP treatment along stationary phase resulted in progressively slower DNA affectation. CIP-induced DSB were not totally irreversible, and a progressive repair activity with time was evident. The magnitude of DNA repair was inversely related to dose. The repair activity was not strictly related to viability. Four E. coli strains with identified mechanism of reduced sensitivity to CIP were assessed with the procedure, obtaining DNA fragmentation levels that were inversely related to MIC dose. Conclusion: The procedure for determination of DNA fragmentation is a simple and rapid test for studying and evaluating the effect of quinolones.

DD028

BIOCHEMICAL EVIDENCES INVOLVE DNA POLYMERASE BETA OF Trypanosoma cruzi IN REPAIR OF OXIDATIVE LESIONS IN MITOCHONDRIAL DNA

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During its life cycle, Trypanosoma cruzi must deal with the deleterious action of reactive oxygen species (ROS) on DNA. ROS can oxidize guanines to generate 7,8-dihydro-8-oxoguanine (oxoG) that has the ability to mispair with adenine. If unrepaired, oxoG can lead to transversions during cell division. Specific biochemical pathways were identified to minimize the mutagenic consequences of oxoG in T. cruzi. We believe that base excision repair (BER) is essential to maintain an equilibrium between generation of genetic variability and genome protection against oxidative genotoxic stress in this parasite. DNA polymerase beta (polβ) is strictly involved in BER, and T.cruzi possesses two mitochondrial polβ (Tcpolβ and TcpolβPAK). We showed that in vitro DNA synthesis capability for both recombinant enzymes was differentially modulated by NaCl and pH. Different from human polβ, Tcpolβ was unable to synthesize DNA across an oligonucleotide containing oxoG or a 3´ mismatch. We immunolocalized Tcpolβ on kinetoplast antipodal sites of replicative epimastigotes and amastigotes cellular forms. To further explore Tcpolβ function in vivo, we isolated clones overexpressing Tcpolβ. Two clones obtained were tested for survival in presence of DNA replicating blocking agent zidovudin (AZT). These clones were more sensitive to AZT treatment compared to control, suggesting that Tcpolβ can incorporate AZT in DNA. This was confirmed through in vitro experiments, suggesting involvement of this enzyme in mitochondrial DNA replication. In addition, overexpressing clones were treated with hydrogen peroxide (H_2O_2). The treatment increased survival rate, and a lower level of 8-oxoguanine was detected in kinetoplast of overexpressing clones compared to control. Curiously, a possible DNA repair focus of Tcpolβ was identified in the vicinity of kinetoplast of epimastigotes wild type CL Brener cells after H_2O_2 treatment. This focus was not observed after methyl methanesulfonate treatment. Lastly, treatment of non-dividing trypomastigotes with crescent doses of H_2O_2 directs the polymerase toward to kinetoplast. The experimental data obtained suggest participation of Tcpolβin replication of kinetoplast and repair of oxidative damage in mitochondrial DNA of T. cruzi.

DD029

USE OF MULTIPLEX OLIGONUCLEOTIDE CLEAVAGE ASSAY ON SUPPORT FOR SELECTION OF DNA REPAIR INHIBITORS BY ROBOTIC SCREENING OF CHEMICAL LIBRARIES

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Aim: Many studies linked chemoresistance and overexpression of DNA repair enzymes. Enzymes from Base Excision Repair pathway and in particular apurinic endonuclease 1 (APE1) are attractive targets for anti cancer drug development as therapeutic modulation of these enzymes could potentiate effects of genotoxic drugs. The aim of our

study was to demonstrate that we could use a multiplex oligonucleotide (ODN) cleavage assay on support together with nuclear extract to screen a compound library and find specific inhibitors of human APE1 and human Uracil-N-glycosylase (UNG). Method: 4 ODN fluorescent duplexes containing tetrahydrofuran (THF) (paired with A), Uracil (paired either with A or with G) and normal base pairs were addressed onto streptavidin coated slides in a 96-well platearray format adapted for robotic handling. Upon action of repair enzymes contained in standard HeLa nuclear extracts, lesions were cleaved and the corresponding fluorescent signal was released. Automated screening of a chemical library of small molecules was performed using extracts at 6 µg/ml and compounds at an average concentration of 200 µM. Results: First, to develop the assay we had to select a specific DNA repair inhibitor as positive control. Several molecules were tested (methoxyamine, nitroindole carboxylic acid, aminouracil, carboxyuracil,…) and hycanthone was chosen, due to its strong inhibitory potential on all targeted activities. The primary screening allowed the identification of about 40 potential hits that exhibited various specificity (specific inhibition of (U/G) or (U/G and U/A) or (U/G, U/A and THF/A)). It is worth noting that, as expected, inhibition of THF cleavage was always associated with inhibition of U cleavage. Conclusion: The identified hits are currently being extensively characterized. Nevertheless we brought here the proof of concept that multiplex ODN cleavage assay using cell extract is a convenient mean for the fast determination of DNA repair inhibitory properties of small molecules.

DD030

ROLE OF THE APOPTOSIS-MODULATORY MOLECULE NF-ΚB IN DNA REPAIR

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Activation of nuclear factor κB (NF-κB) is part of the DNA damage signalling network and regulates a cell survival pathway, which, together with the activation of the cell cycle checkpoints and DNA repair, allows the cell in cases of limited damage to restore a normal life cycle (1). It is well known, that NF-κB is activated upon DNA damage and DNA double-strand breaks (DSBs) in particular via ATM signalling (2), but very little is known about NF-κB downstream targets involved in damage repair. With the use of a fluorescence-based recombination test system we systematically studied a potential role of NF-κB in the repair of DSBs introduced by the meganuclease I-SceI. Expression of the NF-κB family member protein p65, as well as NF-κB activation by TNFα or chemotherapeutic exposure (etoposide, champtothecin and doxorubicin) led to stimulation of DSB repair in different cell types. This effect was fully repressed by concomitant expression of the NF-κB antagonist IκBα super-repressor and was demonstrated to be independent of apoptosis, cell cycle changes, reactive oxygen species, or the chromatin context. To further investigate this NF-κB specific activity, we applied substrates designed to assay distinct DNA repair pathways. Interestingly, we found that homologous recombination (HR) is the DSB repair pathway which is highly up-regulated by NF-κB. To the contrary, NF-κB had a very small impact on microhomology mediated non-homologous end joining (NHEJ) as well as on single-strand annealing (SSA). When we investigated transcriptional NF-κB targets involved in DSB repair by epistatis analysis, our results did not indicate the involvement of BRCA2 or Ku70. Similarly, when checking the dual role apoptosis factors and DSB repair modulators Bcl2, Bcl-xL and Bax, we did not find evidence for an involvement in NF-κB-mediated HR. However, the p65 binding partner BRCA1 was identified to be necessary for NF-κB mediated HR regulation. All in all, our results provide first evidence for a stimulatory role of NF-κB in DSB repair with a specific influence on the pathway of HR mediated by the NF-κB-complex partner BRCA1.

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DD031

GENETIC INSTABILITY IN THE PERIPHERAL LYMPHOCYTES AND BUCCAL CELLS OF HEAD AND NECK CANCER PATIENTS AND THEIR FIRST DEGREE RELATIVES: INFLUENCE OF XRCC1 Arg399Gln POLYMORPHISM?

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It may be important to know the influence of genetic polymorphisms on chromosome damage with respect to the improvement of cytogenetic biomarkers and also to the identification of at-risk groups. We studied the baseline micronucleus (MN) and radiation-induced micronucleus (Ind-MN) frequencies in lymphocytes and the buccal cell MN frequencies and influence of XRCC1 gene polymorphism (Arg399Gln) both for head and neck cancer (HNC) patients (n=59) and their first degree relatives (FDRs) (n=34) as well as controls (n=31).The XRCC1 alleles were detected using PCR-RFLP technique. For the Ind-MN assay, blood samples were exposed in vitro to 2 Gy gamma rays (60Co) at a dose rate of 0.62 Gy/min. The mean(SD)MN and Ind-MN frequencies(‰)in lymphocytes of HNC patients, FDRs and controls were 27.1(9.5), 172.7(44.8),14.1(5.2),169.5(47.1)and 9.0 (6.9), 150.9(43.3), respectively. Significant differences in baseline MN frequencies between studied groups were found ($p<0.05$), however, there were no differences in Ind-MN frequencies between all groups ($p>0.05$). Significant differences in buccal MN frequencies between HNC patients and FDRs as well as controls were found ($p<0.001$). Number of subjects with wild genotype (Arg/Arg), heterozygous subjects (Arg/Gln) and mutant subjects (Gln/Gln) are 23, 12, 12; 21,14,14 and 8,5,5 for patients, FDRs and controls, respectively. Our data suggest that MN frequencies in lymphocytes and buccal cells may be predictive for cancer risk. It might be possible to identify high risk family members by analyzing background MN frequencies in lymphocytes but not in buccal cells. XRCC1 variant genotypes did not significantly modulate the frequency of MN in studied groups. Further work is needed to resolve the importance of other polymorphisms of XRCC1 including codon 194, as well as other DNA repair systems.

DD032

RH1 ENHANCES RADIOSENSITIVITY THROUGH THE ACTIVATION OF ER STRESS AND DNA DAMAGE RESPONSE.

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Background and aims: RH1 (2,5-diaziridinyl-3-(hydroxymethyl)-6-methyl-1,4-benzoquinone), is a novel anticancer agent, which is activated by two-electron reductase NAD(P)H quinone oxidoreductase 1 (NQO1). In this study, we observed that RH1 causes clonogenic cell death in RKO and MDA-MB-231 cells in a NQO1 dependent manner. Methods: RKO.C human cololectal cancer cells, MDA-MB-231 human breast cancer cells overexpressing NQO1 and A549 human lung adenocarcinoma epithelial cells were used. The cytotoxicity of RH1 was assessed with clonogenic survival method or PI (propidium iodide) staining. The effect of RH1 on the response of the cancer cells to irradiation was also determined. The effects of RH1 on the apoptotic machinery or the ER stress response were analyzed with Western blotting or RT-PCR. gamma-H2AX foci formation after the combination treatment with irradiation and RH1 was analyzed with immunocytochemistry. Results and Conclusions: We also observed that RH1 causes activation of caspase-3 and PARP cleavage leading to apoptotic cell death. We investigated the possible involvement of ER (Endoplasmic Reticulum) stress-related unfolded protein in the RH1-induced cell death and found that RH1 increases the expression of CHOP (C/EBP

homologous protein) and activates JNK, but causes no change in the expression of GRP. These results demonstrated that RH1 causes ER stress pathways leading to cell death Importantly, we observed that RH1 is not only cytotoxic but it also causes substantial increase in radiosensitivity of cancer cells. The radiation-induced gamma-H2AX foci formation was significantly increased and prolonged by RH1, indicating RH1 inhibits the repair of radiation-induced DNA damage. RH1 also increased the radiation-induced expression of p53 and p21. In conclusion, RH1 activates caspase and ER stress pathways, thereby inducing apoptosis and clonogeneic cell death. Moreover, RHI enhances the radiation-induced cell death by inhibiting the DNA damage repair. The molecular signaling pathways that we elucidated in this study may provide important information for developing new enzyme-targeting drugs which not only causes cell death but also sensitizes cancer cells to radiation therapy.

DD033

TELOMERE DYSFUNCTION INDUCED FOCI CORRELATES POSITIVELY WITH INCIDENCE OF SISTER CHROMATID EXCHANGES IN CELLS OF FANCONI ANEMIA PATIENTS

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Telomeres consist of repeated DNA sequences and associated proteins that lie at the termini of linear chromosomes and are critical to the protection and stability of internal chromosome sequences, a function known as telomere "capping". In capping chromosomes ends, telomeres restrict chromosome end resection by exonucleases and prevent the improper activation of checkpoint response factors and DNA damage response pathways such as homologous recombination (HR) and non-homologous end joining (NHEJ). Telomeres shorten as cell divide, but also due to action of restriction exonucleases, oxidative damage and inappropriate recombination. When telomere shorten to a critical length, they become uncapped, which can lead to permanent cell cycle arrest. The development of the "TIF" (telomere dysfunction induced foci) assay that is based on colocalization of the DNA repair factors 53BP1 and γH2AX with uncapped telomeres enables measurement of telomere uncapping in different tissues. Our aim was to investigate correlation between homologous DNA recombination processes and function of telomeres. In nine primary fibroblast cell lines, obtained from patients presenting with bone marrow failure syndrome and Fanconi anemia clinical and cellular (diepoxybutane- DEB positive) phenotype, baseline incidence of SCE, T-SCE, γH2AX and TIF were examined. The average incidence of SCE was 4.42+-0.96, ranging from 3.38 to 6.5 per cell, whereas incidence of T-SCE was 1.91+-0.81 ranging from 1 to 3.33. Baseline level of γH2AX foci was 2.27+-1.55 ranging from 0.8 to 4.2, whereas average incidence of TIF was 2.97+-1.47, ranging from 1.44 to 5 per cell. Statistically significant positive correlation between incidence of SCE and TIF was observed (0.77 $p<0.05$) indicating a connection between uncapped telomeres in these cells and homologous recombination, a link which should be further investigated.

DD034

ROLE OF DNA POLYMERASE KAPPA IN THE CYTOTOXIC PROCESSING OF O6-METHYLGUANINE IN HUMAN CELLS

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N-methyl-N-nitrosourea (MNU), a SN1-type alkylating agent, is able to react with both nitrogen and oxygen atoms of DNA bases generating approximately 80 % of N-methyl purines and 10% of O^6-methylguanine (O^6-meGua). O^6-meGua is the primary cytotoxic and mutagenic lesion after MNU exposure in human cells. The detrimental effects of O^6-meGua are due to the erroneous processing of O^6-meGua/T mispairs by mismatch repair (MMR). This pathway was recently found to be involved in the generation of single-stranded DNA (ssDNA) gaps which are recognized by RAD51. The potential involvement of Y-family DNA polymerases in the cytotoxic and mutagenic processing of O^6-meGua has been investigated *in vitro,* but little is known on their role *in vivo*. Down-regulation of DNA polymerase κ (polκ) was achieved by siRNA in the human cell lines HeLa and A2780. Knock-down of pol κ sensitized both cell lines to killing by MNU, although no significant difference in mutagenesis was observed. The association between increased toxicity and the presence of O^6-meGua in DNA, was strongly suggested by *a)* the requirement of O^6-benzylguanine, an O^6-methylguanine-methyltransferase inhibitor, to detect sensitization after MNU and *b)* the less pronounced modulation of killing by methyl-methanesulfonate, a poor inducer of O^6-meGua. After MNU-exposure, a higher fraction of RAD51 foci was observed in polκ-downregulated HeLa cells compared to the native counterpart. These results were not associated to an increase of γ-H2AX foci and SCE levels, markers of homologous recombination pathway, suggesting a non canonical function of RAD51. In addition, polκ-downregulation did not affect the cell cycle profile, as verified by FACS, although a delay in the progression through all cell cycle phases was observed after MNU treatment. It has been recently suggested that translesion synthesis polymerases are also able to assist DNA lesions bypass outside of S-phase, catalysing post-replicative filling-in reactions of ssDNA gaps, left behind the replication fork. All together our data strongly suggest that polκ could have a role in the extension reaction of ssDNA gaps opposite O^6-meGua in a post-replicative manner.

DD035

N-METHYLPURINE DNA GLYCOSYLASE AND ITS POTENTIAL ROLE IN THE MECHANISM BEHIND THE GENOTOXIC THRESHOLD OF ETHYL METHANESULFONATE.

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Background: Alkylating agents are exploited for their DNA damaging properties. At high doses they are used as anticancer agents, while at low concentrations they can be found as residual impurities in drug manufacture. Ethyl methanesulphonate (EMS) and ethylnitrosourea (ENU) are model alkylators used for the study of genotoxicity. With low nucleophilicity, ENU predominantly reacts with both oxygen and nitrogen atoms. EMS, having high nucleophilicity, chiefly reacts with nitrogen, ethylating the N7 position of guanine (N7G). N7G is associated with the induction of chromosomal damage and cytotoxicity. At low concentrations a threshold in clastogenicity was observed with EMS and not ENU in vitro and in vivo. Here we propose the involvement of DNA repair as a mechanism of action behind the threshold response. Aim: DNA repair of N7G is carried out by N-methylpurine DNA glycosylase (MPG). To identify whether DNA repair is responsible for cellular resistance to low levels of EMS, MPG-knockdown cell lines were constructed and tested. Method: shRNA: MPG shRNA clones (Sigma-Aldrich) were transfected into AHH-1 cells and transformants with puromycin resistance were selected. Gene expression studies: AHH-1 cells were seeded for 24 h and dosed with increasing EMS concentrations for one cell cycle. Total RNA and protein were isolated and analysed. Micronucleus assay: AHH-1 cells were cultured/dosed as before. 3µg/ml cytochalasin B was added to each flask during dosing. Micronucleate/Binucleate cells were scored with the Metafer automated system. Results: Gene expression studies using human lymphoblastoid cells revealed EMS but not ENU to induce up-regulation of MPG mRNA and protein. shRNA experiments yielded MPG knockdown of 47% (MPG09I) and 73% (MPG09B), relative to the AHH-1 parent cell line. Toxicology studies showed a loss of a threshold response in both MPG09I and MPG09B cells treated with EMS and no altered response to the clastogenic effects exerted by

ENU. Conclusion: EMS induced DNA damage results in the up-regulation of the MPG repair enzyme. Cells deficient in MPG contain a greater number of clastogenic lesions when compared to MPG proficient cells, suggesting N7G to be the clastogenic lesion responsible for the threshold observed for AHH-1 cells exposed to EMS.

DD036

CYTOTOXIC EFFECTS OF DOXORUBICIN ON XPD-DEFICIENT HUMAN FIBROBLASTS

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Doxorubicin is one of the commonly used chemotherapeutic drugs with a wide spectrum of activities against solid tumors and lymphomas. The cellular metabolism of doxorubicin generates reactive oxygen species (ROS) with potential to damage DNA. DNA repair capacity of the cells is important not only for preventing malignant transformation but also for its influence on the response to chemotherapy. Among several DNA repair pathways, the nucleotide excision repair is the most versatile one in repairing of bulky adducts and other types of damage like oxidative lesions. The aim of this study was to evaluate the role of ROS on the sensitivity of XPD-deficient fibroblasts to doxorubicin. For this purpose, cell lines carrying different mutations in the XPD gene as well as the repair proficient MRC5 cell line were employed. Flow cytometry analysis (FACS) after 48, 72, and 96 h of DOX treatment revealed an intensive arrest at G2 in MRC5 cells and increased sub-G1 population in XPD-deficient cells. The increased apoptosis induction was also confirmed by morphological analysis after acridine orange staining. Pre-treatment with the radical scavenger N-acetylcysteine had no effect on the cell cycle distribution after DOX treatment, indicating that free radicals release does not seem to be involved in apoptosis induction by DOX in our experimental conditions. The oxidative DNA damage induced by DOX was studied in the modified comet assay including incubation with the enzymes formamidopyrimidine DNA-glycosylase (Fpg) and endonuclease III (Endo III). The increment in the DNA damage score after enzymes incubation represents the extension of the oxidative DNA damage. The continuous DOX treatment during 24, 48 and 72 h lead to DNA breaks accumulation. However, the incubation with the enzymes Fpg and ENDO III did not increase significantly the DNA migration, suggesting that the accumulated strand breaks did not result from oxidative damage. Our results indicate that the increased sensitivity of XPD-deficient cells to DOX in our study cannot be related to ROS-induced damage or cell death. Work supported by CNPq, CAPES and FAPESP.

DD037

DNA DAMAGE IN HUMAN HEPATOMA CELL LINE (HEPG2) AND PERIPHERAL BLOOD LYMPHOCYTES AFTER MICROCYSTIN-LR EXPOSURE

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Microcystins (MCs), naturally occurring cyanobacterial hepatotoxic cyclic heptapeptides, present acute and chronic hazards to animal and human health. The primary mechanism of MCs toxicity is specific inhibition of serine/threonine protein phosphatases (PP1 and PP2A). In addition to protein phosphatase inhibition, MCs can cause oxidative stress by increasing the formation of reactive oxygen species (ROS) and by modifying intracellular antioxidant enzymes. MCs are considered to be liver specific toxins, because they enter cells through the multispecific transport system for organic anions and bile acids. In the present study the genotoxic activity of microcystin-LR (MCLR) on human hepatoma cell line (HepG2) and human peripheral blood lymphocytes was investigated. The cells were exposed to non-toxic concentrations of the toxin for 4, 6 and 24 hours and the amount of DNA strand breaks was evaluated using the comet assay. The induction of DNA strand breaks in HepG2 cells was dose (0.01, 0.1 and 1 μg/ml) and time dependent with the maximal DNA damage detected after 4 hours of exposure. The amount of strand breaks declined with further incubation and after 24 hours we did not detect difference in DNA damage between toxin-exposed and control cells. In human lymphocytes higher concentrations of MCLR (0.1, 1 and 10 μg/ml) and longer exposure times were needed to induce DNA damage. The results showed that DNA strand breaks induced by MCLR were transiently present probably as intermediates formed during DNA repair. Human hepatoma cells and human lymphocytes have different sensitivity towards cyanobacterial peptide MCLR.

DD038

THE INFLUENCE OF MICROCYSTIN-LR ON HEPG2 CELL PROLIFERATION AND MICRONUCLEUS FORMATION

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Microcystins (MCs) are hepatotoxic cyclic heptapeptides produced by freshwater cyanobacteria. They are inhibitors of serine/threonine protein phosphatases 1 and 2A and are involved in liver tumour promotion. Several recent studies, including ours, indicated that MCs are genotoxic and may also act as tumour initiators. In the present study the genotoxicity of microcystin-LR (MCLR) was evaluated using the cytokinesis-block micronucleus (CBMN) assay. MCLR (0, 0.01, 0.1, and 1 μg/ml) induced dose dependent increase of MNi formation in HepG2 cells after 16 h of exposure. To our knowledge this was the first study where the effect of MCLR on nucleoplasmic bridges (NPBs) and nuclear buds (NBUDs) formation was evaluated. A slight but insignificant increase in the frequency of NPBs after MCLR exposure was observed, while there were no differences in the number of NBUDs compared to control. The result confirms that MCLR is clastogenic, and the increase of NPBs indicates that MCLR can induce also DNA mis-repair and/or chromosome rearrangement. Exposure of HepG2 cells to MCLR also reduced the mitotic index. With the MTT assay we found that MCLR dose dependently reduced cell growth compared to the control, which was statistically significant after 72 and 96 h of exposure. With the immunostaining with the primary mouse antibody against Ki67 protein, which is a proliferation marker, we confirmed the decrease in cell division after exposure to MCLR. Using flow cytometry we showed that exposure to MCLR affected the cell-cycle distribution in HepG2 cells, resulting in cell arrest in the G0/G1 phase and decreased number of cells in G2/M phase at 24 h exposure compared to the control group. These data together with the data of our previously published studies provide additional evidence that MCLR should be considered as genotoxic toxin. The MCLR induced G0/G1 cell arrest is in line with its DNA damaging potential as it may indicate the arrest of cell-cycle progression, as a response to DNA damage to allow time for DNA repair.

DD039

INTERACTION OF DNA TOPOISOMERASE I WITH DNA INTERMEDIATES AND PROTEINS OF BASE EXCISION REPAIR

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DNA topoisomerases are involved in multiple cellular processes including replication, transcription and recombination. These enzymes are required for a solution of the topological problems arising in the processes of DNA metabolism and influence on their activity.

Topoisomerase I (Top1) performs cleavage and religation of DNA strand within specific cleavable site via the formation DNA-protein covalent intermediate between the catalytic tyrosine residue of the enzyme and 3'-phosphate of the cleaved strand. Yeast Top1 from Saccharomyces cerevisiae was shown recently to interact with nicked and gapped DNA lacking Top1 specific site. Such structures can originate in cell during the processes inducing DNA damages or during DNA repair. In the present work the interactions of human and yeast Top1 with DNA structures imitating base excision repair intermediates were studied in details. DNA duplexes containing nick or short gap were designed. To increase selectivity of the protein modification in cellular and nuclear extracts, circular double stranded DNA containing uracyl residues distributed statistically within one strand was synthesized using M13 mp19 phage DNA. DNA structures with single strand breaks were produced by the treatment of dUMP containing DNA with purified uracyl DNA glycosylase and apurinic/apyrimidinic endonuclease or endogenous enzymes of cellular extracts. Circular DNA was shown to crosslink with cellular extract proteins more selectively than short DNA duplexes. Major modified product in bovine testis nuclear extract was identified as Top1 by immunoprecipitation with polyclonal antibodies against Top1. The level of Top1 modification decreased when purified poly(ADP-ribose) polymerase (PARP1) was added in the extract. The data obtained suggest that PARP1, one of the main nick sensors in mammalian cells, can compete with Top1 for available single strand breaks in DNA, so inhibits the formation of Top1-DNA suicide adduct. This work was supported by a grant from the RFBR (07-04-00389, 09-04-91320), BRHE fellowship (РНП.2.2.2.3.10031) and HRJRG-102.

DD040

GENOTOXIC AND CELLULAR SIGNALLING RESPONSES FOLLOWING EXPOSURE TO ULTRAFINE SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES

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Background: Ultrafine superparamagnetic iron oxide nanoparticles (USPION) have a range of potential medical applications including a contrast enhancing agent for magnetic resonance imaging, targeted drug delivery vehicles and in magnetic tumour ablation (hyperthermia). To enhance their biocompatibility and maximise cell internalisation, these nanoparticles are often coated with dextran (dUSPION). Exposure to dUSPION may therefore occur occupationally (health care practitioners or during manufacture), and clinically (during diagnostic imaging or treatment). The current study investigates the consequences of intracellular dUSPION localisation on iron homeostasis signalling pathways and DNA integrity. Methods: Physico-chemical characteristics of the dUSPION were determined using dynamic light scattering (size distribution, agglomeration); transmission electron microscopy (morphology, size, structure); elemental dispersive X-ray analysis (composition); zeta potential (surface charge). Human MCL-5 lymphoblastoid B cells were then exposed to a dUSPION dose range and subsequent genotoxic effects assessed with the cytokinesis blocked micronucleus assay. Real-time RT-PCR was utilized to examine the gene expression profiles of transferrin receptor 1 (TfR1), ferroportin, hepcidin and ferritin. Results: Following exposure, dUSPION demonstrated intracellular accumulation in a dose- and time-manner that was accompanied by a significant increase in micronuclei at sub-cytotoxic concentrations, and was dependent upon the oxidation state of the iron oxide. Additionally, changes in cellular signalling were observed, with down-regulation of TfR1, up-regulation of ferroportin and a dramatic suppression of hepcidin gene expression. Conclusions: Increased intracellular uptake of dUSPION was associated with significant gross chromosomal damage. Furthermore, given that TfR1 controls iron uptake, ferroportin promotes its export and hepcidin blocks iron export, all gene expression changes in response to dUSPION exposure indicate a cellular response attempting to reduce cellular iron content. Interestingly, the effects observed were a function of the iron oxide oxidation state, indicating the importance of subtle differences in nanomaterial physico-chemical characteristics.

DD041

REPAIR PATHWAY OF O6-ALKYLGUANINE AND O4-ALKYLTHYMINE

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O6-alkylguanines (O6-alkG) and O4-alkylthymines (O4-alkT) in DNA caused by alkylating agents are major lesions that induce mutation. Many types of DNA repair systems in E. coli work to repair alkylated DNA, such as nucleotide excision repair (NER), base excision repair, and O6-alkylguanine DNA alkyltransferase (AGT). The mismatch repair system (MMR) plays an important role in maintaining the high fidelity of genome DNA. However, the contribution of MMR to the repair of alkylated bases is unclear. Among E. coli CC series strains, CC102 is used for the detection of GC to AT transitions that may be driven by O6-alkG lesions. Using CC102 derivatives defective in AGT, NER or MMR, we analyzed the repair system responsible for the repair of O6-alkG. Results showed that O6-methylG was repaired by systems in the order AGT>>MMR>NER, while ethylated G was repaired by NER>AGT>>MMR. Data indicated that O6-alkG lesions were repaired by MMR with low efficiency. Alkylating agents also produce O4-alkT in DNA. The E. coli CC106 strain can be used to detect the reverse-mutation, AT to GC, which should be caused by O4-alkT. Mutant frequencies of CC106 treated with methylating and ethylating agents were more elevated in the AGT-deficient strain than other strains, while those with ethylating agents were higher in the NER-deficient strain. Unexpectedly, mutant frequencies with every alkylating agent tested were lower in MMR-deficient strains than in MMR-proficient strains. This result suggests that the MutS protein could not recognize O4-alkT:G, or rather could recognize the O4-alkT:A base pair. To examine the involvement of MMR in the absence of AGT or NER activity, we constructed E. coli strains deficient in both MMR and AGT or NER. The results obtained using these strains suggest that O6-alkG is recognized by the MutS protein and removed by another system since mutant frequencies increased in MutS-deficient strains, but not in MutL- or MutH-deficient strains. In contrast, the repair of O4-alkT might be inhibited by the presence of MMR even if AGT or NER is deficient. Taken together, a different pathway may contribute to the repair of O6-alkG and O4-alkT.

DD042

THE DETECTION OF ANEUPLOIDY IN PERIPHERAL BLOOD LYMPHOCYTES OF CHILDREN WITH CHRONIC KIDNEY DISEASE

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The incidence of cancer in chronic kidney disease (CKD) found to be higher when compared to the general population. Also the treatment with immune supressors after the transplantation treatment might be another risk factor for cancer development. The mechanism underline has not been totally known yet. Therefore, the investigation of the patients with CKD and their treatment approaches is crucial. Among the CKD patients, children deserve the highest attention as they have to live with the disease longer and vulnerable to the treatment of CKD. Thus, we conducted a study involving children; in pre-dialysis stage (Pre-D) (n=16), on regular hemodialysis (HD) (n=14) and transplanted

(Tx) (n=15). Cytokinesis blocked micronucleus assay combined with fluorescence in situ hybridization technique in peripheral blood lymphocytes by using centromeric probe was used in this study. The results revealed that, centromere positive (C+) and centromere negative (C-) micronucleus frequencies were found to be highest in Pre-D patients. The significant increase was in Pre-D patients (mean±SD; 5.06±1.44) vs. Tx patients (2.93±2.58) for C+ micronucleus frequency (p=0.02). The percentage of C+ micronuclei in total micronuclei was 55%, 65% and 56% for Pre-D, HD and Tx patients, respectively. Considering the preliminary results it can be concluded that the treatment in CKD either by regular hemodialysis or transplantation decreases the genotoxicity. Furthermore, determining the aneuploidy may be advantageous to clarify the mechanisms of chronic kidney disease.

DD043

DNA DAMAGE IN LYMPHOCYTES OF CHILDREN WITH CHRONIC KIDNEY DISEASE AND AFTER TRANSPLANTATION BY COMET ASSAY

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One consequence of chronic kidney disease (CKD) is an elevated cancer risk. There is sufficient evidence to conclude that there is a heightened incidence of at least some cancers in dialysis patients. Cancer risk may be related to an elevated level of genomic damage. The increased risk of cancer in patients who have had renal transplants has mainly been attributed to immune supressive therapy. There is no genomic data which has been demonstrated by some cytogenetic markers in children with CKD and after transplantation. In this study, the oxidative DNA damage in peripheral lymphocytes from 17 children in pre-dialysis stage (Pre-D) , 14 children on regular hemodialysis (HD) and 17 transplanted (Tx) children has been compared by the strand breaks and endonuclease III (Endo III), formamidopyrimidine glycosylase (FPG) sensitive sites in Comet Assay. Gender and age were consistent among the groups. Our results revealed that; strand breaks (tail moment) were significantly increased in Tx children (mean±SD) (1.10±0.41) vs. children on HD (0.79±0.26)(p=0.03). Although the children in Pre-D were found to have higher sensitive sites against the children on HD and Tx children, the only significant increase was in FPG sites for the children in Pre-D vs. children on HD (2.36±3.19 and 0.47±0.54, respectively) (p=0.04). There was no correlation between the duration of treatment and the comet parameters evaluated (p>0.05). Regard to our preliminary results, in children with chronic kidney disease, long-term maintenance HD therapy and renal transplantation may reduce the degree of genomic damage in the name of oxidative DNA damage.

DD044

GENOTOXIC ACTIVITY INDUCED BY VARIOUS H. PYLORI STRAINS IS ASSOCIATED WITH A DOWNREGULATION OF DNA MISMATCH REPAIR GENES EXPRESSION

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Cancer arises from the stepwise accumulation of genetic changes resulting in a loss of the control mechanisms as those governing cell proliferation and genome stability. During its long term persistence in the host, the Helicobacter pylori infection is though to promote such events. Using a mouse mutagenesis assay, we previously demonstrated the induction of a gastric mutagenic effect in H. pylori SS1 infected-mice after 6 months, mainly attributable to oxydative DNA damage related to the chronic gastritis observed. The goals of the present study are i) to identify genetic instabilities events responsible for the genotoxic activity associated to the infection, ii) to investigate the effects of Helicobacter pylori infection on known DNA repair pathways. Big blue transgenic mice were infected for 3, 6 and 12 months with the H. pylori strains SS1 or B38, a MALT lymphoma derivative and the mutation spectra determined. Presence of genetic instabilities was investigated on 3 (CA)n repeats loci of the mouse genome. Mismatch DNA repair (MMR) gene expression was analyzed by reverse-transcription polymerase chain reaction on RNA isolated from mouse stomach and from AGS cells infected either with the H. pylori strains SS1 or B38. Our data demonstrated that as the H. pylori strain SS1, the strain B38 also induced a gastric mutagenic effect in vivo. However the mutation spectra induced by B38 is mainly composed of GC->AT transition events as compared to the strain SS1 which induced mostly GC->TA and AT->CG transversions. After 6 months of infection, deletion/insertion events in the CA repeat runs and decreased level of MMR components are evidenced. Downregulation of MMR genes expression was also confirmed in vitro, either with H. pylori SS1 or B38. It concerned mainly Msh2, Msh3 and Msh6 involved in the first steps of the mismatch repair pathway. In conclusion, H. pylori generates genetic instabilities, and deregulation of DNA repair components as MMR. The induction of genotoxic events appeared as a common properties of the H. pylori strains, underlying the role of genetic instabilities in the promotion of gastric neoplasia during the infection.

DD045

OXIDATIVE DNA DAMAGE INDUCED BY 2-NITROPROPANE IN RAT LIVER: EFFECT OF RESVERATROL ON ANTIOXIDANT DEFENCES

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Resveratrol is a polyphenolic compound with potent free radical scavenging and antioxidant properties. Recently it has also been shown to activate various transcription factors (NF-kB, STAT3, β-catenin and PPAR-α), to inhibit protein kinases and induce antioxidant enzymes. We utilized a single dose of 2-nitropropane (2-NP) (100 mg/kg) to induced oxidative DNA damage in rat liver and to investigate the effect of resveratrol on oxidative stress parameters and on antioxidant defences. Oxidative DNA damage, measured as 8-hydroxy-2'-deoxyguanosine (8-oxodG) levels in the liver was significantly higher (about 40%) in rats sacrificed 15 h after 2-NP treatment relative to controls which received vehicle alone. On the contrary, rats given resveratrol (100 mg/kg/d) in the diet for 14 d and treated with 2-NP had 8-oxodG levels similar to controls. The treatment with 2-NP also induced an increase of antioxidant enzymatic and non enzymatic defences in liver tissue of rats, superoxidedismutase (SOD) activity was about 2.5 fold higher, while total glutathione (GSH) levels were much higher than those measured in controls. The pre-treatment for 14 d with resveratrol inhibited the antioxidant defence response and SOD and GSH levels were similar to controls. Moreover, studying the total plasma antioxidant capacity by the FRAP assay, we observed that 2-NP treatment promoted an important increase of antioxidant capacity partially inhibited when rats were pre-treated with resveratrol. Our findings show that 2-NP induces, at least in the early phase of administration, an antioxidant defence response by both enzymatic and non enzymatic systems, e.g SOD, GSH. The increase in oxidative DNA damage found after 2-NP administration could be explained by the inhibition of DNA repair.

DD046

STUDY OF GENOTOXIC EFFECTS AND GENE EXPRESSION PROFILES IN TK6 HUMAN CELLS EXPOSED TO DNA OXIDIZING AGENTS

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It has been widely accepted in the last few years the existence of thresholds for indirect DNA-damaging agents. In return, DNA-reactive agents have been assumed to act through a non-threshold mode of action, as they directly induce DNA lesions that could be converted into mutations. However, this does not take into account the existence of defence and repair mechanisms that provide protection to a certain extent leading to the possibility of threshold concentration-response curves for genotoxicity induction. Some authors recently proposed the possibility of a threshold for alkylating agents (Doak, 2007 - Müller, 2009). In this context our work was to test the hypothesis that DNA oxidizing agents may also exhibit a thresholded dose-response relationship. Oxidative stress is defined as an imbalance of the cellular redox homeostasis due to an increased production of reactive oxygen species and a decrease in antioxidant defences, and results particularly in DNA damage (such as 8-OH-dG). In order to investigate the different cellular responses involved in the oxidative stress mechanism, our goal was to relate genotoxic effects to changes in gene expression profiles after exposure of human lymphoblastoid TK6 cells to well-known oxidizing agents. For this, the induction of chromosome damage was investigated by using the in vitro micronucleus test. Concurrently, transcriptomic analyses were performed by extracting mRNA from the exposed cells. Results of our genotoxicity assays showed interesting non-linear dose-effect relationship starting with a range of nonmutagenic doses allowing the determination of a No Observed Effect Level (NOEL), and going step-wise up to high doses suggesting the involvement of several and complementary cellular events. Indeed, the gene expression profiles reflected the various cellular and molecular responses to oxidative stress and DNA damage (induction of antioxidant defence systems, DNA repair pathways, redox regulation, cell cycle control, cell death...). Overall, our results suggested the existence of dose thresholds for DNA oxidizing agents and the necessity to combine standard in vitro genotoxicity assays with gene expression profiling technology, particularly for human health risk assessment.

DD047

ROLES OF TRYPANOSOMA CRUZI RAD51 IN DNA REPAIR: REPAIR OF DOUBLE STRAND BREAKS AND OXIDATIVE LESIONS

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Rad51 is the central enzyme of homologous recombination. In this report, we present different roles of Rad51 in the DNA repair of Trypanosoma cruzi, the etiologic agent of Chagas' disease. To achieve this goal, we generated TcRad51 heterozygous knockout cells. Western blot assays showed a decrease in TcRad51 protein induction after gamma radiation in the transfected cells, confirming their Rad51 knockout status. We then submitted these cultures, along with TcRad51 overexpressing cells and wild type cells, to treatment with different genotoxic agents. The results demonstrated that TcRad51 protein levels interfere with T. cruzi's growth after gamma radiation exposure. The lack of one allele of TcRad51 delays the growth of the parasites while TcRad51 overexpression allows a faster growth recovery. In order to better understand this process we performed TcRad51 immunolocalization experiments during the T. cruzi recovery process after gamma radiation in the different cells. In the wild type cells, TcRad51 accumulates in the nucleus immediately after the damage and protein signal increases until it reaches the highest point at 24 hours. However, the TcRad51 heterozygous knockout cells presented a delay in the increase of TcRad51 protein intensity and values reached were not as high as the ones obtained for TcRad51 in wild type cells. On the contrary, the majority of the TcRad51 overexpressing cells presented higher TcRad51 intensities before and after gamma radiation when compared to wild type cells. These results indicate that TcRad51 has an important role in double strand breaks (DSBs) recovery. We also exposed these cells to ultraviolet light and cisplatin, both of which are cross-links agents, but these cells showed a similar sensitivity to that observed for wild type cells. On the other hand, the overexpression of TcRad51 confers a greater resistance to hydrogen peroxide treatment while the disruption of one allele of TcRad51 increases the sensitivity to this agent. Therefore, TcRad51 plays a role in the repair of oxidative lesions in T. cruzi possibly through the restart of stalled replication forks during the S phase.

DD048

DNA STRAND CLEAVAGE AND OXIDATIVE DAMAGE CAUSED BY N-NITROSOPROLINE AND OTHER N-NITROSODIALKYLAMINES WITH UVA IRRADIATION, AND CHARACTERIZATION OF PRODUCTS FORMED IN THE PHOTO-REACTION OF N-NITROSOPROLINE WITH 2'-DEOXYGUANOSINE

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N-Nitrosoproline (NPRO) is endogenously formed from proline and nitrite and occurs in human urine at 0.01–0.1 microM. We have detected the direct mutagenicity of NPRO plus natural sunlight towards *S. typhimurium* and found single-strand DNA breaks in human fibroblast cells on treatment with NPRO plus UVA, as detected by the comet assay. We also have found that other N-nitrosodialkylamines, such as NDMA, NDEA and NMOR, can be converted into a directly mutagenic compound on UVA irradiation. In this study, we treated double-stranded DNA from phage φx174 with nitrosamine plus UVA, and analyzed single strand breaks. We also measured the formation of 8-oxodeoxyguanosine (8-oxodG) in the treated DNA of calf thymus, and studied the characterization of photo-products formed from irradiated 2'-deoxyguanosine (dG) with NPRO. NPRO was irradiated using UVA with dG in an acetate buffer (pH 3.7). HPLC-UV analysis showed that five or more products were formed during the reaction, three of which were purified and characterized by HPLC/ESI-MS/MS as 2'-deoxyxanthosine, 2'-deoxyoxanosine and 8-oxodeoxyguanosine. DNA damages caused by NPRO and UVA merit attention as possible mechanisms leading to increased carcinogenic risk from sunlight-UVA irradiation. The mechanisms of formation of oxidative damage and strand breaks caused by irradiated N-nitrosoproline and other N-nitrosamines were also discussed.

DD049

REACTIVE OXYGEN SPECIES-MEDIATED MITOCHONDRIAL DYSFUNCTION AND CELL DEATH IN RESPONSE TO NANO-SIZED TIO2

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Titanium dioxide (TiO2) has been widely used in many areas including the biomedicine, cosmetics, and environmental engineering. Recently, it has become evident that some of TiO2 particles have shown considerable cytotoxic effect in human normal cells. However, the molecular basis for the cytotoxicity of TiO2 has yet to be defined. We demonstrated in this study that TiO2 induces cell growth inhibition, DNA damage, and mitochondrial dysfunction in human normal liver cells through intracellular ROS generation, and that ROS-dependent caspase 8 and Bax activation is involved in TiO2-induced mitochondrial dysfunction and cell death. Treatment of TiO2 induced an increase in intracellular ROS, caspase 8 activatrion, conformational change and mitochondrial redistribution of Bax, mitochondrial membrane potential loss, and cell death. Inhibition of caspase 8 or Bax with siRNA of Bax significantly attenuated TiO2-induced mitochondrial membrane potential loss and cell death, but did not affect ROS generation. Moreover, inhibition of ROS with an antioxidant, N-acetyl-L-cysteine (NAC), clearly suppressed caspase 8 activation and conformational change and mitochondrial translocation of Bax and subsequent mitochondrial cell death. These results indicate that ROS-dependent activation of caspase 8 and Bax is critically required for mitochondrial cell death in response to TiO2 in human normal liver cells. Elucidating the molecular mechanisms utilized by nono-sized particles to regulate mitochondrial dysfunction and cell death is critical for the development of prevention strategies to protect cytotoxicity of nono-materials.

DD050

DEEPENING IN THE COMPREHENSION OF THE ACTION MECHANISMS OF A NEW METAL-BASED ANTIPROLIFERATIVE DRUG WITH DNA DAMAGING ACTION

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The search for new metal-based drugs is a main topic in experimental oncology. In recent years new metal-complexes have been identified as a very promising class of antitumor active compounds but the targets and the mechanism of action of these molecules have not yet been elucidated. In this context, the metal complex [Ni(S-tcitr)$_2$] (S-tcitr=S-citronellalthiosemicarbazonate) shows interesting antiproliferative characteristics. [Ni(S-tcitr)$_2$] enters the cell and induces G$_2$M cell cycle arrest, p53 independent-intrinsic-apoptosis by down-regulation of Bcl-2, mitochondrial membrane potential loss and caspase-3 activation. A DNA damaging action was observed by the alkaline Comet Assay and the use of specific glycosilases (ENDO III) to detect oxidative damage showed that DNA damage is not preferentially due to oxidative species. We hypothesize that the genotoxic insult by itself or an excessive or altered repair of the DNA damage could produce the observed cell killing, due to the fact that [Ni(S-tcitr)$_2$] treatment did not induce gene mutation in *Salmonella typhimurium* reversion test (with or without metabolic activation) or chromosomal damage (Micronucleus test on human lymphocytes). To better understand the interaction between this molecule and cellular pathways, we have started a genomic phenotyping approach using yeast (*S.cerevisiae*) as a model system which shows a sensitivity against [Ni(S-tcitr)$_2$] toxic effects comparable to human leukemic cells. The whole BY4742 haploid strain deletion collection from the *S. cerevisiae* Genome Deletion project, which includes 4826 single gene deletion mutants in non essential genes, will be screened. This panel includes deletants in non essential genes involved in biological processes that are the main targets of antitumor drugs in human cells. The analysis of the drug effect on the complete collection will permit to evaluate via bioinformatics studies the significance of the results obtained. Furthermore, we are testing our compound against a sub-panel of cell lines derived from the “US National Cancer Institute 60 human tumor cell line anticancer drug screen”. The preliminary data show the strongest effect on proliferation in leukemic cell lines (GI_{50}<30μM).

DD051

EVALUATION OF MUTAGENICITY BY EXPOSURE TO INTERMEDIATE FREQUENCY (2, 10 and 20 kHz) MAGNETIC FIELDS USING IN VITRO GENOTOXICITY TESTS

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OBJECTIVES; Recent years, several devices that used intermediate frequency (IF; from 300Hz to 10MHz) magnetic fields (MFs) such as IH cooking hobs, RFID, EAS, etc, were introduced in our living environment. However, there is not enough scientific research focused on possible health effects of IF MFs, so far. Therefore, in the WHO EHC monograph No. 232 that was published in 2007 stated that further research on intermediate frequencies, as frequencies between 300Hz and 100kHz is required. In this study, we have investigated the mutagenic potential of the IF MFs using mouse lymphoma assay (MLA) and in vitro micronucleus test. Although actual MFs observed in environment are complex (combined, modulated, etc.), sinusoidal MFs were used as fundamental wave model. METHODS; L5178Y tk3.7.2C and V79 were used. The conditions of exposure to sinusoidal IF MFs were 0.8mT at 2kHz, 1.5mT at 10kHz or 0.8mT at 20kHz, respectively. In MLA assay, L5178Y cells were inoculated in T-25 flask filled with 5ml of RPMI1640 medium with 10% horse serum and were exposed to IF MFs for 48hr with single dilution after 24hr. MLA assay was performed in microplate method. In in vitro micronucleus test, V79 cells were exposed to IF MFs for 24hr in T-25 flask. After exposure period, cells were collected and re-inoculated in chamber slides. Finally, cells were fixed after 24hr from the treatment by cytochalasin B and ratios of micronucleus formation rates were estimated. RESULTS; There is no significant difference in the mutation frequency of tk allele (tk to tk-/-) and plate efficiency between IF MF exposed and unexposed cells in 2kHz, 10kHz and 20kHz, respectively. These results suggest that 2kHz, 10kHz and 20kHz, 0.8mT sinusoidal IF MFs did not have mutagenic potential for tk allele of mice lymphoma cell line L5128Y tk 3.7.2C. In addition, there is no significant difference in micronucleus frequency in V79 cells in in vitro micronucleus test. These results suggest that 2, 10, 20kHz sinusoidal IF MFs have no mutagenicity even in its field strength exceeded over 100 times of reference level for public in the guidelines for limiting exposure to time-varying electric, magnetic and electromagnetic fields by the International Commission on Non-Ionizing Radiation Protection (ICNIRP).

DD052

SILENCING OF OXIDATIVELY-DAMAGED GENE IN MAMMALIAN CELLS

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We and others have previously shown that oxidative DNA base lesions, particularly 7,8-dihydro-8-oxoguanine (8-oxoG) induced by photosensitisation reactions, can interfere with transcription resulting in decreased gene expression. This effect is unexpectedly strong and long-lasting, keeping in mind that (i) 8-oxoG has a very week potential to interfere directly with transcription; and (ii) removal of 8-oxoG by DNA repair is fast and highly efficient. Now we demonstrate that damaged plasmid DNA undergoes a strong time-dependent transcriptional inactivation in mouse and human host cells, leading to the complete loss of gene expression in a considerable fraction of the transfected cells. To explain the observed effects, we have quantitatively analysed the survival of damaged and undamaged plasmids after incubation in the host cells, and measured by chromatin immunoprecipitation (ChIP) the histone modifications that are relevant to regulation of gene expression. We show that oxidative DNA damage locally induces a distinct pattern of chromatin that can cause a long-lasting gene silencing. These results identify DNA damage as a factor, capable to alter the gene expression by a chromatin-mediated mechanism, and thus reveal a new aspect of transcriptional toxicity of oxidative DNA lesions.

DD053

STRESS-ACTIVATED PROTEIN KINASE / C-JUN-N-TERMINAL KINASE (SAPK/JNK) IS PART OF THE CELLULAR DNA DAMAGE RESPONSE

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c-Jun-N-terminal kinases/stress-activated protein kinases (SAPK/JNK) are activated by genotoxins, finally regulating gene expression and survival. However, SAPK/JNK are not yet established as part of the DNA damage response. Here, we investigated the effect of the DNA crosslinking drug cisplatin (cDDP) on early (2-6 h) and late (16-24 h) dual phosphorylation (Thr183/Tyr185) of SAPK/JNK (p-SAPK/JNK) in rodent and human fibroblasts deficient in nucleotide excision repair (NER) or non-homolo-

gous end joining (NHEJ). The number of cisplatin-DNA adducts in wild-type MEFs, as measured by inductively coupled plasma mass-spectrometry (ICP-MS), correlated with early and late activation of SAPK/JNK. As compared to UV-C and transplatin, rather low number of cisplatin-DNA adducts were sufficient to trigger this signalling. NER defective human CSB cells exhibited an increased SAPK/JNK phosphorylation at late time point after treatment as compared to the wildtpye. Similar results were obtained by siRNA-mediated knock-down experiments. Increase in late p-SAPK/JNK level after cisplatin treatment was also found in XPA but not in XPC cells. Furthermore, cisplatin-induced late SAPK/JNK activation was more pronounced in NHEJ defective SCID cells, which lack functional DNA-PKcs, as compared to the wildtype. Such effect was not observed in ATM-/- MEFs. Analysing the number of residual cDDP-adducts by ICP-MS and southwestern method, we found that the repair mutants did not differ from their corresponding wildtype. Thus, elevated SAPK/JNK phosphorylation in repair defective cells after cDDP-treatment was not related to the level of primary cDDP adducts. Rather, the late cisplatin-induced gain of p-SAPK/JNK in repair defective cells correlated with an increase in both γH2AX foci formation and checkpoint activation (p-Chk-1 and p-Chk-2). This indicates that secondary DNA lesions, such as DSBs, which are generated as a consequence of error-prone processing of initial cDDP adducts, might contribute to signalling to SAPK/JNK. With the data at hand, we have identified SAPK/JNK as a novel player of the DNA damage response after cisplatin treatment. The detailed molecular mechanisms involved in DNA damage-triggered signalling to SAPK/JNK are currently under investigation.

DD054

BENEFICIAL EFFECTS OF THE HMG-COA REDUCTASE INHIBITOR LOVASTATIN AGAINST GENOTOXIC DRUG-INDUCED CYTOTOXICITY IN VIVO

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Cisplatin (cDDP), Doxorubicin (Dox) and ionizing radiation (IR) are prototypical genotoxins inducing well-characterized types of DNA damage, foremost monoadducts, crosslinks, and double strand breaks (DSB). The HMG-CoA reductase inhibitor lovastatin impacts cellular responses to genotoxic stress. In vitro, lovastatin promotes the survival of human endothelial cells after IR and Dox exposure. Here, we aim to scrutinize these in vitro findings in vivo. To this end, BALB/c mice were exposed to Dox, cDDP, or IR with or without lovastatin co-treatment. Subchronic toxicity was analyzed 3 weeks later. Hematotoxicity and cardio-, nephro-, or hepatotoxicity were analyzed in blood and serum samples, respectively. Additionally, mRNA expression of inflammation and fibrosis markers was analyzed in heart, liver, and kidney by real-time PCR. Genotoxic effects of the treatments was examined by analysis of histone H2AX phosphorylation. Dox-treated animals exhibited elevated plasma levels of troponin I as well as GPT and GLDH, indicative of heart and liver toxicity, respectively. Correspondingly, mRNA expression of the fibrogenic cytokine CTGF was up-regulated in both organs. Lovastatin reduced heart and liver damage and lowered CTGF mRNA expression. cDDP-induced kidney toxicity was reflected by an increase in TNF-α, IL-10, and KIM1 mRNA expression. Lovastatin had only minor effects on cDDP-induced kidney injury. Animals treated with IR suffered from hematotoxicity, as indicated by a decline in the number of thrombocytes and leukocytes. Furthermore, they showed up-regulation of TGF-β, IL-6, and Collagen type I and III mRNA expression in the lung. Lovastatin attenuated thrombopenia as well as IR-induced pro-inflammatory and pro-fibrotic responses. It did not impact the level of IR-induced DNA damage as analyzed by determination of histone H2AX phosphorylation. With the data at hand, we have shown that lovastatin can attenuate the cytotoxicity of individual genotoxins in an agent- and organ-specific manner. In particular, it has beneficical impact on pro-fibrotic signalling pathways stimulated by Dox and IR treatment. Hence, lovastatin may be useful to attenuate delayed cytotoxicity of DNA damaging agents.

DD055

SUBSTRATE SPECIFICITY OF NATURALLY OCCURRING AND PHOSPHOMIMETIC MUTANTS OF HUMAN 8-OXOGUANINE-DNA GLYCOSYLASE

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8-oxoguanine-DNA glycosylase (OGG1) is a base excision repair enzyme that removes oxidized forms of guanine bases, 8-oxoguanine (8-oxoG) and formamidopyrimidine (Fapy-G) from their pairs with cytosine. On the contrary, removal of 8-oxoG from easily formed pairs with A may cause G→T mutations. Mutated forms of *OGG1* gene have been detected in some human cancers, but little information is available on the activity and opposite-base specificity of the respective forms of the enzyme. We have used 8-oxoG:C and 8-oxoG:A oligonucleotide substrates to analyze kinetics of three human OGG1 polymorphic forms, A288V, D322N, and S326C. We have also studied S231E, S232E, S231/232E, S280E, and S326E site-directed mutants of OGG1, which mimic possible phosphorylated forms of the enzyme. OGG1-A288V and OGG1-S326C were similar to the wild type enzyme in terms of the opposite-base specificty, and OGG1-D322N was the most specific form. On the other hand, the absolute activity of OGG1-D322N was the lowest of all forms studied. All phosphomymetic mutants were slightly lower in activity than the wild-type OGG1 but similar to it in the opposite-base specificity. We have compared the ability of OGG1 mutants to remove oxidized guanine bases from γ-irradiated calf thymus DNA. Generally, the activity of the enzymes on this substrate mirrored the situation with the oligonucleotide substrates, and the activity against Fapy-G lesions paralleled the data for 8-oxoG lesions. However, OGG1-S326C removed 8-oxoG from oligonucleotides and Fapy-G from the irradiated DNA its activity against 8-oxoG in the irradiated DNA was much lower than that of the wild-type enzyme. In addition, we have investigated the ability of OGG1 mutant forms to be stimulated by human AP endonuclease APEX1. Unlike with the wild-type OGG1, very low stimulation was observed for all phosphomimetic mutants.

DD056

NEIL GLYCOSYLASES: NOVEL ACTIVITIES AND REGULATION

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Oxidatively damaged DNA is repaired via the base exision repair (BER) pathway initiated by DNA glycosylases. NEIL1 and NEIL2 are mammalian DNA glycosylases that repair some ring-fragmented purines, and oxidized and ring-saturated pyrimidines. The full substrate range of NEIL proteins is presently unknown, and the mechanisms of regulation of their activity are poorly understood. 8-Oxoadenine (8-oxoA) is a major product of DNA oxidation. Mutagenesis experiments suggest that transient 8-oxoA:C pairs form in vivo. We have tested the proficiency of NEILs in repairing 8-oxoA and found that NEIL1 excises it from 8-oxoA:C but not other pairs. The kinetic constants were obtained. We have reconstructed BER of 8-oxoA:C in vitro using NEIL1, AP endonuclease, DNA polymerase β and DNA ligase. NEIL1 caused an abortive initiation of repair, stopping after 8-oxoA removal and DNA strand cleavage, indicating possible involvement of polynucleotide kinase in the repair. Amino groups in proteins are often sites of post-translational modification. They can be easily condensed with aldehyde moieties present in many low-molecular-weight cellular compounds. Pyridoxal 5'-phosphate (PLP), one of such aldehydic effectors, is known to covalently modify and efficiently inhibit many DNA-dependent enzymes. We have examined the effect of PLP modification on DNA glycosylases and have found that NEIL2 was inhibited by PLP. Kinetics of inhibition was consistent with slowly developing covalent modification. PLP-conjugated NEIL2 lost the ability to bind damaged DNA. Mass spectroscopy showed that a single PLP group is attached to NEIL2 at Lys-50, which presumably lies in one of the loops

responsible for DNA binding. Repair of oxidative DNA lesions is known to be impaired *in vivo* by ions of some heavy metals. We have found that NEIL1 is inhibited by submillimolar Cd^{2+}, Zn^{2+}, and Cu^{2+}, with the inhibition at least partly due to formation of metal–DNA complexes. Cd^{2+} and Cu^{2+} caused a loss of preferential binding of NEIL1 to damaged DNA. Therefore, the inhibition of NEIL1 by heavy metal ions may be one of the reasons for their comutagenicity.

DD057

IMPROVING GENOTOXICITY TESTING: COMET ASSAY WITH 3D SKIN MODELS

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Since March 2009 animal testing is prohibited for the safety assessment of cosmetic ingredients due to the 7th Amendment of the Cosmetic Directive. The currently available in vitro tests are of low predictive value due to a high rate of false positives results. One strategy to address the demand of improved in vitro genotoxicity tests is to introduce more relevant test systems into safety assessment. Since the skin is the first site of contact with maximum exposure for many chemicals skin models were introduced into genotoxicity testing within the framework of a COLIPA sponsored joint research project involving four laboratories and three different skin models: An epidermal model: EpidermTM (MatTek) and two full-thickness-models: Phenion®-Full-Thickness-Skin-Model (Henkel), and RealSkin (Skinethic™).
Following technical bases were implemented:
a. Specific protocols for the single cell isolation of keratinocytes
b. A harmonized Comet assay procedure for each skin tissue
c. An SOP for Comet assay slide analysis
d. Statistical criteria to evaluate and define experiments
Based on these standards the effects of genotoxins (MMS, 4NQO) were investigated after topical application to the skin model. A clear dose-dependent increase of the intensity of the comet tail was observed with each skin model. The results obtained with EpidermTM could be confirmed in a second laboratory proving the good transferability of the test system. The harmonized protocol is suited for multiple donors and the inter- and intra-experimental variabilities were within acceptable ranges. This work is funded by the European Cosmetic Industry Association COLIPA and ECVAM.

DD058

EVALUATION OF DNA DAMAGES ON CELLS IN DIFFERENT PARTS OF GLANDULAR STOMACH IN A COMET ASSAY USING RATS

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AIM: The comet assay is a promising technique for evaluating DNA damage in vivo. However, there is no validated testing guideline and experimental techniques have not been optimized. For example, there is no agreement on which part of the cells of glandular stomach should be measured to detect direct mutagenic effects of chemicals. To investigate which cells should be collected to detect genotoxicity of chemicals in glandular stomach, we measured DNA damages on cells from different parts of glandular stomach in a comet assay. METHOD: Male SD rats were treated twice orally at 3 and 24 hours before sacrifice with vehicle only, with ethyl methanesulfonate (EMS, 100 or 200 mg/kg) or with 2,6-diaminotoluene (2,6-DAT, 125, 250 or 500 mg/kg). Then the rats were sacrificed and the glandular stomach was removed. The mucosal layer was divided into three zones in fundal glands and pyloric gland, respectively. 1. Surface epithelial cell zone (SEpC, containing columnar epithelial cells) 2. Proliferating cell zone in isthmus (PrCI) 3. Differentiated secretory cell zone (DSeC, containing differentiated secretory cells). Three target cells of each zone were collected from fundal glands and PrCI cells from pyloric glands and tested in an alkaline comet assay. Whether the target cells were obtained was confirmed by the histopathological analysis. RESULT: As for the sensitivity of the layers in fundal glands, the comet tail intensity of the SEpC in the vehicle control group was higher than those of PrCI and DSeC, as expected. Treatment with EMS showed a marked increase of the tail intensity in any part of mucosa though the differences of tail intensity to the vehicle control in the PrCI and DSeC were slightly lower than that in the SEpC. There is no difference in reactivity between cells from fundal and pyloric glands. 2,6-DAT showed no significant increase of the tail intensity in the PrCI, however, that of the SEpC was significantly increased. CONCLUSION: Our research and proposed method of cell collection will contribute to improve the sensitivity to detect the DNA damage in stomach appropriately.

DD059

THE COMET ASSAY FOR THE EVALUATION OF CISPLATIN AND CURCUMIN GENOTOXICITY IN PC12 CELLS COMPARING FREE INTERNET SOFTWARE AND VISUAL SCORE DATA

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Toxic side effects of the antitumoral agent cisplatin (cDDP) are closely associated with increase of intracellular reactive species, which could damage cells leading to oxidation of essential cellular components, and to eventual genomic instability events, which can be seen as DNA fragmentation. Curcumin is a polyphenolic compound with a broad spectrum of biological activities like neuroprotective properties, strongly related to its antioxidant potential. However, studies have shown that curcumin could also be cytotoxic and genotoxic under specific conditions. Previously studies with the association between cDDP and curcumin showed that curcumin in low concentrations were nontoxic by itself, and decrease cDDP genotoxicity. The aim of our study was to investigate curcumin and cDDP effects on PC12 cells by comet assay, as well, to compare results obtained from visual score analysis and a free internet software for comet analysis. PC12 cells were treated with curcumin (1.0 and 5.0 μg/mL) for 5h; cDDP (0.1μg/mL) for 3h and the pre-treatment with curcumin 2h before 3h of cisplatin treatment, in the associated protocols. To assess the DNA damage induced by cisplatin and/or curcumin, the damage index was used in the visual score and the % DNA, Olive moment and tail moment were used as indexes in the software analysis. Our results indicate that cDDP decrease the extent of DNA migration in comparison with control in PC12 cells. This suggested that cDDP was connected mainly with the presence of DNA and DNA-protein crosslinks. When PC12 cells were pre-treated with 5 μg/mL with curcumin, a significant reduction in the DNA migration was found in comparison with cDDP group, nevertheless, curcumin treatments alone also show reduction in the DNA migration. Thus, there was not possible determine that whether curcumin can protect cells against cDDP-induced oxidative damage. Besides, we could observe that reduction in the DNA migration was easier observed on software analysis than on visual scoring. Other studies could be performed to better understand the interactions between curcumin and DNA. Financial Support: CNPq, CAPES and FAPESP.

DD060

N-NITROSO COMPOUNDS AND COLON CARCINOGENESIS: AN IN VITRO TOXICOGENOMICS APPROACH

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Background: N-nitroso compounds (NOC) may play a role in human cancer development since they have been shown to be genotoxic, and are known to be carcinogenic in animals. Since the human gastro-intestinal tract is an important route of exposure through endogenous nitrosation, we hypothesize that NOC exposure targets genetic processes relevant in colon carcinogenesis. Methods: To investigate the genomic responses following 24 hours of NOC exposure, we analysed the transcriptomic effects of genotoxic concentrations of two nitrosamides, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG, 1μM) and N-methyl-N-nitrosurea (MNU, 1mM), and four nitrosamines, N-nitrosodiethylamine (NDEA, 50mM), N-nitrosodimethylamine (NDMA, 100mM), N-nitrosopiperidine (NPIP, 40mM), and N-nitrosopyrrolidine (NPYR, 100mM), in the human colon carcinoma cell line Caco-2 using microarrays. As a phenotypic marker of effect, cell cycle distribution and apoptosis were analysed by flow cytometry. Results: Gene Ontology gene group, consensus motif gene group and biological pathway analysis revealed that the nitrosamides MNNG and MNU had little effect on gene expression after 24 hours of exposure, whereas nitrosamines had a strong impact on the transcriptomic profile. Analyses showed modifications of cell cycle regulation and apoptosis pathways for nitrosamines which was supported by flow cytometric analysis indicating cell cycle blocks and increased levels of apoptosis. We found additional modifications in gene groups and pathways related to oxidative stress and inflammation, which suggest an increase in oxidative stress and pro-inflammatory immune response upon nitrosamine exposure, although less distinct for NDMA. Furthermore, NDEA, NPIP and NPYR most strongly affected several developmental motif gene groups and pathways, which may influence developmental processes. Conclusions: Many of the regulated pathways and gene groups are implicated in the carcinogenic process and their modulation by nitrosamine exposure may therefore influence the colon cancer risk. Nitrosamide exposure had minimal effect which may be due to the unstable nature of this class of NOC resulting in responses earlier than 24 hours.

DD061

IN-VITRO ANTIOXIDANT BEHAVIOUR OF SOME INDOLE HYDRAZIDE AND HYDRAZONE DERIVATIVES

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Reactive oxygen species (ROS) play an important role in physiological processes in human body, but when in excess, ROS cause oxidative damage to molecules. Under physiological conditions, the production and detoxification of ROS are more-or-less balanced. The damage to animal or plant cells and tissues caused by ROS is called oxidative stress. Oxidative stress has been implicated in the development of neurodegenerative diseases like Parkinson's, Alzheimer's and Huntington's diseases, epileptic seizures, stroke and is also responsible from aging and some cancer types. Melatonin (MLT) and its metabolites successively scavenge ROS/RNS is referred as the free radical scavenging cascade. But despite its possible contribution in the regulation of many physiological processes and antioxidant effects, some problems such as lack of selectivity and very short half-life limit its therapeutic use at present. As a part of our ongoing study, sixteen MLT based analogue indole hydrazide/hydrazone derivatives were synthesized and antioxidant activity was investigated in vitro by measuring DPPH, superoxide radical scavenging and lipid peroxidation (LP) inhibitory activities. The results were compared with MLT. All the analogue compounds were characterized on the basis of 1H and 13C NMR, Mass, FT-IR spectra and elemental analysis. In conclusion, majority of synthesized indole derivatives related to MLT showed very high antioxidant activity in three in vitro assays, revealing differences in their relative potencies probably related to electronic distribution. Lack of methoxy group or introduction of Br in the 5-position of the indole ring did not effect the antioxidant capacity of the new indole derivatives infect the in vitro assays showed that most of the compounds were much more active than MLT itself. Lastly it can be certainly said that introduction of hydrazide or hydrazone side chain containing aromatic halogenated ring increased the antioxidant activity of indoles comparing to MLT.

DD062

IN VIVO COMET ASSAY: UPDATE ON ON-GOING INTERNATIONAL VALIDATION COORDINATED BY JaCVAM

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The in vivo rodent alkaline Comet assay is frequently used worldwide for detecting genotoxic chemicals, and is expected to be recommended in the revised ICH-S2 guidance document as the second in vivo genotoxicity test to use when testing pharmaceuticals. The assay, however, has not been formally evaluated for its reliability and relevance. Thus, JaCVAM (the Japanese Center for the Validation of Alternative Methods) has been coordinating an international validation study to evaluate the in vivo rodent alkaline Comet assay as a potential predictor of genotoxic carcinogens and as an alternative to the currently recommended in vivo rat unscheduled DNA synthesis (UDS) assay. The 1st, 2nd and 3rd phase validation studies successfully finished in four or five leading-laboratories (BioReliance, BSRC, Food and Drug Safety Center, Huntingdon Life Sciences, Merck), and the summary will be presented at the meeting. Now the 4th phase (main) validation study is on-going in 13 laboratories with coded compounds and a positive control, ethyl methanesulfonate, based on outcomes of the previous validation studies.

DD063

DNA DAMAGE PREVENTION FROM VINYL CHLORIDE MONOMER (VCM) OCCUPATIONAL EXPOSURE

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The objective of this study was to determine the genotoxic, anti-genotoxic, therapeutic (protective) effects, radiosensitivity, and induced radioresistance before and following therapy with two Centrum® tablets daily. This study utilized a cytokinesis-block assay in peripheral blood lymphocytes. Thirty-seven study workers were divided into four groups based on vinyl chloride monomer(VCM)concentrations in the work place and were compared to 16 controls from the administrative staff. The following parameters were determined twice, once before and once after termination of treatment: VCM concentrations in the air of the working area, the thiodiglycolic acid(TDGA) level in urine, and the micronuclei (MN) frequency in peripheral lymphocytes. For determination of susceptibility, two blood samples from the same persons were irradiated in-vitro with 1 Gy gamma rays of 137Cs before and after treatment. A clear relationship between VCM exposure, urinary excretion of TDGA, and genotoxic effect was found. The initial MN frequency for the control group was 20.6 ± 1.9. The MN frequencies for each level of VCM exposure were as follows: 23.0 ± 4.7 (VCM = 1.4 ± 0.4 mg/m3), 45.5 ± 5.5 (2.5 ± 0.6 mg/m3), 60.1 ± 1.2 (3.9 ± 0.3

mg/m3), and 71.0 ± 6.8 (4.8 ± 0.5 mg/m3). Following four months of treatment, with similar exposure to VCM, the MN frequency decreased in all groups: 11.6 ± 3.9 (control group), 14.4 ± 4.3 (VCM = 1.4 ± 0.05 mg/m3), 26.5 ± 9.0 (2.7 ± 0.6 mg/m3), 36.5 ± 14.5 (3.7 ± 0.4 mg/m3), and 45.4 ± 13.9 (4.8 ± 0.7 mg/m3). The TDGA levels in urine (mmol/dL ± SD), before and after treatment, were as follows: 0.01 ± 0.008 and 0.01 ± 0.007 (control group); 0.06 ± 0.04 and 0.06 ± 0.02 (lowest exposure); 0.075 ± 0.035 and 0.073 ± 0.03 (second exposure group); 0.132 ± 0.07 and 0.155 ± 0.08 (highest exposure). Prior to treatment, in-vitro irradiation revealed adaptive response for the highly exposed group only. There were clear increases in the frequency of MN between control and exposed groups, which indicate linear dose dependences of genotoxic effect (MN), VCM, and the biological marker TDGA before and after treatment. Thus, Centrum® may be used for anti-mutagenic and anti-carcinogenic prevention in persons occupationally exposed to VCM.

DD064

IS IN VITRO COMET ASSAY A SENSITIVE PROCEDURE TO DETECT GENOTOXICITY?

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Although the comet assay, a procedure for quantitating DNA lesions in mammalian cells, is considered to be sensitive, its sensitivity has not been ever ascertained to be higher than the sensitivity of other genotoxicity procedures in mammalian cells. To know whether the comet assay is sensitive procedure to detect DNA damage, we compared the results of the comet, acellular comet, and micronucleus (MN) assays. p53 mutant TK6 and p53 wild WTK1 human lymphoblastoid cells were exposed to MNU, ENU, MMS, EMS, BLM, and UVC. Immediately after the exposure, slides for the comet assay were prepared. For the MN test, cells were cultured for 48 h with CytB. In the comet assay, cells were treated to each mutagen with araC and hydroxyurea (comet/araC) and without araC and hydroxyurea (comet). In the comet assay and MN test, the lowest genotoxic concentration (LGC) or the studied mutagens was lower in WTK1 than in TK6 cells. For BLM, the order of lowest genotoxic concentration (LGC) was acellular comet << MN < comet = comet with araC/HU, while for UVC, the order was MN = comet/araC < comet. For alkylating agents, the order was comet/araC < acellular comet < MN < comet . Acellular comet shows the induction of SSBs as initial lesions and comet/araC shows the induction of alkylation damages and base adducts. Followings are suggested based on our present results: (1) The sensitivity of the comet assay to detect SSBs is not always higher than that of the MN assay, and (2) comet-undetectable level SSBs, alkylation damages, and base adducts as initial lesions can form MNs, and (3) threshold levels of mutagens are lower in WTK1 cells than in TK6 cells

DD065

INVOLVEMENT OF AP ENDONUCLEASE 1 IN THE REPAIR OF STANNOUS CLORIDE-INDUCED DNA DAMAGE

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Stannous-ion-induced DNA damage was suggested to occur via formation of reactive oxygen species (ROS), as found for other metal ions that compromise genetic stability by inducing different types of oxidative DNA damage. Carcinogenic metal compounds are often comutagenic, that is, they enhance the mutagenicity of other xenobiotics or endogenous factors by inhibiting the repair of induced DNA damage. The aim of our study was to evaluate the expression of some genes involved in the response to oxidative stress in yeast Saccharomyces cerevisiae and the genotoxic effect in Chinese hamster fibroblasts (V79) cells induced by SnCl2 treatment. The expression of the genes SOD1, SOD2, GSH1, YAP1, and APN1 was studied by qRT-PCR. We observed significant up-regulation in the expression of YAP1, SOD1 and APN1 in stannous chloride treated yeast cells. The concentration-related increase in DNA damage was observed in V79 cells, evaluated by comet assay. Incubation with lesion-specific enzymes Fpg and Endo III did not increase significantly the DNA migration, suggesting that the induced DNA damage is preferentially strand breaks and/or apurinic/apyrimidinic (AP) sites. Possible influence of Sn2+ on functionality of DNA repair mechanisms was tested in comet assay, evaluating the effect on the repair of damage caused by the alkilating agent methyl methane sulfonate (MMS). The repair of alkilated bases in living cells occurs mainly via base excision repair, contributing to comet formation via DNA repair-induced intermediates (AP-sites). The increased level of DNA strand breaks observed in MMS-treated cells after post-treatment with SnCl2 could reflect accumulation of AP sites (which are converted in strand breaks in comet assay). These results, in addition to the observed significant up-regulation in the expression of APN1 in stannous chloride treated yeast cells, suggest that AP endonuclease 1 could be involved in the repair of SnCl2-induced lesions. Work supported by CNPq and CAPES.

DD066

COMBINED GENOTOXIC EFFECTS OF A METHYLATING AGENT AND RADIATION ON HUMAN CELLS

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It is important to evaluate the health effects of low-dose-rate or low-dose radiation in combination with chemicals as humans are exposed to a variety of chemical agents. Recently, we reported combined genotoxic effects of low-dose-rate radiation and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), the most carcinogenic tobacco-specific nitrosamine, in the lung of gpt delta transgenic mice (Mutat. Res., 626, 15-25, 2007). Under the conditions used, treatments with NNK significantly suppressed the induction of deletion mutations caused by radiation. To investigate the mechanisms involved, we pretreated human lymphoblastoid TK6 cells and the mismatch-deficient derivative, i.e., MT1 cells, with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) followed by g -irradiation. MNNG was chosen because it is a methylating agent as NNK but does not need metabolic activation for methylation on DNA. MT1 and TK6 cells were treated with MNNG for 24 hours at concentrations that gave similar surviving rates. After the treatments, the cells were immediately exposed to g -ray irradiation at a dose of 1.0 Gy and the combined genotoxicity was assayed. Interestingly, there were marked differences between two cells in the dose-responses in micronucleus (MN) assay, TK gene mutation assay, and Apotosis assay. In TK6 cells, the dose response curves along with the pretreatment dose of MNNG were U-shape, suggesting the apparent adaptive responses. In contrast, the frequencies of MN and TK mutations increased along with the dose of MNNG in MT1 cells. The genotoxic effects of MNNG and g -irradiation seemed additive in MT1 cells. The induction of apotosis was inhibited with pretreatment of MNNG in TK6 cells, but this inhibition effect was not found in MT1 cells. From these results, we suggest that mismatch repair proteins may be involved in the apparent adaptive responses against genotoxic effects of g -irradiation in human cells.

DD067

MICRONUCLEUS AND POLYMERASE INHIBITION ASSAYS TO EVALUATE GENOTOXICITY OF "DMPBD" - THE POTENT ANTIINFLAMMATORY COMPOUND FROM ZINGIBER CASSUMUNAR ROXB.

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Background: (*E*)-1-(3',4'-dimethoxyphenyl) butadiene or DMPBD was firstly isolated and indentified from rhizome oil of *Zingiber cas-*

sumunar Roxb (commonly called "Plai" in Thailand) by Thailand Institute of Scientific and Technological Research (TISTR). It had been proven for excellent anti-inflammatory effect by various pharmacological tests. However, it lacks toxicity data. Aim : We therefore carried out this study to investigate its genotoxic activity that could initiate the mutation and cancer. Methods: In the present study, DMPBD was freshly extracted by steam distillation. Its chemical structure was determined by NMR. Genotoxic property of DMPBD was evaluated in term of mutation induction in p53 DNA in streptavidin-coated 48-well microtitre plate by polymerase inhibition or PI assay whose mechanisim based on the inhibition of DNA polymerases encountering damaged p53 DNA while DNA fragmentation in V79 cells detected by cytokinesis- block micronucleus test. Two known mutagens *N*-methyl-*N*-nitrosourea (MNU) and mitomycin C (MMC) were employed as positive controls for PI assay and micronucleus test, respectively. Results: Results obtained from PI assay did not illustrate potential DNA-damaging effect of DMPBD when tested at 10, 100, 250, 500, 1,000 and 10,000 µg/ml whereas the MNU clearly exhibited genotoxic activity in p53 DNA indicated by a reduction in % PCR inhibition being 34.21,46.25, 65.14 and 83.11% for doses of 10, 25, 50 and 100 µM, respectively. Treatment with MMC for 6 hr revealed a dose-dependent induction of micronuclei in V79 cells which mostly was mono-micronuclei. The frequency of micronuclei (MN) analysed in 1,000 binucleated cells (BNC) per treatment were found at 7.79, 6.73, 8.68 and 12.55% in V79-treated with MMC at 1, 10, 30 and 50 ppm. These figures were not significantly different from that of the negative control cells (10.31%) receiving 1% DMSO. Conclusion : The current results obtained by these two assays suggest that DMPBD is possibly non-genotoxin. However, if it is needed to clarify this observation, further genotoxicity studies with the metabolic activating system are recommended.

DD068

GENOTOXICITY AND SENSITIVITY TO THREE MUTAGENS IN TK6 HUMAN LYMPHOBLASTOID CELLS BY CHROMOSOME ABERRATION AND MICRONUCLEUS TESTS

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Background: During the last decade an extensive amount of published data is available to support the use of TK6 human lymphoblastoid cells genotoxicity tests particularly comet assay. Aim: To investigate the sensitivity of TK6 cells to three known mutagens; mitomycin C (MMC), methyl methanesulfonate (MMS) and etoposide by the cytokinesis-block micronucleus (CBMN) assay and chromosome aberration (CA) assay. Methods: TK6 cells were grown in RPMI supplemented with 5% horse serum (HS). For both CBMN and CA assays, cells ($1x10^5$cells/ml) were exposed for 4 and 24 hr to various concentrations of MMC (1.0, 3.0 and 5.0 µg/ml), MMS (3.0, 5.0 and 10.0 µg/ml) and etoposide (0.10, 1.0 and 3.0 µg/ml). The micronucleus formation was analysed in binucleated cells (BNC) prepared by cytochalasin B treatment after genotoxic exposure. Chromosome aberrations were analysed in metaphase cells for both numerical and structural chromosome abnormalities. Results: Does-dependent increases in micronucleus formation and chromosome abnormality were detected in TK6 cells for the three genotoxic agents studied. However, increasing treatment time form 4 to 24 hr did not produce significant different results. The marked increased frequencies of micronuclei per 1,000 BNC induced by MMC, MMS and etoposide were respectively 43.00 ± 2.00, 18.67 ± 3.51 and 25.00 ± 2.00 when treated at 3 µg/ml for 4-hr. The elevated production of micronucleus after treatments corresponding to the increased sensitivity to chromosomal lesions. This was evident by the percentages of cell containing aberrants induced by MMC (6.00± 2.30), MMS (3.83 ± 1.53) and etoposide (2.29 ± 0.72) which were significantly greater than that of the untreated TK6 cells (0.08 ± 0.01, $p<0.05$). The main chromosome lesions induced by MMC and MMS were chromatid gaps and breaks whereas ring formation and fragmentation were also seen for etoposide. In addition, the rate of chromosomal abnormalities in response to MMS was significantly (LSD, $p<0.05$) greater than micronuclei formation. Conclusion: The sensitivity of TK6 cells to chromosomal abnormalities by MMC, MMS and etoposide is easily and reproducibly detected by the micronucleus test. The results obtained in these two mutagenic assays support the use of TK6 cells for genotoxicity tests.

DD069

CELLS AFTER TREATMENT WITH NONGENOTOXIC, CLASTOGENIC AND ANEUGENIC COMPOUNDS

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The phosphorylated form of histone H2AX (γH2AX) is recognized as a sensitive marker for DNA double-strand breaks (DSBs). Although genotoxic agents do not necessarily initiate DSBs, wide-ranging genotoxic agents are known to cause γH2AX induction. Compared to the extensive literatures on the induction of γH2AX by genotoxic agents, little is known about the γH2AX response to non-DNA-damaging agents. The aim of this study was to construct a whole cell ELISA (Cell-ELISA) system that can detect induction of γH2AX by genotoxic chemicals and to test pure aneugens for γH2AX induction. CHL, CHO and V79 cells seeded in 96-well culture plates were exposed to micronuclei-inducing chemicals with various modes of action including eight clastogens (methyl methanesulfonate, N-ethyl-N'-nitro-N-nitrosoguanidine, mitomycin C, cis-diaminedichloro platinum, irinotechan, etoposide, methotorexate and 5-fluorouracil) and six aneugens (colcemid, vincristine, paclitaxel, griseofulvin, CH3310395 and 17-allylaminogeldanamycin). After incubation for 24 hours, the cells were washed, fixed and reacted with an anti-γH2AX antibody followed by HRP-conjugated secondary antibody and coloring reagents. Absorbance from each treatment was measured for γH2AX induction. All of the clastogens induced γH2AX in a dose-dependent manner with the exception of 5-FU in CHO cells. The Cell-ELISA values increased above 1.5-fold of vehicle control. None of the nongenotoxic compounds or aneugens increased Cell-ELISA values to 1.5-fold. The concentration-relation curves were different between the nongenotoxic and aneugenic compounds. After treatment with the aneugens, the concentration-response curves of γH2AX were mostly flat even in moderately cytotoxic concentration ranges while the nongenotoxic compounds exhibited a cytotoxicity-dependent decrease of γH2AX. The concentration-response curves were classified into three types, concentration-dependent increase, cytotoxicity-dependent decrease and flat. The present study results demonstrate that Cell-ELISA measurement for γH2AX could further elucidate the mode of action of the chemicals. The Cell-ELISA system easy to test many compounds might be a helpful tool for better understanding of the implication of γH2AX in the field of genotoxicology.

DD070

DNA REPAIR DEFECTS AND CHROMOSOMAL FRAGILE SITES EXPRESSION

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Common fragile sites (fs) are specific chromosomal areas that present structural alterations when cells are exposed to various stresses, which affect DNA synthesis. Actually, common fs are mostly induced by incubation with aphidicolin (apc), an inhibitor of DNA polymerases α, β and ε. It has been previously demonstrated that homologous recombination (HR) is involved in the repair of DNA damage induced by apc and that Rad51 is one of the HR components responsible for the stability of fragile sites. Since the mismatch repair system (MMR) has been shown to control HR by aborting strand exchange between divergent sequences, we investigated the influence of MMR and HR on fs expression study-

ing the induction and distribution of fs in a cell line derived from a human colon carcinoma (i.e., HCT15). These cells are defective in MMR due to mutations in both alleles of MSH6 gene. We also used a MMR proficient clone transfected with a vector carrying the wild-type hMSH6 cDNA. Moreover, to analyse the requirement of a functional HR in the repair of fs, HCT-15 cells were transiently silenced for RAD51 using specific siRNA oligonucleotides. Results and conclusions: 1) It has been reported that MMR deficient cells are more sensitive to cytotoxicity induced by inhibitors of DNA synthesis with respect to MMR proficient cells; here we demonstrate that HCT-15 cells are also more sensitive to clastogenicity and fs induction by apc as compared to hMSH6-expressing HCT-15 cells. 2) In HCT-15 cells we have found a fs distribution different from the one previously reported in human lymphocytes. Indeed, it has been found that fs distribution depends on cell differentiation. HCT15 cells show alterations mainly at the fs in 16q23 (FRA16D), an area involved in rearrangements in the colon cancer HCT116 cell line. In 16q23 is also located the WWOX gene, that is mutated in HCT116 and classified as tumour suppressor gene. 3) Transient silencing of RAD51 results in induction of breaks mainly at fs, but with a more widely spread distribution (with a lower frequency in FRA16D) than that observed with apc. Moreover, in this experimental condition many more rearrangements are present (tri-and tetra radials, dicentrics and translocations) than after incubation with apc.

DD071

DNA REPLICATION AT COMMON FRAGILE SITE FRA6E UNDER NORMAL AND APHIDICOLIN-INDUCED STRESS CONDITIONS

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Common fragile sites (CFSs) are expressed as breaks/gaps on metaphase chromosomes after partial inhibition of the DNA replication process, as induced by aphidicolin (APH). Up to date, several studies suggest that the fragility at these loci may be related to the incomplete replication of the region. *FRA6E* is one among the most frequently expressed fragile sites of the human genome, and *PARK2* is the most relevant gene mapped inside. By molecular combing, we have evaluated the replication pattern of *FRA6E* under normal and APH-induced stress conditions in primary human lymphocytes. First, the effect of a relatively high dose of aphidicolin (0.4 mM) on the replication progression was evaluated at whole genome level. After 2 h of exposure, a 10 fold significant decrease of the replication rate was found with respect to the control (APH: 0.24±0.02 kb/min; C: 2.09±0.08 kb/min; $P < 0.001$), an effect balanced by the significant decrease of the inter-origin distance (APH: 54.3±8.1 kb; C: 321.3±15.9 kb; $P < 0.001$). At single-locus level, the replication dynamics was analysed at *PARK2 (FRA6E)* with respect to the early replicating control loci *HPRT* and *LAMIN B2*. Fork speeds and the inter-origin distances were determined and origins were mapped, with particular attention to all possible deregulation events of the replication process. The effect of the replication stress (0.4 mM APH, 2 h) was compared to the control condition. In the control sample, the average fork speed at the fragile site and at early replicating sequences were comparable, ranging from 1.2 to 1.5 kb/min. Unidirectional forks seemed to be overrepresented at the fragile site (16/61, 31%), with respect to control sequences (30/160, 19% at *LAMIN B2;* 5/27, 19% at *HPRT*). After APH treatment, fork rates significantly decreased to about 0.30 kb/min both at *PARK2* and at *HPRT*, a response already observed at whole genome level. At *HPRT* the low replication rate was balanced by a significant decrease of the inter-origin distance occurred (APH: 44.8±11.5 kb; C: 124.4±36.6.9 kb; $P < 0.01$). On the contrary, no origins could be mapped at *PARK2-FRA6E* due to the strong deregulation pattern. It can be concluded that a different response to replication stress may occur at the fragile site with respect to control sequences.

DD072

EVALUATION OF HIGH-THROUGHPUT UMU TEST SYSTEM USING TESTER STRAINS EXPRESSING HUMAN CYTOCHROME P450 ENZYMES

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The SOS/*umu* test is generally employed for screening of potential chemical carcinogens. The aim of this study is to examine the sensitivitiy of *umu*-microplate test system with tester strains OY1002/1A2, OY1002/1A1, OY1002/1B1, and OY1002/3A4 expressing human cytochrome P450 (CYP1A2, 1A1, 1B1, 3A4) enzymes. The strains were established by introducing two different plasmids into *S.typhimurium* TA1535. The one plasmid is carrying human cytochrome CYPs and NADPH-cytochrome P450 reductase cDNAs, and the other plasmid is carrying *O*-acetyltransferase and *umuC"lacZ* fusion genes. We evaluated induction of *umuC* gene expression by 2-aminoanthracene, IQ, MeIQ and Glu-P-1, which expressed genotoxicity when bioactivated by CYPs, and the expression is based on measuring β-galactosidase activity. We obtained the following result with a 96-well microplate method. Genotoxicity of these chemicals with OY1002/1A2 strain was able to detect with high sensitivity under the following pre-incubation condition, which is added with isopropyl β-D-thiogalactopyranoside at three hours point, then continuing incubation one more hour before chemical treatment. We found that the microplate method can show approximately equal sensitivity to the test tube method with opitimized chemical treatment condition, and measurement condition of β-galactosidase activity. However, in the OY1002/1A1, OY1002/1B1, and OY1002/3A4, the sensitivity to the microplate method was lower than that to the test tube method. These results suggest that the OY1002/1A2 strain provides the possibility of high-throughput *umu* test system.

DD073

PHOSPHORYLATION OF SAF-A EXCLUSIVELY BY DNA-PK IN RESPONSE TO DNA DOUBLE-STRAND BREAKS MARKS CELL REPAIR CAPACITY.

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Radiotherapy is an essential mean for the curative treatment of cancer. The production of DNA double-strand breaks (DSB) by ionizing irradiation accounts mostly for its toxic effect on tumor cells and DNA repair represent a major determinant of the cell response to DSB. Therefore, radiotherapy efficacy should benefit from developing drugs that would target specifically the predominant repair mechanism, the Non Homologous End Joining (NHEJ) pathway in cancer cells. In mammals, NHEJ relies on recognition, protection and bridging of the DNA-ends by the DNA-dependent protein kinase complex (DNA-PK) composed of the DNA binding KU70/KU80 heterodimer which recruits the serine-threonine kinase catalytic subunit (DNA-PKcs). DNA-PK also activates end-processing enzymes such as the Artemis nuclease and is required for the stable recruitment of the XRCC4/DNA Ligase IV (LIG4)/Cernunnos-XLF complex that catalyzes the final ligation step. Aiming to identify novel phosphosites in response to DNA double-strand breaks (DSB), we have isolated a 120 kDa protein in cells treated by DNA DSB inducers. We identified this protein as SAF-A, mapped the main phosphosite and showed that this site was exclusively phosphorylated by DNA-PK in response to DNA DSB. In addition, we found that the extent and duration of this phosphorylation was in inverse correlation with the capacity of the cells to repair DSB by NHEJ. With the purpose to improve radiotherapy efficacy, we propose to use SAF-A phosphorylation as a specific signal to screen DNA-PKcs inhibitors and to assess the cellular capacity to repair DSB.

DD074

HUMAN PRECISION CUT LIVER SLICES: A VIEW ON INDIVIDUAL SUSCEPTIBILITY TO CARCINOGEN EXPOSURE

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Background / Aim: Efforts are being taken to develop alternatives to animal testing. Many studies focus on the development of in vitro tests as a possible alternative to in vivo chronic animal studies. The relevance of these new systems for humans is a major issue, since large individual variations between humans in response to xenobiotic exposure are known to exist. Therefore this study aims at evaluating the individual susceptibility to carcinogen exposure, by the use of precision cut human liver slices. Methods: Human precision cut liver slices of 5 donors exposed to aflatoxin B1 (AFB1), acetaminophen (APAP), benzo(a)pyrene (BaP) or 2-nitrofluorene (2-NF) are evaluated for cytotoxicity (LDH + ATP leakage) and genotoxic damage (COMET assay). Individual susceptibility is evaluated by the calculation of No Observed Effect Levels (NOELs). Results: Cytotoxicity: After 6 hours no significant increase in LDH or ATP leakage was seen with any of the substances. At 24 hours, an increase in both LDH and ATP leakage was found in all substances. Only for APAP the leakage was not dose dependant. Genotoxic damage: DNA damage showed a dose dependent increase for all substances but APAP. With APAP genotoxic damage was found, but not in a dose dependant manner. Individual variation: NOELs for DNA-damage were lower in comparison to NOELs from the cytotoxicity tests in every substance but AFB1. Both cytotoxicity tests show large differences in the NOELs per individual, these differences seem to be less pronounced with the genotoxic damage assay. Conclusions: Precision cut human liver slices can be used to measure individual susceptibility to carcinogen exposure. The cytotoxicity tests show larger variability between individuals compared to the DNA damage assay. NOELs show clear individual variation for all substances. Future plans: To gain more insight into what causes individual differences in reaction to carcinogen exposure, future studies will focus on: gene / protein expression by micro-arrays, RT-PCR on some toxicity related genes, CYP450 activity and miRNA expression. Furthermore, for several toxicity related genes, genetics, by genotyping studies (SNP) and epigenetics by studying DNA methylation patterns (CpG islands).

DD075

THE HOMOLOGUES OF BACTERIAL ALKB PROTEIN IN CYANOBACTERIA AND ARABIDOPSIS THALIANA

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Cyanobacteria and higher plants belong to evolutionary distant groups of photosynthesizing organisms. Despite of this fact all of them share homology of *alkB* gene primary discovered and studied in *Escherichia coli*. AlkB protein is a non-heme iron (II) and a-ketoglutarate-dependent dioxygenase that oxidatively demethylates 1meA and 3meC lesions in DNA, with recovery of A and C building RNA or DNA. The aim of this study was to describe the activity of AlkB homologues in both groups of organisms: *Cyanobacteria* and higher plants – *Arabidopsis*. We have chosen ten cyanobacterial strains (*Acaryochloris marina* MBIC11017, *Arthrospira maxima* CS-328, *Cyanothece sp.* PCC7425, *Microcoleus chthonoplastes* PCC7420, *Prochlorococcus marinus* MIT9313, *Synechococcus sp.* BL107, *Synechococcus sp.* CC9311, *Synechococcus sp.* RS9916, *Synechocystis sp.* PCC6803) which genomes have been sequenced and revealed existence of at least one *alkB* homologue. Most of the coded enzymes contain whole AlkB domain. On the other hand the genome of *Arabidopsis thaliana*, a plant model organism, shows at least ten *alkB* homologues that produce proteins with whole or part of the AlkB domain, according to *in silico* analysis. Cyanobacterial and *Arabidopsis* cDNA of *alkB* homologues were inserted into high copy number plasmid pET28a in the frame with His-tag in order to overexpress them in *E. coli* strain BL21. The proteins were purified on metal affinity columns (Ni-Sepharose) and used in enzyme activity assay (demethylation activity on oligonucleotide containing 3-methylcytosine residue). Simultaneously, cDNA of *alkB* homologues were inserted into low copy number vector (pVB1x); the resulting plasmid constructs were used in complementation assays in *E. coli* strain deprived of its own *alkB* gene.

DD076

CONTRIBUTION OF TRANSCRIPTION-COUPLED DNA REPAIR TO MMS-INDUCED MUTAGENESIS IN E. COLI STRAINS DEFICIENT IN FUNCTIONAL ALKB AND MFD PROTEINS

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In *Escherichia coli* the alkylating agent methylmethane sulfonate (MMS) induces defense systems (adaptive and SOS responses), DNA repair pathways, and mutagenesis. We have previously found that AlkB protein induced as part of the adaptive (Ada) response protects cells from the genotoxic and mutagenic activity of MMS. AlkB protein is a non-heme iron (II) and a-ketoglutarate-dependent dioxygenase that oxidatively demethylates 1meA and 3meC lesions in DNA, with recovery of A and C. Here, we studied the impact of transcription-repair coupling factor, Mfd protein, on MMS-induced mutagenesis in *E.coli* strains deficient in functional AlkB protein. Measuring the Fpg sensitive sites in plasmid DNA and the frequency and specificity of MMS-induced *argE3*→Arg$^+$ revertants under transient starvation conditions, we found less effective transcription coupled repair (TCR) in *E.coli alkB*$^-$ than in *alkB*$^+$ strains. On the other hand, introduction of *mfd*-1 mutation into the *alkB*$^-$ strains resulted in complete loss of TCR and extremely high induction of the SOS response, not observed in the *alkB*$^+$ *mfd*$^-$ strain. Determination of the specificity of *mfd-1* mutations in CC101-106 strains showed a great prevalence of MMS-induced GC→AT transitions in the CC102 strain, whereas the introduction of *mfd*-1 mutation into both sets of *alkB*$^-$ strains resulted mostly in GC→TA transversions in the CC104 strain. The involvement of 1meA/3meC lesions and also of AP sites in the induction of SOS response and MMS-induced mutagenesis has been postulated.

DD077

LETHAL AND MUTAGENIC EFFECT OF MMS ON E.COLI CELLS DEFICIENT IN BER AND ALKB-DIRECTED REPAIR

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In *Escherichia coli* the alkylating agent methylmethane sulfonate (MMS) induces defense systems (adaptive and SOS responses), DNA repair pathways, and mutagenesis. We have previously found that AlkB protein induced as part of the adaptive (Ada) response protects cells from the genotoxic and mutagenic activity of MMS. AlkB protein is a non-heme iron (II) and a-ketoglutarate-dependent dioxygenase that oxidatively demethylates 1meA and 3meC lesions in DNA, with recovery of A and C. Here, we studied the impact of transcription-repair coupling factor, Mfd protein, on MMS-induced mutagenesis in *E.coli* strains deficient in functional AlkB protein. Measuring the Fpg sensitive sites in plasmid DNA and the frequency and specificity of MMS-induced *argE3*→Arg$^+$ revertants under transient starvation conditions, we found less effective transcription coupled repair (TCR) in *E.coli alkB*$^-$ than in *alkB*$^+$ strains. On the other hand, introduction of *mfd*-1 mutation into the *alkB*$^-$ strains resulted in complete loss of TCR and extremely high induction of the SOS response, not observed in the *alkB*$^+$ *mfd*$^-$ strain. Determination of the specificity of *mfd-1* mutations in CC101-106 strains showed a great prevalence of MMS-induced GC→AT transitions in the CC102 strain, whereas the introduction of

mfd-1 mutation into both sets of *alkB*⁻ strains resulted mostly in GC→TA transversions in the CC104 strain. The involvement of 1meA/3meC lesions and also of AP sites in the induction of SOS response and MMS-induced mutagenesis has been postulated.

DD078

ASSESSING OF RADIOSENSITIVITY OF PERSONS EXPOSED TO IONIZING RADIATION DURING FLIGHTS

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One of the important problems of modern medicine is revealing groups of risk among people professionally exposed to gamma irradiation. Pilots and astronauts are in the special risk group, during the flight they are affected by ionizing radiation, vibration, noise and low gravity. In this paper the problem of low level radiation, possible influence on health and implication for the risk assessment will be discussed. We have used some biological markers that can serve as an early significance of future disease. DNA damages, aberrations of chromosome (unstable), radiosensivity and adaptive response were investigated on human blood lymphocytes. It was shown that in pilot's lymphocytes initial DNA damages are increased in comparison with control donors; the radiosensitivity is higher too (in the mean). But the very important fact – the high level of individual variability in the initial DNA damage and in radiosensitivity is observed. The frequency of lymphocytes with some aberrations and the whole amount of chromosome aberrations is higher in pilots after additional irradiation in control population. The adaptive response is decreased and determined in 14% of pilots in comparison with 69% in control donors. Among the astronauts the adaptive response or the tendency to adaptive response was observed.We can conclude that in 1-10 years after flying it is possible to determine DNA damages, alterations in radiosensivity and capability to adaptation. Perhaps some nontarget effects, for instance genomic instability after low dose irradiation induce these late effects and then pilots and astronauts with enhanced radiosensitivity are a group of risk.

DD079

APOPTOSIS IS THE ONLY CAUSE OF THE PHOSPHORYLATION OF H2AX IN TK6 CELLS TREATED WITH COLCEMID, TAXOL AND VINBLASTIN

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It is important to understand that a micronucleus (MN)-inducing compound has a direct or indirect effect on DNA. Increases in MN determined by staining with centromere or kinetochore probes are generally accepted as evidence of the existence of an indirect mode of action but does not necessarily suggest a lack of direct activity on DNA. The phosphorylated form of histon H2AX (γH2AX) is a sensitive and quantitative biomarker for DNA double-strand break (DSB) formation. Recent studies have revealed that H2AX is phosphorylated via DNA repairing pathways after treatment with wide-ranging genotoxic agents including the compounds that do not initiate DSBs. The aim of this study was to examine the potential use of γH2AX measurement to evaluate the lack of DNA direct activity. Human lymphoblastoma TK6 cells were exposed to a clastogen (methyl methansulfonate, MMS), three aneugens (colcemid, taxsol and vinblastin), and etoposide which has both aneugenic and clastogenic activities. After 24 hours of treatment, the cells were fixed, double-stained with anti-γH2AX and anti-caspase-3 antibodies as an apoptotic cell marker, and analyzed by flow cytometry. Concentration-dependent increase of γH2AX was seen in both caspase-3 positive and negative cells after treatment with MMS, etoposide, taxol and vinblastine whereas colcemid induced γH2AX only in the caspase-3 positive cells. Then, we re-defined the apoptotic cells using MitoTracker-detecting alteration of the mitochondrial membrane, an early event of apoptosis. MMS and etoposide increased γH2AX in a concentration-dependent manner in both apoptotic and non-apoptotic cells. Contrarily, all three aneugens induced γH2AX in the apoptotic cells only. The results demonstrated that apoptosis entirely contributed to the induction of γH2AX after treatment with the three aneugens. It must be noted that γH2AX is not an endpoint directly corresponding to harmful events such as mutations, structural aberrations of chromosomes or genome instability. It remains questionable whether the consideration of genotoxicity merits measurement of γH2AX. However, the present study result suggests that a combination of γH2AX with an early apoptotic marker may be a helpful tool for better understanding genotoxic modes of action.

DD080

OCCUPATIONAL EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBONS IN WOOD DUST

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Background: Malignant naso-sinusal cavity (NSC) tumors represent approximately 3% of Otolaryngology cancers. NSC adenocarcinoma has been accepted as an occupational disease for certain specialist wood workers such as joiners and cabinetmakers. It is estimated that wood workers are subjected to a 50-100 times higher risk for NSC adenocarcinoma than the general population. Several chemical substances have been identified as potentially involved in the guesses of this cancer, such as the tannin in hardwood, formaldehyde in plywood and generally Polycyclic Aromatic Hydrocarbons (PAHs) in wood. PAHs most likely play a central role in the guesses of NSC adenocarcinoma development in wood workers. This study aims to clarify the role of PAHs in the genotoxicity of wood dust. Methods: In a 10 m^3 experimental chamber the typical work processes like sawing, planning or sanding were analyzed. The size distribution of the wood dust particles was evaluated by microcopies analysis. The level of PAHs was measured by Gas Chromatography Mass spectrometry. The following three wood types were tested: rough fir tree, impregnated polyurethane (PU) oak and rough oak. High Performance Liquid Chromatography was used to monitor urinary 1-hydroxypyrene, a metabolite of PAHs. The level of genetic damage due to wood dust exposure was determined by comet assay in peripheral blood lymphocytes by micronucleus assay in nasal and buccal epithelial cells and by the antioxidant level in peripheral blood. Results: The results showed the presence carcinogenic PAHs in wood dust at level of mg/g or ppm. The dust generated by sanding operation of PU impregnated wood contained 100 times higher PAHs levels than the dust of rough wood. The wood working tools such as sander, circular saw or plane could create dust with a particle size distribution centered around of 10 µm or more, therefore the resulting dust deposition is expected in the naso-sinusal cavity. No difference in PAHs content between softwood (fir tree) and hardwood (oak) was detected. Conclusions: The high temperature induced by sanding or cutting tools results in a considerable exposure to PAHs. Excessive heating of the surface of the wood or the PU impregnated surface could be the cause of the measured level of PAHs.

DD081

CORRELATION BETWEEN CHROMOSOME ABERRATION ANALYSIS AND MICRONUCLEUS ASSAY IN SAMPLES OF MEDICAL EMPLOYEES OCCUPATIONALLY EXPOSED TO IONIZING RADIATION

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Ionizing radiation can cause harmful genetic mutations by damaging DNA. Chromosome aberration analysis and micronucleus assay are the most preferable methods to determine those damages. They can be used as complement or substitute to each other. Aim of this study was to evaluate correlation of these tests. The study is conducted over 41 samples of medical employees occupationally exposed to ionizing radiation. Peripheral blood was cultivated 72 hours for micronucleus cytokinesis-block assay and 48 hours for chromosome aberrations analysis. For each sample 100 metaphase and 1000 binuclear cells were analyzed. Within observed chromosome aberrations 33% are minute fragments, 29% acentric fragments, 29% chromatide, and 7% chromosome breaks. We also found 2 dicentrics and 1 translocation figure. In order to determine linear correlation among results of conducted cytogenetic tests, Pearson correlation coefficient was calculated. Significant positive correlation was determined for frequencies of structural chromosome aberrations and micronuclei (r = 0,341, p=0,029). The same was determined for frequencies of all – structural and numerical - chromosome aberrations and micronuclei (r = 0,321, p=0,0407). Significant correlation was not determined for frequencies of numerical chromosome aberration and micronuclei. The gender of participants did not significantly affect frequencies of observed cytogenetic parameters. Results of this study affirm validity of usage of both tests in genotoxicological screenings.

DD082

IMPACT OF POLY (ADP-RIBOSE) POLYMERASE 1- DEFICIENCY IN DNA AND CHROMOSOME DAMAGE FOLLOWING MOUSE WHOLE-BODY GAMMA IRRADIATION

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Poly (ADP-ribose) polymerase1 (Parp1) is a chromatin-associated protein that recognizes and is activated by DNA strand breaks. Parp1 involvement in base excision repair and in double strand break repair has been extensively studied in vitro, but few studies have tried to ascertain its role in DNA damage repair at a whole-organism level. The present work aimed at investigating the effect of Parp1-deficiency on DNA and chromosome damage in several organs of gamma-irradiated mice. Groups of Parp1-proficient (WT) and deficient mice were exposed to 1 Gy whole-body gamma radiation and spleen, liver and bone marrow were collected at 120 min post-irradiation to measure DNA damage using the alkaline Comet assay. Blood samples were collected at 10, 90 and 180 min post-irradiation for the comet assay and at 48 h for analysis of micronucleus in reticulocytes. A time-related variation in the level of DNA damage (percentage of DNA in the tail) was observed in peripheral blood cells of irradiated mice, reaching its maximum at 90 min, independently of the Parp1 status. In bone marrow, a differential response was detected between WT and Parp1-deficient mice, consisting of a significantly higher level of radiation-induced DNA breaks in WT mice. This apparently weaker induction of DNA damage in bone marrow of Parp-1 deficient mice might be due to a stronger cytotoxicity, which was evidenced by a dramatic decrease in peripheral blood reticulocyte number, 48 h after irradiation. Despite this effect, a significantly increased frequency of micronucleated reticulocytes was found in both groups of irradiated mice in comparison to controls, reflecting radiation-induced chromosome breaks in bone marrow cells, which were not affected by Parp1 status. No significant radiation-induced DNA damage was detected in spleen and liver of both mouse groups.In summary, in response to 1 Gy of ionizing radiation, the most prominent effects of Parp1 abrogation in vivo were a decreased level of DNA damage and an increased cytotoxicity in bone marrow cells. The later is in agreement with the recognized higher susceptibility of Parp1-deficient cells to death following a genotoxic stress, probably preventing further accumulation of DNA damage.

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DD083

EVALUATION OF DAMAGE INDUCED IN VITRO BY TAXOTERE AND FARMORUBICIN IN BREAST CANCER PATIENTS DURING NEOADJUVANT CHEMOTHERAPY AND HEALTHY FEMALES

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Drug resistance is considered to be the main impediment to successful cancer chemotherapy. Clinical responses to chemotherapy and toxicity vary among individuals. In this study, we evaluated the basal damage and the cellular response to DNA damage induced in vitro by Taxotere and Farmorubicin in lymphocytes of seventeen female patients between 25 and 70 years with advanced breast cancer before and after chemotherapy and forty age-matched healthy females. The Micronucleus Test and Comet Assay were performed in peripheral blood lymphocytes (PBL) according to a standard protocol. Five treatment groups were analyzed: negative control (25µL of sterile water in the culture medium, because this was the solvent used to prepare the Farmorubicin), solvent control (5µL of ethanol and water solution (13%) in the culture medium, because this was the proportion used to dilute Taxotere), Taxotere (0.5µg docetaxel (Aventis Pharma, England) in the culture medium), Farmorubicin (0.25µg epirubicin (Pfizer) in the culture medium) and drug association (Taxotere + Farmorubicin). In the Comet Assay, three cell samples were collected: T0 (immediately before the cell culture treatment), T1 (immediately after completion of the cell culture treatment) and T2 (four hours after completion of the cell culture treatment). No differences in micronucleus basal frequency were observed between breast cancer patients and healthy females. The Comet Assay and Micronucleus Test determined that patients and healthy females responded with the same intensity to damage induced by Taxotere and Farmorubicin chemotherapeutics. Breast cancer patients showed a reduced repair capability and/or partial elimination of the damaged cells as compared to healthy females. We also observed that DNA damage in breast cancer patients is accumulated during the various cycles of chemotherapy and persists until the end of treatment. Dividing the samples according to age (≤45 years and > 45 years), we observe that increasing the age, the group of patients becomes more susceptible to the damage.

DD084

PROTECTIVE EFFECT OF N-ACETYLCYSTEINE ON RADIATION-INDUCED ACUTE DNA DAMAGE INDUCED BY INTERVENTIONAL CARDIOVASCULAR PROCEDURES

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Background: Invasive cardiovascular procedures involving radiation exposure are associated with the induction of chromosomal DNA damage (Andreassi et al. Eur Heart Journal 2007). N-acetylcysteine (NAC) is frequently used for preventing contrast-induced nephropathy, but it also has weel recognized radioprotective properties. The aim was to assess the effect of NAC on levels of chromosomal DNA damage after interventional catheterization procedures. Methods: Sixty-eight patients (52 males, age 64.6+11.8 years) undergoing invasive cardiovascular procedures (peripheral transluminal angioplasty, n= 48; cardiac resynchronization therapy, n =15 and ablation therapy n=5) were enrolled: 38 patients received the standard hydration protocol consisting of intravenous isotonic saline for 12 h after catheterization (Group II), and 30 patients (26 males, age 65.6+12.9 years) received a double

intravenous dose of NAC (6 mg/kg/h diluted in 250 mL of NaCl 0.9%) for 1 hour before and a standard dose (6 mg/kg/h diluted in 500 mL of NaCl 0.9%) for 12 hours following catheterization (Group II). Micronucleus assay (MN) was used as biomarker of chromosomal damage and intermediate endpoint in carcinogenesis. Dose–area product (DAP; Gy cm2) was assessed as physical measure of radiation load. Results: DAP was higher in NAC-treated patients (I=126.2±79.2 vs II=54.7±23.6 Gy cm2, p<0.0001). MN frequency showed a significant rise at 2 h and 24 h after procedures only in Group I, saline-treated patients (see figure; *=p<.05 vs baseline). Conclusion: NAC treatment may exert a radioprotective effect in patients submitted to radiation-intensive interventional cardiological procedures, possibly through its known direct and indirect (GSH precursor) antioxidant properties.

DD085

DNA REPAIR POLYMORPHISMS XRCC1 Arg399Gln IS ASSOCIATED WITH ELEVATED LEVELS OF CHROMOSOMAL DNA DAMAGE AND TELOMERE DYSFUNCTION IN PATIENT WITH ATHEROSCLEROSIS

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Background: Telomere shortening and chromosomal DNA damage in circulating leucocytes have been shown to be associated with coronary artery disease (CAD). DNA repair is a central factor for maintaining genomic stability, including telomere maintenance. The aim was to test whether genetic polymorphisms in DNA repair capacity (XRCC1 Arg399Gln, Arg194Trp, XRCC3 Thr241Met) are associated with increased levels of chromosomal DNA damage and telomere dysfunction in patient with CAD. Methods: The study population comprised 85 patients (57 male; 66.2±10.0 years; mean ±S.D.) with angiographically-proven CAD. Micronucleus assay (MN) was used as biomarker of chromosomal damage. Telomere length was assessed with quantitative real-time PCR. PCR-RFLP analysis was performed for each genetic variant. Results: MN was higher in female (p=0.04) and telomere length was inversely correlated with age (p=0.03). Carriers of at least one variant allele of XRCC1 Arg399Gln had higher levels of MN (19.0±7.1 Arg/Arg vs 22.9±8.6 Arg/Gln Gln/ Gln p=0.03) and shorter telomere length (0.911±0,20 Arg/Arg vs 0.822±0.19 Arg/Gln Gln/Gln p=0.03). No difference was evidenced about other polymorphisms. Conclusions: Our findings demonstrated that the XRCC1 Arg399Gln polymorphism is associated with both chromosomal DNA damage and telomere dysfunction, suggesting that repair DNA single-strand breaks may have a critical role in the genetic instability-induced atherosclerosis.

DD086

SYSTEMATIC ANALYSIS OF SIGNALING PATHWAYS THAT CONTROL THE DNA-DAMAGE RESPONSE IN EMBRYONIC STEM (ES) CELLS

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Exposure of cells to DNA-damaging agents activates signal transduction cascades that affect DNA repair, cell cycle progression, and cell survival. Different cellular strategies exist to cope with DNA damage: damage is tolerated (with the risk that cells acquire malignant properties), damage is repaired, or cells with damaged DNA are removed from the tissue. The latter strategy involves a complex of signal transduction pathways that can induce various types of cell death, including apoptosis. In a highly simplified model DNA-damage causes activation of the sensor kinases (ATM, ATR, DNA-PK) that activate p53 and the checkpoint kinases (Chk1, Chk2), which in turn regulate effector pathways that lead to DNA-damage, cell survival, or death. In reality, the process is much more complex with many additional regulators, extensive cross-talk, and multiple positive and negative feedback loops. In this project, we make use of gene family short interference (si)RNA libraries to identify kinases and phosphatases that are involved in the apoptotic response to various DNA-damaging drugs in ES cells. Automated high content live cell imaging analysis is used to qualitatively and quantitatively follow the onset of apoptotic cell death. We will unravel the signaling cascades in which identified kinases and phosphatases operate and employ micro-array studies to link them to transcriptional changes upon DNA-damage response.

DD087

ROLE OF POSTTRANSLATIONAL PROTEIN MODIFICATIONS IN THE DNA DAMAGE RESPONSE

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Maintenance of genomic stability is of outmost importance for cells to avoid malignant transformation. The exposure of cells to genotoxic agents (as well as different stresses like hypoxia, UV or radiation) leads to activation of the DNA damage response which ensures that no corrupted DNA is kept or passed to daughter cells. This cellular response comprises a complex signaling network which integrates the damage signal and finally leads to an appropriate cellular outcome. This outcome can be tolerance toward the DNA damage which bears the risk of malignant transformation, the repair of the DNA, or the removal of the cell in which the damage occurred from the tissue which is generally achieved by regulated death of the cell (apoptosis). In recent years much data about the response to DNA damage has been gained using microarray technology providing knowledge about the regulation of genes on the transcriptional level. We will perform a functional genomics screen, which will go beyond the level of gene regulation and will help us to clarify the role of different factors for the DNA damage response. Posttranslational modifications are of great importance to transmit the damage signal, making the enzymes which confer these modifications interesting candidates to screen for. We will make use of siRNA based libraries to silence genes encoding all cellular kinases and phosphatases, as well as ubiquitinases and deubiquitinases, sumoylases and desumoylases. Following siRNA-mediated knockdown, embryonic stem cells are treated with the chemotherapeutic cisplatin. As a bioassay we use live imaging for the binding of fluorophore-labeled annexin V as a marker of apoptosis. In contrast to an endpoint based analysis this allows us to capture the kinetics of the apoptotic response. Further, we use staining of fixed samples for markers of DNA repair, like 53BP1 and g-H2AX which appear in discrete foci after the occurrence of DNA double strand breaks. These assays have been used in preliminary small scale experiments are currently being optimized for the intended large scale screens. Using these different readouts will allow us to identify hits in DNA repair and/or apoptosis pathways.

DD088

POLYMORPHISMS IN DNA BASE EXCISION REPAIR (BER) GENES AND NEURODEGENERATIVE DISEASES

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BACKGROUND: There is indication that terminally differentiated neurons can re-enter the cell division cycle to allow the repair of DNA damage. Indeed, if not properly repaired, damage to both nuclear and mitochondrial DNA can result in neuronal death. In this context several studies indicate that oxidative DNA damage is critical for neuronal survival, and that the DNA base excision repair (BER) pathway is impaired in neurodegenerative diseases such as Alzheimer's disease

(AD), Parkinson's disease (PD) and Amyotrophic Lateral Sclerosis (ALS). Moreover BER seems also involved in the somatic instability of CAG repeats in Huntington's disease (HD). AIMS and METHODS: we are currently searching for association between polymorphism in BER genes and any of the above neurodegenerative diseases by means of PCR/RFLP technique. RESULTS: We observed association between the *OGG1* Ser326Cys polymorphism and sporadic ALS risk in Italian males ($P = 0.01$). On the contrary, the *APE1* Asp148Glu polymorphism was not associated with increased ALS risk. We are currently investigating *XRCC1* Arg194Trp, Arg280His and Arg399Gln polymorphisms in over 200 sporadic Italian ALS patients and 200 matched controls, and preliminary results indicate association between *XRCC1* Arg399Gln and disease risk in males ($P < 0.05$). Our recent analysis of more than 150 Italian AD patients and 150 matched controls failed to find association between the *OGG1* Ser326Cys polymorphism and risk for sporadic AD. OGG1 has been recently implicated in somatic CAG repeat expansion of the HD gene and we observed association between the *OGG1* Ser326Cys polymorphism and both the number of CAG repeats in blood cells and disease age at onset in 91 HD individuals ($P = 0.01$). We are currently genotyping a large Italian case-control group of over 400 subjects to test for a possible association between the *OGG1* Ser326Cys polymorphism and sporadic PD. CONCLUSIONS: Overall results from our group indicate a possible role for polymorphisms in BER genes in different neurodegenerative processes.

DD089

CELLULAR RESPONSES TO CISPLATIN ASSOCIATED WITH LY294002 (INHIBITOR OF PHOSPHATIDYLINOSITOL 3-KINASE) IN GLIOMA CELL LINES

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Gliomas are the most common tumor of the central nervous system. In spite of the combined therapies (surgery, radio- and chemotherapy) the prognosis for these patients is still discouraging. In this work, we tested whether LY294002 (PI3K inhibitor) can enhance the cytotoxicity of cisplatin in human glioma cell lines (U343 and U87). Aiming to analyze the cellular responses to cisplatin plus LY294002, as a first step, we performed survival assays and apoptosis detection; in addition, the expression of several proteins (PCNA, H2AX, p53 and ATR) was analyzed by Western blot. We also tested whether DNA-PK mutant (MO59J) and proficient (MO59K) cell lines would provide results according to the DNA-PK (member of PI3K family) status. Cisplatin (5 to 75 µM) caused a pronounced reduction in cell survival after five days of treatment in U343 and U87 cells. While U343 cells underwent apoptosis (50.1%) after treatment (25µM cisplatin+LY294002; 72h), U87 cells did not show a significant apoptosis induction. Therefore, LY294002 significantly increased cisplatin effects in survival and apoptosis assays for U343, but for U87 cells, LY294002 increased cell death only in survival experiments. MO59K cells were resistant to cisplatin (5, 10, 25, 50 and 75 µM; 72h) while MO59J cells were highly sensitive to the drug, presenting a significant apoptosis induction (43%). While PCNA expression was reduced in cisplatin-treated U343 cells, compared to controls, γ-H2AX was expressed in cell treated with cisplatin and cisplatin+LY294002. Phospho-p53 was expressed only in cisplatin and cisplatin+LY294002 treated cells (24h). The combined treatment (24h) also increased ATR protein expression. Therefore, the results demonstrated that LY294002 plus cisplatin showed significant effects on cell proliferation and cell death, probably due to the inhibition of PI3K, and possibly DNA-PK, which is known to be involved in DNA repair. Moreover, the effects of the combined treatment were observed for proteins related to DNA damage responses. Thus, the inhibition of DNA repair proteins proved to be a promising strategy in cancer therapy. Experiments to confirm the inhibition of DNA-PK are currently under way. (Financial support: FAPESP (Proc. 05/02900-2; 04/15611-6), CNPq and CAPES).

DD090

EVALUATION OF THE PROTECTIVE EFFECT OF BIXIN ON CISPLATIN-INDUCED GENOTOXICITY IN PC12 CELLS

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The neuropathy induced by chemotherapeutic drugs is a complication in the treatment of cancer and other diseases, because this kind of treatment is often painful and requires discontinuation of the therapy. Cisplatin has been commonly used against many forms of cancer, however, its application is associated with many toxic effects such as neurotoxicity, nephrotoxicity, hearing loss and vomiting. These adverse effects have led to the development of specific agents to lessen the drug toxicity. Some studies have suggested that antioxidants administration is able to reduce the damage and protect tissues. Thus, the carotenoids are an important option to be evaluated, because they are considered to be effective antioxidants. In the present study, the genotoxicity and antigenotoxicity of bixin carotenoid on the cisplatin-induced toxicity in PC12 cell cultures was assessed. Cytotoxicity was determined by the MTT assay, chromosomal damage by the Micronucleus test and the extent of primary damage to the DNA by the Comet assay. Bixin was first assessed with respect to its genotoxicity. Bixin concentrations of 0.05, 0.08 and 0.10 µg/ml were neither cytotoxic nor genotoxic to PC12 cells. Thus, these concentrations were used in experiments to verify the protective effect of bixin against damage induced by cisplatin. Although the protective effect of bixin was not evident in the results obtained by the Comet assay, effective inhibition of cisplatin-induced chromosomal damage (Micronucleus test) was shown. The results indicated that bixin could be considered as an agent capable of prevent *in vitro* cisplatin-induced mutagenic damage, and also help in the modulation of oxidative stress developed during chemotherapy with this drug. Further studies should be carried out to better understand the mechanisms involved in the activity of this protective agent.

DD091

REPLICATION FORK STABILIZATION PROTEINS TIMELESS AND TIMELESS-INTERACTING PROTEIN (TIPIN) MAINTAIN GENOMIC STABILITY

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Timeless (Tim) and Timeless-interacting protein (Tipin) form a heterodimeric complex that participates in DNA replication and the ATR-dependent intra-S checkpoint response enforced by ATR phosphorylation of Chk1. In yeasts, orthologs of Tim/Tipin interact with replisome factors and maintain fork stability in the presence of DNA damage. To further characterize the functions of mammalian Tim and Tipin, normal human fibroblasts (NHF) were transiently depleted of these and other proteins via siRNAs. Depletion of Tim, Tipin, and Chk1 reduced viability in a colony formation assay, and Tim- and Chk1-depleted NHF were hypersensitive to UVC exposure. Depletion also reversed UVC-induced inhibition of origin firing, which was accompanied by attenuated phosphorylation of Chk1 in Tim- and Tipin-depleted NHF. Also, DNA fiber immunostaining showed that the rate of fork displacement was reduced with loss of Tim or Chk1, and that fork displacement failed to slow in Tipin-depleted cells exposed to UVC. These observations indicate that Tim and Tipin may mediate intra-S checkpoint responses to UVC exposure. In the absence of exogenous DNA damage, depletion of Tim, Tipin, and Chk1, but not ATR, compromised BrdU incorporation in a manner suggestive of replication stalling and collapse. ATR-dependent P-Chk1 was apparent at 24, but not 48 h, after introduction of Tim and Tipin siRNAs. Co-depletion of ATR and Tim

attenuated P-Chk1; however, BrdU incorporation was similar to Tim depletion alone, indicating that P-Chk1 was not the cause of suppressed replication in Tim-depleted NHF. Depletion of ATR, Tim, Tipin, and Chk1 also was associated with increased frequencies of chromosomal aberrations, and a striking defect of sister chromatid cohesion (SCC) accompanied Tim depletion. Tim interacts with Claspin, also a mediator of the ATR-dependent intra-S checkpoint. Although depletion of Claspin attenuated UVC-induced P-Chk1 in NHF, it did not result in genomic instability or a defect in SCC. Taken together, these findings indicate that Timeless and Tipin contribute to several aspects of genomic stability, including mediation of the essential functions of ATR and Chk1, stabilization of replication forks that encounter natural barriers, and SCC. PHS grants ES014635 (WKK) and ES015856 (MCS).

DD092

POTENTIAL GENOTOXIC RISKS OF SINGLE WALLED CARBON NANOTUBES

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Introduction: Nanotechnology, a fast growing industry applied in 800 manufacturer-identified consumer products is surrounded by uncertainty about possible health hazards. Carbon nanotubes (CNTs) raise a great concern about the risk of occupational exposure. CNTs resemble fibres, hence, the fibre pathogenicity paradigm, based on high aspect ratio, which holds true for asbestos, might apply to CNTs. Length, geometry and surface chemistry, therefore, play an important role in the toxic potential of CNTs. Thus, the validity of the asbestos paradigm with respect to SWCNTs of 3 lengths (0.4-0.8µm; 1-3mm; 5-30mm) were evaluated after thorough physico-chemical characterisation including; surface area (BET), morphology (SEM, AFM), cell uptake (TEM), zeta potential, size distribution (DLS), & impurity (EDX) analyses. Methods: Human bronchial epithelial (BEAS-2B) cells were treated with SWCNTs at 1, 5, 10, 15, 20, 25, 50 & 100 µg/ml for 24 & 48hr along with control and reference (asbestos) controls. The genotoxic potential of these treatments was determined using the cytokinesis blocked micronucleus assay (CBMN) to evaluate chromosomal damage. The same samples were exposed to MCL_5 human lymphoblastoid $TK^{+/-}$ cells to investigate SWCNT induced mutagenic changes using the *hprt* forward mutation assay. Results: After purification SWCNTs were ≈ 97% pure. Under test conditions, they mainly existed in small agglomerates & as bundles of parallel aligned tubes. When applied to BEAS-2B cells, no significant cytotoxic effects were observed at any time point. Significant increase in MN frequency was seen in a concentration and time-dependent manner. Culture medium supplemented with 2% serum showed higher genotoxicity compared to 10% serum requiring careful attention to potential confounding factors in classical test systems to avoid false negative results. SWCNTs also gave rise to a clear and concentration-dependent increase in *hprt* mutations at non-cytotoxic levels in the MCL_5 cells. A significant number of mutant cells were observed at 25, 50, & 100 µg/ml doses with mutagenic potency comparable to that of the established carcinogen crocidolite asbestos. Conclusions: This study demonstrates that SWCNTs induce significant chromosomal damage at sub-cytotoxic concentrations. Furthermore, the *hprt* results support the hypothesis that SWCNTs are mutagenic in mammalian cells which might have a bearing on their possible carcinogenicity.

DD093

GENOTOXIC EFFECTS OF THE ANTINEOPLASTIC DRUGS CISPLATIN AND GEMCITABINE ON URINARY BLADDER CANCER CELLS

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The antineoplastic drugs cisplatin and gemcitabine are commonly used to treat bladder cancer. While cisplatin has the ability to crosslink to DNA, gemcitabine is incorporated into cellular DNA blocking further elongation of the strand. The present study was designed to evaluate levels of DNA damage in two urinary bladder carcinoma cell lines (5637 and T24) *in vitro* treated with cisplatin (0.5 µM, 1µM, or 2µM), gemcitabine (0.78 µM, 1.56 µM or 3.12 µM), or with combination of both drugs. These cell lines were established from high-grade tumors and have mutated *TP53* gene; 5637 cells contain two mutations, one at codon 280 (Arg>Thr) and other at codon 72 (Arg>Pro), and T24 cells display a *TP53* allele encoding an in-frame deletion of tyrosine 126. Cells were collected at 0, 6 and 24 hours after treatment with the chemotherapeutic compounds to evaluate genotoxicity by the comet assay. Increased level of DNA damage (tail intensity) induced by cisplatin was only observed in 5637 cells 24 hours after exposure to the concentration of 0.5 µM. No significant genotoxicity was detected in T24 cells treated with this drug. For gemcitabine, at the three concentrations (0.78 µM, 1.56 µM, and 3.12 µM), increased DNA damage were detected in both cell lines, 6 and 24 hours after exposure. In 5637 cells, higher level of DNA damage was observed at 24 hours when compared to 6 hours protocol. Simultaneous treatment with cisplatin and gemcitabine showed statistically significant increase of DNA damage in both cell lines at 6 and 24 hours, except for the 0.5 µM cisplatin + 0.78 µM gemcitabine protocol at 6 hours (5637 cells). In conclusion, our data confirmed low sensitive of the comet assay to detect DNA damage induced by the crosslinking agent cisplatin, and showed the genotoxicity of gemcitabine on mutated *TP53* bladder cancer cell lines, even when used simultaneously to cisplatin. Supported by FAPESP and CNPq.

DD094

GENOTOXIC EXPOSURE ASSESSMENT IN TANNERY WORKES WITH EVALUATION OF ANTIMUTAGENIC PROPERTIES OF CENTRUM®

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The objective of this study was to identify the genotoxic effect of occupational exposure to genotoxicants typical for tannery workers and to evaluate the anti-mutagenic and DNA protective properties of Centrum®. Twenty-one occupationally exposed tannery workers were studied at four different times: before and directly following completion of four months of therapy with two tablets of Centrum® daily, as well as at the third and fourth months following discontinuation of therapy. The cytokinesis-block micronucleus (MN) test in peripheral blood lymphocytes and the buccal exfoliated cell (BEC) test were utilized to determine genotoxic end points. The treatment group was made up of workers in the finishing department where the main genotoxicants are Cr III, solvents, dyes, and pigments. The results of cytogenetic analyses from exposed workers were compared to 21 controls from the administrative department who were matched for age, years of employment, and smoking status. The MN were recorded per 1000 CB cells, and the BEC test results were recorded per 1500 cells, per person. The initial frequency of MN in the control group was 22.6 ± 2.6 for CB cells and 0.22 ± 0.13 for the HBEC test. After four months of treatment, an anti-genotoxic effect was observed with MN (CB cell) frequencies decreased to 18.5 ± 1.8 and BEC cell frequencies to 0.12 ± 0.01, respectively. The final three and four-month post-therapy examinations showed that MN frequencies for both endpoints overlapped with the initial determination. An anti-genotoxic effect was clearly observed after treatment of the exposed group. The initial frequency of MN was 65.3 ± 6.8 (CB cells) and 1.9 ± 0.4 for the BEC test. After four months of treatment, the MN in CB cells decreased to 35.3 ± 2.6 and the BEC test to 0.7 ± 0.2. The three and four-month post-therapy analyses demonstrated statistically significant lower frequencies for both endpoints compared to initial levels. In addition, clinical examination demonstrated that leukoplakia resolved in four exposed persons after therapy, but reappeared in two persons four months after treatment discontinuation.

DD095

IMPACT OF ARSENIC EXPOSURE ON LEVELS OF SHORT PATCH BASE EXCISION REPAIR TRANSCRIPTS IN LACTATING AND ADULT MICE

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Arsenic (As), a common environmental contaminant present in the drinking-water of a number of global regions, is associated with a range of physiological disorders and induces DNA-damaging levels of intracellular reactive oxygen species (ROS). We examined whether adult or maternal exposure to low or moderate arsenate concentrations in drinking water could influence the levels of short-patch base excision repair (BER) transcripts in adult or neonatal mice, respectively. Lactating mothers and two- or six- month old mice were exposed (24 h or two weeks) to 0, 2 or 50 ppm arsenate ($NaAsO_2$) in drinking water. Lung tissue was harvested from the adult mice and from neonatal mice feeding from the lactating mothers. Levels of short-patch BER transcripts in treated and untreated mice were quantified by real-time PCR and compared between the groups. Levels of transcripts encoding short-patch BER proteins decreased in untreated mice with age, and this effect was not attenuated by As exposure. Increased transcript levels for most genes were observed in neonatal mice treated via maternal exposure to drinking water containing As. Only transcripts of certain BER genes were increased in adults directly exposed to As in drinking water, but not consistently for both As concentrations. In conclusion, expression of short-patch BER genes decreases with age in mice but can be increased in adult mice by exposure to low levels of arsenic in drinking water, and in neonates by maternal exposure to As.

DD096

DNA DAMAGE RESPONSES IN IRRADIATED GLIOBLASTOMA CELL LINES

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Glioblastoma multiforme (GBM) is among the most lethal of all human tumors. The genetic heterogeneity presented by different cell lines derived from GBM seems to be an important cause of diverse signaling pathways following irradiation. We studied four Glioblastoma cell lines, mutated (T98G and U251MG) and wild-type (U343MG-a and U87MG) for TP53 gene under conditions of irradiation with gamma-rays. The objective of the present study was to carry out several kinds of experiments aiming to characterize cellular and molecular signaling pathways underlying responses to ionizing radiation. Parameters as clonogenic survival, apoptosis, comet assay, cDNA microarrays, real time qPCR and Western blot were used. Survival rates analyzed after 10-12 days demonstrated that cell death was significantly induced, and U87MG cells were the most radioresistant cell line. TP53 mutated cell lines showed high frequencies of apoptotic cells, mainly for 48 and 72h after treatment. GBM cells irradiated with 8 Gy presented similar repair kinetics, showing almost 100% of repaired DNA for recovery time of 1-2 h. Correlation fits were found for the following comparisons: DNA damage and the initial time after irradiation (r2=0.92-0.99) and SF8 versus apoptosis induction at 72h (r2=0.88). U343MG-a and T98G cell lines showed similar basal levels of non-phosphorytated TP53 expression (control and irradiated group), but irradiated T98G cells did not show any increase in phospho-Ser15-TP53, in contrast with U343MG-a cells, whose expression was increased following 6h post-irradiation. Gene expression profiles were studied by the cDNA microarray. Several functions were found affected, such as cell differentiation, cell adhesion, cell cycle, apoptosis, DNA repair, signal transduction, etc. Many of them were common or unique to TP53 mutant or wt cells analyzed at 30 minutes and 6 h. Among all GBM cell lines, U87MG presented modulation of several genes belonging to functions related to stress responses, which could be related to radioresistance. The information obtained in this work provides an important contribution towards the elucidation of GBM responses to ionizing radiation, and point out some signaling pathways, which may be tested as targets for molecular or pharmacological inhibition.

DD097

MITOTIC CATASTROPHE INDUCED BY OVEREXPRESSION OF RAD2P UNRELATED TO ITS ENDONUCLEASE ACTIVITY IN SACCHAROMYCES CEREVISIAE

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Nucleotide excision repair (NER) is a major DNA damage repair process. Among eight known complementation groups, the xeroderma pigmentosum (XP) group G gene (XPG) encodes an endonuclease that has a additional function in transcription elongation. Mutations in the XPG gene cause XP and Cockayne syndrome depending on the nature of the mutation. Here, we show an unknown function of *RAD2*. The Rad2p overexpression causes cell cycle arrest in the expression level dependent manner. However, the apoptotic markers were not observed in the Rad2p overexpressed cells. Further analysis revealed that the Rad2p induced cell cycle arrest is not the results of cell death but the consequences of mitotic catastrophe. Moreover, the C-term region of the gene was responsible for the cell cycle arrest while the endonuclease activity of Rad2p was dispensable. Our results imply that the drastically increased skin cancer incidence in XP could arise from the synergistic effects between mitotic catastrophe and the accumulation of damaged DNA.

DD098

RAD2 AND PUF4 REGULATE NUCLEOTIDE METABOLISM RELATED GENES, HPT1 AND URA3

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Yeast *RAD2*, a yeast homolog of the human XPG gene, is an essential element of nucleotide excision repair, and its deletion confers UV sensitivity and nucleotide excision repair deficiency. 6-Azauracil sensitivity of certain *rad2* mutants revealed that *RAD2* has transcription elongation function. However, the fundamental mechanism by which the *rad2* mutations confer 6-Azauracil sensitivity was not clearly elucidated yet. Using an insertional mutagenesis, *PUF4* gene encoding a yeast pumilio protein was identified as a deletion suppressor of *rad2*Δ 6AU sensitivity. Microarray analysis followed by confirmatory RT-qPCR disclosed that *RAD2* and *PUF4* regulated expression of *HPT1* and *URA3*. Overexpression of *HPT1* and *URA3* rescued the 6AU sensitivity of *rad2*Δ and *puf4*Δ mutants. These results indicate that the 6AU

sensitivity of the *rad2* mutants was partly ascribed to impaired expression regulation of genes in the nucleotide metabolism.

DD099

GENOTOXIC AND ANTIGENOTOXIC EFFECT OF TWO VARIETIES OF ACEROLA FRUIT (MALPIGHIA GLABRA L.) AT TWO STAGES OF MATURITY

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Acerola or Barbados cherry is a plant originated in Central America that has been propagated to South America including Brazil due to its good adaptation to soil and climate. Brazil is the major worldwide producer, consumer and exporter of acerola. It is nutritionally important; mainly due to its very high level of vitamin C. Green mature acerola fruit contains a higher amount of vitamin C than red mature fruit. Ripening of the fruit involves a series of complex biochemical reactions such as production of carotenoids, anthocyanins and phenolics and the formation of volatile compounds. The distinction of genetic variability among varieties of acerola can be observed in commercial orchards. The aim of this study was verify the genotoxic and antigenotoxic effect *in vitro* of two varieties of acerola fruit at two stages of maturity. Fruit pulp lyophilized extract of acerola was obtained from two stages of maturity, green and mature, and two varieties, 13/2 and SP-19, from Ceará, Brazil. To assess acerola genotoxic and antigenotoxic activity was performed *in vitro* Comet Assay (CA) in blood samples from four mice. Results were expressed in damage index (DI), according damage classes. Mice blood cells were exposed to different acerola extracts concentrations: C_1= 2mg/ml; C_2= 1mg/ml; and C_3= 0,5mg/ml; during two hours at 37°C; except to C_2, that was exposed also during 4h. For a negative control (C-) we used blood without the addition of extracts but incubated under the same conditions. Four slides were prepared per dose and per animal. To evaluate antigenotoxicity induced by acerola extract, two slides from each dose and each blood sample were immersed in H_2O_2, including C-. Data were submitted to variance analysis (ANOVA) one way and to Dunnet test. The results showed that 13/2 and SP-19 extracts did not present genotoxicity in the Comet assay in peripheral blood cells in relation to C-. Both varieties at green stage showed antigenotoxic activity at C_2 (P<0.01), in relation to same mature variety, independent of exposure time. Thus, green mature acerola fruit presented protective activity in mice blood cells in relation to H_2O_2 at both varieties that can be due the presence of higher content of vitamin C in relation to red mature fruit, besides other compounds.

DD100

GENOTOXICITY AND ANTIOXIDATIVE EFFECTS OF LEAVES AND FLOWER RECEPTACLE EXTRACTS FROM ARTICHOKE (CYNARA SCOLYMUS L.)

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Many natural products referred to as dietary chemo-preventive compounds offer a great potential in the fight against cancer by inhibiting multi-step processes of carcinogenesis, through a different range of mechanisms including antioxidant and antimutagenic activities. The artichoke (*Cynara scolymus*) is widely consumed as tea and food and exerts important biological activities. Such extracts from artichokes have been used for their hepatoprotective, ant carcinogenic, antioxidative and hypercholesterolemic activities, among others. This study investigates the genotoxicity and antioxidant action of artichoke leaf (LE) and flower receptacle (FE) extracts using the Comet assay and the micronucleus test in mice. The doses for LE were 3X500mg/kg, 3X1,000mg/kg, and 3X2,000mg/kg; and for FE 3X2,000mg/kg. The results showed that leaf and flower receptacle extracts did not present mutagenicity in the micronucleus test in peripheral blood and bone narrow cells, nor induced genotoxicity in the Comet assay, except the leaf extract at the highest dose. Different tissues were employed and just liver and brain presented increased DNA damage. The oxidative stress was evaluated by TBARS and CAT tests, and neither test produced positive results. Both extracts of artichoke exhibited antioxidant activities in the DPPH and hipoxantine/xantine oxidase tests (leaves>flower receptacle). The protective ability against the oxidative stress induced by the hydrogen peroxide Comet assay was demonstrated for leaf extract in bone narrow cells. Phytochemical screening detected the presence of flavonoids, phenolic compounds and saponins in leaves and flower receptacle. Thus, artichoke extracts produced positive genotoxicity results and antioxidant capacity in leaf extracts in mice. It is important to take into account that the mechanisms and the conditions that mediate the biological effects of artichoke should be clarified before considering the species as therapeutically useful plant.

DD101

ASSESSMENT OF CELLULAR AND MOLECULAR RESPONSES MEDIATED BY TEMOZOLOMIDE COMBINED WITH METHOXYAMINE, AN INHIBITOR OF DNA REPAIR, IN GLIOBLASTOMA CELL LINES

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The resistance of glioblastoma multiforme (GBM) to the antitumoral drug temozolomide (TMZ) has been reported to be partially due to an efficient base excision repair (BER) pathway. Methoxyamine (MX) is an effective BER inhibitor, which has been investigated as a conceivable treatment for different kinds of tumor. In the present study, the cellular responses to TMZ treatment associated or not with MX were evaluated in GBM cell lines. Among five GBM cell lines (U87, U343, U251, U138 and T98G) treated with TMZ (100-1000 μM) and analyzed after 120h, T98G was the most resistant cell line. In addition, T98G showed significant ($p \leq 0.05$) differences for cells treated with TMZ combined to MX, relatively to single treatments. Furthermore, TMZ (100-800μM) reduced the clonogenic efficiency of T98G cells, while TMZ plus MX significantly increased the cytotoxic effects, even for the lowest concentration. DNA damage analyzed by the comet assay showed significant differences between treatments following 2h of recovery. After 12 and 24h, the amount of DNA damage reached control levels, indicating the repair of DNA breaks. Apoptosis induction in T98G cells showed the highest frequency (24.2%) at 72h (600μM), while the highest apoptosis induction (47.7%) was observed for the same concentration combined to MX. Western blot analysis demonstrated that APE1 was less expressed for TMZ plus MX, probably due to AP-sites blockade caused by the inhibitor. FEN1 showed low levels of expression at 48h and 72h, indicating the inhibition of BER pathway downstream to the AP removal by APE1. PCNA expression was higher for TMZ plus MX (24h and mainly 48h), probably due to an increased DNA damage. The present results demonstrated that the association of TMZ plus MX interfered with the expression of proteins involved in BER, thus reducing the clonogenic efficiency of T98G cells, probably as a consequence of the high production of unrepaired DNA-MX adducts, leading to cell death, including apoptosis. The modulation of BER is a promising strategy for magnifying the therapeutic impact of TMZ; this strategy may embrace the option to establish novel and efficient therapy protocols for the treatment of patients with gliomas. [Financial support: FAPESP (Proc. nº 06/01947-8), CNPq, and CAPES].

DD102

LIFE CYCLE OF MICROCNUCLEUS ANALYZED BY CONFOCAL LIVE CELL IMAGING

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Micronucleus (MN) is widely utilized in biomonitoring for genetic instability of humans exposed to clastogens and aneugens. However, the impact of MN on the cell and the MN life cycle are still less clear. To explore exactly when, where, and how MN originate from and finally conclude in living cells, we first constructed dual-color fluorescent TK6 cells in which histone H3 and alpha-tubulin were differentially expressed as fusion to mCherry (red) and EGFP (green) fluorescent proteins, respectively. We recorded the emergence and behavior of micronuclei (MNi) during one cell cycle (especially M-phase) by using confocal time-lapse imaging to further understand the formation properties and fate of MN derived by exposure of MN-inducing agents, mitomycin C (MMC; crosslink), γ-ray (strand break), or vincristine (VC, spindle poison). The frequency of MN formation in the live cell analysis was corresponding to that in conventional MN test. In live cell analysis, all control cells were normally divided; no MN formation was observed. Using MMC as a MN-inducing agent, 7.3 % of cell division promoted MN induction from lagging chromosome fragments just after chromosome segregation at early anaphase. When □-ray was used, MN formation (8.1%) was frequently observed during multipolar division at late anaphase and the MN originated from lagging pieces of chromosome broken by the abnormal division associated with supernumerary centrosomes. The process of MN formation induced by γ-ray was different from that by MMC. While, MN formation process for VC was similar as that for MMC and the MN frequency was 3.4%. In addition, another source letting the MN frequency for VC increased was recognized as misaligned chromosomes (whole chromosome) which did not align in metaphase plate. Thus, the formation process of MNi induced by MMC, γ-ray, and VC, were strikingly different one another, indicating that there are several mechanisms to produce MN. In addition, we show the direct evidence that nuclear envelope reassembly plays a critical role in MN formation and MN re-entered daughter cell nucleus by attaching to mitotic chromosome during next cell division.

DD103

INFLUENCE OF VITAMIN B12, FOLIC ACID AND POLYMORPHISMS IN FOLIC ACID METHABOLIC PATHWAY ON DNA STABILITY IN LYMPHOCYTES FROM WORKERS OCCUPATIONALY EXPOSED TO LOW LEVELS OF IONIZING RADIATION

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Interindividual variability in DNA damage response after exposure to ionizing radiation could be explained by inherited differences in DNA genes of folic acid methabolic pathway. For this reason, associations between genetic polymorphisms in TS (thymidylate synthase), MTHFR (methylenetetrahydrofolate reductase exon 4 and 7), RFC (reduced-folate carrier), MTR (methionine synthase), SHMT1(serine hydroxymethyltransferase)and MTRR (methionine synthase reductase) genes and the number of spontaneously induced micronuclei in peripheral blood lymphocytes were investigated. Folic acid and vitamin B12 plasma concentrations were also measured. In addition, by the use of alkaline comet assay, kinetics of DNA repair after exposure to 2 and 4 Gy of gamma radiation were also studied. The exposed group comprised of 70 individuals occupationally exposed to low levels of ionizing radiation (41.99±11.05 years, 434.69±134.37ng/l of vitamin B12, 6.96 µg/l of folic acid) and 44 controls (39.68±10.91 years, 479.59±157.64 ng/l of vitamin B12, 7.65±3.27µg/l of folic acid). Folic acid and vitamin B12 concentrations were higher in controls, and also among women in both exposed and control group. Although DNA repair kinetic followed the same pattern and the values of tail length, tail intensity and tail moment were similar before exposure, statisticaly significantly lower values were observed in the exposed group in all time interval (15, 30, 60, 120 minutes and 24 hours). The micronuclei frequency observed in the exposed group was two –fold higher than in the control (6.90±5.93, 0-24 vs. 12.60±8.68, 1-37). All the analysed allelic frequencies were in agreement with the Hardy-Weinberg Equilibrium, and there was no difference between the two groups, except for the SHMT1 gene. Detailed statistical analysis will be done for confirmation whether the two-fold higher micronucleus frequency in exposed group and differences in DNA damage response after exposure to ionizing radiation are due to the differences in genotyping.

DD104

DNA DAMAGE AND CHECKPOINT RESPONSES IN HUMAN PROSTATE EPITHELIUM

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Cellular DNA damage triggers the DNA damage response pathway, and leads to enforcement of cell cycle checkpoints. These are essential for the maintenance of genomic integrity, and are activated in early stages of tumorigenesis. A special feature of prostate cancer is its high incidence and multifocality. To address the functionality of DNA damage checkpoints in the prostate, we analyzed the responses of human primary prostate epithelial cells (HPEC) and freshly isolated human prostate tissues to gamma-irradiation. We find that gamma-irradiation activates the ATM-associated DNA damage response pathway in the HPECs, but that the clearance of gamma-H2AX foci is delayed. Surprisingly, gamma-irradiated HPECs were unable to enforce cell cycle checkpoint arrest and had sustained Cdk2-associated kinase activity due to lack of inhibitory Cdk-phosphorylation by Wee1A tyrosine kinase. We further show that HPECs express low levels of Wee1A, and that ectopic Wee1A efficiently rescues the checkpoints. We recapitulate the absence of checkpoint responses in epithelium of ex vivo irradiated human prostate tissue, despite robust induction of gamma-H2AX. The findings show that prostate epithelium has a surprising inability to control checkpoint arrest, the lack of which may predispose to accrual of DNA lesions. Further studies indicate which types of conventional drugs are able to induce DNA damage response in fresh human prostate tissues. Special interest is focused on cell type specific damage responses.

DD105

MARINE PRODUCTS AS ANTICANCER AGENTS: INTERACTION OF THE ECTEINASCIDINS WITH DNA AND MACROMOLECULAR MACHINES.

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Yondelis, zalypsis and tryptamicidin are ecteinascidin derivatives (ETs) originally isolated from the Caribbean sea squirt Ecteinascidia turbinate, pointing to marine products as a potential new source of molecules with original chemical structures and activities. Yondelis and zalypsis have antitumor activity toward otherwise chemoresistant

tumors including soft tissue sarcoma, ovarian cancer and multiple myeloma. ETs form monofunctional adducts with the exocyclic guanine in the minor groove of DNA. The current study aims to elucidate the interaction of the 3 ETs with DNA and DNA-associated functions. The ETs show potent cytotoxic activity in the low nM range. Cellular exposure to the 3 ETs is accompanied by rapid formation of DNA double strand breaks (DBSs), a particularly deleterious genotoxic lesion, in agreement with our previous observations for yondelis (Soares et al., Proc Natl Acad Sci USA 104:13062-13067,2007). Interestingly, even at elevated doses, DSBs is not observed for all cells, pointing to a dependence on the cell cycle. In agreement, biparametric flow cytometry demonstrates that DSBs is particularly pronounced for S phase cells, suggesting that collision between the ET adducts and the replication fork lead to formation of DSBs. In marked contrast, neither double nor single strand breaks are observed in resting lymphocytes, suggesting that the transcriptional machinery does not contribute to the formation of DSBs, at least not in quiescent cells. DSBs triggers the DNA damage response and activates p53. However, p53 status has no detectable effect on the cytotoxic activity of the drug. Cells deficient in homologous recombination repair show pronounced sensitivity to ETs, whereas deficiency in non-homologous end-joining has no detectable influence on the cytotoxicity. In conclusion, these studies characterize the interaction of 3 ETs, yondelis, zalypsis and tryptamicidin, with DNA and DNA associated functions, and identify factors likely to play a role in the clinical response to these agents, such as proliferation status and the capacity for homologous recombination repair. This study was supported by the CONTICANET EU program no LSHC-CT-2005-018806, by PharmaMar, the CAPES-COFECUB program and Association pour la Recherche sur le Cancer, France.

DD106

IDENTIFICATION AND CHARACTERIZATION OF MutT FAMILY ENZYMES IN CAENORHABDITIS ELEGANS

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Nucleic acid bases are subject to modification when present in dNTPs as well as in DNA. Modified dNTPs may be mutagenic since polymerases incorporate them into DNA. 8-oxodGTP, for example, which is major source of oxidised guanine in DNA (1), may be incorporated opposite adenine, leading to AT to CG transversions (2). In E. coli, 8-oxodGTP incorporation is prevented by the MutT protein, which dephosphorylates it to 8-oxodGMP (3). The importance of this type of DNA damage avoidance is highlighted by the fact that MutT homologue (MTH1)-knockout mice show higher incidence of lung, liver and stomach cancers (4,5) and that human MTH1 was found to be over-expressed in many human diseases, including cancer. In our research, we use C. elegans as a model system to study DNA damage responses (DDR) (6). Our goal is to develop an experimental system to study whole organism response to endogenous oxidative DNA damage through transiently manipulating the composition of the dNTP pool by depleting MutT enzymes by RNAi. To do so, we first needed to identify and characterize C. elegans MutT homologues. We identified 11 ORFs in the C. elegans genome with sequence similarity to the E. coli MutT. Based on initial bioinformatic analyses we chose 6 candidates, that were cloned and subjected to functional and biochemical characterisation. Here, we will present biochemical and functional characterization of the first functional MutT homologue in C. elegans.

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DD107

ERROR-PRONE TRANSLESION SYNTHESIS IN MMS2RAD5 DOUBLE MUTANT IS ENTIRELY DEPENDENT ON PCNA SUMOYLATION.

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Rad5 and Mms2 function in concert with Ubc13 in polyubiquitination of proliferating cell nuclear antigen (PCNA). PCNA polyubiquitination is responsible for activation of the error-free subpathway of DNA damage tolerance pathway. This error-free mechanism is based on avoidance of DNA damage, causing DNA replication block, by temporary use of homologous newly synthesized DNA strand of sister chromatid as a template. Both Rad5 and Mms2 defects inactivate this mechanism and cause mutator phenotypes explained by channeling of DNA lesions from error-free processing, by DNA damage avoidance pathway, to error-prone translesion synthesis (TLS). However, the nature of this TLS has never been explored. Here we show that spontaneous mutator phenotype caused by mms2 deletion is predominantly dependent on activity of Siz1, the SUMO ligase, which in cooperation with Ubc9 specifically attaches SUMO to lys164 of PCNA. The rad5-mediated mutator effect is only in part dependent on Siz1. UV induced mutagenesis is also partially dependent on Siz1 in both mms2 and rad5 single mutants. However, in mms2rad5 double mutant both spontaneous and UV induced mutations entirely depend on Siz1 mediated PCNA SUMOylation. Altogether, the results indicate that in the absence of DNA damage avoidance pathway big part of spontaneous and UV-induced DNA lesions is processed by SUMOylation dependent TLS. Additionally, the results show the new, independent of their function in PCNA polyubiquitination, and redundant role of Rad5 and Mms2 in promoting TLS when PCNA SUMOylation is compromised.

DD108

ANTI-CANCER EFFECTS OF POLY-METHOXYLATED FLAVONES EXTRACTED FROM GARDENIA COLLINSAE

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Many flavonoid derivatives have a broad spectrum of biological activities including anti-tumor. Recent research reveals that these effects are dependent on the poly-phenolic structural groups of the compounds. The aims of this study is to evaluate the anti-cancer effect of poly-methoxylated flavone derivatives; 5-hydroxy-6,7,3',4',5'-petamethoxyflavone and 5,3'-dihydroxy-6,7,4',5'-tetramethoxyflavone, on human colon cancer (Caco-2) and liver cancer (HepG2) cell lines. The cells were cultured in appropriated medium with additive supplements in 5% CO_2 incubator, at 37° c, to confluence. Cells were treated with 5-hydroxy-6,7,3',4',5'-petamethoxyflavone or 5,3'-dihydroxy-6,7,4',5'-tetramethoxyflavone at various concentrations and control cells were treated with DMSO (0.2%) for 48h. Cytotoxic effect (CC50) of each compound was determined by MTT assays. Cell death was evaluated from morphological damages of nuclei, and biochemical alteration of enzyme involved in apoptosis. The results revealed significant growth inhibition action and induction of DNA fragmentation effect of the compounds in both cancer cell lines. The mechanism of cell death was exhibited via up-regulation of mitchondial caspases enzyme activities. The presenting efficacy of 5-hydroxy-6,7,3',4',5'-petamethoxyflavone on inhibition of these cancers was higher than 5,3'-dihydroxy-6,7,4',5'-tetramethoxyflavone. These results suggested that the number and position of methoxyl group are significant in compound action and that 5-hydroxy-6,7,3',4',5'-petamethoxyflavone could be considered to future benefit for chemotherapeutic agent.

DD109

DETECTION OF LIPID PEROXIDATION-INDUCED DNA ADDUCTS CAUSED BY 4-OXO-2-NONENAL AND 4-OXO-2-HEXENAL IN HUMAN AUTOPSY TISSUES

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DNA adducts are produced exo- and endogenously via exposure to various DNA-damaging agents. It is important to identify major DNA adducts formed in human tissues to understand their possible impact on human health. Recently, we have developed a new technique to detect numerous known or unknown DNA adducts simultaneously using liquid chromatography tandem mass spectrometer (LC-MS/MS). This novel approach, named as "DNA adductome", monitors the neutral loss of 2'-deoxyribose from positively ionized 2'-deoxynucleoside adducts over a certain range of transitions. An adductome map showing LC retention times, mass-to-charge ratio, and relative peak intensity of potential DNA adducts can then be created to present various DNA adducts detected in DNA samples. In this study, we applied the "DNA adductome" to human autopsy tissues, and discovered that several major DNA adducts seen on the adductome map of one human lung were identical to 4-oxo-2-nonenal (4-ONE)- or 4-oxo-2-hexenal (4-OHE)-related DNA adducts. 4-ONE and 4-OHE are the end products of lipid peroxidation of ω-6 and ω-3 unsaturated fatty acids, respectively. They may induce the formation of substituted etheno-DNA adducts, such as heptanone-etheno-2'-deoxynucleoside adducts (HεdC, HεdG, HεdA), or butanone-etheno-2'-deoxynucleoside adducts (BεdC, Bε5-MedC, BεdG, and BεdA). Since no DNA adduct levels of these DNA adducts have been reported in human tissues, we analyzed 4-ONE- and 4-OHE-related DNA adducts in 68 organs of different individuals using LC-MS/MS analysis, and adduct levels were quantified using isotope dilution method. Analytical results revealed that the amount of targeted DNA adducts varied largely among individuals or organs. The median levels (detection rates) of HεdC, HεdG, HεdA, BεdC, BεdA, Bε5-MedC, and BεdG were 10.55 (97.1%), 16.07 (94.1%), 13.94 (73.5%), 2.05 (41.1%), 4.59 (8.8%), 3.40 (5.9%), and 0.47 (5.9%) adducts/10^8 bases, respectively. In addition, while no correlation could be seen between HεdC and 8-oxo-2'-deoxyguanosine (R^2=0.0234), one of the most famous oxidative DNA adducts, the adduct level of HεdC was found to be strongly correlated to other lipid peroxidation-derived DNA adducts, such as BεdC (R^2=0.9367) and 1,N^6-etheno-2'-deoxyadenosine (R^2=0.7042).

DD110

FORMALDEHYDE DOES NOT INDUCE SYSTEMIC GENOTOXICITY

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Formaldehyde (FA) is genotoxic in vitro. It induces DNA-protein cross-links (DPX) which may lead to the formation of mutations. Due to its high reactivity, FA may lead to local genotoxic effects in vivo but systemic effects are unlikely. To investigate potential systemic genotoxic effects as part of a comprehensive toxicological study, male Fischer-344 rats were exposed to FA by inhalation for 4 weeks (6 h/d, 5 d/w). Groups of 6 rats each were exposed to 0, 0.5, 1, 2, 6, 10 and 15 ppm. At the end of the exposure period, peripheral blood samples were obtained and coded to ensure blind evaluation. Blood samples were used for the comet assay, the sister chromatid exchange test (SCE test) and the micronucleus test (MNT). DNA migration in the comet assay was measured both directly and after irradiation of the blood samples with 2 Gy gamma irradiation. The latter modification of the comet assay was included to increase its sensitivity for the detection of DPX. For the comet assay, four slides were analysed from each blood sample, two without and two with irradiation. From each slide, 50 randomly selected cells were measured by image analysis and tail intensity (% tail DNA) and tail moment were evaluated. For the SCE test, blood was cultured for 56 h in the presence of BrdU (10 µg/ml for the last 35 h) and SCE were counted in 30 second division metaphases per sample. The MNT with peripheral blood was performed according to the instructions for the micronucleus analysis kit MICROFLOW (Litron Laboratories). Approximately 20000 cells per sample were analysed by flow cytometry and the percentage of reticulocytes with micronuclei (MN) was determined. Positive control substances induced a significant effect in the genotoxicity tests and thus demonstrated the sensitivity of the test systems. FA did not induce any significant effect in any of the genotoxicity tests performed. It can be concluded that inhalation of FA in a 28 days study with FA concentrations up to 15 ppm does not lead to systemic genotoxic effects in rats. This comprehensive study supports previous investigations suggesting that genotoxic effects of FA at distant sites are highly unlikely and confirms the view that positive results reported in some human biomonitoring studies are not related to FA exposure.

DD111

INDUCTION AND REPAIR OF DNA-PROTEIN CROSSLINKS AND CHANGES IN GENE EXPRESSION IN HUMAN NASAL EPITHELIAL CELLS EXPOSED TO FORMALDEHYDE IN VITRO

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Formaldehyde (FA) is an important industrial compound and also a naturally occurring biological compound present in all cells. FA is rapidly detoxified via a multi-step pathway yielding formate and CO2. Formaldehyde dehydrogenase (FDH or ADH3) and other enzymes involved in this pathway seem to be ubiquitous enzymes. If FA is not metabolically inactivated and reaches the nuclear DNA, it can form DNA-protein crosslinks (DPX). DPX are efficiently repaired in all cell types studies but incompletely repaired DPX can lead to the formation of mutations. Using the alkaline comet assay modification for the detection of DPX, we measured induction and repair of FA-induced DPX in primary human nasal epithelial cells (HNEC) in vitro. Using a full-genome human microarray (Affymetrix U133 PLUS 2.0), we also determined changes in gene expression in FA-exposed cells. HNEC exposed to FA (100 and 200 µM) for 1, 4 and 24 h showed complete removal of DPX when treated with 100 µM FA, whereas in cells treated with 200 µM FA, a significant amount of DPX persisted. Gene expression was only moderately affected in cells treated with 50 or 100 µM for two or four hours. In particular, expression of genes involved in the metabolic inactivation of FA were unchanged. A pathway analysis indicated that none of the 186 genes related to DNA repair showed any significant change despite marked DNA repair activity. Exposure to 100 or 200 µM FA for 4 or 24 hours caused significant changes in the pattern of gene expression. The effect of FA on gene expression was more pronounced after 4h exposure than after 24 h. Treatment with 200 µM FA for 4 h caused up-regulation of 307 genes and down-regulation of 745 genes (2-fold change or more compared to the untreated control). After exposure for 24 h, 31 genes were up-regulated and 31 genes were down-regulated. Interestingly, FDH and other genes mainly involved in the detoxification of FA were not affected in any of the experiments. In summary, our experiments show that the determination of changes in gene expression is not a more sensitive indicator for the detection of FA-induced genotoxicity than the measurement of DNA damage by the comet assay. Genes involved in detoxification of FA and DNA-repair genes are not induced by FA in HNEC.

DD112

INTER-LABORATORY COMPARISON OF NER CELL PHENOTYPE ASSAYS

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Inter-individual susceptibility to mutagens/carcinogens can be assessed by genotyping or phenotyping at molecular or cellular level for genes and/or pathways involved in key events regulating the exposure-effect responses. Due to a great number of DNA repair genes identified and the interactions among the pathways (BER, NER, DSB, MS) responsible for the repair of DNA adducts and breaks, phenotyping is becoming an attractive approach to integrate the repair capacity resulting from many genes involved. As far as BER is concerned, various approaches have been applied. However, for the assessment of nucleotide excision repair (NER) only few methods are available. Within the ECNIS network of Excellence (www.ecnis.org) we aimed at developing and validating phenotypic assays for NER, since it is known that an inherited defect in one of various proteins involved in NER causes the disease xeroderma pigmentosum, and greatly increases the risk of skin cancer following sun exposure. The major aim was to validate NER phenotyping assays applicable for large scale biomonitoring studies within the framework of an inter-laboratory-ECNIS collaboration. The participating laboratories (VUB, ISI and UM) compared their available/new methodologies for phenotypic *in vitro* assessment of DNA repair activity in order to measure the inter-individual differences in the repair capacity of bulky DNA adducts (induced by 0.5 μM BPDE) in peripheral blood mononucleated cells (PBMC) obtained from the same donors with known XPA and XPD genotypes. Therefore PBMCs were isolated from 10 young, healthy female donors. In a first part, VUB performed a recently developed aphidicolin block assay to asses NER on the freshly isolated cells. In a second phase, ISI received frozen cells from VUB to perform the same cellular challenge assay as VUB but on cryopreserved cells. And in a third phase, UM applied their modified comet assay (Langie *et al*., 2006) in order to measure the capacity of human lymphocyte extracts. The results from the collaboration will be presented. This work was financed by ECNIS (Environmental Cancer Risk, Nutrition and Individual Susceptibility), a network of excellence operating within the European Union 6th Framework Program, Priority 5: “Food Quality and Safety” (Contract No 513943)

DD113

CELLULAR ADAPTIVE RESPONSE TO OXIDATIVE STRESS INDUCED BY EXHAUSTIVE EXERCISE: ROLE OF NRF2-DRIVEN HEME OXYGENASE-1 INDUCTION

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It has been speculated that intense exercise may cause oxidative injury through generation of reactive oxygen species. However, our body tends to adapt to oxidative stress. This study was aimed to test a hypothesis that exhaustive exercise induces the expression of heme oxygenase-1 (HO-1), a key enzyme in cellular antioxidant defence. HO-1 upregulation was mediated by antioxidant response elements (ARE) which are under control of the redox sensitive transcription factor NF-E2-related factor-2 (Nrf2). Human peripheral blood mononuclear cells (PBMCs) were obtained from the venous blood of fifteen healthy volunteers before, immediately after, and 1 h after an exhaustive exercise. Isolated human PBMCs were subjected to the electrophoretic mobility gel shift assay and Western blot analysis. The Nrf2-ARE binding activity and HO-1 expression, measured immediately after exercise, were more pronounced than those achieved before exercise. After 1 h recovery, Nrf2-ARE binding activity and HO-1 expression returned fully to the basal levels. In follow-up studies with mice, HO-1 expression in muscle and colon, analyzed 1 h after exhaustive exercise, dramatically increased in comparison with that observed both before and immediately after exercise. In contrast, there was no significant increase of HO-1 expression in heart and lung. Taking all these findings into account, we conclude that exhaustive exercise transiently induces Nrf2 activation and subsequent expression of HO-1 as part of the adaptive cytoprotective response against oxidative stress.

DD114

NO COORDINATES TO SH- AND [4Fe-4S] 2+ CENTERS TO FORM DINITROSYL-IRON COMPLEXES (DNIC) TO REGULATE THE E.COLI ADA GENE EXPRESSION.

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Background. Ada DNA repair response protects E.coli from alkylating damages. A source of endogenous alkylation is amino acids and peptides nitrosylation by endogenous NO, being produced in hypoxic cells under Fnr [4Fe-4S] $^{2+}$ protein control. The aidB gene expression is served mostly to protect E.coli from alkylation during anaerobic growth. We suppose that namely Fnr [4Fe-4S] $^{2+}$ serves both as NO target and NO-sensor and regulator of aidB gene expression in anaerobic conditions. Aims. Experimental verification of our original hypothesis - a “quasi-adaptive response” (quasi-Ada) to alkylating agents; study if S-nitrosylation (instead of S-alkylation in the true Ada) of the Ada sensor protein serves a signal for quasi-Ada activation and contribute to cell resistance to MNU; establish the structure of NO signaling molecule; obtain data consistent with our suggestions in the mechanism of the aidB regulation and new NO signaling function. Methods: E. coli mutants bearing (alkA::lacZ; alkB::lacZ and aidB::lacZ) operon fusion [Volkert, 1998] were used. Di – and tetranitrosyl iron complexes were tested as NO donating agents. EPR-resonance spectroscopy was applied to detect intracellular anisotropic EPR signal with g-factor 2,03. Results: Quasi-Ada DNA repair response in the cell treated with NO- donors was quantified by 3-5 fold increasing in the levels of alkA and alkB gene expression and 1,5-2,5 fold decreasing in the rate of mutations and lethal lesions, induced by MNU. Intracellular iron was indispensable for NO-signaling: Fe- chelator OP prevented the phenomenon. NO treatment led to the appearance of EPR signal with g 2,03, which disappeared after OP pretreatment. A positive up- regulation of aidB in hypoxic cells and a negative aidB down-regulation in the cells after NO pretreatment were found. The specific changes in the level and form of the EPR-signals were found. We quantified sulfide released from Fnr during [4Fe-4S]$^{2+}$ to [2Fe-2S]$^{2+}$ cluster conversion. Conclusions: We demonstrate at the first time a new mechanism regulating the Ada sensory protein. The quasi-Ada DNA -repair response and the aidB expression regulation extends the functional range of NO as a signal molecule in signal transduction and DNA repair. The work was supported by RFBR (08-04-00228).

DD115

STUDY OF CELL cycle, Apoptosis and DNA damage, in human K562 leukaemia cells: Effect of the Alkenylbenzene Myristicin

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Myristicin (1-allyl-3,4-methylenedioxy-5-methoxybenzene) is a naturally occurring alkenylbenzene, it is the principal aromatic constituent of nutmeg volatile oil and mace, and is used as a flavouring in food and non-alcoholic drinks. Previous studies performed by us in Chinese Hamster Ovary Cells (CHO) indicated that myristicin was a strong apoptosis inducer and a weak genotoxicant. The aim of this study was to investigate the possible cytotoxicity and genotoxicity of myristicin in a human cancer cell line of Chronic Myeloid Leukaemia (K562).

First we evaluated the cytotoxicity of myristicin at different incubation times using the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxy-methoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) test. The results obtained suggest that cell viability decreases to 50% after a 48 hour period and with concentrations higher than 500μM. Induction of apoptosis, with the TUNEL assay, was also confirmed after a 48 hour incubation period. We then evaluated DNA lesion effects with γ-H2AX and the Alcaline Comet Assay and myristicin showed no genotoxicity after one hour incubation in γ-H2AX assay and weak genotoxicity in the comet assay. To fully evaluate the genotoxic activity of this molecule, cells were monitored with the trypan blue exclusion and with the Comet assay for 24, 48 and a 72 hour periods and there was no increase in cell number and no genotoxicity associated. To evaluate the effects of myristicin on the cell cycle, we use flow cytometry, and observed time dependent G2/M arrest. These findings suggest that the cytotoxic activity of this molecule in K562 cell line might be due to the inhibition of the cell cycle following by apoptotic events.

DD116

ARTEMIS NUCLEASE FACILITATES APOPTOTIC CHROMATIN CLEAVAGE

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One hallmark of apoptosis is DNA degradation, first as high molecular weight (HMW) fragments followed by extensive internucleosomal fragmentation. During apoptosis, the DNA-dependent protein kinase (DNA-PK) is activated. DNA-PK is involved in the repair of DNA double strand beaks (DSB) and its catalytic subunit is associated with the nuclease ARTEMIS. It is reported here that upon apoptosis elicited in human cells by agents causing DNA DSB but also staurosporine and other inducers, ARTEMIS binds to apoptotic chromatin together with DNA-PK and other DSB repair proteins. ARTEMIS recruitment to chromatin showed a time- and dose-dependency, relied on DNA-PK protein kinase activity and was blocked by antagonizing the onset of apoptosis with a pancaspase inhibitor or upon overexpression of the anti-apoptotic BCL2 protein. In the absence of ARTEMIS, no defect in caspase-3, PARP-1 or XRCC4 cleavage or in H2AX phosphorylation was noticed and DNA-PKcs was still phosphorylated on S2056 in response to staurosporine. However DNA fragmentation including HMW fragmentation was delayed in ARTEMIS-deficient cells as compared with cells expressing ARTEMIS. In addition, ARTEMIS also enhanced the kinetics of the MLL gene cleavage at the breakage cluster breakpoint frequently translocated in acute or therapy-related leukemias. These results highlight a facilitating role for ARTEMIS in the early site-specific chromosome breakage during apoptosis possibly by recognizing and cleaving secondary DNA structure like those present in the Matrix Attachment Regions.

DD117

TRANSPLACENTALLY-INDUCED CENTROSOMAL AMPLIFICATION AND ANEUPLOIDY IN PRIMATES EXPOSED IN UTERO TO ANTIRETROVIRAL DRUGS.

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Antiretroviral therapy, used to treat Human Immunodeficiency virus (HIV-1), reduces maternal-fetal HIV-1 transmission and saves the lives of thousands of children yearly. However, the nucleoside reverse transcriptase inhibitor (NRTI) zidovudine (AZT) is a transplacental carcinogen in mice, and the NRTI combination of AZT plus lamivudine (3TC) induces genotoxic effects in offspring of CD-1 mice and patas monkeys exposed in utero. Here, genomic integrity in the offspring of patas dams given human-equivalent NRTI protocols during gestation was evaluated. Mesenchymal cells were cultured from bone marrow of patas (newborn, 1 and 2 years of age) exposed in utero to no drug (n=2), or human-equivalent doses of AZT/3TC (n=3), AZT/3TC /Abacavir (ABC) (n=1), or AZT/3TC/ Nevirapine (NVP) (n=3). Cells were examined by immunohistochemistry for: micronuclei (MN) with or without kinetochore material; centrosomes; and localization of nuclei. Scoring of ~5,000 cells revealed MN that contained no kinetochore signal, and MN that contained staining for kinetochore (CREST +) signal, indicating the ability of the NRTIs to act as both clastogens and aneugens. In 0.9, 2.3, 3.1 and 6.5% of cells from patas exposed to no drug, AZT/3TC, AZT/3TC/NVP and AZT/3TC/ABC, respectively, there were MN with no kinetochore material. In 0.2, 0.8, 1.1 and 3.2 % of cells from patas exposed to no drug, AZT/3TC, AZT/3TC/NVP and AZT/3TC/ABC, respectively, there were MN containing kinetochore material. Thus, there was a drug-induced abnormal chromosomal segregation as evidenced by the presence of MN containing kinetochores. Subsequently, cells were examined for centrosomal amplification. Supernumerary (>2) centrosomes were 1.26 % of cells in an unexposed monkey at birth, and 2.0 % of cells in an unexposed monkey at 3 years of age and 4.5, 5.3 and 4.6 % in cells from patas exposed to AZT/3TC, AZT/3TC/NVP and AZT/3TC/ABC, respectively. These studies show the existence of an NRTI-induced persistent malfunction in chromosomal segregation occurring in fetal bone marrow of patas monkeys exposed transplacentally to antiretrovirals. Therefore, NRTI-induced genotoxicity induced occurs not only because of incorporation into DNA, but also by interaction with the mitotic spindle resulting in aneuploidy.

DD118

COMPARATIVE ANALYSIS OF THE DNA REPAIR GENES IN THE ACETIC ACID BACTERIA GLUCONACETOBACTER DIAZOTROPHICUS AND GLUCONOBACTER OXYDANS

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Gluconacetobacter diazotrophicus is a Gram-negative alpha-proteobacterium, tolerant to acid environments, nitrogen fixing and was the first endophyte diazotrophic bacterium isolated from sugarcane. *Gluconobacter oxydans*, also Gram-negative alpha-proteobacterium, has the ability to incompletely oxidize a great variety of alcohols, carbohydrates and related compounds. Belonging to the family *Acetobacteriaceae*, both are interesting for industries and biotechnology, and had their genomes sequenced recently. The aim of this work was to analyze and compare their DNA repair genes once they are very important for maintain the genome integrity considering the adversities of the environment. Regarding this, the DNA repair genes were identified in both organisms, using as bait the ones from *Escherichia coli* and *Caulobacter crescentus*. For this study, a database of the DNA repair orthologs sequences was built and comparative analyses were made *in silico* using the packages Blast, Clustal and Paup. The analysis has shown the presence of the main repair paths in both organisms – excision repair, direct repair, recombinational repair and SOS response – most of the times with good similarity to those from *E. coli*. Interestingly, some gene duplications were found to be in the chromosome and in the plasmid, as for *uvrD*, *dnaE* and *ssb*. This observation and the phylogenetic trees interpretations are indicatives for events of lateral gene transfer. A great novelty was the identification of orthologs for *recD* in *G. diazotrophicus* and *recB* in both bacteria. Until now, there were no reports for the presence of the recombinational repair RecBCD initiation path in alpha-proteobacteria.

DD119

A NUCLEAR FORM OF GAMMA-TUBULIN IS PART OF A DNA DAMAGE SIGNALLING PATHWAY

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Double strand breaks are the most deleterious lesion in living organisms. They occur not only spontaneously as consequences of meiosis or replication block, but also from exogeneous events such as ionizing radiation or chemical exposure. These lesions lead to a series of signalling pathways that allow the cells to repair or tolerate the damage. Recombination repair catalysed by Rad51 protein plays an essential role in repair of DNA double-strand break and DNA cross-linking adducts. γ-tubulin, a centrosomal protein involved in the assembly of microtubules, was recently shown to be part of the same nuclear complex with Rad51, either in mammalian cells in S phase of the cell cycle or following DNA damaging treatment. We show here that Rad51 was not required for γ-tubulin nuclear foci formation. However both proteins were part of a complex with γH2AX, each protein being able to immunoprecipitate the other two. A monoubiquinated form of nuclear γ-tubulin was found to be associated to this complex in extracts of S phase or asynchronous cells. A decrease of γ-tubulin monoubiquitination was observed if BRCA1 was knocked down by siRNA. In addition, chromatin immunoprecipitation shows that γ-tubulin is present at an induced unique DNA double-strand break. Our results suggest that nuclear γ-tubulin might have a role in DNA damage signalling pathway in which it could participate through its modified form, possibly contributing in linking DNA repair and mitosis.

DD120

OLIGONUCLEOTIDE MICROARRAYS ELABORATION USING A CLICK CHEMISTRY APPROACH: APPLICATIONS TO MISMATCHES AND DNA REPAIR ACTIVITIES DETECTION

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DNA microarrays have recently known a growing interest because of their enormous potential applications like diseases' diagnosis or other genome analyses (1, 2). The most popular approach used for the DNA microarrays elaboration consists in a post-synthesis immobilization of functionalized DNA fragments (3, 4). To reach that goal, a large set of bioconjugate chemistries were proposed and explored (3). Here we present the site-specific immobilization of alkyne-modified oligonucleotides on an azide-terminated glass slide via an efficient click chemistry approach (5, 6). The first application of the latter DNA microarrays concerns mismatch detection. Different oligonucleotides containing mismatches or not were grafted onto the slide and then hybridized with a cy3-labelled complementary DNA target. Using a fluorescent detection, we were able to discriminate the perfect match from one or two mismatches. A second application of these biosensors that concerns the detection of enzymatic DNA repair activities (7, 8) was also developed. Indeed, several damaged oligonucleotides (each containing a defined altered nucleobase such as 8-oxoguanine, uracil and abasic site) were spotted on activated supports and hybridized with their complementary DNA target. The resulting functionalized slides containing a panel of double-stranded DNA substrates were incubated with different enzymes involved in the DNA repair machinery (namely DNA-glycosylases and AP-endonucleases). The enzymes activities induced a fluorescence decrease by a selective cleavage at the damaged site. Such original microar could be used in miniaturized quantitative assays for diagnosing DNA repair deficiencies in cellular extracts together with the screening of DNA repair modulators agents.

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DD121

SYNTHESIS, CHARACTERIZATION AND MUTAGENIC PROPERTIES OF N7-GUANINE ADDUCTS FORMED BY EPOXIDES

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Ethylene oxide (EO), propylene oxide (PO) are important volatile industrial chemicals, but there still an ongoing debate on the risks associated with their occupational exposure.[1] Although the International Agency for Research on Cancer (IARC) has classified EO as a known carcinogen in humans (Class 1) and PO as a possible human carcinogen (Class 2B), their mechanism of carcinogenicity is not well characterized, but is thought to involve the formation of DNA adducts. These epoxides are directly acting agents, which react with DNA forming primarily N7-alkylguanine derivatives (*N*7-(2-hydroxyethyl)guanine for EO and *N*7-(2-hydroxypropyl)guanine for PO).[2]. The aim of the present research project is to wholly identify the genotoxic and mutagen potential of the N7-hydroxyalkylated guanine adducts.[3] Emphasis was particularly placed on the assessment of biological response of enzymatic repair and replication machineries towards the latter alkylguanine derivatives. For the first time a set of oligonucleotides (from tetranucleotides up to 13-mer DNA) containing a unique N7-alkylguanine adduct with different steric hindrance of the alkyl moiety (respectively a methyl-, a hydroethyl- and a hydroxypropyl group) have been prepared. The suitable modified oligonucleotides have been synthesized upon a direct treatment with different alkylating reagents (dimethylsulfate, ethylene oxide or propylene oxide) using distinct experimental procedures, specific of each reagent. The resulting modified DNA oligomers have been purified and then characterized by chromatography, electrophoresis and mass spectrometry analyses. Several biochemical studies have been performed using the latter damaged oligonucleotides substrates (adduct stability, enzymatic digestion, *in vitro* repair studies…). Such DNA oligomers that insert a set of targeted N7-alkylated guanine lesions are promising candidates to investigate *in cellulo* mutagenic properties after incorporation into plasmid probes and then transfection into cells.[4]

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DD122

MODULATION OF DNA REPAIR BY MONOTERPENES FROM SAGE IN Escherichia coli

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A set of *E. coli* K12 strains, constructed for detection of antimutagens and estimation of the mechanisms of antimutagenesis, was used to investigate antimutagenic potential of monoterpenes from sage: Camphor (C), α/β Thujone (T) and Eucalyptol (E). Genetic endpoints such as spontaneous and induced mutations, recombination, and SOS response were studied in repair proficient strain, and NER and MMR mutants. To detect antimutagenic effect resulting from modulation of DNA repair and replication, UV-irradiation and 4NQO (UV mimetic) were used as mutagens. The spontaneous and induced mutagenesis was monitored by reversion tests (*argE3*→Arg^+). The SOS response kinetics in repair proficient strain was studied by measuring activity of β-galactosidase expressed from *sfiA*::l*acZ* fusion; the effect on protein synthesis was determined by measuring activity of constitutive alkaline phosphatase. The spontaneous and induced recombination was measured in recombination proficient *lacMS286* Φ80dII*lacBK1* strain by scoring Lac^+ recombinats. Neither of the monoterpenes was mutagenic in repair proficient and MMR deficient strains, but T caused a two-fold increase in the number of Arg^+ revertants in NER deficient strain. All

tested monoterpenes significantly reduced UV- and 4NQO-induced mutagenesis in repair proficient strain, while this effect was diminished in NER deficient strain. Neither of the monoterpenes alone could induce SOS response, but they differently affected SOS response following UV-irradiation. While in control cultures the level of β-gal reached maximum 100 minutes following UV and rapidly subsided, in cultures with C induced enzyme levels were maintained long after the treatment. E slightly reduced the level of β-gal and the maximum enzyme induction was 20 minutes delayed compared with the control. Similar reduction was obtained with T, but with no effect on enzyme kinetics. In addition, T slightly reduced the growth rate and the levels of alkaline phosphatase. Spontaneous and UV-induced recombination was increased by C and E, respectively, while T had no effect. Although monoterpenes could affect SOS response and recombination, obtained results indicated modulation of NER as common mechanism of their antimutagenic action.

DD123
COMPARISON OF THE REPAIR KINETICS OF UVA VERSUS UVB-INDUCED CYCLOBUTANE PYRIMIDINE DIMERS IN HUMAN KERATINOCYTES

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Worldwide, one in three cancers is skin-related and the WHO expects the skin-cancer epidemic to increase. Solar ultraviolet radiation (UVR) by inducing DNA photo-lesions, has become the prime cause of most skin cancers. These cancers could be prevented if we protect ourselves from UVR. UVR comprises three main regions: UVC is absorbed by the ozone layer and does not affect the skin. UVB is directly absorbed by DNA and induces different forms of lesions like cyclobutane pyrimidine dimers (CPDs). In contrast with UVB, UVA is indirectly absorbed by DNA. UVA is suspected to play a key role in induction of skin tumors and may be even more important than UVB in mutagenesis. CPDs have been found to be induced in human skin cells exposed to UVA through a different mechanism but the mechanism of CPD induction by UVA is not clearly identified. Most sunscreens filter out UVB absorption, but they cannot block most of UVA, so they do not help to prevent skin cancer. A better assessment of the routes by which UVA and UVB induce CPDs, may lead to prevention of skin cancer. In the this study, by use of the highly sensitive single cell gel electrophoresis (T4endoV modified comet assay), the yield of CPDs in HaCaT keratinocyte cell line was assessed. Firstly, we optimised the enzyme conditions for comet assay technique on Keratinocytes (HaCaT cell line). Then, formation of UVA-induced CPDs versus UVB-induced CPDs was compared. Finally, the repair rate of CPDs induced by UVA and UVB was compared. Following irradiation, levels of CPD and ALS were significantly higher in the cells irradiated with UVB, compared to UVA. However, for the first time, we noted the rate of repair for UVA-induced CPD to be much faster than the rate of UVB-induced CPD.

DD124
A SIMPLE, RELIABLE AND VERSATILE MEDIUM-THROUGHPUT COMET ASSAY

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The comet assay is widely used to measure different lesions in DNA, by incubating the gel embedded nucleoids with damage-specific endonucleases. It can also be used to monitor DNA repair. Applications include basic research, testing of chemicals for genotoxicity, and human biomonitoring. In the conventional format, 1 or 2 gels (approximately 20 mm square) are placed on a microscope slide. The number of samples that can be processed at one time is relatively small; it is determined by the number of slides that can easily be handled and the capacity of the electrophoresis tank. To increase throughput of the assay we have designed a system with 12 small gels on one slide. With a silicone gasket clamped over the slide, this approach allows the incubation of individual gels with different genotoxic chemicals, enzymes or cell extracts. Thus several times more samples can be analysed per electrophoresis run, and smaller volumes of test solutions are required. Examples of the application of this modified method include the simultaneous analysis of different lesions using a range of enzymes; the analysis of several samples for DNA repair activity; and fluorescent in situ hybridisation (FISH) of the DNA with specific, labelled probes. This procedure is cost effective, since it reduces the amount of reagents needed, and can easily be applied in any laboratory. Scoring of comets is greatly accelerated by using the PathfinderTM automated image analysis system. We have tested the new method for possible problems such as leakage between wells, and 'edge effects', i.e. distorted or anomalous comets near the gel boundary. Incubations with different doses of lesion-inducing chemicals, or enzymes in each well, give results that correlate closely with those obtained using the conventional method. This new comet assay technique is robust, reliable, and very convenient to use. Supported by EC contract LSHB-CT-2006-037575

DD125
DNA DAMAGE MEASURED IN LYMPHOCYTES FROM MOTHERS AND NEWBORNS WITH THE COMET ASSAY

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We are investigating the levels of DNA oxidation in lymphocytes from maternal and cord blood as part of the NewGeneris project. The hypothesis is that exposure during pregnancy to damaging or protective agents, especially in the diet, can influence the risk of disease in children as a consequence of oxidative damage. Lymphocytes have been collected in cohorts from different countries, and analysed using the comet assay in combination with lesion-specific endonucleases to measure oxidised bases. The approach has been validated by studying inter-laboratory variation in measurements of the same samples, as well as reproducibility within one laboratory. Positive and negative reference standards are employed as a quality control. A high throughput version of the comet assay facilitates the assay of large numbers of samples but requires automated scoring. We will give a preliminary report on the levels and range of damage in different cohorts and the comparison between mothers and children. We will also describe problems that we have encountered, that should be taken into account when carrying out large-scale international studies of this kind.
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DD126
SATURATED EXOCYCLIC HYDROXYALKANO DNA ADDUCTS ARE NEW SUBSTRATES FOR ESCHERICHIA COLI ALKB PROTEIN

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The AlkB-type proteins are repair enzymes which remove alkyl lesions from bases *via* an oxidative mechanism restoring native DNA structure. Recently, it was found that they also repair DNA etheno(ε)-adducts. Exocyclic adducts (ε-adducts among them) can originate endogenously from reaction of lipid peroxidation products: α, β-unsaturated- and epoxy- aldehydes with DNA bases. Their source are also industrial carcinogens: *e.g.*, vinyl chloride metabolites, chloroethylene epoxide and chloroacetaldehyde (CAA), or dietary and environmental toxin acrolein (ACR). We reacted 5-mer oligodeoxynucleotides, TTXTT, where X = C or A, with CAA, to modify cytosine and adenine to 3,N^4-ethenocytosine (εC) and 1,N^6-ethenoadenine (εA), respectively, and

with ACR to modify cytosine to $3,N^4$-α-hydroxypropanocytosine (HPC). The reaction of cytosine with CAA proceeds *via* relatively stable intermediate, $3,N^4$-α-hydroxyethanocytosine (HEC), what allowed to prepare 5-mer containing this adduct. Modified 5-mers containing exocyclic adducts, εC, HEC, HPC and εA, were studied as substrates for *E. coli* AlkB protein. Using HPLC technique, which allows to separate the modified from unmodified (repaired) oligomer, we have found that all studied exocyclic adducts are repaired by AlkB protein, although less efficiently than the reference 3-methylcytosine (3meC). The repair process requires higher Fe(II) and lower α-ketoglutarate concentrations in comparison of 3meC repair. The pH optimum for the repair decreases with a decrease of pKa value of the adduct. These results suggest that in active centre of AlkB enzyme the substrate is bound in the protonated form. On the basis of the published structure of AlkB protein it can be concluded that positively charged substrate interact with negatively charged carboxylic group of Asp135. Our results show that *E. coli* AlkB repairs exocyclic adducts of different structures, unsaturated etheno, εA and εC, and also saturated hydroxyalkano, HEC and HPC. Therefore, we suppose that the substrate specificity of AlkB-type proteins may be broad and that these proteins can be engaged in reversal of various kinds of DNA lesions, also those caused by lipid peroxidation products in mammalian cells.

DD127

EXPRESSION OF HEPATIC GENES INVOLVED IN DNA DAMAGE RESPONSE DURING THE LIFE CYCLE OF THE RAT

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Susceptibility to adverse health events caused by environmental agents, including pharmaceutical drugs, may be dependent on the suite of genes expressed in the liver at any given time. The expression of different sets of genes is modulated during the life cycle, and this may influence the biological responses to xenobiotics and chemicals. By understanding the natural dynamics of the transcriptome at the various life stages, the assessment of health risk versus benefit can be more rationally determined. Our hypothesis is: During the life cycle of the rat, there are changes in the expression of genes in the liver that are potentially associated with susceptibility to disease and adverse effects of drugs and chemicals. To address this question, microar were used to quantify the relative expression levels of hepatic genes in both male and female F344 rats at the following ages: 2 wks, 5 wks, 6 wks, 8 wks, 15 wks, 21 wks, 52 wks, 78 wks, and 104 wks. Gene expression profiles were determined in the median lobe of the livers of 5 male and 5 female rats at each of the nine ages using Agilent one-color 4 x 44K rat whole genome ar. Principal components analysis demonstrated both sex differences and age differences in overall gene expression patterns. Examination of genes involved in DNA repair, the DNA damage response, and xenobiotic metabolism showed significant sex and age differences, including Msh3, Gadd45b, and Cyp2c. Overall, the expression of more than 300 genes was affected by sex and age.

DD128

INFLUENCE OF SIDE SUBSTITUENTS IN PHTHALOCYANINES ON THEIR PHOTO-CHEMICAL PROPERTIES FOR APPLICATION IN PHOTODYNAMIC THERAPY

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Phthalocyanines containing metal ions Zn(II) and Al(III) have a great potency as photosensitizers in photodynamic therapy of malignancies and other diseases. In this way phthalocyanines are investigated for the development of second and third generation of photosensitizers. Main aim in the design of such reagents is increasing of the selectivity of action and the solubility without deterioration of photo-physical properties of phthalocyanines. These features could be achieved by introducing of the antisense oligonucleotides, DNA binding ligands or small charged substituents in peripheral positions of the phthalocyanine ring. In our work we have investigated the photo-physical properties and phototoxicity of Zn(II)-phthalocyanine and its derivatives with moieties mentioned above. Times of fluorescence decay were measured using the time-correlated single-photon-counting method. Lifetimes of singlet and triplet oxygen, as well as quantum yields of singlet oxygen generation and energy transfer were measured using time-resolved singlet oxygen luminescence. It was shown that side moieties influenced on photo-physical properties of phthalocyanines. But it was found that this influence on the most important parameter, quantum yield of singlet oxygen generation, was slight. The toxicity of compounds under study was tested on Jurkat cells (human acute T-cell leukemia). Cell samples containing photosensitizers were irradiated using diode laser with emission at the wavelength 668 nm. After irradiation the fresh medium was added and cells were incubated up to three days. It was shown that irradiation time during 6 minute was enough for the appearance of phototoxic effect. Maximal effect of cytotoxicity was detected at 3-4 hours after irradiation. Supported by a Grant from the Russian Foundation for Basic Research (08-04-00334-a) and President Grant (652.2008.4).

DD129

RADIATION-INDUCED H2AX PHOSPHORYLATION IN BONE MARROW AND GERM CELLS OF PARP-1 KNOCKOUT MICE

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Recently, a role of poly(ADP-ribosyl)ation was shown in the ATM-initiated DNA repair pathway, with PARP inhibited and PARP-1 defective cells showing, for instance, compromised H2AX phosphorylation in response to ionizing radiation and MNNG. Here we report the results of a study in which H2AX phosphorylation was measured in bone marrow and germ cells of PARP-1 knockout (KO) or wild type (WT) mice between 1 and 48 h after exposure to 4 Gy X . In WT bone marrow the induction of cells with microscopically detectable foci followed a kinetic similar to that measured in mammalian cells irradiated in vitro, with the peak at 1 h and a sharp decrease between 1 and 4 h post irradiation. While no difference was detected between unirradiated cells of the two genotypes, after irradiation, the formation of γH2AX foci was delayed in PARP-1 KO mice by about 1 h; the flow cytometric analysis of γH2AX content/cell suggested that delayed phosphorylation could partly explain the delay in the detection of foci. In spermatocytes and spermatids of WT irradiated mice the percentage of cells with foci increased rapidly after irradiation, as in somatic cells, but remained almost the same up to 48 h later, in spite of the fact that levels of comet assay measured DNA breaks were back to control by 2 h post-treatment. The flow cytometric measurement of γH2AX content/cell showed that in spermatocytes and spermatids basal levels of γH2AX were much higher than in somatic cells. These observations suggested that, in germ cells, H2AX phosphorylation may have functions additional to that in the DNA break signalling pathways, possibly related to chromatin remodeling. Immunohistochemical analysis of anti-γH2AX labelling in unirradiated and irradiated testicular sections can contribute to characterize the distribution and function of H2AX phosphorylation in the various germ cell stages. The same rapid induction and long time persistence of foci observed in the germ cells of WT mice was observed in PARP-1 KO animals. When compared to the PARP-1 KO bone marrow phenotype, data on germ cells suggest a different involvement of the PARP-1 protein in the activation of H2AX phosphorylation in somatic and germ cells. Further studies should clarify the role of ATM in the determination of this difference.

DD130

K63-LINKED UBIQUITIN CHAINS CONJUGATED TO PCNA CONTROL RECOMBINATION REPAIR OF SS-GAPS

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DNA Replication hindered by bulky lesions poses a serious challenge to cellular proliferation. Yet, cells defective in excision repair of such DNA adducts survive high doses of damage. An arsenal of universally conserved mechanisms termed DNA damage tolerance (DDT) is believed to deal with stalled forks, to circumvent this problem. In eukaryotes, DDT is under the control of the RAD6 epistasis group. Previously, our group has shown that about half of the genes of the RAD6 pathway encode enzymes involved in ubiquitylation, and that PCNA, the replication processivity factor, is the key target. We showed that PCNA can be modified either by a single ubiquitin moiety, by a noncanonical (K63-linked) polyubiquitin chain, or by SUMO. PCNA is constitutively SUMOylated during S-phase in order to prevent unwanted recombination between sister-chromatids by the recruitment of the anti-recombinogenic helicase Srs2. On the contrary, PCNA ubiquitylation is specifically triggered by the presence of DNA damage. DDT can operate either in an error-prone manner that involves monoubiquitylated PCNA and the recruitment of specialized translesion polymerases (TLS), or an error-free, triggered by polyubiquitylated PCNA by a mechanism that is largely unknown. Here we describe S.cerevisiae mutants that lead to spontaneous hyper-induction of PCNA ubiquitylation in the absence of any exogenous DNA damage. The strongest inducers localized on Polymerase δ. Using a chromatin fractionation approach we propose that ubiquitylated PCNA mainly arises at immature Okazaki fragments during lagging-strand synthesis. Such ss-gaps must be filled to allow proliferation, and PCNA polyubiquitylation plays a critical role in coordinating their repair. Based on this finding we designed a robot-based synthetic lethality rescue screen in yeast that identified genes required downstream of polyubiquitylated PCNA. The anti-recombinogenic helicase Sgs1, linked to the Bloom cancer predisposition syndrome in humans, engages late in the reaction, promoting the completion of recombination initiated by K63-linked ubiquitin chains on PCNA. In conclusion, we present a novel tool for the study of eukaryotic DDT, providing a new perspective of the activation and molecular mechanism of DDT.

DD131

RESPONSE OF MUTYH- AND MSH2- DEFECTIVE CELLS TO DNA DAMAGE INDUCED BY COMBINED 6-THIOGUANINE AND UVA TREATMENT

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Background The thiopurine antimetabolite azathioprine (Aza) is used in the treatment of cancer, inflammatory conditions and as an immunosuppressant. Systemic treatment with Aza results in high levels of DNA 6-thioguanine (6-TG). Exposure of cells containing DNA 6-TG to low doses of UVA produces reactive oxygen species (ROS) and photochemical oxidation of DNA 6-TG into cytotoxic and mutagenic lesions. *Aims* We examined the role of the Base Excision Repair DNA glycosylase Mutyh and the Mismatch repair (MMR) protein Msh2 in the cellular response to 6-TG/UVA-induced DNA damage. The contribution of an oxidized dNTP pool to 6-TG/UVA cytotoxicity was also investigated. *Methods* Mutyh- or Msh2-defective mouse embryo fibroblasts (MEFs) were exposed to a non-toxic concentration of 6-TG and irradiated with a low dose of UVA. Survival was determined by clonal assays. DNA 6-TG and 8-hydroxydeoxyguanosine (8-oxodG) were measured by HPLC and HPLC/EC, respectively. *Results* 6-TG and UVA were synergistically toxic to wild-type MEFs - neither 6-TG or UVA alone detectably affected survival. Mutyh- or Msh2-defective cells were more resistant than wild-type MEFs to killing by 6-TG/UVA. The combined treatment significantly increased the levels of DNA 8-oxodG. The dNTP pool contributed to both the increased levels of DNA 8-oxodG and to the enhanced toxicity of combined 6-TG/UVA. Wild-type cells grown for 24hrs in 6-TG and UVA irradiated immediately contained more DNA 8-oxodG and were more sensitive than the same cells irradiated after a further 24h growth in medium without 6-TG to deplete the pool of 6-TG nucleotides. *Conclusions* The combination of 6-TG and UVA introduces DNA 8-oxodG and causes cell death. Surprisingly, the absence of either Mutyh or Msh2 confers tolerance to 6-TG/UVA. The molecular mechanism underlying this phenotype is currently under investigation.

DD132

THE INFLUENCE OF LIPOPOLYSACHARIDE-INDUCED INFLAMMATION ON THE EXPRESSION OF DNA REPAIR GENES IN YOUNG RATS

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Oxidative DNA damage may be a risk factor for development of various pathologies, including malignancy. We studied inflammation triggered modulation of repair activity in the intestines of three-week-old rats injected i.p. with *E. coli* or *S.typhimurium* lipopolysacharides (LPS) at doses of 1 and 10 mg/kg. The control group consisted of the animals not treated with LPS. Subsequent formation in these animals of colonic preneoplastic lesions, aberrant crypt foci (ACF) was also investigated. Five days after LPS administration no differences were observed in repair rate of 1,N^6-ethenoadenine (εA), 3,N^4-ethenocytosine (εC), 8-oxoguanine (8-oxoG) and AP-endonuclease (APEX) in intestines of these rats, as measured by the nicking assay. However, a significant increase in all repair activities was found within one and two months after *S.typhimurium* LPS treatment. *E. coli* LPS significantly increased only the 8-oxoG repair. *S. typhimurium* LPS stimulated mRNA transcription of pro-inflammatory proteins, lipooxygenase-12 and cyclooxygenase-2, as well as some DNA repair enzymes like AP-endonuclease (Ape1) and εC-glycosylase (Tdg). mRNA level of DNA glycosylases excising εA (Mpg) and 8-oxoG (Ogg1) was also increased by LPS treatment, but only at the highest dose. Transcription of all enzymes increased for up to 30 days after LPS, and subsequently decreased, with the exception of Ape1, which remained elevated even two months after LPS administration. Thus, the repair efficiency of εA, εC and 8-oxoG depends on the availability of Ape1, which increases Ogg1 and Tdg turnover on damaged DNA, as well as presumably stimulates Mpg. One and two months after administration of *E. coli* or *S. typhimurium* LPS, the number of aberrant crypt foci in rat colons increased in a dose- and time-dependent manner. Thus, inflammation stimulates the repair capacity for εA, εC and 8-oxoG, but simultaneously triggers the appearance of preneoplastic changes in the colons. These results suggest that the oxidative stress related to bacterial infections long-lastingly stimulates the transcription of enzymes responsible for oxidative DNA damage repair, which can be one of the defence mechanisms of the organisms against the effects of infections.

DD133

ABNORMALITIES IN THE ORDER OF GENE-SPECIFIC REPAIR, CHROMATIN CONDENSATION AND TRANSCRIPTION ACTIVITY IN DIFFERENT GENOMIC LOCI PREDICT CLINICAL OUTCOME IN MULTIPLE MYELOMA

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Background/aims: In recent years, there has been an exponential increase in research concerning changes in epigenetic mechanisms regulating gene expression and chromatin condensation that occur during carcinogenesis. In this report, the effect of differential epigenetic alterations on the clinical outcome in multiple myeloma (MM) patients is presented. Methods: Chromatin condensation (using micrococcal nuclease digestion), transcription activity (steady-state levels using RNA slot-blots and rates of transcription using run-off assay) as well as *in vitro* melphalan-induced damage formation/repair (monoadducts and interstrand cross-links using Southern blot) were measured in four genomic loci (b-actin, p53, N-ras, d-globin), in peripheral blood mononuclear cells (PBMC) taken from thirty-two MM patients (23 responders, 9 non-responders) before therapeutic treatment, as well as from twelve healthy volunteers. Results: In all patients examined, a close association was observed between the locus-specific DNA repair efficiency, transcriptional activity and chromatin condensation. Strikingly, the order of variation of these three markers between different genes was b-actin>p53>N-ras>d-globin in all healthy volunteers and in 95% of responders to chemotherapy, while a perturbation of this order was found in 80% of non-responders. Conclusion: Knowledge about the epigenetic networks that control transcriptional activity, chromatin structure and the region-specific repair of different genomic loci may help us to derive novel approaches to cancer chemotherapy. Acknowledgements: This work was partly supported by the EU ECNIS Network of Excellence (Contract No 513943).

DD134

DEVELOPMENT OF A MULTIPLEX, LONG QUANTITATIVE PCR-BASED METHOD TO MEASURE P53-SPECIFIC MELPHALAN INTERSTRAND CROSS-LINKS AND ITS POSSIBLE CLINICAL APPLICATION

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Background: We have previously found that the extent of p53-specific damage formation/repair in peripheral blood mononuclear cells (PBMC) from multiple myeloma (MM) patients following *in vitro* exposure to melphalan correlates with the respective results obtained *in vivo*, and is of value in predicting clinical outcome. Here we present the development and validation of a rapid, sensitive and non-radioactive method for measuring DNA damage following exposure to melphalan, with potential for application in the clinic. Methods: Cell cultures, as well as human whole blood and PBMC, were treated with melphalan (0-1000 μg/ml) for various time-periods. Genomic DNA was isolated and the conditions for multiplex, long, quantitative PCR to simultaneously amplify a 7 kb fragment (part of the p53 gene) and a 500 bp fragment (part of the IFN-β1 sequence; internal standard) were developed and optimized. Results: The extent of PCR amplification of the p53 fragment was conversely proportional to the treatment concentrations of melphalan, implying dose-related inhibition by the DNA adducts formed. Comparison with adduct levels measured by Southern blot analysis showed that the the adducts measured by Q-PCR corresponded to interstrand cross-links, while monoadducts induced by melphalan could not be measured by Q-PCR. Application of the assay to *in vitro*-treated human blood samples showed inter-individual variation which reflects DNA repair differences. A strong correlation was found between the *in vitro* melphalan-induced damage in PBMC from MM patients and their clinical response. No such correlation was obtained using whole blood. Conclusion A simple PCR-based methodology to quantify melphalan-induced p53-specific ICL formation/repair can be used to select those patients with MM who are more likely to benefit from high-dose melphalan therapy. Acknowledgements: This work was partly supported by the EU ECNIS Network of Excellence (Contract No 513943).

DD135

DEVELOPMENT AND VALIDATION OF A QUANTITATIVE, PCR-BASED ASSAY FOR THE MEASUREMENT OF PLATINUM DNA DAMAGE FORMATION/REPAIR IN OVARIAN CARCINOMA. CLINICAL APPLICATION

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Background: DNA damage formation/repair plays an important role in the mechanism of action of genotoxic drugs and may therefore determine individual patient clinical outcome. However, the assays used are time-consuming, as they require extensive purification of the DNA, quantification, enzymatic digestion and complex experimental procedures. We present here the development and validation of a gene-specific, rapid, quantitative, non-radioactive, sensitive method for measuring damage formation/repair following exposure to platinum-based anticancer drugs used in the treatment of ovarian carcinoma. Methods: Cell cultures and peripheral blood mononuclear cells from healthy volunteers were treated with cisplatin or carboplatin for various time-periods. Following genomic DNA isolation, gene-specific damage formation/repair was examined using Southern blot as well as a multiplex long quantitative PCR (Q-PCR) carried out in a 7kb fragment (part of the p53 gene) and a 0.5kb fragment (part of the IFN-β1 sequence; internal standard). Results: Using both platinum drugs, the kinetics of intra- and interstrand cross-links formation/repair was examined using Southern blot. Cisplatin-induced intrastrand cross-links levels reached a plateau within ~3h of treatment, while peak interstrand cross-links were observed at ~24h of exposure. Carboplatin-induced maximal levels of both intra- and interstrand cross-links were observed within 24h of drug incubation. The extent of Q-PCR amplification of the p53 fragment was conversely proportional to the treatment concentrations of both platinum-based drugs examined, implying dose-related inhibition by the DNA adducts formed. Parallel analysis of the same samples using both Southern blot and Q-PCR, showed that the adducts measured by Q-PCR correspond to total platinum-induced lesions. Conclusion: Using the current protocol of the Q-PCR method it is feasible to measure gene-specific damage formation/repair in readily accessible biological material from humans exposed to platinum-based drugs and to examine, at the level of individual patients, the relationship between the formation/repair of cytotoxic DNA damage and clinical outcome. Acknowledgements: This work was partly supported by the ECNIS Network of Excellence (Contract No 513943)

DD136

REPAIR OF MELPHALAN ADDUCTS SHOWS A GENERAL 5' TO 3'-END GRADIENT EFFECT IN TRANSCRIBED GENES: CLINICAL IMPLICATIONS

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Background: Melphalan is a nitrogen mustard employed in the treatment of multiple myeloma (MM). In this report, the mechanistic basis of melphalan adduct repair, as well as its implication for clinical out-

come in MM, were investigated. Methods: Human primary fibroblasts with different repair backgrounds, as well as peripheral blood lymphocytes (PBMC) from 5 human volunteers and MM patients (9 responders, 7 non-responders) prior to therapeutic treatment were exposed to melphalan. The kinetics of DNA adduct formation/repair were evaluated in four gene loci with varying transcriptional activity and local chromatin condensation (b-actin, p53, N-ras, d-globin). Results: In repair-deficient fibroblasts, adducts accumulated to similar levels in all four genes studied, indicating that the state of transcription and local chromatin condensation did not affect adduct formation. In repair-active fibroblasts, the operation of very rapid repair ($t_{1/2}$~15-120min, depending on the gene sequence) was observable leading to the removal of ~60-75% of the adducts formed. To investigate if the rapidly repaired adducts are located in specific domains in the gene, we measured adduct kinetics along the length of N-ras and p53. A gradient of repair efficiency of their transcribed strands was found, with faster repair at the 5'-end. A similar 5' to 3'-end gradient effect was seen in PBMC from healthy volunteers and in all MM patients. In the N-ras gene, non-responders to chemotherapy showed greater repair activity than responders. Interestingly, this difference appears to be greatest at the 5'-end of the gene and to decrease towards the 3'-end of the gene. No difference between responders and non-responders was evident in regions upstream to the N-ras as well as at the overall genome repair. These observations imply the possibility that clinical response may be related to DNA repair occurring in specific sub-regions. Conclusions: Melphalan adducts showed a gradient of repair efficiency with faster repair at the 5'-end of transcribed genes, while the clinical outcome in MM patients may be related to differential removal of adducts located in specific domains of the genome. Acknowledgements: This work was partly supported by the EU ECNIS Network of Excellence (Contract No 513943).

DD137
INTER-INDIVIDUAL VARIATION IN NUCLEOTIDE EXCISION REPAIR ACTIVITY IN HEALTHY YOUNG ADULTS: ASSOCIATION WITH DNA REPAIR POLYMORPHISMS AND GENE EXPRESSION

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In the recent years several studies have investigated polymorphisms in DNA repair genes and their possible links to the risk of various cancers. Sequence variants in DNA repair genes are assumed to modulate DNA repair capacity and, therefore, are associated with the altered cancer risk. Inter-individual susceptibility to mutagens/carcinogens can be assessed by genotyping or phenotyping at molecular or cellular level for genes and/or pathways involved in key events regulating the exposure-effect responses. Due to a great number of DNA repair genes identified and the interactions among the pathways (BER, NER, DSB) responsible for the repair of DNA adducts and breaks, phenotyping is becoming an attractive approach to integrate the repair capacity resulting from many genes involved. In the present report we attempt to investigate associations between DNA repair genetic polymorphisms (ERCC1, XPD, OGG1, XRCC1, XRCC3 genes) and individual DNA repair activity in a healthy population, assessing in vitro BPDE-induced DNA damage. PBMCs were isolated from 225 young healthy donors: 149 were women and 76 were men; ages ranged from 18 to 48 years (mean 24.9 years and median 23.1 years). Smoking habits were investigated by a questionnaire subministration: 72.89 % resulted never, 9.78% former and 16.44% current smokers. DNA repair capacity was not affected by smoking habits, although the population is made-up mainly by young people light smokers. We did not observed any significant influence on BPDE-damage repair capacity in ERCC1 and XPD polymorphisms. Neverthless NER capacity was significantly decreased in individuals with the homozygous variant (CC) in OGG1 rs293795 than those with the wild-type (TT) and heterozygous (TC) genotypes. Moreover DRC was significantly higher in individuals with the homozygous variant (AA) in XRCC3 rs861530 and was significantly lower in individuals with the homozygous variant (GG) in XRCC3 rs1799796. The association between genes of different repair pathways suggests important crosstalk mechanisms.
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DD138
BIOINFORMATIC ANALYSIS OF THE CELL DEATH INDUCED BY N-METHYL-N-NITROSOUREA, A MODEL SN1 METHYLATING AGENT, IN TWO LUNG CANCER CELL LINES OF HUMAN ORIGIN

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Since lung cancer is the most frequent cause of cancer death, new therapeutic approaches are eagerly needed. Methylating agents constitute a widely used class of anticancer drugs, the effect of which on human non small-cell lung cancer (NSCLC) has not been adequately studied. N-methyl-N-nitrosourea (MNU), a model SN1 methylating agent, induces cell death through a distinct mechanism in two human NSCLC cell lines studied, A549(p53wt) and H157(p53null). In A549(p53wt), it induces a non-apoptotic cell death whereas in H157(p53null), MNU induced apoptotic cell death, confirmed by cytofluorometry of DNA content and immunodetection of apoptotic markers. Thus, the mechanism of the cell death induced by SN1 methylating agents is cell type-dependent and must be assessed prior treatment. To further investigate the differential effect of MNU in the above lung cancer cell lines we performed a time course gene profiling study using DNA microar (Illumina platform at 24, 48 and 72h of treatment. The number of statistically significant differentiated genes (ANOVA, $p<0.01$) presenting a minimum of 1.5 fold alteration in their expression greatly varied between the two cell lines. Namely, in H157 cells, 2219 genes were found as significantly altered among the three treatment times, whereas in the case of A549 cells the relevant number was only 530. In both cell lines the most pronounced alterations in gene expression between MNU treated and non-treated cells, were observed in 48h. By applying GO-based meta-analysis we further associated gene expression changes with modulation of several biological processes and functions. It is important to note that the altered functions derived through this analysis differ between the two cell lines. Regarding H157 cells functions related to apoptosis, cell cycle and proliferation but also cholesterol biosynthesis were among the significantly altered while in A549 cells the altered functions were mainly related to cytoskeleton organization, integrin signalling and response to stress. The results are currently under further investigation and validation.

DD139
EVALUATION OF HIGH-THROUGHPUT UMU TEST SYSTEM USING TESTER STRAINS EXPRESSING HUMAN CYTOCHROME P450 ENZYMES

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The SOS/*umu* test is generally employed for screening of potential chemical carcinogens. The aim of this study is to examine the sensitivitiy of *umu*-microplate test system with tester strains OY1002/1A2, OY1002/1A1, OY1002/1B1, and OY1002/3A4 expressing human cytochrome P450 (CYP1A2, 1A1, 1B1, 3A4) enzymes. The strains were established by introducing two different plasmids into *S.typhimurium* TA1535. The one plasmid is carrying human cytochrome CYPs and NADPH-cytochrome P450 reductase cDNAs, and the other plasmid is carrying *O*-acetyltransferase and *umuC''lacZ* fusion genes. We evaluated induction of *umuC* gene expression by 2-aminoanthracene, IQ, MeIQ and Glu-P-1, which expressed genotoxicity when bioactivated by CYPs, and the expression is based on measuring β-galactosidase activity. We obtained the following result with a

96-well microplate method. Genotoxicity of these chemicals with OY1002/1A2 strain was able to detect with high sensitivity under the following pre-incubation condition, which is added with isopropyl β-D-thiogalactopyranoside at three hours point, then continuing incubation one more hour before chemical treatment. We found that the microplate method can show approximately equal sensitivity to the test tube method with opitimized chemical treatment condition, and measurement condition of β-galactosidase activity. However, in the OY1002/1A1, OY1002/1B1, and OY1002/3A4, the sensitivity to the microplate method was lower than that to the test tube method. These results suggest that the OY1002/1A2 strain provides the possibility of high-throughput *umu* test system.

DD140

COVALENT BINDING OF AGT TO DNA THROUGH A BIS-FUNCTIONAL AGENT AND ITS POSSOBLE IMPLICATION TO HUMAN CARCINOGENESIS

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1, 2 dibromoethane (DBE) belongs to a large class of potentially hazardous compounds, the haloalkanes and is known to be both mutagenic and carcinogenic in experimental animals and probably carcinogenic to humans. The cytotoxicity of DBE was increased in bacteria overexpressing the human or bacterial AGT. AGT repairs the DNA damage produced by n-nitroso compounds by removing the alkyl groups, mainly from the O6 position of guanine in a stoichiometric and autoinativating way and transfers it to a Cys-residue in its active site. In vitro studies showed that DBE is a substrate for AGT but it can also react with the DNA via its second nucleophilic site. Thus, a DNA-DBE-AGT complex is formed which might be toxic and mutagenic for the cells. We have used a Hela tet-on system that leads to a 10-fold overexpression of AGT in the presence of doxycycline in order to verify that the AGT context in isogenic cell lines is a major determinant of the cytotoxicity of DBE. Cell viability methods (MTT and Trypan Blue Assay) were used to test the growth retardation that DBE provoked to cells and showed that the LD20 of cells overexpressing the AGT was 60% lower than those that didn't express it at all. To study the formation of the DNA-DBE- AGT complex three methodologies were used: a) Dot blot analysis where DNA was extracted and hydrolyzed and the AGT protein was detected by an anti-AGT antibody (ab), b) Immunoprecipitation of AGT followed by DNA detection using an anti-DNA ab and c) DNA isolation followed by western blot analysis for AGT detection. Coincubation of DNA, purified AGT and DBE gave a strong positive signal for the formation of the complex utilizing all the above mentioned methods. Dibromopropane also mediated DNA-AGT complex formation though to a lesser extent while dibromopentane did not show any measurable interaction. Detection of the DNA-DBE-AGT complex after treatment of HeLa cells has so far not been achieved but efforts to do so are continuing. It is hoped that this will permit us to explore the biological implications of the formation of AGT-DNA crosslinks.

DD141

PROTECTION OF STRIATAL CELLS EXPRESSING A MUTANT HUNTINGTIN GENE BY THE HYDROLASE HMTH1

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Huntington disease (HD) is an autosomal dominant neurodegenerative disease in which a high level of the 8-oxo-7,8-dihydroguanine (8-oxodG) oxidized DNA base has been reported in affected neurons. This can occur either through direct oxidation of DNA guanine or *via* incorporation of the oxidized nucleotide during replication. hMTH1 is the major human hydrolase that degrades oxidized purine nucleoside triphosphates to the corresponding monophosphates to minimize their incorporation. We previously demonstrated that mice overexpressing hMTH1 are protected from the mitochondrial toxin 3-nitropropionic acid (3-NP) induced neurodegeneration in a mouse model for HD (DeLuca G. et al, PLoS Genet. 2008). To further clarify the relationship between oxidative stress and neurodegeneration we examined the effects of hMTH1 in an *in vitro* model of HD. SV40 large T antigen-immortalized progenitor striatal cells from mutant knock-in mice expressing the expanded CAG repeats of the *huntingtin (htt)* gene (Hdh^{Q111}) were transfected with the *hMTH1* cDNA and compared to Hdh^{Q7} cells containing a CAG repeat of wild-type length. In clonal assays hMTH1 provided a strong protection against the selective vulnerability of 3-NP towards Hdh^{Q111}cells. A similar protection was observed against cytotoxicity induced by H_2O_2. To investigate the selective mechanism(s) underlying these protective effects, we measured oxidative DNA damage at nuclear level and in mitochondria (mt). In all cell lines, basal levels of 8-oxodG were found to be 1.5-2 fold higher in mtDNA in comparison to nuclear DNA. Expression of the mutant *htt* gene in Hdh^{Q111} cells was associated with a 1.5 fold increase in mtDNA oxidation and hMTH1 decreased this to wild-type levels. Following exposure to 3-NP, increased oxidation of mtDNA was observed in Hdh^{Q111} cells in comparison to wt cells (1.5-fold), while levels of 8-oxodG were sensibly lower in nuclear DNA. hMTH1 expression in Hdh^{Q111} cells decreased mtDNA oxidative damage induced by both these oxidants. In accordance with a limitation of mtDNA oxidative damage provided by hMTH1 expression, an increase in the efficiency of amplification of mtDNA was observed in Extra Long-PCR experiments.

DD142

EFFECT OF ATM AND DNA-PK INHIBITION IN THE FORMATION OF CHROMOSOMAL ABERRATION IN PROFICIENT AND DEFICIENT DSB REPAIR CHO CELLS

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DNA double strand break (DSB) is the main DNA lesion responsible for the formation of chromosomal aberrations (CA). Cell-cycle checkpoints and DNA repair mechanisms take part in signaling and converting DSBs into CA. There are two major DNA repair mechanisms of DNA double-strand break (DSB). Homologous recombination (HR) is an error free system that takes advantage of the homologous sequence to repair DSBs. Non Homologous End Joining (NHEJ) is an error prone process that constitutes the main DSB repair pathway in eukaryotic cells, whose principal component is the DNA-dependent protein kinase (DNA-PK). ATM, a member of the phosphatidylinositol (PI) 3-kinase-related family of protein kinases, plays a key role in regulating the genotoxic stress response by sensing DNA damage, activating cell-cycle checkpoints and participating in DNA repair processes. In order to get an insight into the role played by ATM and DNA-PK in the formation of CA, the general PI3-kinase inhibitor Wortmannin (WM) as well as the specific ATM inhibitor KU-55933 (KU) were used in DSB repair proficient and deficient CHO cells exposed to X-. WM or/and KU inhibitors were added one hour prior to X-irradiation and kept for three hours after. A BrdUrd pulse after X-irradiation was given in order to analyze CA only in cells treated in G1 stage. Western blot analysis employing an antibody that recognizes the phosphorylated serine at 1981 site of ATM (pATM) showed a lower inhibition of pATM in WM treated cells than in KU treated ones. A significant increase in the frequency of chromosomal type aberrations (fragments and exchanges) was found after X-irradiation in the presence of WM or KU in comparison with X-irradiation alone in normal CHO cells, while a slight increase was observed in DNA-PK deficient cells. The increase of exchange type of aberrations followed by ATM and NHEJ inhibition indicate that mechanisms other than these two could also be involved in the formation of chromosomal rearrangements during G_1 phase. Few G_1 x-irradiated cells with both chromosome and chromatid type of aberrations were observed in normal cells exposed to WM or KU, while a higher frequency of cells with both types of CA were found in DNA-PK deficient cell line either exposed or not to WM or KU.

DD143

PROTEOMICS AND SPR IMAGING APPLIED TO PLATINATED DNA INTERACTOME STUDIES.

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Background / Aims: many anti-cancerous treatments directly alter tumour cell DNA by forming covalent addition products with its bases, as well as DNA-DNA and DNA-protein crosslinks. Questions still need to be addressed regarding which proteins are involved in the recognition, repair or bypass of these chemical lesions. The goal of our study is to establish a molecular cartography of proteins involved in these processes for a specific damage. DNA lesions caused by *cis*-diamminedichloroplatine (II) (cisplatin) are well-characterized, with a majority composed of intrastrand GpG crosslinks. In addition, several proteins such as nucleotide excision repair components or high-mobility-group proteins are already known for their capability to get directly or indirectly bound to cisplatin adducts. We therefore chose cisplatin as our model of damaging agent. Methods: we have adapted a simple and straightforward ligand-fishing method to capture DNA-binding proteins. It consists of linearized plasmid DNA linked to magnetic beads by a streptavidin-biotin interaction. Following exposure of this system to a DNA-damaging agent, it will serve as a trap for proteins contained in cellular lysates and which are associated with chemical lesions. A subsequent proteomics analysis on the recovered proteins will allow their identification. A complementary SPR imaging approach has been optimised to study in real time and simultaneously the interaction of purified proteins with various platinated DNA probes. Results: our ligand-fishing and SPR imaging approaches allowed the analysis of proteins such as HMGB1 and DDB2. In particular, we were able to show dissimilar binding characteristics of HMGB1 with double-stranded control DNA, single-stranded control DNA, cisplatin damaged DNA and oxaliplatin damaged DNA. Conclusion: the original tools developed in this preliminary study showed their usefulness to improve our understanding of damaged DNA / protein interactions. They will be applied to other DNA lesions biologically less characterized than those generated by cisplatin.

DD144

ROSUVASTATIN PROTECTS FROM ANGIOTENSIN II - INDUCED OXIDATIVE STRESS AND GENOMIC DAMAGE

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Rosuvastatin (RSV) is a synthetic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, widely available for use in the management of hyperlipidaemia. It has a more potent lipid-lowering efficacy than the other available statins, and significantly more patients receiving RSV achieve LDL-C goals. Many clinical trials have shown that statins substantially decrease cardiovascular morbidity and mortality in patients with and without coronary disease. Many of the actions of statins are thought to be mediated by decreasing reactive oxygen species (ROS) formation. Mainly ROS derived from NADPH oxidase play a critical role in Angiotensin II- (Ang II) mediated hypertension, cardiac hypertrophy, fibrosis and remodeling in the heart and vasculature. Epidemiological studies exploring the connection between hypertension and cancer incidence found higher cancer mortality in hypertensive patients and an increased risk to develop kidney cancer. Recently we have reported that Ang II induces genomic damage in mammalian cells and isolated mouse kidney, most likely via oxidative mechanism. We have also shown that DNA damage induced by unphysiologic oxidants like phorbol myristate acetate (PMA) was reduced to control values by RSV concentrations starting from 10 nM. rosuvastatin restored glutathione levels in cells treated with PMA. Now we report that rosuvastatin protects Ang II induced superoxide anion radicals by dihydroethidium (DHE) staining and DNA damage by single cell gel electrophoresis (SCGE, comet assay). Further in molecule level, rosuvastatin protects the formation of Ang II induced 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) adduct in LLC-PK1 cells by LC-ESI-MS/MS method. Most interestingly RSV prevents chromosomal damage (micronucleus; MN) in LLC-PK1 at 1 µM concentration.

DD145

FLOW CYTOMETRIC ANALYSIS OF MICRONUCLEI IN RAT PERIPHERAL BLOOD: AN INTERLABORATORY EVALUATION

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Concern over selective removal of micronucleated red blood cells from the peripheral circulation by the spleen has limited the use of peripheral blood measurements in micronucleus test using rats. Flow cytometric scoring methods have been reported indicating that measurements in rat peripheral blood may in fact be reliable if scoring focuses on the youngest reticulocytes and if a sufficient number of cells are scored. An interlaboratory study was performed to determine if replicate samples analyzed at BSRC and Litron were different or similar. Sprague-Dawley rats were treated with cyclophosphamide (5, 10 or 15 mg/kg/day) by a single oral administration. A blood sample was collected from each rat at 48hrs after dosing. The samples were prepared in duplicate using the Rat MicroFlow^{PLUS} Kit, and one replicate of each was analyzed in both laboratories. The interlaboratory data of flow cytometric analysis results showed good correlation ($r = 0.97$), although the instrument used at each site was different. In addition, the flow cytometric data were similar to data obtained from scoring peripheral blood samples stained supravitally with acridine orange, using microscopy. Based on the above results, if the instrument settings are adjusted using the malaria biostandard, samples can be analyzed by flow cytometry at different laboratories without problems.

DD146

EFFECTS OF DIFFERENT GENOTOXIC SUBSTANCES ON THE PHOSPHORYLATION OF P53 IN HEPG2 CELLS

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p53 and p21 are key proteins in the reaction of cells to genotoxic stress, initiating, and or modulating key pathways such as apoptosis and cell cycle. In various publications the dysregulation of expression, and or phosphorylation of these proteins by chemicals which are also genotoxic are described. Therefore differences of chemicals in alteration of expression of p21 and expression and phosphorylation of p53 at different sites might be a tool to help predicting the genotoxic mode of action of a chemical. In expression profiling studies in the p53 competent cell line HepG2 we found no alteration of expression for p53 independent of the genotoxic treatment whereas p21 was induced by Etoposide (EP), Griseofulvin (GF), Bleomycin (BM) Benzo[a]pyrene (BP) and MNU. Whether p53 phosphorylation sites were also phosphorylated differently by genotoxins acting by different mechanisms, was therefore tested with the chemicals EP, GF, BM, BP and MNU representing different genotoxic modes of action. The substances were tested in the in vitro micronucleus assay and the alkaline COMET assay to determine concentrations genotoxic in HepG2 cells. Phosphorylation of p53 was measured at serines 15, 46, and 392 at concentration clearly posi-

tive in genotoxicity assays. Commercially available antibody kits specific for the respective phosphorylated serine were used for testing. Micronucleus and or COMET assays revealed the expected results for all test substances. However significant differences between the substances were found in the phosphorylation tests. BM was the most potent inducer of phosphorylation of p53 at serines 15, 46, and 392. MNU significantly induced phosphorylation of p53 only at serine 392. EP mainly induced phosphorylation at serine 15 on p53. GF induced phosphorylation of serine 46 only whereas with BP no significant effect was observed at any phosphorylation site of p53. This result suggests that phosphorylation of different serines on p53 seems to be substance and probably mechanism dependent in HepG2 cells. Whether these effects might be useful in future to help characterize the genotoxic mode of action of substances with unknown genotoxic mechanisms will have to be further investigated.

DD147

THE ROLE OF NER DAMAGE RECOGNITION FACTORS IN THE REPAIR OF OXIDATIVE DNA LESIONS

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Several lines of evidence indicate that functional overlap between different repair pathways occurs frequently to guarantee genomic stability. Base excision repair (BER) is the strategy of election for oxidative damage repair. Nucleotide excision repair (NER) has been evolved to excise mutagenic UV photoproducts but is able to detect a wide range of DNA modification by detection of abnormal DNA fluctuations. The recognition of damage is accomplished by XPC and XPA in Global genomic repair (GGR) and by XPA, CSB and CSA in Transcription coupled repair (TCR). Several lines of evidence indicate that endogenous/oxidative lesions are NER substrates but this information is far from being complete and/or lacks of mechanistic insights. To further clarify the role of the different NER players in oxidative DNA damage repair, we took advantage of a set of mouse embryo fibroblasts (MEF) defective in one or two NER factors. HPLC/ED analysis showed that CSB as well as XPA MEF, treated with the oxidizing agent potassium bromate, are defective in the repair of 8-oxoguanine (8-OH-Gua) and that MEF lacking both proteins accumulate more damage than the single mutants. These data indicate that XPA and CSB both participate to the repair of 8-OH-Gua and suggest that they are involved in two different repair pathways. The accumulation of 8-OH-Gua was also confirmed in potassium bromate treated XP-A primary fibroblasts when compared with normal fibroblasts (50% and 70% repair at 2 hr after treatment, respectively). Moreover XP-A fibroblasts showed a marked hypersensitivity to the toxic effect of oxidation. To investigate whether XPA plays a role in DNA damage recognition by interacting with OGG1, the main 8-OH-Gua DNA glycosylase, double XPA/OGG1 human fibroblasts were constructed by shRNA. Experiments are in progress to characterize their repair ability and mode. A role of XPA in the oxidative damage recognition/repair could account for neuronal degeneration that characterizes XP-A patients as well as mice lacking both XPA and CSB.

DD148

THE ROLE OF COCKAYNE SYNDROME PROTEINS IN THE REPAIR OF ENDOGENOUS DNA DAMAGE

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Cockayne syndrome (CS) patients present neurological abnormalities with demyelination as predominant damage. CS proteins have a main role in transcription-coupled repair (TCR) by assisting RNA polymerase II in dealing with DNA damage-induced transcription blocks. Recent evidence suggest that CSA and CSB proteins might play additional function(s) in transcription and in the removal of oxidative damage such as 8-oxoguanine (8-OH-Gua) and cyclopurines and that these alterations might be of relevance in the type and severity of CS clinical symptoms. In this study we identified another class of lesions that involve CS proteins in their repair, namely DNA single-strand breaks (SSB). CS-A and CS-B human fibroblasts treated with alkylating or oxidizing agents presented a higher number of SSB that persisted for longer times than in normal fibroblasts, as measured by the comet assay. This defect was fully complemented by transfection with the wild type genes. To gain mechanistic insights, oxidative DNA damage repair was analysed in a battery of *Csa*- or *Csb*-null mouse embryo fibroblasts (MEFs) with additional deficiencies in nucleotide excision repair or base excision repair (BER) genes. Wild-type MEF fully repaired 8-OH-Gua within 2 hr after treatment with potassium bromate. The repair kinetics of mutant MEF was monitored within the same time frame. *Csa* and *Csb* MEFs accumulated 8-OH-Gua in their genome at a similar extent as *Xpc*-null MEFs. The lack of OGG1 caused a more significant accumulation of 8-OH-Gua confirming that this is the major repair mechanism for this oxidized guanine. *Csb* MEFs with an additional deficiency in XPC showed increased DNA oxidation levels as compared with *Csb* single mutant MEFs, suggesting that the involvement of CSB protein in 8-OH-Gua repair is XPC-independent. The absence of OGG1 together with the additional defect in CSB did not lead to a further increase in DNA oxidation levels when compared to *Ogg1* mutant MEFs. A plausible interpretation of these data is that the cleavage by OGG1 is required to affect CSB-mediated DNA repair of 8-OH-Gua. All together, these findings identify the involvement of the CSB protein in the processing of AP sites/SSB that are transcription-blocking lesions, thus suggesting a possible mechanistic link between TCR and BER.

DD149

SIGNALLING AND REPAIRING DNA DAMAGE IN DIFFERENT PHASE OF CELL CYCLE

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Cosmic represent a serious risk for humans during space travels outside the Earth's magnetic field. About 90% of the cosmic are hydrogen protons, about 9% are alpha particles, and all of the rest of the elements make up only 1%. Protons, as well as other particles and photons, induce DNA strand breaks, and in addition abasic and oxidized bases. The DNA double-strand breaks (DSBs) represent the most severe damage for the genome integrity and are the main lesion induced by ionizing radiation. There are two major pathways of DSB repair: non-homologous end joining (NHEJ) and homologous recombination (HR). In *Saccharomyces cerevisiae* cells, DSBs induced by exposure to protons were predominantly repaired via HR and postreplication repair pathways. The aim of this work is to analyse the contribution of HR and NHEJ in DNA DSB repair of mammalian cells, after irradiation with different doses of γ-rays and low-energy protons. We used a human cell line of lung fibroblasts (CCD-34Lu) in which we studied the kinetics of induction and repair of DSB by *in situ* immunofluorescence, following the formation and disappearance of ionizing radiation-induced foci (IRIF) of γ-H2AX and 53BP1. We analysed by confocal microscopy the presence of IRIF in relation to an S/G2/M phase marker, CENP-F, a protein of the nuclear matrix that is expressed in

late S, G2 and M phase cells. After irradiation with 0.5 and 5 Gy of γ-rays, CENP-F^{+} cells presented more foci than CENP-F^{-} cells at all times analysed, without differences between high and low dose. Moreover, the percentages of cells with different number of IRIF (0, 1-4, 5-9, 10-19, 20-30 foci/nucleus) show that in G2 cell population are present cells (near 5%) with a high number of foci/nucleus (20-30) also after 24h from irradiation as well as in non-irradiated cells. In CENP-F^{-} cell population the percentage of cells without foci or with very few foci was about 85% after 24h from irradiation. The results obtained after irradiation with γ-rays show that both HR and NHEJ contribute to DSB rejoining and the cells with a high foci number are probably due to the presence of DNA replicating cells. After proton irradiation with 0.5 Gy the results are similar to that of γ-irradiation; the experiments with 5 Gy are in progress.

DD150

POLO-LIKE KINASE - REGULATION OF DNA REPLICATION UNDER STRESSED CONDITIONS

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Complex mechanisms coordinate DNA replication, DNA repair and chromosome segregation to maintain genomic stability. If these mechanisms are failing cells can loose their identity and organisms are prone to tumour development. A very powerful system to study the biochemistry of the major pathways involved in the maintenance of genomic stability is Xenopus laevis egg extract. This model system was previously proved to discover insights in the function of ATM and ATR in preventing the accumulation of DNA double strand breaks during DNA replication. Another protein playing an important role during chromosomal DNA replication is Polo-like kinase (Plk) 1. Plk1 is a multifunctional protein, essential for cell cycle progression. In many tumours Plk1 is overexpressed. A role for Plk1 during mitosis has been established by many studies showing absence of Plk1 impairs mitotic progression. However, although Plk1 is expressed throughout the cell cycle, its function during S-phase is unknown. Using Xenopus laevis egg extract as model system, our findings reveal an unanticipated role for Plx1 in the regulation of S-phase progression under stressful conditions. We demonstrated that Plx1, the Xenopus orthologue of Plk1, is required for DNA replication in the presence of stalled replication forks, which can arise spontaneously during unchallenged replication. When a replication forks is stalled, Plx1 is located to the chromatin by binding to the Mcm complex through its Polo-box domain. For this recruitment of Plx1 to the chromatin ATR-dependent phosphorylation of serine 92 of Mcm2 is required. Plx1 then suppresses the ATM/ATR-dependent intra-S-phase checkpoint that inhibits origin firing. This function of Plx1 ensures the completion of DNA replication and prevents the loss of genetic information. Additionally, depletion of Plx1 leads to accumulation of chromosomal breakage as shown with the comet assay. These data suggest that Plx1 promotes genome stability by regulating DNA replication under stressful conditions.

DD151

CITOTOXICITY ANALYSIS AND APE1/REF-1 EXPRESSION INDUCED BY OXIDATIVE STRESS IN NUCLEOTIDE EXCISION REPAIR PROFICIENT AND DEFICIENT CELLS

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Reactive oxygen species (ROS) such as hydroxyl radical, hydrogen peroxide and singlet oxygen are the most abundant toxic endogenous agents in aerobic organisms. Also, the exposure of some pigments, such as methylene blue and riboflavin, to light is able to generate ROS by the absorption of light energy. These ROS are involved in oxidative stress and can oxidize biomolecules, including proteins and DNA. The DNA damage repair by the base excision repair (BER) occurs by the action of many enzymes that remove oxidative lesions. It was described that ROS promote the induction of endonuclease APE1/Ref-1, an important BER enzyme, which plays an essential role as a regulator of cellular response to oxidative stress. However, the molecular mechanisms involved in this induction and its regulation have not been well elucidated yet. Furthermore, the involvement of nucleotide excision repair (NER) proteins in removing oxidative damage and stimulating APE1/Ref-1 repair activity has been previously reported. In order to evaluate the citotoxicity and APE1/Ref-1 expression in NER-proficient and deficient cells, different cell lines were treated with different concentrations of H_2O_2, riboflavin plus 33.3 J/cm^2 and methylene blue plus 22.2 J/cm^2. Cell viability was determined by trypan blue dye exclusion assay and APE1/Ref-1 levels were determined by immunoblotting. Our results showed that NER-deficient cell lines appeared to be more sensitive to both agents when compared to proficient cells. Also, differential expression of APE1/Ref-1 according to agent, time after treatment and cell line could be observed. According to the results, it may be concluded that oxidative stress induced by H_2O_2 and photosensitization of riboflavin and methylene blue affect the viability of both cell lines and cells deficient in NER are more sensitive, indicating the involvement of NER enzymes in repair of oxidative damage. Moreover, APE1/Ref-1 expression can be induced after oxidative damage in a differential way, according to the oxidative agent and cell line. Supported by: CNPq, PADCT, Redoxoma, CAPES, UFRN.

DD152

CHARACTERIZATION OF MUTY-GLYCOSYLASE ACTIVITIES IN DNA REPAIR

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Reactive oxygen species (ROS) are byproducts of cellular metabolism which are able to react with biomolecules, such as proteins, lipids and nucleic acids, causing deleterious modifications to cell. Methylene blue (MB) is a photosensitizer that can intercalate on DNA molecule and block replication, transcription and DNA repair. Also, MB can lead to DNA interstrand crosslink.The exposure of MB to light produces ROS, such as singlet oxygen ($^1O_2$), one of the most reactive forms of molecular oxygen. $^1O_2$ carries out the oxidation of guanines, inducing DNA lesions, such as 7,8-dihydro-8-oxoguanine (8-oxoG), the most common oxidation product that during replication may misspair with adenine leading to mutations. The aim of this study was to evaluate citotoxicity, mutagenic potential and pattern of proteins expression during oxidative stress induced by MB plus light. For that purpose, *Escherichia coli* proficient and deficient in MutY-glycosylase (MutY) strains were used. Both strains were treated with MB plus light and mitomycin C (MC), a DNA interstrand crosslinking agent. The growth, survival, mutagenesis rates and protein expression patterns were analyzed. The treatment with MB plus light lead to cell death, mutagenesis and alteration in the protein synthesis patterns in both *E. coli* strains. However, the MutY deficient strain showed a higher sensibility than the proficient one. Interestingly, the treatment with MB in the dark and MC affected the bacterial growth in MutY-deficient strain. This result was not expected, since MutY has not been related to crosslink repair. Taken together, these results suggest the involvement of MutY in the correction of uncharacterized lesions and its absence induces changes in the pattern of protein expression. *Supported by*: CNPq and CAPES.

DD153

ASSESSMENT OF DNA DAMAGE BY THE COMET ASSAY: COMPARATIVE EVALUATION OF METHODS FOR PREPARATION OF SINGLE-CELL SUSPENSIONS FROM MULTIPLE TISSUES OF RATS.

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The comet assay is a rapid, sensitive and inexpensive screening tool for measuring early DNA damage in single cells. The assay is gaining acceptance as a standard technique for evaluating DNA damage in vivo; however, there is no internationally acceptable guideline for the methods used including dissociation of tissue into single cells. This suggests the need for fine-tuning the methods to make them acceptable for regulatory submissions. Thus, we are currently conducting rodent studies not only to gain experience and expertise within FDA in conducting the comet assay but also to address some of the methodological issues. Groups of male F344 rats were treated with 0 or 100 mg/kg MMS by gavage and DNA damage in liver, lung, kidney, bladder, bone marrow and stomach was measured at 3 and 24 hr sampling times using the Perceptive Assay IV Image Analysis System. Single-cell suspensions were prepared from liver, lung and kidney by, a) mincing only using a pair of sharp scissors, b) homogenization or enzyme treatment and c) mincing followed by “Tissue press” dissociation. Cell scrapping and Tissue press methods were used for stomach and bladder. Cell viability was assessed by Trypan blue. Approximately, 10,000 cells were embedded in low melting agarose on microscopic slides and used for comet analysis. For each tissue, 100 cells per slide were analyzed to determine the extent of DNA damage using % DNA in comet tail. The preliminary results indicated that the mincing method displayed positive results in all the tissues and sampling times examined and that the MMS-induced DNA damage was 4-20-fold higher than that found for the untreated controls ($p \leq 0.01$). Untreated control samples showed remarkable background variability in DNA damage with the stomach and kidney displaying the highest damage. All tissues examined at 24 hr by mincing showed around 20-50% reduction in the MMS-induced DNA damage compared to DNA damage measured at 3 hr sampling. The reduction in the damage measured at 24 hr is probably reflective of DNA repair. Statistical analysis of DNA damage from single-cell preparations by homogenization and “Tissue press” are ongoing and these results may help us identify the best method for sample preparation for the comet assay.

DD154

SOMATIC STEM CELLS OF *ATM*-NULL MICE ARE HYPERSENSITIVE TO X-RAYS FOR MUTATION BUT RESISTANT FOR CELL KILLING

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Atm (ataxia telangiectasis mutated) is a gene that plays an important role in DNA repair and apoptosis after double strand breaks in DNA. However, little is still known about its role in stem cells. In the present study, we investigated phenotype of *Atm*-null allele using a system that enables us to in situ monitoring of not only mutations at specific locus in somatic stem cells, but also cell death after ionizing radiation. The *Dlb-1* mutation assay systems homozygous (-/-) for disrupted *Atm* allele, and homozygous for the wild-type allele (+/+) were constructed by mating C57BL, *Dlb-1*^{b}/*Dlb-1*^{b}; *Atm* (+/-) males with SWR, *Dlb-1*^{a}/*Dlb-1*^{a}; *Atm* (+/-) females. Resulting tester mice were irradiated with an X-ray dose of 4 Gy at a dose-rate of 50 cGy/min immediately after birth or at a postnatal age of 10 weeks. Irradiated neonates and adults were subjected to the mutation assay ten and two weeks after irradiation, respectively, where evidence of mutations at the *Dlb-1* locus in small intestinal stem cells was detected as mutant clones on the epithelial tissue of villi, and the frequency (F) and the mean size (S) of mutant clones were determined. F value in the untreated, control *Atm* (-/-) mice relative to that in the wild-type mice in the control was 3.6 ±0.5. The relative F vales for induced clones in the *Atm* (-/-) mice irradiated as neonates and adults were 2.3±0.7 and 7.9±0.9, respectively. These data demonstrate hyper mutability of the *Atm* (-/-) mice both before and after irradiation. S values for clones from the control wild-type and *Atm* (-/-) were 0.08±0.11 and 0.16±0.11, respectively. In comparison with these size data, S values from mice irradiated as adults showed no evidence of stem cells death after irradiation, irrespective of the *Atm* genotype. In contrast, S values from irradiated wild-type and *Atm* (-/-) neonates were 0.30±0.09 and 0.60±0.01, respectively, demonstrating hypersensitivity of *Atm* (-/-) stem-cell precursors to killing effect of radiation. These results support our conclusion that intestinal stem cells of *Atm*-null mice are hypersensitive to X-rays for mutation but resistant for cell killing, and suggest that transition of stem-cell precurcres to stem cell accompanys shutdown of *Atm*-dependent apoptosis pathway in stem cells.

DD155

COMBINING SHAPLEY VALUE AND STATISTICS TO THE ANALYSIS OF GENE EXPRESSION DATA IN CHILDREN EXPOSED TO AIR POLLUTION

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In gene expression analysis, statistical tests for differential gene expression provide lists of candidate genes having, individually, a sufficiently low p-value. However, the interpretation of each single p-value within complex systems involving several interacting genes is problematic. In parallel, in the last sixty years, game theory has been applied to political and social problems to assess the power of interacting agents in forcing a decision and, more recently, to represent the relevance of genes in response to certain conditions. In this paper we introduce a Bootstrap procedure to test the null hypothesis that each gene has the same relevance between two conditions, where the relevance is represented by the Shapley value of a particular coalitional game defined on a microarray data-set. This method, which is called Comparative Analysis of Shapley value (shortly, CASh), is applied to data concerning the gene expression in children differentially exposed to air pollution. The results provided by CASh are compared with the results from a parametric statistical test for testing differential gene expression. Both lists of genes provided by CASh and t-test are informative enough to discriminate exposed subjects on the basis of their gene expression profiles. While many genes are selected in common by CASh and the parametric test, it turns out that the biological interpretation of the differences between these two selections is more interesting, suggesting a different interpretation of the main biological pathways in gene expression regulation for exposed individuals. A simulation study suggests that CASh offers more power than t-test for the detection of differential gene expression variability. CASh is successfully applied to gene expression analysis of a data-set where the joint expression behavior of genes may be critical to characterize the expression response to air pollution. We demonstrate a synergistic effect between coalitional games and statistics that resulted in a selection of genes with a potential impact in the regulation of complex pathways.

References: Moretti S. et al.(2008) Combining Shapley value and statistics to the analysis of gene expression data in children exposed to air pollution. BMC Bioinformatics, 9:361.

DD156

FUNCTIONAL SIGNIFICANCE OF WERNER SYNDROME PROTEIN PHOSPHORYLATION BY ATR FOR FRAGILE SITE STABILITY

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Background: Common fragile sites (CFS) are DNA regions prone to breakage and found rearranged in many tumours. Although the precise mechanism of fragile site expression is still elusive, recently, it has been proposed that these sites are DNA regions where DNA replication forks spontaneously pause or arrest and that their stability largely depends on intact ATR-dependent checkpoint. WRN is the protein mutated in the human Werner syndrome (WS) and belongs to the RecQ family of DNA helicases. We recently established that WRN is a key regulator of fragile site stability and that WRN and ATR act in a common pathway avoiding accumulation of DNA breaks at CFS. Aims: The aim of this study is to elu-

cidate the functional significance of ATR-dependent phosphorylation of WRN for the maintenance of fragile site stability and whether WRN affects checkpoint response at naturally-occurring replication fork stalling sites. Methods: We produced an ATR-unphosphorylable form of WRN (WRN6A) by site-directed mutagenesis and generated WS fibroblasts stably expressing the unphosphorylable protein. FISH analyses were performed to assess the induction of the most frequently expressed CFS in wild-type, WS and WRN6A fibroblasts. Immunofluorescence and Western immunoblotting were carried out to investigate the role of WRN in checkpoint signalling upon perturbation of replication at CFS. Results: Alanine substitutions at putative ATR consensus sites actually abrogated phosphorylation of WRN by ATR following replication arrest or aphidicolin-induced replication slowing at CFS. Furthermore, expression of WRN6A did not rescue the characteristic WS sensitivity to HU or CPT treatment. WRN phosphorylation by ATR was required to prevent accumulation of DNA gaps and breaks at CFS. Even though loss of WRN function did not affect DSB formation at early time-points after aphidicolin-induced replication delay at CFS, checkpoint activation in WRN-deficient cells appeared to occur more precociously than in the wild-type and the effect of WRN6A expression is currently under investigation. Conclusions: This study provides additional insights into the cooperation between WRN and ATR to prevent chromosomal abnormalities in human cells.

DD157
THE WERNER SYNDROME PROTEIN PARTICIPATES IN THE RESPONSE TO ONCOGENE-INDUCED REPLICATION STRESS

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Background: Oncogene activation determines a chronic state of replication stress in human cells. Consistently, precancerous and cancer cells have to face elevated levels of replication fork stalling and collapse. The response to replication perturbation is controlled by the ATR kinase that ensures correct recovery of stalled/collapsed replication forks, in order to avoid accumulation of chromosomal alterations. To promote recovery of stalled/collapsed forks, ATR controls many downstream proteins, included the WRN RecQ helicase/exonuclease, a key genome caretaker. Aims: The aim of this study is to investigate whether the WRN protein and its ATR-regulated function are required to deal with replication perturbation induced by oncogene expression in human cells. Methods: Using adenoviruses to over-express the CyclinE and E2F1 oncogenes, we analysed activation of WRN and DNA damage accumulation. We also performed cell viability assays to determine the role of WRN in cellular recovery after oncogene expression. We investigated whether recovery from replication arrest is affected by loss of ATR-dependent phosphorylation of WRN and the involvement of WRN-alternative pathways. Finally, we performed FISH analysis to evaluate the importance of WRN function, upon oncogene expression, to grant stability at common fragile sites, which are genomic loci that are naturally-sensitive to replication perturbation. Results: Oncogene expression in human cells triggered WRN relocalisation in nuclear foci that colocalised with RPA, but not with the DSB marker g-H2AX histone. Loss of WRN or expression of an ATR-unphosphorylable mutant form of WRN determined an enhanced cell death in response to oncogene-induced replication stress. Furthermore, WRN is essential to ensure chromosomal integrity after oncogene expression. Finally, loss of WRN function led to the requirement of alternative pathways to grant cellular recovery from oncogene-induced replication stress. Conclusions: These results show an essential function of WRN in dealing with oncogene-induced replication stress in order to ensure cell viability and genome integrity. Furthermore, this study provides additional insights into the mechanisms underlining replication for recovery in oncogene-expressing precancerous lesions.

DD158
B[a]P INDUCED S-PHASE ARREST: A DOUBLE-EDGED SWORD

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Background: Benzo(a)pyrene (B[a]P) is an environmental pollutant with known carcinogenic properties. Previous studies have documented that an S-phase arrest occurs in HepG2 cells following exposure to B[a]P. However, neither the exact mechanism nor the consequences of this cellular response are adequately understood. Methods: HepG2 cells were treated with 3 μM B[a]P for 96 hours and subsequently followed for up to 8 days. Cell cycle kinetics and DNA damage responses were analysed using FACS, western blot analysis and immunofluorescence-based confocal microscopy. Results: Following cell treatment with B(a)P, we noted evasion of the G1/S phase checkpoint, followed by induction of S phase arrest, S phase recovery, and induction of the G2/M checkpoint. Western blot analysis showed induction first of the ATR-dependent and subsequently the ATM-dependent pathways, roughly coinciding with the appearance of the above mentioned cell-cycle checkpoints. Confocal microscopy using anti-BPDE-DNA adduct antiserum showed that induction of DNA damage was followed by accumulation of γH2AX foci and subsequent formation of ATM and ATR foci. These observations, which were in accordance with our data derived from the western blot analysis, also revealed accumulation of severe DNA damage on the chromosomes, a fact that may be related to the observed genetic instability and appearance of abnormal mitotic events at high frequency. Moreover the abrogation of S-phase arrest by the chemical chk1 inhibitor UCN-01, instead of increasing the frequency of abnormalities caused by B(a)P, as might have been expected, triggered a strong apoptotic signal while the surviving cells surprisingly showed greatly decreased genetic instability. Conclusions: Although S-phase arrest is considered to be a mechanism by which the cell can reduce its load of genetic damage, our results suggest that it may have an unpredictable impact on the balance between cell death and heritable genetic damage. Acknowledgement: This work was partly supported by the EU ECNIS (Environmental cancer risk, nutrition and individual susceptibility) Network of Excellence

DD159
TISSUE-SPECIFIC ACCELERATED AGING: THE IMPACT OF DNA DAMAGE IN THE WHITE ADIPOSE TISSUE OF PROGEROID ERCC1-/- MICE

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Background. Genome instability may causally contribute to ageing, as exemplified by the premature appearance of multiple age-related features in a growing family of human syndromes and associated mouse models. ERCC1 is an endonuclease required for nucleotide excision repair (NER) and the repair of DNA interstrand crosslinks (ICLs). Ercc1-/- mice develop normally but rapidly display numerous age-related features, including ataxia, kyphosis, loss of subcutaneous fat, weight loss, skin atrophy and sarcopenia. Methods. To delineate the causal role of DNA damage in (accelerated) aging, we sought to restrict ERCC1-driven progeria in a single tissue such as the white adipose tissue (WAT), a major energy reservoir in mammals that plays a central role in the regulation of mammalian longevity. Using the Cre-LoxP system, we crossed the Ercc1-floxed mice with the Fabp4-Cre mice to obtain the WAT-specific Ercc1-/- animals. Results. At the organismal level, the one-month old WAT-specific animals appear healthy with no pathological symptoms. The great majority of animals were lean and occasionally they also presented with reduced body weight. As expected, Ercc1 mRNA levels were substantially reduced in the adipose tissue of WAT-specific Ercc1-/- animals as compared to controls. However, the Ercc1 gene expression levels in the adipose tissue of either WAT-specific or wt littermate controls were significantly higher than those seen in the liver, kidney or spleen derived from the same ani-

mals. In combination with the absence of adipose tissue in Ercc1-/- animals, this further supports the relevance of the Ercc1 gene to the WAT physiological function. At the cellular level, exposure of wt preadipocytes to mitomycin-induced ICLs suppressed substantially their differentiation into mature adipocytes as evidenced by the suppression of known WAT differentiation markers and the complete lack of stored triglycerides. Conclusions. The generation of tissue-specific progeroid animals appears to be an invaluable approach to systematically delineate the complex, age-related pathology that rapidly develops in NER progeria. Future experiments will focus on the proteome and genome-wide expression analysis of Ercc1 adipose tissue coupled to a number of physiological and in vitro assays.

DD160

UNDERSTANDING THE CELL DECISION PROCESS IN RESPONSE TO THE DNA LESION O6METHYLGUANINE

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The cellular response to DNA damage is determined by the coordinated activity of signaling molecules from a number of pathways including stress response, survival, checkpoint signaling, DNA damage repair, and apoptotic pathways. How a cell deciphers this complex intracellular signaling network to yield a par-ticular phenotypic response is unclear. The goal of this project is to understand the response to O6methylguanine, a DNA base lesion commonly induced by chemotherapeutic agents, at the molecular and cellular level by integrating experimental and computational approaches. The signal transduction network downstream of this damage is largely regulated by dynamic protein phosphorylation events. Experiments have been done to monitor the time-dependent activation of a number of proteins in the DNA damage network following treatment with the alkylating agent MNNG. IP kinase activity assays revealed that the DNA damage kinase Chk2 and stress activated protein kinase JNK are activated in response to MNNG. In addition, the proteins ATM(S1981), Chk2(T68), Chk1(S317), p53(S15 and S20), and H2AX(S139) were found to be phosphorylated at sites which are linked to their activation. In the same manner cell cycle progression and cell death have been dynamically monitored using a variety of experimental techniques. MNNG induces an arrest in the second S phase after treatment with the drug followed by apoptotic cell death. Both the cell cycle response and cell death response are dependent on O6MeG and mismatch repair. Interpretation of this data through experimentally-based computational models will allow us to identify combinations of signals which are most predictive of the cellular response to the O6MeG base lesion. To test model predictions we will perturb the network by knocking down key signaling network proteins. Comparison between in silico and experimental perturbations of the network will permit us to optimize the predictive power of our computational model.

DD161

SEARCH FOR DNA REPAIR INHIBITORS: RATIONAL DESIGN AND PROPERTIES STUDY OF INHIBITORS FOR HUMAN OGG1 AND APE1 ENZYMES

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In mammalian cells, reactive oxygen species, alkylation and other processes and/or exogenous drug toxicity can cause endogenous mutagenic and cytotoxic DNA base lesions that are predominantly repaired by the base excision repair (BER) pathway. BER is initiated by lesion-specific glycosylases (particularly hOgg1) that excise the damaged base from the sugar-phosphate backbone, resulting in a potentially cytotoxic apurinic/apyrimidinic (AP) site intermediate that becomes the substrate for the major human AP endonuclease (Ape1). Evidence for Ape1 and Ogg1 as attractive therapeutic targets in anticancer drug development recently has been demonstrated by studies that link expression of these enzymes in many cancers to resistance of tumor cells to radio- and chemotherapy. Moreover, Ape1 also shows a protective effect in several cancer cell models to a variety of DNA damaging agents. In this work we present the rational design of selective small-molecule hOgg1 and Ape1 inhibitors. We have suggested to use 6-amino-4-chloropirazolo-[3,4-d]-pyrimidine as inhibitor for human Ogg1. By using in gel separation of enzyme's reaction products was shown, that the compound effectively interacts with hOgg1 and already in low concentration can inhibit enzyme activity. About twenty potential compounds, capable to modulate catalytic activity of Ape1 were designed and synthesized. Primary screening of compounds was carried out and some of them were selected for the further studying. In summary, we have identified small molecule inhibitors for the Ape1 and Ogg1 DNA repair enzymes. The data obtained allow us to conclude that the new generation of compounds for probing the BER process in general in vitro and in vivo were designed. This work was supported by grants from the Russian Foundation of Basic Research (07-04-00191, 08-04-00334, 08-04-12211), Russian Ministry of Education and Science (NSch-652.2008.4.), and the Siberian Branch of the Russian Academy of Sciences (28, 48, 90, 21.22).

DD162

DNA REPAIR MECHANISMS IN PAEDIATRIC INFLAMMATORY BOWEL DISEASES: POTENTIAL CLINIC IMPLICATIONS

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Inflammatory Bowel Diseases (IBD) are relapsing forms of chronic inflammatory disorders associated with predisposition to colorectal carcinoma (CRC). Both microsatellite (MSI) and chromosomal instability (CIN) have been observed in IBD cases and can be both detected early in dysplastic, premalignant tissues. Although MSI is a well known molecular feature of mismatch repair (MMR)-defective tumours a new mechanistic link between MSI and another DNA repair mechanism, the base excision repair (BER), has been recently suggested. In IBD colon cells, the overexpression of specific players of BER, the major pathway responsible for the removal of oxidative DNA lesions, might be a defence response to the "oxyradical overloaded microenvironment" generated by inflammatory response. Fresh biopsies from macroscopically normal and inflamed areas of colon tissues have been collected from 66 paediatric IBD patients during routine colonoscopies including 52 patients with ulcerative colitis (UC) and 14 patients with Crohn's disease (CD). MSI was observed in 40 % of paediatric IBD cases. As expected, preliminary data of gene expression analysis indicate that inflammation related gene expression levels, such as INF□, PTGS2, NOS2A were able to provide a statistically significant discrimination between inflamed and normal mucosa. In contrast to what observed in familiar and sporadic CRC, MSI phenotype observed in IBD patients is completely independent of deregulation of MMR genes. The potential contribution of BER-dysfunction to the IBD associated-MSI phenotype is currently under investigation. In order to evaluate the levels of oxidative DNA damage in IBD patients, the 8-hydroxydeoxyguanosine (8-OHdG) residues in DNA extracted from inflamed colon tissues compared to the normal counterpart of the same patient have been measured by HPLC-EC. A trend towards increased 8-OHdG levels in inflamed versus normal tissue has been observed suggesting a possible role of 8-OHdG as inflammation marker. In order to identify valuable prognostic markers predictive of response to therapy and risk of cancer development, association studies between molecular and clinical characteristics of IBD patients are currently in progress.

DD163

THI1 ARABIDOPSIS MUTANT REVEALS IMPAIRED TOLERANCE TO H2O2 TREATMENT.

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The A. thaliana thi1 gene is involved in thiamine biosynthesis and was firstly described as having a potential role in genome stability. A. thaliana thi1 dual role was investigated in this work with a collection of physiological, biochemical and molecular approches, using a wild-type and a previously characterized mutant line. Expression analysis demonstrates that the gene is responsive to light and possibly under the control of the circadian clock. Seed stocks of mutant line presented lower germination rate and higher sensitivity to flooding stress comparatively with the wild-type (w.t.). Proteomic analysis showed an overproduction of phosphoglycerate dehydrogenase (PGDH) and enolase in the mutant line compared to the w.t.. PGDH is involved in antioxidants synthesis while enolase blocks cellular growth and redirect cell metabolism to programmed cell death. Chloroplast genome DNA damage was investigated and reveals that the mutant line is less tolerant than the wild type genotype to the presence of hydrogen peroxide. All these data together strengthen the hypothesis that THI1 is involved in DNA repair/tolerance since plants with defective THI1 are more susceptible to cpDNA damage.

DD164

A NEWLY IDENTIFIED XP-E PATIENT HAS MUTATION IN DDB1-DOCKING MOTIF AND IMPAIRED UV-DDB-BASED UBIQUITIN LIGASE ACTIVITY

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Ubiquitination has emerged as an important regulatory mechanism in the initiation of NER in non-transcribed DNA. Attachment of either a single ubiquitin (Ub) or a poly-ubiquitin chain to a target protein is a multi-step process involving Ub-activating enzyme (E1), Ub-conjugating enzyme (E2), and Ub-ligase (E3). The UV-DDB-based ubiquitin ligase (DDB1-CUL4A^{DDB2}) has a central role in cellular response to UV-irradiation. DDB1-CUL4A^{DDB2} co-localized with UV-damaged DNA and targets for ubiquitination the proteins that are involved in recognition of UV-distorted DNA and the surrounding histones. The mono-ubiquitination of the histone is postulated to relax the chromatin structure around the lesion and facilitates formation of the preincision complex. UV-DDB is comprised of DDB1 and DDB2 proteins and mutations in DDB2 cause the XP-E phenotype, which has a partial deficiency in global genome repair. We report the identification of a XP-E patient (PX31PV), with a novel mutation in the DDB2 gene. The patient carries a genomic DNA deletion, which results in several cDNA alterations: One of the products is DDB2 protein with a loss of Lys268 (delK268). Ectopically expressed delK268 protein fails to make heterodimer with DDB1 protein and, consequently, abrogates the formation and UV-induced binding of the DDB1-CUL4ADDB2 ubiquitin ligase to the chromatin. The XP31PV cells have impaired mono-ubiquitination of histone H2A and poly-ubiquitination of XPC after UV-treatment. The final consequence of dysfunctional ubiquitin ligase is deficient NER in the XP31PV cells. This finding, in addition to the previously characterized R273H DDB2 mutant (in XP2RO patient) offers very important experimental support for the recent prediction that a conserved "WDXR" motif within the WD40 domain in DDB2 is required in the formation of the interface between DDB1 and DDB2 proteins. Importantly, our crystal structures of the human UV-DDB complex, confirms the roles of Lys268 and Arg273 in the formation of the DDB1-docking surfaces on DDB2. Our finding demonstrates that DDB2, like the other DDB1 and CUL4 binding proteins, contains the "WDXR" motif which facilitates interaction with DDB1.

DD165

ROLE OF NER GENES IN THE DNA DAMAGE RESPONSE OF TERMINALLY DIFFERENTIATED CELLS EXPOSED TO DNA SINGLE STRAND BREAKS INDUCING AGENTS

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NER (nucleotide excision repair) as well as BER (base excision repair) are generally down-regulated in terminally differentiated cells. However, cells maintain active NER in the genomic domains where transcription is active and this repair relies upon the GGR (global genome repair) genes. No information is available on the mechanism of BER in transcription domains.Murine muscle satellite cells were isolated from CSB -/-, XPA-/- and XPC-/- mutant mice. These cells were able to differentiate in vitro thus providing a tool for studying the effects of specific NER defects in the DNA damage response in terminally differentiated cells. The downregulation of XRCC1, a marker of decreased BER activity, was confirmed in these NER defective myotubes. For this study two model agents were selected, namely H2O2 and camptothecin. They both induce DNA ssb but in the case of camptothecin, which is an inhibitor of DNA topoisomerase I, they are specifically targeted to the template strand of transcribed genes. H2AX phosphorylation is an early event in DNA damage signalling and plays a major role in DNA repair and apoptosis. Gamma H2AX foci were monitored after treatment to SSB inducing agents of wild-type and NER defective myotubes. Exposure to H2O2 induced H2AX phosphorylation in all myotubes independently of the DNA repair defect, although to a different extent. Conversely, a defect in either TCR (transcription coupled repair, for CSB, XPA) or GGR (XPA, XPC) led to a lack of H2AX posphorylation following exposure to camptothecin in myotubes. Under the same treatment conditions wild-type myotubes displayed the expected activation of H2AX. All together these findings suggest that NER damage recognition factors are involved in the signalling of SSB induced by camptothecin. Work is in progress to clarify the underlying molecular mechanisms.

DD166

FUNCTIONAL CHARACTERIZATION OF MUTANT MUTYH PROTEINS ASSOCIATED WITH FAMILIAL ADENOMATOUS POLYPOSIS.

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A defective human adenine DNA glycosylase (MUTYH) activity results in accumulation of G to T transversions and is associated with a recessive form of colorectal adenomatous polyposis (MUTYH-associate polyposis, MAP). With the aim to set up a suitable assay for predicting the contribution of rare MUTYH variants to the disease, a real time assay has been set up. Surface plasmon resonance (SPR) spectroscopy has been applied for real-time detection of the binding properties of wild type and some MUTYH variants to an 8-oxoG:A DNA substrate. In this respect the properties of three MUTYH mutants car-

rying a missense (R171W), a deletion (E466del) and an insertion (ins137IW) mutation were compared to the wild type MUTYH enzyme. The study was also applied to the widely studied Y165C and G382D variants. The SPR results are here discussed together with those obtained with the more conventional gel electrophoresis mobility shift (EMSA) and glycosylase activity assays. Studies are also in progress to set up the SPR method to study the functional properties of DNA-glycosylases in cell-free extracts.

DD167

DNA DAMAGE INDUCED BY OXIDATIVE STRESS IN HEPATIC IRON OVERLOAD DISEASE, OESOPHAGEAL AND PROSTATE CANCERS

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Consumption of food contaminated by mycotoxins in conjunction with hepatitis B and C and excessive intake of dietary iron as a result of drinking home-brewed in iron pots constitutes are important factors leading into hepatocellular carcinoma (HCC) which constitutes indeed a major public health problem in developing countries where the incidence is estimated at 23-26%. Due to the *Helicobacter pylori* infection and other non elucidated factors, squamous cell carcinoma of the oesophagus (SCCO) has become the most common cancer in black Southern African men (incidence: 14.5%). Prostate Cancer (PC) is the second leading cause of cancer related death in men in Western countries with the highest incidence among Black Americans. This study aims to demonstrate the implication of oxidative stress in the extent of DNA damage during the progression of these types of cancer. Oxidative stress was estimated by measuring specific biomarkers: Lipid peroxides (LPO), Thiobarbituric acid reactive substances (TBARs), Isoprostane (ISO) were evaluated using classical methods whereas Superoxide dismutase (SOD), and Glutathion peroxidase (GPx) activities were assessed using kits commercially available. DNA damage was evaluated by measuring the level of 8-hydroxy-2'-deoxy-guanosine (8-OH-dG) using a commercial kit based on a competitive *in vitro* enzyme-linked immunosorbent assay (ELISA) for quantitative measurement of the oxidative DNA adduct 8-OH-dG in tissue, serum and plasma. 4-Hdroxy-2'-nonenol (4-HNE) was also evaluated by ELISA method. Consistent correlation has been observed between DNA damage by the presence in situ (biopsies) of 8-OH-dG, the level of this metabolite in serum/plasma and the extent of oxidative stress biomarkers in SCCE, HCC and PC. Despite our progress in earlier detection of these types of cancer, their aetiology still remains not very well understood resulting in a partial efficiency of their prevention. The capacity of Free radicals to damage DNA molecule and to overexpress some oncogenes suggests their implication in the aetiology of these types of cancer and justifies the use of antioxidant compounds modulating the expression of these genes for their prevention.

DD168

IN VITRO STUDY OF THE EFFECTS OF DIFFERENT DOSES OF VISFATIN ON ANTIOXIDANT ENZYMES ACTIVITIES WITH OXIDATIVE DNA DAMAGE RELATION IN HUMAN MELANOMA ME45 MEDIA CELLS.

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Background: Visfatin is a novel adipokine, secreted by visceral and subcutaneous fat, could be involved in the development of obesity-associated insulin resistance and carcinogenesis. The goal of the study was to examine the effects of visfatin on antioxidant enzyme activities, such as: catalase (CAT), gluthation peroxidase (GSH-Px), gluthation reductase (GR) and manganese and copper zinc- containing superoxide dismutase – MnSOD and Cu/ZnSOD. A oxidative DNA damage level in Me45 cell culture, were also studied. Induced by ROS (Reactive Oxygen Species) *oxidative damage* of *DNA,* were measured by Komet Assay. We tried to verified the hypothesis that elevated visfatin plasma level from morbidly obese patients may influence on antioxidant enzymes activities and oxidative DNA damage level in tumor cell *in vitro*. Material and Methods: Human *melanoma* cells Me45 were cultured in DMEM medium with 15% fetal bovine serum, 100 U/ml penicillin and 0.1 mg/ml streptomycin under standard culture condition. MnSOD and Cu/Zn SOD isoenzyme activities were estimated according to Oyanagui method. GSH-Px activity was measured following the method of Paglia and Valentine. CAT activity was measured according to the kinetic method of Aebi. GR assay was based on the oxidation of NADPH to $NADP^+$. Komet Assay were performed on the Me45 cells, after 24 hours incubation with 0.04 µg/ml visfatin and 5 minutes incubation with genotoxic factor (100 µM H_2O_2), by Olive protocol. Human visfatin was added to cell culture in concentration 0.04 µg/ml. Results: After 24 h in the visfatin treated group, MnSOD, Cu/Zn SOD, CAT and GSH-Px media activities significantly increased when compared to the control. GR media activity was significant decreased in visfatin treated group when compared to the control. Incubation with visfatin resulted with similar to the control DNA protection in human melanoma cancer cells. Conclusion: Although of strong influence of visfatin on the antioxidative systems its action on the repair systems of damaged DNA is still unclear. Decreases of free radicals level, in treated with visfatin cells, didn't protect from the single and double breaks in nucleic acids. Repair of damaged DNA in Me45 is stronger dependent on the another than hormonal control.

DD169

CERECQ HELICASES ARE INVOLVED IN DNA DAMAGE RESPONSES

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RecQ helicases are highly conserved from *E. coli* to humans, and several homologues of the helicases have been identified in various multicellular organisms. For example, five homologues have been found in humans to date: RECQ1, BLM (RECQ2), WRN (RECQ3), RTS (RECQ4), and RECQ5. Mutations in human RecQ helicases result in rare diseases of aging and cancer characterized by genome instability. RecQ helicases play essential roles in maintenance of genomic stability and interact with proteins involved in DNA metabolic pathways such DNA repair, recombination, and replication. In the nematode *Caenorhabditis elegans*, four RecQ homologues are predicted from the genomic DNA sequence. We purified recombinant *C. elegans* WRN-1 and BLM proteins and investigated their substrate specificities in vitro in order to better understand their function in vivo. We found that CeRecQ proteins are a 3'-5' helicase. Further, we characterized their responses to DNA damage using immunostaining and COMET assay. Results indicate that CeRecQ proteins are required for responding to DNA damage. Thus, the abilities of CeRecQ proteins to unwind a variety of DNA structures and respond to DNA damage may play an important role in preventing the accumulation of abnormal structures, and eventually contributing to genome stability.

DD170

COMPLEX REGULATION OF THREE PRIME EXONUCLEASE 1 (TREX1) BY GENOTOXIC STRESS: FOS-DEPENDENT GENE INDUCTION, NUCLEAR TRANSLOCATION, AND INTERACTION WITH DNA POLYMERASE ETA

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Cells respond to genotoxic stress with the induction of DNA defense genes. Aimed at identifying novel players involved in this response, we analyzed the genotoxic stress-induced expression of DNA repair genes in mouse fibroblasts. Microarray analysis revealed a strong UV mediated induction of the three prime exonuclease I (trex1), which was verified by semi-quantitative PCR and real-time PCR. Induction of TREX1 was also observed on protein level. Experiments using the transcriptional inhibitor actinomycin D showed that induction of trex1 is caused by de novo RNA synthesis, which was further substantiated by promoter analysis studies showing inducibility of the trex1 promoter by UV light. Promoter dissection experiments showed that a 730 Bp fragment containing a potential AP-1 site is sufficient for UV inducibility. This AP-1 binding site is recognized by c-Fos (AP-1) in vitro and in vivo, as revealed by chromatin immunoprecipitation and electromobility shift assays. In accordance, induction of TREX1 was not observed in c-Fos deficient cells, supporting a role for c-Fos in the induction of TREX1. Besides UV light, TREX1 was induced by other DNA damaging genotoxins such as benzo(a)pyrene and hydrogen peroxide. Upon genotoxic stress, TREX1 was translocated into the nucleus where it physically interacts with the translesion polymerase eta. This interaction was greatly stimulated by UV light and benzo(a)pyrene, suggesting that TREX1 participates in the processing of mutagenic DNA damage. Induction of TREX1 was also observed in human fibroblasts upon exposure to UV light and benzo(a)pyrene. Further, we show that the human trex1 promoter is targeted by AP-1. Overall, we identified trex1 as a novel DNA damage inducible gene and demonstrate a complex TREX1 response involving gene induction, nuclear translocation and stimulation of interaction with the translesion polymerase eta.

DD171

FUNCTIONAL GENOMICS SCREEN TO IDENTIFY TRANSCRIPTION FACTORS INVOLVED IN DNA DAMAGE, REPAIR AND APOPTOSIS

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Cancer is one of the leading causes of death which is primarily due to inability of cells to maintain their genomic stability. Cells in our body are continuously exposed to various sources of DNA damaging conditions such as UV light, metabolites, or genotoxicants which cause various kinds DNA damage. If the damage is repairable, cells have the innate machinery to repair and progress through normal cell cycle. In contrast, if the damage is beyond correction, the cell is directed towards apoptosis, which is an important mechanism to clear the organism from cells that carry the risk of becoming malignant. A large number of signaling transduction pathways including transcription factors and proteins involved in a variety of post translational protein modifications are involved in this process. Our research is focused on delineating signaling pathways involved in DNA damage recognition, repair and apoptosis upon exposure to genotoxic compounds by functional high-throughput siRNA screening. For this purpose, mouse embryonic stem cells will be transfected with various siRNA gene-family libraries and the focus in this project will be on transcription factors. Subsequently, cells will be exposed to cisplatin and DNA damage recognition, repair and apoptosis will be accessed using live cell imaging on a high-throughput microscope system. By combining validated hits with already obtained transcriptomics data, we expect to be able to derive a detailed map of the transcriptional machinery involved in the DNA damage response in stem cells.

DD172

THE RESPONSE TO DNA DAMAGE OF TERMINALLY DIFFERENTIATED CELLS

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Post-mitotic cells are expected to be provided of a tight mechanism of genome integrity control to guarantee the maintenance of their correct functioning along the life span. We recently used mouse satellite cells as an *in vitro* differentiation model to study base excision repair (BER) regulation in terminally differentiated muscle cells (myotubes). A clear impairment of BER efficiency in myotubes versus their proliferating counterpart (myoblasts) was observed. At molecular level BER attenuation was ascribed to lower levels of XRCC1 and DNA ligases and was mirrored by an accumulation of ssb and phosphorylated H2AX nuclear foci upon exposure to hydrogen peroxide and methylmethane sulfonate (MMS). We further extended the analysis of BER capacity to a wide range of DNA glycosylases. No major difference in the expression level of these enzymes was detected in myotubes versus myoblasts with the exception of UNG2 and MYH which were strongly downregulated in post-mitotic cells. Both DNA glycosylases deal with DNA lesions formed at replication. Despite the accumulation of damage, myotubes are extremely resistant to the cytotoxic effects of DNA damaging agents such as ionizing radiation, MMS and campthotecin. In contrast, myotubes are extremely sensitive to doxorubicin, a topoisomerase II inhibitor, indicating that some of cell death pathways are still functional in terminally differentiated cells. Interestingly, we observed that autophagy, a cell mechanism which can paradoxically act by promoting both cell survival and cell death, is activated during *in vitro* muscle cell differentiation. Nevertheless, it does not seem to be essential for the differentiation program itself. In the case of myoblasts the MMS-induced cell death was due to a p53-dependent induction of an autophagic pathway. Further studies are currently in progress to investigate the potential involvement of autophagy in cytotoxic response to DNA damage in terminally differentiated myotubes.

DD173

LYMPHOBLASTOID CELL LINES AND CRYOPRESERVED ISOLATED LYMPHOCYTES DO NOT CORRELATE FOR γ-RAYS-INDUCED CHROMOSOME DAMAGE AND DOUBLE STRAND BREAKS.

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Mutagen sensitivity assays with peripheral blood mononuclear cells (PBMC) have been widely applied to assess individual susceptibility to genotoxic agents and its association to cancer risk or to DNA repair polymorphisms. For this aim, immortalized lymphoblastoid cell lines (LCL) might be considered as a convenient alternative to PBMC: LCL may offer practical advantages with respect to PBMC, due to their unlimited availability. However, a systematic evaluation of the suitability of EBV transformed LCL to act as surrogate of PBMC has not yet been performed. In this work we investigated the response elicited by γ-radiation in PBMC and in the respective immortalized LCL of 20 healthy subjects. Radiation sensitivity was evaluated by the cytokinesis block micronucleus (CBMN) and G_2 chromosome aberration assays, and by histone H2AX phosphorylation/dephosphorylation kinetics detected by cytofluorimetric analysis. In the CBMN assay, PBMC were treated either in a quiescent (G_0) or in a proliferating status. The results obtained show similar levels of γ-H2AX and dephosphorylation kinetics in LCLs and PBMC after irradiation, indicating the maintenance of DNA repair capacity after immortalization. Data however highlight different patterns of response in the two cell types as well as in resting and proliferating PBMC: i) basal frequencies of micronuclei (MN) and nucleoplasmic bridges (NPB) were much higher in LCL than in PBMC; ii) induced MN were higher in PBMC treated in G_0 than in LCLs or in proliferating PBMC; iii) induced NPB were much higher in LCLs than in PBMC; iv) chromosome damage in G_2 was higher in

PBMC than in LCLs, where more telomere associations were observed. At the individual level, no correlation was observed between results in PBMC and LCL with any of the biomarkers. Interestingly, in PBMC a significant inverse correlation was observed between MN formation and intensity of γ-H2AX fluorescence, suggesting that an efficient DSB signalling may prevent MN formation. In conclusion the results of this work do not support the use of LCL as an alternative to PBMC for studies on individual susceptibility. The different sensitivity to radiation in PBMC and LCL observed may be related to epigenetic changes associated to immortalization. Supported by the Italian Space Agency (ASI-MoMa) and the Italian Association for Cancer Research (AIRC).

DD174

ROLE OF BIM IN SPINDLE POISON-INDUCED APOPTOSIS

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Apoptosis is a well known cellular response to damage caused by a variety of environmental and therapeutic agents to DNA or other cellular structures, as the mitotic spindle. In this respect, spindle poisons which cause mitotic arrest act as powerful apoptotic agents, though the mechanism leading from microtubules depolymerization to cell death remains not elucidated. Data in the literature show that Bim, a BH3-only protein pro-apoptotic member of the Bcl-2 family, is sequestred on the microtubule-array by interaction with the light chain of dynein. In a similar way, p53 another well known pro-apoptotic molecule, is associated with cellular microtubules and is transported to the nucleus by dynein. Based on these data, we have investigated whether in response to tubulin depolymerization, Bim and p53 could be involved, and eventually in which manner, in the first steps of apoptosis. For this purpose H460 lung tumor cells were treated with 7,5 nM of the spindle poison combretastatin-A4 (CA-4) for 8-48h and the following parameteres were investigated: 1) variations of Bim-dynein interaction 2) regulation of Bim expression 3) relocalization of Bim at mitochondria 4) effect of silencing of Bim with specific siRNA 5) transcription-indipendent pro-apoptotic activity of p53. In agreement with data in literature, we found that Bim is linked to dynein motor complex and in response to CA-4 treatment and hence in increasing conditions of microtubules depolimerization, Bim was released from dynein. In addition, a QT-PCR analysis assay showed that drug treatment could also increase the transcriptional level of Bim. Together, the release of Bim and its increase at transcriptional level determined the engagment of the mitochondrial apoptotic pathway, due to mitochondrial relocalization of Bim. In addition, Bim silencing strongly reduced apoptotic commitment. Concerning the role of p53 in CA-4-induced apoptosis, we found that treatment determined its release from cytoskeleton and re-localization to mitochondria. As a next step, we are investigating the cross-talk between Bim and p53 in apoptosis caused by spindle poisons. Furthermore, it remains to be investigated the involvement of Bim in aneuploidy at drug concentration lower than those able to achieve complete mitotic spindle disassembly.

DD175

THE HISTONE ACETYLTRANSFERASE P300 IS INVOLVED IN THE DNA SYNTHESIS STEP OF NUCLEOTIDE EXCISION REPAIR

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Exposure to genotoxic agents may induce the formation of a variety of DNA lesions that cells must remove in order to avoid genomic instability, and to prevent cancer formation. Recent findings have shown that cell cycle checkpoints and DNA repair systems cross-talk each other. However, the role and the molecular mechanisms underlying these connections are not yet well understood. The cell cycle inhibitor $p21^{CDKN1A}$ is directly involved in nucleotide excision repair (NER), thanks to its interaction with PCNA, and with the histone acetyltransferase (HAT) p300. Previous studies suggested that p300 HAT activity might be required in NER, by providing chromatin accessibility to DNA repair factors. In addtion, it was shown that p300 interacts with PCNA, and that p21 regulates p300 HAT activity by dissociating interaction with PCNA. Since p300 may also associate with other NER factors, such as XPA, or DDB2, it has been suggested that p300 may play an additional role by regulating directly some step of the repair process. To further understand the involvement of p300 in DNA repair, we have investigated the influence of p300 knock-down by RNA interference (RNAi) on NER efficiency. We have assessed NER activity after treatment of primary human fibroblasts with siRNA to silence p300, and/or its homolog CBP, in order to analyze the specificity of the process. NER has been evaluated by analyzing unscheduled DNA synthesis (UDS) activity through autoradiography of ^{3}H-thimidine incorporation, after UV irradiation. Concomitantly, cell viability has been determined with the MTT test. The results have indicated that p300, but not CBP silencing, reduces significantly the number of autoradiographic grains. In addition, cells treated with siRNA to p300 have shown a reduced viability after UV irradiation, as compared with cells treated with control siRNA molecules. To clarify whether p300 is required in a particular step of NER, we have analyzed the recruitment of PCNA at sites of local UV-irradiation, after p300 silencing. No significant difference in the recruitment of PCNA, has been found in p300-siRNA treated cells, suggesting that p300 activity is required at a downstream step, thereby impairing UDS activity. (Work supported by AIRC).

DD176

CHARACTERISATION OF THE MRE11-DNA COMPLEX IN DOUBLE-STRAND-BREAK REPAIR COMBINING X-RAY CRYSTALLOGRAPHY, SFM AND COMPUTATIONAL MODELING.

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Mre11 forms the core of the multifunctional Mre11-Rad50-Nbs1 (MRN) complex that detects DNA double-strand breaks (DSBs), activates the ATM checkpoint kinase, and initiates homologous recombination (HR) repair of DSBs. Two crystal structures of Pyrococcus furiosus Mre11 bound to different DNA-hairpins substrate, published on Cell by R.S. Williams et al., elucidate how *pf*Mre11 coordinates ds-DNA and process it in the nuclease active sites. These structures cannot explain the mechanism of the endonucleolytic activity of Mre11, for that reason one of our aims is to crystallize the complex between Mre11 and ss-DNA. Another aim is to investigate the characteristic of this complex using other techniques such as SFM and computational modeling to test the crystallographic deductions and to reveal the structural organization of Mre11 responsible for endonucleolytic activity. In an evolutionary analysis we will compare three different Mre11 homologues, respectively archaeal Mre11, a prokaryotic Mre11-like protein from *Thermotoga Maritima* and human MRE11. We present preliminary results of an SFM analysis of the reaction between Mre11 and three different DNA substrates respectively a 1.8 Kb blunt-ended ds-DNA, a 3 Kb ds-DNA with 4 nt overhang ends and an 800 nt long 500 bp ds-DNA followed by 300 nt ss-DNA 3' overhang. Our results show that Mre11 alone bound with strong affinity to longer ss-DNA, possibly as multimers, and with less affinity the short 4 nt overhangs of the ds-DNA substrate, confirming the X-ray data. Surprisingly Mre11 bound well to double stranded parts of all substrates. To explain this behavior we present a computational investigation using combined docking and molecular dynamic simulations based on approach the crystal structures. This suggested that Mre11 could bind DNA ends

and internal sites through domain rearrangements. These combined methods are aimed at identifying key residues involve in the interactions with DNA and the possible implications for evolution of domain organization. These predictions will be combined with 'a posteriori' mutagenesis work to modify activity and to disrupt the complex.

DD177

THE ROLE OF BER AND NER PATHWAYS IN DNA RECOVERY OF HEAD AND NECK SQUAMOUS CELL CARCINOMA

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Head and neck squamous cell carcinoma (HNSCC) comprise about 6% of all malignant neoplasms. One of the major risk factor of HNSCC is tobacco smoking. There are approximately 4 thousand chemical compounds identified in tobacco smoke. Many of them like polycyclic aromatic hydrocarbons (PAHs) and aromatic amins (AA) are well known carcinogens which cause variety of DNA damage including base modification and DNA cross-links. Cells remove those lesions mainly by base excision (BER) and nucleotide excision (NER) repair pathways. The aim of our research was to evaluate the efficiency of DNA repair by NER and BER in head and neck squamous cell carcinoma. We examined peripheral blood lymphocytes obtained from HNSCC patients, healthy subjects as well as HTB-43 larynx and CRL-1628 tongue cancer cell lines. DNA repair efficiency was estimated by in vitro assay with cells extracts and plasmid substrates of heteroduplex with AP site in BER or UV-irradiated plasmid in NER. The level of [γ^{32}P]-dAMP incorporation during strand resynthesis as equivalent of DNA repair was detected by autoradiography and quantified using densitometry. Our results indicate that there is significant decrease in the efficacy of DNA excision repair pathways in lymphocytes from patients and HNSCC cancer cells as compared to healthy controls. In conclusion, we suggest that BER and NER pathways may be critical for DNA recovery of HNSCC cells what in turn may be responsible for their higher susceptibility to mutagenesis and cancer transformation.

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DD178

EFFECTS OF THE HIV TREATMENT DRUGS NEVIRAPINE AND EFAVIRENZ ON BRAIN CREATINE KINASE ACTIVITY, DNA DAMAGE AND ANXIETY AND MOOD-RELATED BEHAVIOUR IN MICE.

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Introduction: Nevirapine (NVP) and Efavirenz (EFV) are antiretroviral drugs belonging to potent class of non-nucleoside reverse transcriptase inhibitors (NNRTIs) widely used for the treatment human immunodeficiency virus (HIV) infection as part of highly active antiretroviral therapy (HAART). Although the HAART is able to bring down viral load to undetectable levels and restore immune function, their prolonged use causes several adverse effects. It has been demonstrated that both NVP and EFV are able to cross the blood-brain barrier, and then causing important central nervous system (CNS) related side effects. Considering that the exact mechanisms responsible for CNS toxicity associated with NVP or EFV remain unknown and that creatine kinase (CK) plays an important role in cell energy homeostasis, and also the DNA damage may be involved in the adverse effects observed in brain tissue, in the present work we evaluated CK activity and DNA damage by Comet assay in brain of mice after chronic administration of EFV or NVP. In addition, this study investigated the effects of drugs chronically administrated in mice on elevated plus-maze, forced swimming and open-field tests. Material an Methods: Male CF-1 mice were treated orally, once a day for 36 days, with EFV 10 mg/kg or NVP 3.3 mg/kg or vehicle. Behavioral tests were conducted one hour after drug injection. On day 28, 32 and 36 of drug treatment, mice were evaluated on elevated plus-maze test, open-field test, and forced swimming test, respectively. The animals were killed by decapitation 3 h after the last administration of the drugs. The brain was immediately removed and hippocampus, striatum, cerebellum, cortex and prefrontal cortex were isolated for CK and Comet assay analyzes. Results and Conclusions: Our results demonstrated that NVP or EFV significantly inhibited CK activity in cerebellum, hippocampus, striatum and cortex of mice. In addition, the behavioral results demonstrated that EFV, but not NVP, reduced the exploration to open arms in the elevated plus-maze test. Neither NVP nor EFV altered mouse behavior in the forced swimming and open-field tests. At the same way, the comet assay results showed that only EFV increased the DNA damage in brain. Thus, our findings point to a genuine anxiogenic-like effect of EFV since it reduced exploration to open spaces of elevated plus-maze test. Although it is difficult to extrapolate our findings to the human condition, the inhibition of brain CK activity by NVP and EFV and the increased DNA damage caused by EFV may be associated with the neurological adverse symptoms of these drugs. Additionally, these observations are important to understand the mechanisms by which NNRTIs could induce side effects and could contribute to the development of novel strategies to attenuate these adverse effects in patients under chronic regimens with HAART.

DD179

LYMPHOCYTES FROM INDIVIDUALS CARRYING THE OGG1-S326C POLYMORPHIC VARIANT PRESENT A LOWER CLEAVAGE ACTIVITY OF 8-OXOGUANINE: EFFECTS OF GENE EXPRESSION AND OXIDATION STATUS.

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OGG1 is the major DNA glycosylase for the removal of the mutagenic lesion 8-oxoguanine (8-oxoG). A frequently found polymorphism, a serine to cysteine substitution at position 326 of the OGG1 protein (S326C), has been associated with cancer risk in several population-based studies. A genotype-phenotype correlation study was carried out in 225 healthy volunteers. Protein extracts were prepared from peripheral lymphocytes and OGG1 activity was measured by means of an in vitro repair assay. Cell extracts were incubated in the presence of an oligonucleotide containing a single 8-oxoguanine and labelled at the 3'end with a fluorescent dye, and OGG1 activity was monitored by quantification of the cleaved products as detected by PAGE. The average cleavage activity was 2.25+/-0.52 fmoles/ug of protein. A significantly lower activity ($p<0.001$) was detected in extracts from subjects homozygous for the Cys allele when compared with those homozygous for the Ser allele. OGG1-Cys homozygotes displayed an approximately 26% lower basal 8-oxoG glycosylase activity than the OGG1-Ser variant homozygotes. OGG1 gene expression was measured by RT-PCR in the same samples. A significant correlation between cleavage activity and gene expression was recorded ($R=-0.30$, $P<0.001$) but no association was found between OGG1 gene expression and the S326C SNP. Recent data revealed that the oxidation status of human OGG1 S326C polymorphic variant is the main determinant of the cellular repair activity (Bravard et al., 2009). To gain mechanistic insights into

this phenomenon, the wild type and S326C polymorphic variant were purified and their sensitivity to reducing/oxidizing agents was characterized in vitro. The analysis by SDS PAGE of the wild type and polymorphic proteins revealed significant differences in the sensitivity to conformational changes with the polymorphic variant being most sensitive to the absence of reducing agents. However, the cleavage activity of the two proteins was comparable when normalized for the relative yield of the 39 kDa band suggesting that the protein is active only in this monomeric conformational status. The characterization of the effects of oxidation on the cleavage activity of the wild type and polymorphic variant by functional assays as well as by mass spectrometry is currently under progress.

DD180
MITOCHONDIRAL DNA REPAIR IN DIFFERENTIATED NEURONS

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Recent studies expanded known DNA repair pathways in mammalian mitochondria to include mismatch repair and the long-patch base excision repair (LP-BER). Mitochondrial LP-BER likely plays an important role in protecting mitochondrial DNA (mtDNA) by removing toxic oxidative lesions. LP-BER in mitochondria depends on FEN1 flap endonuclease, a protein that is also essential for nuclear DNA repair and replication, and for cellular proliferation. However, in terminally differentiated cells such as neurons and muscle cells, FEN1 is down-regulated as the cells stop proliferating. The decreased levels of FEN1 and perhaps other proliferation-associated proteins may limit mtDNA maintenance in terminally differentiated cells. The problem may be especially acute for neurons, which are highly dependent on mitochondria but must often maintain function for decades. Accumulated mtDNA oxidative damage may underlie age-related neurodegeneration and neurodegenerative disorders. Using mouse CAD neuroblastoma line as a model system, we found that FEN1 level is significantly decreased when the cells were induced in vitro to differentiate into neurons. DNA2, another flap endonuclease involved in mtDNA repair, was also down-regulated in differentiated CAD cells. Using RNA interference technology, we found that artificial down-regulation of FEN1 in HeLa cells led to decreased DNA2 levels. In contrast, down-regulation of DNA2 did not diminish FEN1 expression. This result and the observations with neurons suggest that DNA2 and FEN1 may functionally interact. Because these two critical LP-BER proteins are down-regulated following differentiation, we are examining the sensitivity of mtDNA to oxidative damage in differentiated neurons compared to proliferating cells.

Environmental mutagenesis

EM001
MUTATION STUDY IN VITRO ON RECLAIMED WATER IN CITY
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BACKGROUND & AIM: To explore the mutation effect of reclaimed water in Tianjin. MATERIAL AND METHODS: reclaimed water in low water and high water periods were fetched. Reverse phase C-18 solid-phase extraction (RP-C18SPE) was used for the extraction of organic compounds from water samples. Ames test, Cytokinesis-block Micronucleus test and plasmid DNA breaking test was used to detect the damage of water samples on genetic material. Moreover, PCR-DNA gene sequencing method was used to examine the mutation effects of water samples on some exons of P53 gene. RESULTS: Ames test showed that both untreated reclaimed water and treated reclaimed water, except untreated reclaimed water of high water period after metabolism by S9, could induce mutation of TA98 and TA100 strains with dose-response relationship, which demonstrated the frameshift and base replacement mutation effects of municipal reclaimed water. There were three doses: 16.7, 33.3 and 66.7ml/ml (the denominator was original water) in micronucleus test, and the micronucleus number was only slightly increased in the dose of 66.7ml/ml, but there was no significant difference compared with control group. DNA breaking test demonstrated that with the increasing doses of municipal reclaimed water, the plasmid damage was also obviously enhanced. In addition, linear DNA appeared when plasmid was treated by low water period samples, and this phenomenon might attribute to the different compounds of water sample extraction. Gene sequencing method testified that organic extraction of municipal reclaimed water can induce the mutation of p53 gene in human embryo liver cell(L-02 cell). CONCLUSION: municipal reclaimed water, which showed mutation effects in some extent, might has potential hazards to environment and health.

EM002
INTEGRATION OF METABOLIC ACTIVATION OF XENOBIOTICS INTO A BIOSENSOR FOR THE DETECTION OF GENOTOXIC EFFECTS
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Assays based on the bacterial SOS-response are frequently used as rapid low-cost methods for the detection of genotoxicity. These test systems are mostly combined with a bioactivation of the sample in order to mimic the metabolization of xenobiotics that facilitates renal excretion. The SOS-umu-test is well established and standardized but its use is restricted to the laboratory due to a lack of a portable device allowing field toxicity analysis. As an answer to this need, a whole cell biosensor based on the bacterial SOS-response combined with an electrochemical signal detection is under development; the device will include a metabolic activation module, based on the use of stabilized hepatic microsomal preparations (S9-fraction). The construction of such a whole cell biosensor would open a new spectrum of possibilities for rapid and portable water genotoxicity testing. The characterization of activity and stability of the S9-fraction and the development of a long term stabilization protocol for the complete xenobiotics metabolic activation system (S9-mix = S9-fraction + activating cofactors) will be presented. Furthermore, the performance of the electrochemical signal detection that will be implemented in the biosensor is compared to the well established and ISO-standardized SOS-umu-protocol. Special attention is paid to a possible interference between electro-active species in the metabolic activation-system and the chrono-amperometric signal detection. In summary, (1) for the first time a long term stabilization of S9-mix at room temperature is demonstrated and (2) the compatibility of the system for the metabolic activation of xenobiotics and electrochemical signal detection is shown. The findings indicate the feasibility of the construction of a biosensor for the detection of genotoxic effects with an integrated metabolic activation of xenobiotics. ACKNOWLEDGMENT We gratefully acknowledge the funding by the BMBF-MOST Cooperation in Water Technology Research grant number WT 601 (Project 02WU0844) supported by the German Federal Ministry of Education and Research (BMBF) and the State of Israel Ministry of Science, Culture and Sport (MOST).

EM003
CLUSTERING OF UPPER GASTROINTESTINAL CARCINOMAS AND GEOGRAPHIC VARIATION IN TOPOGRAPHY ARE INDICATIVE OF COMMON ENVIRONMENTAL CARCINOGENS IN HIGH-RISK AREAS IN NORTHERN CHINA
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Aim In the past, striking geographic variation was noted only for esophageal cancer (EC) in the high-risk region in Northern China. This paper aims to note the clustering of upper gastrointestinal cancers and geographic variations of gastric cardia cancer (GCC) and gastric non-cardia cancer (GNCC) as well. Methods Cixian, Yangcheng, Linxian and Shexian are four counties in the high-risk region. They were previously shown to have the highest mortality rates of EC (≧80 /100,000) from 1974 to 1976. Their combined population was 2.4 million in 2002. Geographically, Shexian is entirely mountainous, Linzhou and Yangcheng are mostly moutainous, and Cixian is with one-third mountain, one-third hill, and the other third plains. To show the geographic variation, we compared the incidence rates of EC, GCC, and GNCC from 1998 to 2002 between the four counties. Result. The total incidence rates of EC, GCC, and GNCC ranges from 173.5 to 287.1 for the males, and 111.5 to 145.7 for the females, accounting for 70 to 80 percents of the all-body malignancies in the four counties. Geographically, the percentages of GCC and GNCC increase from Cixian to Yangcheng and Linzhou, and further to Shexian, while that of EC decreases in the same pattern. Conclusion Clustering of EC, GCC, and GNCC in the region, together with development of EC on local chickens, suggest the existence of common environmental carcinogens. The geographic variation in topography of upper gastrointestinal cancers may be related to regional variation in environmental carcinogens, as suggested by past animal experimental carcinogenesis tests. As for carcinogenesis, local carcinogens may have sustained a high susceptibility by initiating, selecting, and promoting genetic alterations for many years, or even building-up epigenetic mutation over generations. This hypothesis can satisfactorily explain the findings of immigration studies. Because nitrosamines are widely exclaimed as a main risk factor, and water is the main source for nitrosamines to enter into human body, to improve water supply condition may be a good primary prevention, although according to immigrants study, a generation's time is needed before meaningful decrease in incidence become clear.

EM004
FORMATION, DNA REPAIR, AND TLS OF 3-NITROBENZANTHRONE-DERIVED DNA ADDUCTS
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AIM: The urban air contaminant 3-nitrobenzanthrone (NBA) is a suspected human carcinogen. NBA forms several DNA adducts through metabolic activation in vivo. Some DNA polymerases are known to commit translesion DNA synthesis (TLS) across DNA adduct. Error-prone replication during TLS results in mutation, and mutations are thought to cause cancer. In the present study, we analyzed formation of DNA adducts of NBA, DNA repair of the adducts, and TLS across the adducts. METHODS: DNA adducts formation in HepG2 human hepatoma cells exposed to NBA was measured with LC/MS/MS using stable isotope-labeled internal standards. DNA repair efficiency was estimated by measuring remaining adducts in the cells after appropriate repair time. TLS mechanism was investigated using E. coli cells and plasmids that have site-specifically a single NBA-derived adduct. RESULTS AND CONCLUSIONS: LC/MS/MS detected dG-N2-C2-aminobenzanthrone (ABA), dG-C8-N-ABA and dA-N6-C2-ABA adducts, and dG-N2-C2-ABA adduct was predominant in HepG2 cells. Removal of dG-N2-C2-ABA by DNA repair was slower than those of dG-C8-N-ABA and dA-N6-C2-ABA in HepG2 cells. DNA replication of site-specific modified plasmid with a dG-N2-C2-ABA was blocked severely in E. coli. On the other hand, dA-N6-C2-ABA hardly inhibited the replication. An extent of inhibition by dG-C8-N-ABA was in-between. Base sequence analysis of progeny plasmids revealed that dG-N2-C2-ABA was the most mutagenic adduct among the others. The results of replication in E. coli that lacks one of the TLS polymerases indicated that both Pol IV and Pol V were involved in error-prone TLS across dG-N2-C2-ABA adduct. On the other hand, TLS across the other adducts was carried out by multiple DNA polymerases, and the TLS was error-free. In conclusion, NBA formed dG-N2-C2-ABA predominantly, this adduct repaired slowly and severely inhibited DNA replication. However, once TLS occurred across this adduct, it frequently induced mutation. ACKNOWLEDGEMENT: We sincerely thank Dr. R. P. P. Fuchs (CNRS, France) for his kind advices.

EM005

INDUCTION OF DNA-ADDUCTS, OXIDATIVE STRESS AND APOPTOSIS AFTER EXPOSURE TO SELECTED POLYCYCLIC AROMATIC HYDROCARBONS IN RAT LIVER 'STEM-LIKE' CELLS

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Polycyclic aromatic hydrocarbons, such as dibenzo[a,l]pyrene (DBalP), benzo[a]pyrene (BaP) and its methylated derivatives, and methylated derivatives of benzo[a]anthracene (BaA) belong to group of various environmental contaminant with strong genotoxic effects. In this study, we evaluated their genotoxic effects in rat liver epithelial cell line WB-F344, in vitro model of liver oval cells. The analyzed endpoints included formation of DNA-adducts, induction of oxidative stress, phosphorylation of Chk1, p53, modulation of S-phase of cell cycle and apoptosis. Formation of DNA-adducts was measured by the 32P-postlabeling method. We found that DBalP and 7,12-dimethylbenz[a]anthracene (DMBA) were the most potent inducers of DNA adducts formation. Other methylated BaAs induced only low levels of DNA adducts in comparison with DMBA. In the group of BaP and its methylated derivatives, all tested compounds induced formation of DNA-adducts, with 1-MeBaP being the most potent inducer. Formation of reactive oxygen species (ROS) was detected by flow cytometry. The strongest tested inducers of oxidative stress were DMBA, BaP and 6-MeBaP. Phosphorylation of p53 protein was detected by Western blotting, and it was significant only in case of DBalP, DMBA, 10-MeBaA, BaP and 1-MeBaP. Activation of Chk1 protein was measured after exposure to BaP and its methylated derivates. These results corresponded with activation of p53 protein. The delay of cells in S-phase of the cell cycle was caused by 10-MeBaA, DMBA, BaP, DBalP and 10-MeBaP. Apoptosis was detected by flow cytometry and fluorescent microscopy. The results suggested that apoptosis was induced mainly by compounds inducing high levels of DNA adducts, with exception of 10-MeBaA, which induced significantly higher apoptosis than expected from the 32P-postlabeling data. In this case, formation of ROS leading to oxidative stress might be involved in its toxicity, alternative to formation of stable DNA adducts. The present results suggest that liver epithelial cells might be a useful model for studies of genotoxicity of PAHs and related organic pollutants. [This work was supported by the Czech Science Foundation, grant No. 525/08/1590].

EM006

EFFECTS OF POLYCYCLIC AROMATIC HYDROCARBONS IN HUMAN PROSTATE CARCINOMA LNCaP CELLS

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The prostate carcinoma is presently one of the most prevalent cancer diseases. Epidemiologic studies suggest a possible link between genotoxin exposure and prostate carcinogenesis. In this study, we investigated effects of potent genotoxic polycyclic aromatic hydrocarbons (PAHs) - dibenzo[a,l]pyrene (DBalP) and benzo[a]pyrene (BaP) in human prostate androgen-responsive adenocarcinoma cell line LNCaP. Our results based on Western blot and RT-PCR analyses showed that PAHs are metabolically activated and form significant levels of PAH-DNA adducts in LNCaP cells as detected by 32P-postlabeling technique. However, the DNA damage failed to activate cellular defense mechanisms including cell cycle arrest and apoptosis (nuclear DNA fragmentation identified morphologically by fluorescent staining). Therefore, in the second part of the study, we further investigated possible mechanism(s) contributing to the survival of LNCaP cells exposed to genotoxic PAHs. We also examined effects of the test compunds on overall profile of gene expression in the prostatic cells by microarray technology. The preliminary analyses of microarray data revealed that responses to BaP were similar to the effects of a potent aryl hydrocarbon receptor (AhR) agonist 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). A series of genes associated with DNA damage response (RAD21, RAD51, ERCC6, BRCA1), cell cycle control (CCNA2, CDK2, E2F2, E2F1) and or mitosis progression (CENPA, AURKA), seem to be downregulated, while some anti-apoptotic genes, such as BCL6 were upregulated in LNCaP cells. Future studies should establish the role of BCL6 and/or possible other anti-apoptotic signals in the survival of prostatic cancer cells exposed to genotoxic PAHs. [Supported by the Czech Science Foundation, project No. 310/07/0961].

EM007

THE COMPARISON STUDY ON CHROMOSOMAL ABERRATIONS IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES INDUCED BY 18.8MeV PROTON AND ^{60}Co-γ RADIATION

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Background and aims: Proton is the main component of space radiation and may bring risk to astronaut's health. In order to understand the radiation biological effect of proton, the dose-effect curves of chromosomal aberrations in human blood lymphocytes induced by proton and ^{60}Co-γ ray were established and relative biological effects between both were compared. Methods: Human blood *in vitro* was exposed to 0.5, 1, 2, 3, 4 and 5Gy 18.8MeV proton produced by accelerator of China Institute of Atomic Energy and ^{60}Co-γ ray of same doses respectively, dose rate was 0.5Gy/min. Conventional chromosome preparation was accomplished. The chromosomal aberrations were counted and dicentrics and rings were used as establishing dose-effect curves. RBE of 18.8MeV proton radiation was obtained by comparison of chromosomal aberration frequency with ^{60}Co-□ ray. Results: The frequencies of chromoasomal dicentrics and rings induced by 0.5-5Gy proton and ^{60}Co□ ray showed better dose dependence.

Both dose-effect curves fitted to linear-square model. RBE of proton showed negative correlation with dose and were from 1.87~1.01, 1.41 average in range of 0.5-5.0 Gy. Conclusions; 18.8MeV proton produced higher relative biological effect in low dose range compared with ^{60}Co-γ ray.

EM008

RELATIONSHIP BETWEEN TP53 TUMOR SUPPRESSOR GENE MUTATIONS AND SMOKING-RELATED BULKY DNA ADDUCTS IN A LUNG CANCER STUDY POPULATION FROM HUNGARY

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Background/Aim. Lung cancer rate in Hungary is one of the highest in the world among men and also very high among women, for reasons not clearly understood yet. The aim of the study was to explore characteristics of DNA damage and TP53 gene mutations in lung cancer from Hungary. Materials and methods. Tissue samples from 104 lung resections for lung cancer patients, both men and women, operated on for non-small cell lung cancer, specifically, primary squamous cell carcinoma or adenocarcinoma were studied. Of the cases, 37% smoked up to the surgery, 24% stopped smoking within one year before the surgery, 26% stopped smoking more than a year before the surgery, and 13% never smoked. TP53 mutations were detected by automated capillary electrophoresis single strand conformation polymorphism and sequencing. Bulky DNA adduct levels were determined by 32P-postlabelling in non-tumorous lung tissue. Results. In total, 45% (47/104) of the cases carried TP53 mutation. The prevalence of TP53 mutations was statistically significantly associated with duration of smoking, tumour histology and gender. Smokers had approximately twice as high bulky adduct level as the combined group of former and never smokers (10.9 ± 6.5 vs 5.5 ± 3.4 adducts/108 nucleotides). The common base change G→T transversion (8/43; 19%) was detected exclusively in smokers. Conclusions. For the first time, we demonstrate that most carriers of G→T transversions had also a high level of bulky DNA adducts in their non-tumourous lung tissue. Our study provides evidence for a high burden of molecular alterations occurring concurrently in the lung of lung cancer patients.

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EM009

EFFECTS OF MULTIPLE STRESSORS EXPOSURES ON PLANT POPULATIONS

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An assessment of the state of plant and animal populations inhabiting polluted territories and the analysis of mechanisms of their adaptation to adverse environmental conditions undoubtedly has general biological importance. Consequently, studies that examine biological effects on non-human biota in natural settings provide a unique opportunity for obtaining information about the potential biological hazard associated with pollution of the environment. Nevertheless, up to now there is a distinct lack of quantitative data on the real long-term biological consequences of chronic multipollutant exposure lasting a long period of time. Actually, few studies exist that are directly relevant to understanding the responses of plant and animal populations to multipollutant exposure in their natural environments. The results of long-term field experiments in the 30-km Chernobyl NPP zone, Ukraine, in the vicinity of the radioactive wastes storage facility (Leningrad Region), at radium production industry storage cell territory (the Komi Republic), in the Bryansk Region of Russia affected by the Chernobyl accident, and in Semipalatinsk Test Site, Kazakhstan that have been carried out on different species of wild and agricultural plants are discussed. Although overwhelming majority of pollutants cause primary damage at the molecular level, there are emergent effects at the level of populations, non-predictable solely from the knowledge of elementary mechanisms of the pollutants' influence. The chronic low dose exposure appears to be an ecological factor creating preconditions for possible changes in the genetic structure of a population. Plant populations growing in areas with relatively low levels of pollution are characterized by the increased level of both cytogenetic disturbances and genetic diversity. Under conditions of chronic exposure in pine tree populations were developed seed descendants with significantly lower reproductive ability than in the reference population. Contamination of the plants environment activates genetic mechanisms, changing a population's resistance to exposure. However, in different ecological situations, genetic adaptation to extreme edaphic conditions in plant populations could be achieved with different rates.

EM010

A MULTI-BIOMARKER APPROACH FOR ASSESSING PRODUCED WATERS TOXICITY AND GENOTOXICITY IN GAMBUSIA AFFINIS AS A MODEL FISH SPECIES

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The aim of this study was to develop and validate a multi-biomarker approach in Gambusia affinis, proposed as a model fish to assess the toxicological and genotoxic impact of produced water (PW). PW is seawater mixed with hydrocarbons and derives from the extraction of oil and gas from under the seabed; it contains residual hydrocarbons, trace elements, naturally occurring radioactive material and potentially toxic treatment chemicals such as biocides, dispersants, detergents and scale inhibitors used in oil production. Female mosquito fish (Gambusia affinis) were exposed for 8 and 30 days in the laboratory to different PWs from Mediterranean on-shore oil and off-shore gas installations. Specimens were also exposed to water and sediment from an Italian ship canal (Navicelli Channel). DNA damage was evaluated in erythrocytes by single cell gel electrophoresis (Comet assay) and erythrocytic nuclear abnormalities (ENA) assay. Biotransformation and oxidative stress biomarkers responses were also investigated to obtain a correct interpretation of complex results and a clear indication of the degree of stress syndrome induced by PWs: induction of phase I (EROD, BPMO activity) and phase II (GST activity) biotransformation systems, FACs, LPO, enzymatic antioxidants (GPX, GR, CAT), a non-enzymatic antioxidant (glutathione-GSH). AChE activity and porphyrins were assessed too. G. affinis was found to be a suitable test organism for laboratory studies to investigate the potential toxicity of PWs. Genotoxicity biomarkers were found to be sensitive responses in exposed organisms, in particular, increase of Comet assay and ENA assay values after 8 days of exposure demonstrated genotoxic effects of PWs. On the other hand, increase in tail DNA (%) and absence of alteration in ENA frequencies allowed to hypothesize that organisms are able to repair the DNA damage after 30 days. These data, compared to other test results, allowed to validate the multi-biomarker approach for the integrated assessment of exposure and toxicological and genotoxic hazard due to petroleum derivatives. The multiparametric approach and Gambusia affinis as model fish species offer a powerful combination of low cost, rapid response time and high response sensitivity.

EM011
GENOTOXICITY EVALUATION OF ONE HIGHLAND ASTERACEAE

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Ambrosia arborescens shrub originally from South America has long been used in traditional medicine, distributed in high areas (2500 to 3800 m above sea level). In phytochemical investigations, studies of Ambrosia arborescens revealed the presence of alkaloids, flavonoids, guanilosides and sesquiterpen lactones, besides several active principles from its essential oil. Considering the interest in natural components, this study wants to assess the genotoxic and antigenotoxic potential of three extracts obtained from it. Three Ambrosia arborescens extracts: brome ethanol, dichloromethane and petroleum ether, were assessed using whole blood cells incubated 2 hours at 37ºC with these extracts in different concentrations, all the extracts were diluted in 1% DMSO. Determination of DNA damage by comet assay was conducted according to Tice et al. 1999 with some modifications; slides were stained with a silver nitrate solution. Fifty cells were evaluated per slide considering cell damage frequency (ID) and two slides were evaluated for treatment. Brome ethanol extract was used in 5 concentrations between 2000mg/ml and 250mg/ml, Petroleum ether extract was also used in 5 concentrations from 66.55mg/ml to 8.31 mg/ml. In both cases the three highest concentrations, showed a statistical difference with the negative control whereas the 2 lowest, did not. Finally dichloromethane extract was used in 10 concentration because of the active principles found in it, from 14.1 mg/ml to 0.0025 mg/ml. As a result the three highest concentrations showed an increase in the ID, despite the others seven that did not have a genotoxic activity. For the antigenotoxicity assessment the three not genotoxic concentrations from each extract were evaluated against 1.5% H2O2. Brome ethanol extract as well as petroleum ether extract had an important reduction in the ID, compared with the inductor. In contrast dichloromethane extract only shows a statistical difference in the highest concentration. The Ambrosia arborescens extracts possess genotoxic activity in high concentration nevertheless low concentrations do not have genotoxic activity. However in antigenotoxic assessment the highest chosen concentrations present more activity decreasing the ID respect to the H2O2.

EM012
GENOTOXIC ASSESSMENT OF TWO SESQUITERPEN LACTONES

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Sesquiterpene lactones are natural products distributed widely in the Asteraceae family, especially in genders such as Artemisia and Ambrosia, these substances are usually bitter, and their distribution can be different depending on the plant since 0.01% to 8% in dry material, but the highest concentration is frequently found in leaves. Sesquiterpene lactones biological activity has been reported, including cytotoxic, antibacterial, antifungal, antitumoral, analgesic and antiprotozoal effects. Numerous essential oils have been obtained from Bolivian plants. However, studies related to the biological activity of those metabolites are scanty. Asteraceae family, essential oils are rich in monoterpenes, oxygenated sesquiterpenes, some sesquiterpene hydrocarbons, and a high quantity of sesquiterpene lactones especially in the aerial part, product of them, two molecules Damsina and Coronofilina were obtained. Damsina and Coronofilina were assessed using whole blood cells incubated 2 hours at 37ºC in five concentrations from 200µg/ml to 25µg/ml, both molecules were diluted in 1% DMSO. Determination of DNA damage by comet assay was conducted according to Tice et al. 1999 with some modifications; slides were stained with silver nitrate solution. Fifty cells were evaluated per slide considering cell damage frequency (ID) and two slides were analyzed. In the genotoxic assessment concentrations of 25µg/ml, 50µg/ml and 100µg/ml did not show a statistical difference with untreated cells for both molecules; also Coronofilina at 150µg/ml did not present genotoxic potential, but it did in the highest concentration. On the other hand Damsina showed a statistical significance since 150µg/ml. For the antigenotoxic assessment the three non genotoxic concentrations were considered, in this assessment, both molecules showed a decrease in the ID in 100µg/ml and 50µg/ml, but in the lowest concentration 25µg/ml the statistical difference was not significant. Therefore, low concentrations are not genotoxic, however they posses less restorative capacity than the others concentrations.

EM013
GENOTOXICITY OF AGRICULTURAL SOILS DEDICATED TO CABBAGE (BRASSICA OLERACEA VAR. CAPITATA L) CROP GROWING IN HUARICANA, LA PAZ, BOLIVIA.

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Vicia faba micronucleus test was used to assess the genotoxicity of soils dedicated to cabbage crop growing in Huaricana, an agricultural village located about 26 km south of La Paz city. Soils samples were collected during dry season (April - September) in two different times: before "sowing period" and after "harvest period" from three agricultural plots and a "negative control plot" dedicated to grow organic vegetables for about ten years. Interviews were realized to the farmers and owners of the plots sampled in order to determinate chemical products and agricultural practices used during the current campaign and previous campaigns. The genotoxicity in terms of micronuclei (MCN) frequencies in the root tip cells of Vicia showed that soils sampled from two fields (H1 y H2) were genotoxic before beginning the cabbage crop growing. This indicates that the pesticides or others contaminant residues applied during previous campaigns maintained their genotoxic effect on soils. The third soil sample (H3) did not present genotoxic potencial, due to be used as fallow land during the last two agricultural campaigns. After the "harvest period" both H1 and H2 maintained their genotoxic condition due to the application of α- cyano-pyretroides, pesticides that have previously shown clastogenics effects in commercial formulations. Likewise, H3 also showed a significant increasing in the MCN frequency respecting to the negative control, although any kind of chemical control were used during the campaign. This behavior can be explained through the effect of some agricultural practices used during dry season, like farmland irrigation with wastewater arising from Choqueyapu River, which goes through the city accumulating industrial effluents and municipal sewage from its many tributaries.

EM014
GENOTOXICITY BY MERCURY IN FRESHWATER FISHES FROM MADRE DE DIOS RIVER

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Mercury is an environmental polluting agent of high toxicity, widely used in operations of gold extraction. This heavy metal is disposed directly into aquatic ecosystems, causing serious pollution problems in the aquatic fauna as well as in human being through consumption of contaminated fish and river sediments, and water. Mercury in sediments undergoes a methylation process, becoming one of the most toxic forms of this element, methylmercury, which has strong teratogenic, nephrotoxic and neurotoxic potential. Considering the danger which populations may be exposed, and mercury compounds are able to induce genetic damage, it was done a preliminary assessment with native fish (n = 9) of the river Madre de Dios through the erythrocyte micronucleus test, due to the assay is sensitive to both clastogens and aneugens. As a result micronuclei can contain either chromosome fragments or whole chromosomes. A total of 4000 cells were assess per individual. Similar behavior was observed for the frequency of micronuclei (MNC ‰) and damage index (ID) pacupeba> catfish> blanquillo (MNC ‰ 28167> 26750> 15667 and ID 0.39> 0033> 0018) respectively. Although these values are low, it must be consider that

both the pacupeba and catfish could be good bioindicator of mercury contamination. However, further research is required in this regard, increasing the number of individuals; also assess if the parasites (myxosporidios) that were found in catfish, generate additional damage by mercury contamination.

EM015
COMET ASSAY AND MICRONUCLEI TEST ON WILD BIRDS TO ASSESSMENT THE MUTAGENIC EFFECT OF HEAVY METALS POLLUTION IN TOWNS WITH MINER ACTIVITY

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Mines activity pollutes heavy metals from the ground and water of the surrounding areas. This may cause the gathering of some bio-toxines. The chronic gathering of this would provoke poisoning and even death. The environmental surveillance use bio-indexes to evaluate the damage on the environment as well as some genotoxines. Most of heavy metals have been shown as genotoxic for in-vitro cells. However, there are not any studies on the relation between the exposition to heavy metals and genotoxic effects on wild animals. Micronuclei test in blood and comet assay in liver, on adult specimens of Nothorprocta ornate and Nothura darwinii. The species used for this research were captured within the range of studies based on tisular bio-gathering of As, Cd, Pb, and Sb. These birds came from two miner towns where it was proved the edafic and aquatic pollution of heavy metals, and two towns without miner activity. In addition, four Nothorprocta ornate specimens were taken out of an experimental farm. The comet assay was standardized on the composition of the liver of these species. The visualization of nuclei was done by silver nitrate staining. None of these approaches revealed a significant difference between birds from towns with miner activity and towns without this activity. Thus, we conclude that no damage is done in these species by the miner activity. Although the species that were part of our experiment showed a great biogathering, they did not show a close relation between concentration of heavy metals and genotoxic damage

EM016
MUTAGENIC EVALUATION OF ETHANOLIC EXTRACT TO Baccharis genistelloides BY AMES TEST

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Baccharis genistelloides is a slightly shrubby plant commonly used in the folk medicine in Bolivia and in other countries in South America. This plant has many therapeutical properties as medicinal herb such as liver-protective properties, and stomach disorders; also to treat diabetes, ulcers, angine, anaemia, diarrhoea and inflamatory processes. B. genistelloides has many phytochemical compounds, such as essential oils, diterpenes (clerodane), saponins and their glycosides, and several flavones. Different biological properties for the extracts of B. genistelloides has been reported such as antioxidant, anti-inflamatory and antimutagenic activities against of 3-amino-1-methyl-5H-pyrido[4,3-b]-indole. The Salmonella typhimurium mutagenicity assay or Ames test (Maron and Ames 1983) is the most widely used test for assessing the genetic toxicity of chemicals. The bacterial strains used in this test have mutations in genes coding for enzymes required for the biosynthesis of the aminoacid histidine. Several of these strains contain frameshift mutations and thus respond to chemicals that induce frameshift mutations; others have base pair substitutions and are used to detect chemicals that induce this type of mutation. We tested the mutagenic potential of ethanolic extract from the air parts of B. genistelloides by the Ames test with the plate incorporation methodology and with the strains TA98, TA100 and TA102; with and without S9. The concentrations evaluated were 5, 2.5, 1.25, 0.625 and 0.3125 µg/plate; we used dimethilsulfoxide (DMSO) as diluent and negative control. The results showed that all the concentrations evaluated of B. genistelloides ethanolic extract are not mutagenic to any of the tested strains (- S9 and + S9) and in the conditions of our assay.

EM017
TOXIC AND GENOTOXIC ACTIVITY TO CLORPIRIFOS BY SMART TEST

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Chlorpyrifos as an active ingredient found in various pesticides, is a broad spectrum organophosphate and is used in agriculture and household pest control, is water soluble making contact with the organic phase environment. These components inhibit acetylcholinesterase (AChE), an enzyme of the nervous system of a number of species, including humans, that is activated in the human liver by cytochrome P-450, through oxidative desulphurization to toxic forms similar to oxygen, as well as non-toxic metabolites, which did not inhibit AChE. In this study toxic and genotoxic capacity to pesticide chlorpyrifos were evaluated using Drosophila melanogaster. Larvae of third instar from the standard (ST) and high bioactivation (HB) crosses were fed with concentrations between 7 to 0.0015 parts per trillion (ppt) of the pesticide. Lethal 50 dose was determined by counting survivors and genotoxic evaluation test was conducted by the somatic mutation and recombination test in Drosophila wings with genetic markers mwh and flr3. The organophosphate pesticide chlorpyrifos with trade name Lorsban 48E presents toxic activity at concentrations between 0.2 and 7ppt and subtoxic activity between 0.0015 and 0.1 ppt, this may be because of chlorpyrifos neurotoxicity by the inhibitory action of the enzyme acetylcholinesterase and the decrease of haemocytes . In relation to genotoxicity, we followed the protocol established by Graf et al. (1984), in the standard cross, Lorsban 48E showed inconclusive answer, however in the high bioactivation cross, genotoxic activity was found at concentrations of 0.1, 0.05, and 0.015 ppt. This reaction could be produced by the trichloropiridinol a secondary metabolite, and components of formulation of the pesticide: chlorpyrifos, xylene and emulsifiers.

EM018
GENOTOXIC EVALUATION OF MILKS BY SMART TEST

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Aflatoxins (AF) are secondary metabolites of fungi mainly of the Aspergillus genus with all the aflatoxins AFB1 is considered the most carcinogenic compound, followed by AFM1 which is a product of the biotransformation of AFB1, the AFM1 is excreted in milk by cows that have eaten food contaminated with AFB1 due to food mishandling that is given to cattle, generating a proliferation of fungi due to favorable conditions of humidity and temperature. Milk is a fundamental food for children, and the possibility that this contaminated with AFM1 should be a concern. In this research we have evaluated 15 samples of raw milk, 12 milk ultrapasteurized and 6 powdered milk to the level of contamination AFM1 by the ELISA method (Test Ridascreen Fast Aflatoxin M1), then the genotoxic assessment by Somatic mutation and recombination test (SMART) in wings to Drosophila melanogaster of the sample with the highest concentration of the AFM1. We used standard and high bioactivation crosses. Larvaes to third instar from both crosses were fed with different sample concentrations; the surviving individuals were counted and then the hairs of the wings were analyzed according to the protocol of Graf et al. (1984) to observe the frequency of mutations using genetic markers mwh (multiple hairs) and flr3 (wide base hairs). All milks were positive for the presence of AFM1 with values ranging from 40, 94 up to 804.02 ppt of AFM1. The results for the most contaminated milk showed genotoxic activity for standard cross in 50, 200 and 800 ppt, whereas the high bioactivation cross did not. In brief milk assessment revealed a genotoxic potential.

EM019

NITRO-COMPOUNDS INVOLVEMENT TO THE MUTAGENICITY OF URBAN PM2.5 AND PM10 IN TURIN.

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Fine particles may be active carriers of toxic compounds into the lung alveoli. The direct acting mutagenicity per unit mass of fine PM is significantly higher than those of the coarse particles especially in urban area, where the nitro-compounds seem to play a predominant role on the total mutagenicity. In this study the mutagenic properties of urban PM2.5 and PM10 were evaluated and the nitro-compounds role was estimated. PM2.5 and PM10 samplings were daily performed during the 2007 in Turin and a consolidated in vitro test - the Salmonella assay - is conducted with PM2.5 and PM10 organic extracts. The mutagenic properties were assessed for each month of sampling. S. typhimurium TA98 and TA98 derived strains were used. Nitroreductase-less mutant TA98NR and YG1021 carrying a nitroreductase-producing plasmid assays were performed to define the nitro-compounds burden on the mutagenic properties. The annual measured mean levels of PM2.5 and PM10 were 34±20 and 48±18 µg/m3. The PM2.5/PM10 ratio ranged from 0.36 to 0.89. The Salmonella assay showed higher mutagenicity in autumn/winter (20±15 TA98NR, 54±39 TA98, 173±161 YG1021 net revertens/m3) respect to spring/summer (2±2 TA98NR, 7±8 TA98, 24±27 YG1021 net revertens/m3)($p<0.01$). There is also statistical significant seasonal difference in the gravimetric analysis data. The ratio between the TA98 net revertants per PM2.5 µg is 6.5 folder than per PM10 µg. Moreover bioassays results showed an amplified response in the YG1021 strain and a reduced response in the TA98NR strain. The net revertant ratio TA98NR/YG1021 is equal to 11±4 for PM2.5 and 13±6 for PM10 organic extracts ($p<0.01$). There is a significant correlation with NO concentration especially for YG1021 net revertants obtained after PM10 organic extract exposures. These results highlight the critical role of the urban fine and ultrafine PM pollution and show up the predominant role of the bacterial nitroreductase on the induced mutagenicity. The reduction of the NO2 functional groups and the generation of more reactive intermediates is fundamental to produce DNA mutations. These findings can describe a relevant role of the nitro compounds and they underline a primary prevention improvement in order to reduce nitrated molecules air pollution.

EM020

DETECTION OF INDUCED GENOMIC POLYMORPHISMS IN LUNG CELLS AFTER IN VITRO EXPOSURE TO PM2.5 ORGANIC EXTRACTS.

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Background: The mutagenic and genotoxic properties of the particulate matter is widely demonstrated especially for fine particles. Various DNA damages have been well proved both in prokaryotic and eukaryotic systems after in vitro exposition. Among these injures there are base insertion, deletion and substitutions, cross links and pyrimidine dimer formation, single and double stand breaks. These kinds of damage could be promoted by compounds carried by the PM, which are able to interact directly with the DNA or after modifications mediated by cell enzymes (e.g. oxidation, alchilation and DNA bulky adduct formation). A simple way to detect DNA modification could take advantage from the comparison of amplification profiles obtained with random or semi-random PCR techniques. The goal of this work is to propose a new-method to describe the genotoxic properties of fine PM. Such method, based on a bio-molecular approach, involves the study of genetic fingerprint of exposed and control cells. Materials and Methods: PM2.5 was sampled during the winter season. Embryonic human lung fibroblast (HELF) cultured cells were exposed to increasing amounts of PM2.5 organic extracts. Cell proliferation and viability were evaluated, and the XTT assay was performed to estimate the mitochondrial activity of live cells. Parallel series of HELF cells were used for total DNA extraction and AFLP (Amplified Fragment Length Polymorphism) characterisation and their electrophoretic profiles were then compared with those of non-exposed cells. Results and Conclusions: At the maximum dose of exposure (250 µg of PM2.5 / ml of culture medium) we observed 56% cell viability reduction, 83% of mitochondrial activity conservation and the 9% of polymorphic amplification products, respectively, in treated cells compared to the controls. These preliminary data show the practicability of this method in detecting appreciable PM-induced polymorphisms levels and its suitability in evaluating genotoxic effects of air pollutants.

EM021

CLONING AND EXPRESSION OF THE TUMSTATIN ACTIVE PEPTIDES-T7 AND ITS DERIVANT-T7-NGR

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Aim To enhance the role targeting, design to link NGR sequence with tumstatin active peptides-T7's C-terminal,the derivant called T7-NGR. Methods The cloning vector pMD-T7 and pMD-T7 N were constructed by PCR and gene synthesis methods, respectively, identified by digestion and DNA sequencing. After the digested plasmids were solated by the low melting point agarose electrophoresis,the target-fragment was cut off and mixed with the recovery of the digested vector pET28a. Expression vector pET-T7 and pET-T7 N were constructed in low melting point agarose, identified by digestion and DNA sequencing, transformed into competent Escherichia coli BL21 (DE3), induced by IPTG. Results Identification result shows that pET-T7 and pET-T7 N were correct. Tricine-SDSPAGE results showed that IPTG concentration of 1 mM,after the induction of 25_C, 8 h, T7 peptides and T7-NGR peptides have achieved the optimum conditions of expression. Conclusions In conclusion, the expression vectors of the two peptides has been successfully constructed, and got product, no coverage at home and abroad, laid the foundation for further activity experiments.

EM022

EVALUATION OF MICRONUCLEUS INDUCTION BY AIRBORNE PM2.5 AND COMPARISON WITH SALMONELLA ASSAY RESULTS

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Background: fine PM is an important health risk for citizens. Arpa Emilia Romagna created in 1997 a regional network for continuous monitoring of mutagenicity of PM2.5 in different urban sites of the region using Salmonella typhimurium reversion bioassay.
Aim: mutagenicity assessment of urban PM by in vitro micronucleus assay compared with Salmonella typhimurium assay (Ames).
Methods: PM2.5 particulate samples are collected in February, July and December from 2004 to 2006 in Parma, Bologna, Ferrara and Rimini and from Feb 2005 in Ravenna. The sampling is continuous during 24h (sampling flow 1m3/h) and PM mass is determined by gravimetric analysis; monthly filters are extracted with acetone by Soxhlet apparatus. Genotoxicity is evaluated using cytokinesis-block micronucleus assay (CBMN) in human lymphocytes from whole blood of healthy donors and using two Salmonella strains (TA98 and TA100) with and without metabolic activation (S9). In the same extracts we determined Polycyclic Aromatic Hydrocarbons (PAHs) concentrations, too.
Results: CBMN assay results show the presence of clastogenic/aneugenic substances, adsorbed on PM, only in 2 winter samples of Bologna, Feb 2004 and 2005. However some samples show toxicity at higher doses (1.6 and 3.2 m3/ml). Data from Salmonella assay show presence of mutagenic substances with a prevalence of direct-acting mutagens, in a typical seasonal trend, with higher levels in autumn-winter and lower in warmer period of the year. In this study, induction

of micronuclei is not correlated with PAHs and PM2.5 concentrations ($R2 < 0.4$). Comparing the bioassays we found the best correlations (revertants/m3 vs MN/m3) in Ferrara and Ravenna samples, that show Pearson's r varying from 0.5 to 0.6. Conclusions: in this study micronuclei induction seems not to be related to PAHs and PM2.5 levels, highlighting that mutagenicity can be associated to other classes of substances and that is determined by PM quality rather than its quantity. The use of several mutagenicity assays, with different endpoints, gives a better knowledge of PM genotoxicity. In fact the poor correlation between CBMN and Salmonella assay results shows that one bioassay cannot be used as a powerful marker of genotoxicity.

EM023

COMPARISION OF MUTAGENICITIES AND GENE EXPRESSION PROFILES OF COMFREY AND RIDDELLIINE IN RAT LIVER

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Pyrrolizidine alkaloids (PAs) are constitutes of over 6000 plants, and about half of the identified PAs are genotoxic and many are tumorigenic in humans and experimental animals. Riddelliine is one of the tumorigenic PAs and has been studied as a prototype of PA. Comfrey (*Symphytum officinale*) is a perennial plant containing 9 PAs and has been consumed by humans as a vegetable, a tea and an herbal medicine. In this study, we evaluated the mutagenicities and gene expression profiles of comfrey and riddelliine using a rat model to examine whether the PAs in comfrey were responsible for the plant's carcinogenicity. The treatment schedules were based on previous protocols that resulted in liver tumors in rat. Groups of 6 Big Blue Fisher 344 rats were treated with riddelliine at 0.1-1 mg/kg body weight by gavage five times a week for 12 weeks or fed a diet containing 2-8% comfrey root for 12 weeks. Animals were sacrificed one day after the last treatment and the livers were isolated for the *cII* mutation assay and gene expression analysis. The riddelliine treatments produced a significantly dose-dependent increase in mutant frequency (MF) in the *cII* gene in rat liver. Comfrey treatment also significantly induced MFs with a similar level at the different doses. The mutational spectra induced by comfrey and riddelliine treatments were very similar, in which the major type of mutations were G:C → T:A transversions with a high frequency of tandem base substitutions. Comparison of the differentially expressed genes and biological processes altered by the high-dose treatments of comfrey and riddelliine showed that there were a number of common genes and functional processes that were related to PA carcinogenesis. There was a strong correlation between the two treatments for expression alterations of the genes related to the drug metabolisms and carcinogenesis of PAs. The mutation result suggests that the mutagenicity of comfrey is associated with the PAs contained in the plant and the similarity of carcinogenesis-related gene expression patterns from the treatments of comfrey and riddelliine indicates that PAs contained in comfrey are the main active components responsible for the carcinogenicity of the plant.

EM024

IS 4-VINYLPHENOL A GENOTOXIC AGENT?

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4-Vinylphenol (4-VP) is an environmental pollutant, a constituent of tobacco smoke and a food flavour. Furthermore, 4-VP is a minor metabolite of styrene (IARC 2B) arising from 3,4 epoxidation of the aromatic ring. Experimental studies in rodents have shown that 4-VP is even more toxic for the liver and lung than styrene and its principal genotoxic metabolite styrene 7,8-oxide (7,8-SO). The results of an European study on styrene-exposed workers highlighted a significant correlation between micronuclei (MN) and the urinary levels of 4-VP. Aim of this work is to establish if 4-VP could exert genotoxic effects on human cells in vitro. Both Comet Assay on fresh leukocytes and Micronucleous test (CBMN assay) on EBV-transformed human lymphocytes were performed to identify if 4-VP could affect DNA integrity and/or chromosomal structure or number. Leukocytes of four subjects were treated with increasing doses (0-2000 µM) of 4-VP and analyzed by alkaline Comet assay; none of them showed an increased DNA migration. The EBV-transformed cell line was characterized for its spontaneous frequency of MN and showed a stable MN frequency among independent experiments. It was possible to detect an induced damage of clastogenic and aneugenic known chemicals, such as bleomycin, colcemid, diethylsilbestrol and 7,8-SO. The induction of micronuclei of increasing concentration of 4-VP (0-400 µM) on EBV-transformed cells was analyzed. We observed a significant increase of MN in cells treated with 4-VP; the lowest effective dose ($p<0.05$) was 100 µM. In cells treated with 400 µM the MN increase (29 MN/1000; $p<0,001$) was comparable with the Colcemid induced one but lower than that induced by 7,8-SO (48 MN/1000); at this concentration, 4-VP caused a cell division inhibition comparable to that induced by treatment with 7,8-SO (200 µM). The use of a lymphoblastoid line allows us to highlight an in vitro genotoxic activity of 4-VP, only hypothesized until now. The significant MN increase and the complete absence of DNA damage induction could support an aneugenic mechanism of action. Further studies are in progress to identify the nature of the genotoxic activity (aneugenic or clastogenic ?) of 4-VP.

EM025

POTENTIAL GENOTOXICITY OF NANO-SIZED TiO2 PARTICLES IN ISOLATED BOTTLE-NOSE DOLPHIN (Tursiops truncatus) LEUKOCYTES

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Nanotechnology is a developing field, however our knowledge on the environmental fate and impact of nanoparticles (NPs) is still limited. Many studies on NPs toxicity have been focused on their potential effects on human health, but scarce information are available on the susceptibility of endangered species, such as marine mammals. A possible accidental way of exposure to NPs during their industrial production is the skin; TiO2 NPs, present in solar creams are able to migrate to the corneal tissue and known to interfere with the immune system. This study is aimed to assess the profitability of isolated leukocytes for evaluating the potential genotoxic effects of NPs on toothed cetaceans. The bottle-nose dolphin Tursiops truncatus was selected as study species being commonly reared in captivity. Blood samples from four males and a female specimens were achieved by the Adriatic SeaWorld "Oltremare" (Riccione, Italy). Leukocytes were isolated by the lyses procedure and exposed in vitro to TiO2 (rutile and anatase) NPs. Three experimental times of exposure (4, 24, 48h) and three doses (20, 50, 100 µg/ml) were tested. Hydrogen peroxide was used as positive control. Genotoxicity was assessed by the Comet Assay (detecting DNA strand breaks) and the Micronucleus Test (detecting chromosomal alterations). Cell viability was also assed by the Trypan Blue test. Comet Assay preliminary results regarding 4h exposure indicate no genotoxic effect of TiO2 nanoparticles. However, for two of the five animals examinated, at high doses (100 µg/ml) rutile titanium dioxide seems to induce an increase in DNA fragmentation, respect to controls. Datas on the other times of exposure, and the ones coming from the other tests are still to be analysed.

EM026

GENOTOXICITY AND CELLULAR BIOMARKERS IN THE FRESHWATER BIVALVE UNIO PICTORUM MANCUS (MOLLUSCA, UNIONIDAE). A CASE-STUDY FROM CECINA RIVER (TUSCANY, ITALY).

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Rivers are receptacles for industry, agriculture, urban wastes containing mixture of pollutants, whose biological effects are hardly predictable. The EU highlighted the importance of water pollution within the 2000/60 WFD, which demands a good ecological status for rivers by the 2015. In this respect, a main challenge is to implement monitoring strategies to assess the biological impact of pollution in river basins. The use of resident or caged sentinel species is a valuable choice, especially when chemical data and biomarkers are integrated. The aim of this research was to use a panel of biomarkers based on genotoxic, lysosomal and antioxidant responses in the freshwater bivalve Unio pictorum mancus to assess the environmental quality of Cecina river (Tuscany Italy) mainly impacted by metals. Metal bioaccumulation was also investigated. Bivalves were collected from an unpolluted site and translocated (4 weeks) to seven sites characterized by different levels of chemical disturbance. Specimens translocated downstream the main source of metal pollution exhibited higher Hg and As bioaccumulation accompanied by the modulation of biomarker responses including either modified levels or activities of antioxidants studied and TOSC-HO.. In addition, increased micronuclei frequency (indicating chromosomal damage) and decreased Neutral Red Retention Time (indicating lysosomal alterations) were also detected in the most impacted sites. Otherwise, the result of the Comet assay did not evidence a clear trend of DNA damage among sites. In conclusion, U. pictorum mancus was proven to be a profitable sentinel species for biomarker approach and caging procedure to assess environmental quality in freshwater ecosystems. Moreover, data obtained by maintaing samples under experimental controlled conditions in tanks with sediments taken from the same stations of the river basin used for translocation studies contributed to better characterize the environmental impact.

EM027

MODULATION OF CYTOCHROME P450 ACTIVITY BY BIOTIN.

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Cytochrome P450 (CYP) is a superfamily of enzymes that metabolize the majority of xenobiotics in several organisms including mammals. Metabolism of xenobiotics plays an important role in chemical carncinogenesis1; CYP activity may result in the generation of electrophilic metabolites which can react with proteins and DNA, forming adducts and increasing the risk of developing cancer. On the other hand, Biotin is a water-soluble vitamin that acts as cofactor of carboxylases which catalyzes key reactions in gluconeogenesis, fatty acid metabolism and amino acid catabolism. Biotin has been proposed as a possible safe alternative in the treatment of hyperglicemia2; thereafter, it's mandatory to realize toxicity tests, including the possibility of drug-drug interactions due to modulation in the basal activity of CYP. Pioneering studies by Dakshinamurti3 provided evidence that biotin affects gene expression. Since then, researchers have identified more than 2000 human genes susceptible of being modulated by biotin4, 5. Nevertheless, with the exception of CYP1B1 in Jurkat cells6, there are no data concerning enzymes involved in Phase I metabolism. This study was focused on the capacity of Biotin to modulate the expression of CYP1A1 and CYP1A2 in the liver of rats. These enzymes are involved in the metabolism of several procarcinogens and commonly-used drugs. Male Wistar rats were divided into 8 groups, 4 control and 4 experimental (4 animals per group). The experimental groups were treated daily with Biotin (2mg/kg, i.p.) and sacrificed after 1, 3, 5 and 7 days of treatment; control groups were treated exactly as experimental ones, but they were injected with distilled water. Livers were removed and microsomes were prepared according to the procedure described by Maron and Ames7. CYP1A1/A2 activities were measured spectrofluorometrically by monitoring the formation of resorufin according to Burke et al 8. Gel electrophoresis and immunoblot analysis were carried out to assess protein concentration. Our results show that CYP1A2 enzyme activity but not protein concentration was significantly increased 24 h after administration of 1 dose of Biotin. Possible drug-drug interactions could be anticipated in the use of Biotin as a therapeutic drug in humans.

EM028

DO PROTEIN CONJUGATES OF POLYCYCLIC AROMATIC HYDROCARBONS INDUCE GENOTOXIC EFFECTS *IN VITRO* OR *IN VIVO*?

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Polycyclic aromatic hydrocarbons (PAH) belong to the most extensively studied groups of ubiquitous environmental pollutants, because they cause a broad spectrum of toxic and genotoxic effects in cells and organisms, including DNA mutations, carcinogenesis, teratogenesis, and immune dysfunctions. The primary routes of human exposure to PAH are inhalation of polluted air and tobacco smoke, as well as ingestion of contaminated food and water. One of the protective reactions of the organisms against negative effect of various agents is a production of antibodies, especially neutralizing antibodies, which can catch and neutralize the agents. In our project, we follow the induction of antibodies against PAH after short immunization. PAH are small molecules. Generally, small molecules become an immunogens after conjugation with height-molecular proteins, e.g. KLH (keyhole limpet hemocyanine) and can cause the production of antibodies after short-time immunization. Some PAH, especially benzo(a)pyrene (B(a)P), are genotoxins and are able to induce DNA adducts after their metabolic activation. For that reason, our research interest was focused on genotoxic/non-genotoxic effect of PAH-protein conjugates, which will be used for immunizations of experimental animals. In *in vitro* experiments, HepG2 cells were incubated with PAH-KLH conjugates (B(a)P-, pyrene-, anthracene-,and chrysene-KLH), and in *in vivo* experiments, laboratory mice were immunized by nine PAH-KLH conjugates. During immunization, the production of specific antibodies against PAH in blood serum of mice was detected. PAH-DNA adducts in HepG2 cells (in vitro experiments) or in hepatic cells from immunized mice (in vivo experiments) were detected by ^{32}P-postlabeling. As a positive control was used B(a)P.We found, that none from tested PAH-KLH conjugates induced DNA adducts in HepG2 cells as well as in hepatic cells from immunized mice. Moreover, DNA adducts was not detected in HepG2 cells, which were incubated with anti-PAH antibodies-enriched serum from immunized mice. Presented PAH-KLH conjugates could be used for immunizations without negative genotoxic effect in organisms.
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EM029

THE USE OF GENOTOXIC BIOASSAYS TO EVALUATE THE ENVIRONMENTAL QUALITY IN A REGION UNDER THE INFLUENCE OF URBAN WASTE IN GUAÍBA LAKE BASIN (BRAZIL).

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The mussel species Limnoperna fortunei was chosen as biomonitoring

organism in Guaíba Lake Basin, based on population data, distribution, and sensitivity. Previous in vitro and in vivo studies with Single Cell Gel Assay (SCCGA) and Micronuclei test (Mn) on this freshwater mussel showed that it is successful in biomonitoring studies, especially in urban pollution monitoring. This was observed using this model to assess genotoxicity in samples collected from the rivers that flow into Guaíba Lake and from the Lake. Urban influence was identified as the main contamination source of genotoxins in this river basin region. In this context, this study evaluated two sampling sites in the Guaíba Lake (Guaíbe PC e Guaíba BR), near urban waste discharges, and a control site (Itapuã) insight a preserved area, using L. fortunei individuals. Mussels were sampled in situ and exposed to laboratory conditions, and tested by SCGA and MN using haemolymph cells. In order to understand environment mutagenic contamination, surface water and sediment interstitial water were tested by the Salmonella/microsome assay. Comparing to the control site, the Guaíba BR sample induced DNA damage in haemocytes of mussels sampled both in situ ($P< 0.05$) and exposed to laboratory ($P< 0.05$) conditions, whereas MN only in in situ ($P< 0.05$) collected mussels. This sample also presented the only surface water mutagenic sample with TA98 (MI= 2.90) mutagenicity in the presence of metabolic activation. Guaíba PC samples increased MN frequency in situ ($P< 0.01$) and in laboratory ($P< 0.05$) conditions comparing to the Itapuã results. Metal influence seems to be less important than organic influence in genotoxic induction. These results confirm the strong urban influence in this region, showing that biomonitoring is a powerful tool to detect this kind of contamination in water bodies.

EM030
A POLYMERASE-TAUTOMER MODEL FOR UV TARGETED SUBSTITUTION MUTATIONS

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The polymerase-tautomer model for ultraviolet mutagenesis is based on formation of rare tautomeric bases in cis-syn cyclobutane pyrimidine dimers and that fact that during error-prone or SOS synthesis the induced DNA polymerase inserts canonical bases opposite the dimers in a complementary way in contrast to uncomplementary one in the conventional models. Error-prone and SOS replication of double-stranded DNA having cis-syn cyclobutane cytosine and thymine dimers, with one or both bases in a rare tautomeric conformation, results in targeted transitions, transversions and one-nucleotide gaps. There are same types of potential mutagens damages in cis-syn cyclobutane cytosine and thymine dimers. They correspond to 5 basic types of rare tautomeric conformations of thymine [Grebneva, 2006] and 7 of cytosine. The structural analysis indicates that three types of cis-syn cyclobutane cytosine dimer containing a single tautomeric base can cause G:C→A:T transition or G:C→C:G homologous transversion. Another two dimers can result only in G:C→T:A transversion. The one type of dimer can cause G:C→A:T transition, G:C→C:G homologous transversion or G:C→T:A transversion. The two types of the dimers may result in one-nucleotide gaps. It will be found that A-rule and the given model of UV mutagenesis mutually supplement each other. Grebneva HA. 2006. A model for targeted substitution mutagenesis during SOS replication of double-stranded DNA containing cis-syn cyclobutane thymine dimers. Environ Mol Mutagen 47:733-745.

EM031
OXIDATIVE DAMAGE IN HUMAN EPITHELIAL ALVEOLAR CELLS EXPOSED IN VITRO TO OIL FLY ASH TRANSITION METALS

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Among particulate matter emissions from combustion processes, oil fly ash (OFA) displays a marked oxidative and inflammogenic reactivity, due to the high content of bioavailable transition metals. In the present study, we evaluated the biological effects of an OFA water solution, composed of the transition metals Fe (57.5%), V (32.4%), and Ni (10.1%), in human epithelial alveolar cells (A549 line). The fluorimetric analysis by 2',7'-dichlorofluorescein showed a significant, dose- and time-dependent induction of intracellular reactive oxygen species (ROS) triggered by OFA metal components at subtoxic doses. The metal chelator deferoxamine and the radical scavenger dimethylsulfoxide attenuated the metal-induced generation of ROS. Confocal microscopy observations strengthened these findings and showed an intense cytoplasmic fluorescence with perinuclear thickenings in A549 cells, in the absence of morphological damage. Metal-induced generation of ROS was significantly correlated with a dose- and time-dependent DNA damage, as assessed by single cell gel electrophoresis (comet assay). Catalase was able to decrease dramatically DNA damage. Fluorimetric analyses by diphenyl-1-pyrenylphosphine showed a parallelism between generation of ROS and formation of lipid peroxides. The results obtained in the experiments evaluating the effects of individual metal solutions did not show any significant difference in DNA damage between Fe(III) and V(IV), but highlighted the higher capability of V(IV) to increase ROS in the cytoplasmic compartment. The different behavior of these two elements, confirmed by the weak Fe-induced lipid peroxidation, may be ascribed to the presence of Fe-binding proteins, such as ferritin, in the cytoplasm. Finally, Ni(II) had negligible effects on ROS production. On the whole, the results obtained in this study show the strong capability of transition metals adsorbed to OFA to cause widespread damage to biological macromolecules, and suggest potential health effects resulting from exposure to power plant emissions in industrialized sites.

EM032
BIOMONITORING OF DNA DAMAGE IN PERIPHERAL BLOOD LYMPHOCYTES OF SUBJECTS WITH DENTAL RESTORATIVE FILLINGS

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Dental fillings provide a major iatrogenic exposure to xenobiotic compounds due to the high prevalence of surface restorations in developed countries. Experimental data suggest that both amalgams, which contain mercury, and resin-based dental materials cause an impairment of the cellular pro- and anti-oxidant redox balance. The aim of this study was to assess the potential genotoxicity of dental restorative compounds in peripheral blood lymphocytes of young exposed subjects compared with controls. The study examined, by use of the comet assay, 68 carefully selected subjects taking into account the major known confounding factors. In the 44 exposed subjects, the mean numbers of restored surfaces was 3.0 and 3.8 in males and females, respectively. Tail length, percentage of DNA in the tail, tail moment or Olive tail moment were twofold higher in the exposed group than in unexposed controls, with significant differences. No significant difference was observed between amalgam and composite fillings. Furthermore, as shown by multivariate analysis, the association between dental fillings and DNA damage was enhanced by the number of fillings and by the exposure time. Among the lifestyle variables, a moderate physical activity showed a protective effect, being inversely correlated to the DNA damage parameters evaluated. On the whole, the use of DNA-migration allowed us to detect for the first time the potential adverse impact on human health of both kinds of dental filling constituents, the amalgams and the methacrylates. The main mechanism underlying the genotoxicity of dental restorative materials of various nature may be ascribed to the ability of both amalgams and methacrylates to trigger the generation of cellular reactive oxygen species, able to cause oxidative DNA lesions.

EM033

FORMATION OF DNA ADDUCTS BY THE ENDOCRINE DISRUPTOR BISPHENOL A IN VITRO AND IN MOUSE LIVER AND EPITHELIAL MAMMARY CELLS

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Endocrine disruptors (ED) represent a major toxicological and public health issue, and the xenoestrogen bisphenol A (BPA) has received a tremendous amount of attention due to the high production volume and widespread human exposure. Also due to its similarity to diethylstilbestrol, a known human carcinogen, BPA has been investigated for its genotoxic and carcinogenic properties, but the results have been either inconclusive or controversial. Metabolically activated BPA has previously been shown to form DNA adducts both in vitro and in rat liver. The present study was designed (a) to assess the sensitivity threshold of DNA adduct detection by 32P postlabeling in an acellular system and (b) to evaluate formation of DNA adducts in both liver and mammary epithelial cells of female CD-1 mice receiving BPA with the drinking water (200 mg/kg body weight) for 8 consecutive days. The reaction of BPA with calf thymus DNA, in the presence of S9 mix, resulted in a dose-dependent formation of multiple DNA adducts, with a detection limit of □10 ng of this ED. Administration of BPA to mice resulted in the evident formation of DNA adducts not only in the liver (3.4-fold over controls) but also in target mammary cells (4.7-fold). Although DNA adducts do not necessarily evolve into tumors or other chronic degenerative diseases, the presence of these molecular lesions in target mammary cells may bear relevance for the potential involvement of BPA in breast carcinogenesis.

EM034

EXPOSURE TO ELECTROMAGNETIC FIELDS AND EFFECTS ON GENOMA.

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The interaction between electromagnetic fields (EMF) and living being represents a current and complex issue. Main studies deal with mutational effects, environmental exposure, biological damage, epidemiological evidences. The non ionising nature of EMF does not support a classical action on macromolecules, however, studies reported changes in MN test or comet assay. New perspectives raised from molecular biology and analysis of gene transcription. EMF are an almost ubiquitous environmental factor, whose exposure affects large populations worldwide. In the last decades, several EMF frequencies have been considered in complex studies, including ELF, radiofrequencies and microwaves. Most recently, attention is focusing on WiFi applications.

To the aim of describing the state of the art on environmental exposure and mutagenesis risk, epidemiological evidences and experimental achievements are briefly reviewed. In addition, to consider possible mechanisms of interaction between EMF and living being, a new exposure system developed to study WiFi exposure is introduced. Experimental models and data on gene transcription in vivo and in vitro are shown. Methods include an exposure setup made of a GTEM 250 cell, powered by a signal generator with proper amplifiers. Background magnetic flux density was measured and exposure conditions were optimized. Dosimetry and specific SAR was calculated by numerical dosimetry. Micronucley test were performed by standard protocols and RNA analysis by microarray. Data were analysed by a bioinformatics approach. To confirm expression profiles, oligonucleotides were designed and candidate genes were verified by real time pcr. Results do not support evidences for a mutational effect of EMF. Presence of major rearrangements in gene transcription was not confirmed. However, several studies report transcriptional changes at selected genes, suggesting a possible action of EMF on cell pathways and genome functions. Interpretation of results requires extreme prudence due to the novelty of the issue and the presence of contradictory results in the literature. The large environmental exposure of population and the differences in the risk perception require rigorous evaluation of data and consciousness in result communication.

EM035

UPREGULATION OF STEM CELL ANTIGEN-1 IN THE LUNG OF NEONATAL MICE EXPOSED TO ENVIRONMENTAL CIGARETTE SMOKE

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There is adequate reason to suspect that perinatal exposures to carcinogens contribute to both childhood cancers and cancers appearing later in life, and certain adult diseases may have their origins early in life. In particular, early age smoking has been viewed as an independent risk factor for lung cancer, and adolescence may constitute a critical period during which tobacco carcinogens can induce fields of genetic alterations. Mice are particularly susceptible to carcinogens when exposure starts early in life. We evaluated the expression of stem cell antigen-1 (Sca-1) gene in the lung of variously aged CD-1 mice, either untreated or exposed to environmental cigarette smoke (ECS) and/or to a light source. Sca-1 expression progressively decreased with age. The expression of Sca-1 gene and the amount of Sca-1 protein, which was exclusively localized in endothelial cells of the pulmonary vasculature, were significantly upregulated in mice exposed either to ECS or ECS plus light throughout the weaning period, starting at birth. Therefore, the observed variations of Sca-1 in mouse lung, as related to exposure of neonatal mice to ECS, are very likely to reflect the amounts of bone marrow-derived cells in the pulmonary vascular endothelium. This conclusion is consistent with our previous findings that exposure to ECS upregulates vascular endothelial growth factors (VEGFs), endothelin, and other angiogenesis-related genes or proteins in rodent tissues. These findings may contribute to explain the high vulnerability of mouse lung early in life.

EM036

HIGH SUSCEPTIBILITY OF NEONATAL MICE TO MOLECULAR, BIOCHEMICAL AND CYTOGENETIC ALTERATIONS INDUCED BY ENVIRONMENTAL CIGARETTE SMOKE AND LIGHT

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Our recent studies have shown that both cigarette smoke and UV-containing light, which are the most widespread and ubiquitous mutagens and carcinogens in the world, cause systemicgenotoxic damage in hairless mice. Further studies were designed with the aim of evaluating the induction of genotoxic and carcinogenic effects in Swiss albino mice exposed to smoke and/or light since birth. We observed that a 4-month whole–body exposure of mice to mainstream cigarette smoke, starting at birth, caused an early and potent carcinogenic response in the lung and other organs. Our further experiments showed that exposure of mice to environmental cigarette smoke, during the first 5 weeks of life, resulted in a variety of significant alterations of intermediate biomarkers, including cytogenetic damage in bone marrow and peripheral blood, formation of lipid peroxidation products, increase of bulky DNA adduct levels, induction of oxidative DNA damage, and overexpression of OGG1 gene in lung, stimulation of apoptosis, hyperproliferation and loss of Fhit protein in pulmonary alveolar macrophages and/or bronchial epithelial cells, and early histopathological alterations in the respiratory tract. Moreover, exposure of mice to UV-containing light, mimicking solar irradiation, significantly enhanced oxidative DNA damage and bulky DNA adduct levels in lung, and synergized with smoke in inducing molecular alterations in the respiratory tract. The baseline OGG1 expression in lung was particularly high at birth

and decreased in post-weanling mice. Oxidative DNA damage and other investigated end-points exhibited differential patterns in post-weanling mice and adult mice. The findings of these studies provide a mechanistic clue to the general concept that the neonatal period and early stages of life are critical in affecting susceptibility to carcinogens.

EM037

REGULARITIES OF A JOINT EFFECT OF STABLE STRONTIUM, CALCIUM AND FLUORINE ON PLANTS AT A CYTOGENETIC LEVEL

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In 1909 E.A. Mitcherlich, a German agrochemist, had formulated the law of a joint effect of factors. This law is usually associated with a crop yield and we have tried to test its validity at a cellular level and to interpret in terms of the current state of cell biology and molecular genetics. Our experiments seek to study a separate and a combined effect of stable strontium, calcium and fluorine ions using Allium test technique. The effect is assessed from mitotic indices in root meristem cells as well as aberration cell (ACF) frequency and spectrum. The following solution concentrations are used:
• SrCl2 7 mg/l (average level in ground water in the Central Russia), 70 mg/l (in strontium);
• CaCl2 24 mg/l (content in natural water); 32 mg/l (in calcium);
• NaF 1.2 mg/l (in fluoride).
It is shown that only at strontium concentration equal to 70 mg/l (and at 32 mg/l in calcium) the mitotic index is really below the reference value. At strontium concentrations of 7 mg/l the fraction of aberrant cells, however, is higher as compared to the experiment (4.26 versus 1.80). In case of a joint effect of Sr+Ca the fraction of aberrant cells increases as compared to reference values. The coefficient of a joint effect of two factors is found from K=ACFΣ / (ACF1+ ACF2) where ACFΣ is at a joint effect of factors 1 and 2 minus ACF in a reference experiment, ACF1 is at a separate effect of factor 1 minus in a reference experiment, ACF2 is in case of a separate effect of factor 2 minus ACF in the reference experiment. If K>1, one may speak about mutual intensification (synergism), if K<1, there is a mutual attenuation of effects (antagonism), if K=1, it is a simple addition of two effects (additivity). In our case, for a joint effect of strontium, calcium and fluoride ions the following K coefficient values are obtained:
• for Sr + F: K = 0,09;
• for Ca + F: K = 0,24;
• for Sr + Ca: K =0,68.
Hence, in all three cases there is antagonism, i.e. calcium and fluoride remove the adverse effect of strontium on plant genes and cells. Strontium ions probably stimulate apoptosis in onion cells and fluoride and calcium ions prevent this process.

EM038

CYTOTOXICITY AND GENOTOXICITY OF PALYTOXIN-LIKE COMPOUNDS FROM OSTREOPSIS SPECIES.

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During the last decade, the occurrence of harmful algal blooms has increased both in frequency and in geographic distribution in many regions of the world. Benthic dinoflagellates of the genus Ostreopsis have expanded their distribution from tropical-subtropical to temperate waters, such as the Mediterranean Sea. Among the thousands of species of microscopic algae at the base of the marine food chain, there are a few dozen which produce potent toxins that impact human health through the consumption of contaminated fish or shellfish, or through water or aerosol exposure. Palytoxin (PLT) is one of the most potent marine biotoxins known to date, exhibiting extreme toxicity in mammals. Analyses of plankton samples collected along the Ligurian coasts (Italy) during a massive bloom of the tropical microalga Ostreopsis ovata indicated the presence of putative palytoxin (p-PLT) together with a palytoxin-like molecule which was named ovatoxin-a. The aim of our study was to characterize the toxic and potential genotoxic properties of algan toxins accumulated in shellfish tissues. A number of extracts from mussel samples, collected in the framework of a specific monitoring program for marine biotoxins in Liguria, were tested in vitro in human hepatoma HepG2 cells. Genotoxicity was evaluated using the comet assay and micronucleus test. A dose-response curve for cell-death was observed for 1 and 3 hours of exposure with 100% of mortality at the concentration 20 ng/ml p-PLT. Preliminary data revealed an increase of DNA fragmentation index and of micronuclei frequency.

EM039

LONG-TERM EXPOSITION BY DOMESTIC RADON RADIATION AND GENOTOXICAL EFFECTS IN CHILDREN FROM WESTERN SIBERIA

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Results of chromosomal aberration level and spectrum study in 48-hours peripheral blood lymphocytes cultures of 8-18 years old children-teenagers (n=211, mean 13,3 ± 0, 17 years old) living in the south part of Kemerovskaya area - Gornaya Shoria (boarding school, Tashtagol town) are presented. Mean metaphases with aberrations were 5,3 ± 0,16 % in studied group that is significantly higher (p < 0,01) than background level of this index in this region (Kemerovskaya area) - 2,62 ± 0,29%. Aberrations frequencies of separate classes were 3,91 ± 0,15 for single fragments; 1,29 ± 0,07 for pair fragments; 0,03 ± 0,01 for chromatide exchanges and 0,24 ± 0,03 for chromosome type exchanges. Furthermore in 4 individuals (1,9 %) were found Rogue cells that were contained polycentric, ring chromosomes and multiple double minutes. Radon concentration in living space and classrooms' air (total 36 measurements conducted in different seasons) was 68-1285 (mean 395 Bk/m3). Reasons of chromosomal aberrations frequency increasing in this mountain area inhabitants are discussed (ultrahigh radon radiation doses influence are included). This study was supported by the RFBR grant, 07-04-96031-r_ural_a; by the Russian Federal Agency for Science and Innovations (contract 02.512.11.2233).

EM040

CYTOGENETIC AND MOLECULAR CYTOGENETIC MONITORING OF NUCLEAR POWER PLANT WORKERS APPLYING CHROMOSOMAL ABERRATION ASSAY, MICRONUCLEUS TEST AND FLUORESCENT IN SITU HYBRIDIZATION (FISH)

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The results of long term biomonitoring of nuclear power plant workers with proper physical dosimetry control are presented. Aim: To evaluate the relationship between the accumulated dose and the frequency of chromosomal aberrations, micronuclei and stable translocations (FISH). Material and methods: The study comprises 294 subjects, employees in NPP "Kozloduy", 238 of them are exposed workers and 56 control subjects from the administrative staff. The exposed workers are divided into 7 groups according to the radiation doses received. The accumulated doses for 107 of the workers are above 200 mSv, as measured by film dosimeters. Chromosomal aberration assay was performed in peripheral lymphocytes of 205 exposed workers and 51 controls. Micronucleus test was applied in samples from 211 workers and 38 control subjects. FISH analysis for stable translocations comprises 77

samples, 30 of them controls. FISH analysis with pancentromeric DNA probe was apply to 32 MNT samples. Data regarding the age, time of NPP employment, life stile exposures were obtained. Results: The frequency of stable, as well as unstable chromosomal aberrations and micronuclei is significantly increased in the peripheral blood lymphocytes of exposed workers compared to the controls. This increase is observed when accumulated doses are above 100 mSv for unstable and stable chromososmal aberrations and higher than 200 mSv for micronuclei. Linear dose-effect relationship is estimated for the biomarkers used by applying multiple regression analysis. The frequency of stable translocations detected by FISH showed more expressed relationship with the accumulated doses long term radiation exposure as compared to the dicentrics frequency (R2 = 0,31 and 0,10) respectively. No difference between centromere-positive and centromere-negative micronuclei was found. Conclussion: Molecular cytogenetic technique (FISH) for scoring of stable translocations is the most useful approach for estimation of genetic damage after prolonged radiation exposure.

EM041

THE SELF-FERTILIZING MANGROVE KILLIFISH *KRYPTOLEBIAS MARMORATUS* : A POTENTIAL MODEL SPECIES FOR MOLECULAR CARCINOGENESIS AND ECOTOXICOGENOMICS

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Kryptolebias marmoratus is the only known self-fertilizing hermaphrodite vertebrate. Because of this uniqueness, there has been great interest in its biology, physiology, developmental biology, and genomics along with other basic and applied areas of environmental genomics and molecular carcinogenesis. In laboratory studies, *K. marmoratus* has shown susceptibility to a number of xenobiotics including carcinogens. Toxicologists are interested in the mechanisms of action of environmental chemicals of contemporary interest such as endocrine-disruptors and may consider this as a suitable fish model for study of chemically-induced carcinogenesis. Single short-term exposure to model chemical carcinogens is sufficient to induce tumorigenesis in *K. marmoratus* in a relatively short span of time. In recent years, a bulk of data has been generated about gene sequences of *K. marmoratus*. Laboratory maintenance of *K. marmoratus* is relatively easy. Gene bank data are readily available, though not as extensive as for zebrafish (*Danio rerio*) or Japanese madaka (*Oryzias latipes*). The expressed sequence tag (EST) data, differential display real time polymerase chain reaction (DD-RT-PCR), and Gene Sequencer 20^{TM} (GS-20) sequencing data have recently been helpful in understanding the genomics and proteomics of this species. Recently, sequences of the oncogenes, *ras* and tumor suppressor gene, *p53* have been reported in *K. marmoratus*. The expression of these important genes which play significant roles not only in carcinogenesis but also in other signal transduction pathways was observed to be modulated by environmental endocrine disrupting chemicals (EDCs). Having a large sequence database (cDNA sequences and ESTs) it is expected that the use of a microarray in the near future will open new vistas for this fish with applications in cancer research, aquatic toxicology, endocrinology and genomics. This review discusses potential uses of *K. marmoratus* in new biotechnological approaches of aquatic toxicology.

EM042

GENE EXPRESSION PROFILING OF COPPER-INDUCED RESPONSES IN THE INTERTIDAL COPEPOD *TIGRIOPUS JAPONICUS* USING 6K OLIGOCHIP MICROARRAY

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The intertidal copepod *Tigriopus japonicus* has shown promising results in classical acute and chronic toxicity tests. Besides, it is one of the most extensively used copepod species for gene expression studies involving environmental chemical exposure. The *T. japonicus* genomics appear to be promising area for study of mechanistic aspects of marine environmental pollutants. In this study, a 6K oligochip for *T. japonicus* was constructed and 5,463 spots (2,313 upregulated and 3,150 downregulated) were identified on microarray by hieratical clustering of genes after exposure to copper (10 g/L for 6,12 and 24 h). Finally, by gene annotation, 138 and 375 genes were observed to be upregulated and downregulated, respectively at all the time of exposure. Most of the changes of expression were observed at the short exposure of 6 h and the response persisted up to 24 h. The repertoire of cupper-induced gene expression is complicated, and several modes of function such as growth, physiological functions, metabolic functions, reproductive and hormonal regulation functions, detoxification functions, and antioxidant functions were involved in modulation. Cu specifically upregulated some isoforms of cytochrome P450 (CYP). On the other hand, a majority of downregulated genes encoded for proteins important for growth and development. The expression profile of selected genes was also confirmed by quantitative real time RT-PCR. These results demonstrate suitability of oligochip microarray for risk assessment of trace metals in *T. japonicus*. The expression profiles also provide insight into the mechanism of action of trace metals. Till today, major breakthroughs in invertebrate toxicogenomics have mainly been in *Daphnia* and *Drosophila*. However, *Daphnia*'s use is limited to freshwater ecotoxicogenomics. Here we propose an oligochip microarray-based approach for risk assessment of trace metals in a potential model marine test species.

EM043

EXPRESSION OF DNA DAMAGE RESPONSE GENES IN THE MARINE COPEPOD *TIGRIOPUS JAPONICUS* EXPOSED TO UVB

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Marine harpacticoid copepod *Tigriopus japonicus* which mainly inhabit a rock pool of intertidal zone is frequently exposed to extreme UVB. UVB may induce detrimental effects such as lower survival, reproduction and development rate of copepod. To evaluate the effect of UVB on *T. japonicus*, we conducted the acute toxicity test with extensive UVB dose range (0-20 or 0-36 kJ/m^2) in the condition of two UVB intensities (50 and 100 μW/cm^2) and also examined the expression pattern of DNA repair related genes. To investigate the gene expression pattern induced by UVB, we conducted real time RT-PCR with the copepod which was irradiated to UVB (19 kJ/m^2 at 50 □W/cm^2 intensity). When the copepod was exposed to UVB with the intensity of 50 and 100 μW/cm^2, levels of LD_{50} were 19 and 21 kJ/m^2, respectively. Expression patterns of DNA repair related genes were shown significant modulation according to time course. Also we checked activated form of p38 kinase and Jun N-terminal kinase (JUNK) to see what kind of signal transduction pathway would be involved in DNA damage by UVB. Upon DNA damage by UVB, those kinases were up-regulated with up-regulated expression pattern of relevant genes. Therefore, *T. japonicus* can be considered as a promising marine species for ecotoxicity testing and risk assessment of UVB in coastal marine environments.

EM044

MODULATION OF OXIDATIVE STRESS GENES OF THE INTERTIDAL COPEPOD *TIGRIOPUS JAPONICUS* AFTER EXPOSURE OF SEVERAL KINDS OF CHEMICALS

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To use the intertidal copepod *Tigriopus japonicus* as a testing model system for evaluating estuarine environmental contamination, we ana-

lyzed 87,638 expressed sequence tags (ESTs) and 1.4 Gb of genomic DNA, and subsequently made 12K *Tigriopus* oligoChip for multipurpose use. Firstly, to check oxidative stress genes after exposure of several kinds of chemicals (heavy metals, biocide, endocrine-disrupting chemicals), we tested key genes of oxidative stress genes along with use of *Tigriopus* 12K oligoChip. Also we checked cellular defense mechanism using TUNEL and BrdU assay systems. Here we found that copper would induce oxidative stresses even though low concentrations. Also both apoptosis and cell proliferation occurred in *T. japonicus* after exposure of copper for 96 hrs. In this presentation, we show that *Tigriopus* would be a good model to test effects of several chemicals on the cellular defense and environmental adaptation at the molecular and cellular levels. This is new attempt to use the marine copepod for evaluating the chemicals along with elucidating molecular mechanisms and environmental adaptation, and also possibly useful to compare with other aquatic organisms on those aspects.

EM045

THE COPEPOD *TIGRIOPUS*: A PROMISING MARINE MODEL ORGANISM FOR ECOTOXICOLOGY AND ENVIRONMENTAL GENOMICS

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There is an increasing body of evidence to support the significant role of invertebrates in assessing impacts of environmental contaminants on marine ecosystems. Therefore, in recent years massive efforts have been directed to identifying viable and ecologically-relevant invertebrate toxicity testing models. *Tigriopus,* a harpacticoid copepod has a number of promising characteristics which make it a candidate for worthy consideration in such efforts. *Tigriopus* and other copepods are widely distributed and ecologically important organisms. Their position in marine food chains is very prominent, especially with regard to the transfer of energy. Copepods also play an important role in the transportation of aquatic pollutants across the food chains. In recent years there has been a phenomenal increase in the knowledge base of *Tigriopus* spp., particularly in the areas of their ecology, geophylogeny, genomics and their behavioural, biochemical and molecular responses following exposure to environmental stressors and chemicals. Sequences of a number of important marker genes have been studied in various *Tigriopus* spp., notably *T. californicus* and *T. japonicus*. These genes belong to normal biophysiological functions (e.g. electron transport system enzymes) as well as stress and toxic chemical exposure responses (heat shock protein 20, glutathione reductase, glutathione *S*-transferase). Recently 400,740 expressed sequenced tags (ESTs) from *T. japonicus*, have been sequenced and of them, 9,004 ESTs showed significant hit (E-value, >8.0 x E-04) to the mouse genome database. Metals and organic pollutants such as antifouling agents, pesticides, polycyclic aromatic hydrocarbons (PAH) and polychrlorinated biphenyls (PCB) have shown reproducible biological responses when tested in *Tigriopus* spp. Promising results have been obtained when *Tigrioups* was used for assessment of risk associated with exposure to endocrine-disrupting chemicals (EDCs). Application of environmental gene expression techniques has allowed evaluation of transcriptional changes in *T. japonicus* with the ultimate aim of understanding the mechanisms of action of environmental stressors.. Through a better understanding of toxicological mechanisms, ecotoxicologists may use this ecologically-relevant species in risk assessment studies in marine systems. The combination of uses as a whole-animal bioassay and gene expression studies indicate that *Trigriopus* may serve as an excellent tool to evaluate the impacts of marine pollution throughout the coastal region. The purpose of this review is to illustrate the potential of using *Tigriopus* to fulfil the niche as an important invertebrate marine model organism for ecotoxicology and environmental genomics. In addition, the knowledge gaps and areas for further studies have also been discussed.

EM046

CONSTRUCTION OF A WHOLE CELL ELECTRICAL BIOSENSOR FOR THE DETECTION OF GENOTOXIC EFFECTS

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In the field of environmental chemistry and ecotoxicology there is a growing demand for lab-independent devices based on whole cell biosensors for the detection of genotoxic compounds. Such biosensors can open a new spectrum of possibilities for rapid and portable water toxicity testing, potentially useful for a variety of monitoring applications. Here we describe the design and construction of such a sensor, consisting of genetically tailored bacterial tester strains that are co-immobilized together with an enzymatic system for the metabolization of xenobiotics on a bio-microelectromechanical system (bio-MEMS). Bioactivated xenobiotics with a (pre)genotoxic potential induce the bacterial SOS-response, which – in turn - drives the expression of a reporter gene, either phoA or lacZ. Thus, either alkaline phosphatase or β-galactosidase are expressed in response to the genotoxic effect of the sample in a dose dependent manner. The activity of the reporter is monitored electrochemically by the oxidation of the product p-aminophenol. The general architecture of the biosensor together with important aspects of the development process of the sensor (implementation of bio-activation, strain construction, design of mirco-electrodes) will be presented. ACKNOWLEDGMENT We gratefully acknowledge the funding by the BMBF-MOST Cooperation in Water Technology Research grant number WT 601 (Project 02WU0844) supported by the German Federal Ministry of Education and Research (BMBF) and the State of Israel Ministry of Science, Culture and Sport (MOST).

EM047

COLIPA VALIDATION OF THE RECONSTRUCTED HUMAN SKIN MICRONUCLEUS ASSAY (RSMN): A NOVEL MICRONUCLEUS ASSAY IN A 3D HUMAN SKIN MODEL

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Current mammalian cell in vitro genotoxicity assays induce a high level of false positive results leading to a large number of costly and time consuming follow-up in vivo genotoxicity studies. As of March 2009, the 7th Amendment to the EU Cosmetics Directive prohibits the use of in vivo genotoxicity tests in safety assessments for cosmetics, greatly impacting the assessment of genotoxicity of new ingredients. To address this, the European Cosmetic Toiletry and Perfumery Association (COLIPA) initiated an international project to establish and evaluate more predictive in vitro genotoxicity assays using 3D human tissues. One focus has been on the 3D human skin micronucleus assay (RSMN) in EpiDermTM.. Since skin is the first site of contact with maximum exposure to many different products including cosmetics, the RSMN assay offers the potential for a more realistic application/metabolism of test compounds for evaluating genotoxicity (1,2,3). The COLIPA RSMN project is a multi-lab initiative involving Procter & Gamble (US), L'Oreal (France), Henkel (Germany), and the Institute for Invitro Sciences (IIVS, US). Intra-laboratory and inter laboratory reproducibility have been investigated with model genotoxins mitomycin C and vinblastine sulfate as well as a variety of chemicals that

require metabolic activation. In addition studies with coded chemicals are in progress. This model is a promising new in vitro method for detecting micronuclei induction in human skin. This work is funded by the European Cosmetic Industry Association COLIPA.

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EM048

ARSENIC EFFECTS ON IMPORTANT NUCLEOTIDE EXCISION REPAIR (NER) KEY PLAYERS: FUNCTION, PROTEIN LEVEL AND GENE EXPRESSION

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The human carcinogen arsenic is an ubiquitous distributed metalloid. Our dietary intake, especially drinking water, provides the majority of this element in the global population. Ingested inorganic arsenic is metabolized to methylated derivatives which are today partly considered to be more toxic than inorganic species. Thus it is reasonable to conclude that some adverse health effects seen in humans chronically exposed to inorganic arsenic are caused by these metabolites. One important mechanism of arsenic-induced carcinogenicity seems to be DNA repair inhibition. Especially trivalent arsenicals strongly inhibit the repair of bulky DNA lesions. This fact has already been demonstrated before by different research groups. Therefore this present study focused on different molecular aspects of the inhibition of NER, which is involved in the removal of such adducts induced by environmental mutagens. During our current research we first investigated the impact of arsenic on protein function via local UVC irradiation combined with fluorescent antibody labelling following protein level and gene expression studies of the NER key players XPC and XPA. Here we report that arsenite (≥ 1 µM) and MMA^{III} (monomethylarsonous acid) (≥ 0.1 µM) strongly reduced gene expression of the important NER genes *XPC* and *XPA* after 24 h preincubation with the respective arsenical compound. This modified gene expression lead to diminished XPC protein level, whereas down regulated *XPA* expression did not show a lowering effect on the protein level of XPA. In case of XPC, the principle initiator of global genome NER, the protein reduction was sufficient to repress DNA damage association of XPC. Additionally we observed a disturbed association of XPA, which is likely to be essential to build a platform for the assembly of the pre-incision complex to UVC damaged DNA. Since total XPA protein amount was not altered significantly we suggest a functional coherency between XPC association to damaged DNA and diminished XPA damage association. Summarized our data indicate that arsenite and more distinct MMA^{III} interact with expression of NER genes resulting in disturbed assembly of the NER machinery.

EM049

ASSOCIATION BETWEEN GENETIC POLYMORPHISMS IN DNA REPAIR GENES AND MINISATELLITE INSTABILITY IN A POULATION FROM THE SEMIPALATINSK NUCLEAR POLYGON REGION, KAZAKHSTAN

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We have investigated 2 DNA-repair gene polymorphisms: XRCC1 (194 Arg/Trp, 399 Arg/Gln), XRCC3 (241Trp/Met) to reveal genetic susceptibility to mutagens and to correlate the frequencies of minisatellite gene mutations with the frequency of polymorphic variance in DNA repair genes. Isolated DNA of 174 samples from people living around the Semipalatinsk nuclear polygon area and 107 DNA samples from frequency-matched control group was used. The determination of polymorphisms in RCC1, XRCC3 genes was carried out by restriction analysis of amplified fragments. We tested the association between variant polymorphic genes with expression of minisatellite instability using $\chi2$ statistics for each generation in total and stratified by exposure vs. control groups. In the stratified analysis, $\chi2$ statistics was used to test the association in each stratum and Cochran Mantel Haenszel test was used to test the association between polymorphic genes and minisatellite instability, adjusting for exposure status. Beslow-Day Test was used to test the homogeneity of the association between variant polymorphic genes and minisatellite instability expression in control and exposed groups. The results indicate that there were no statistical significant differences in the frequencies of variant polymorphic genes between the exposed and control populations in each generation (P0, F1 and F2). The only significant finding on XRCC3 Trp241 Met (P2 generation) could due be to type I error because of the large number of tests performed. The insignificant results could be due to insufficient sample sizes (OR=0, 39; CI=0.17-0.89, P=0, 02). Beslow-Day Tests show that the associations between variant polymorphic genes and minisatellite instability expression in control and exposed groups are homogeneous. The level of frequencies of minisatellite mutation rates in F1 from exposed group was increased in those who had the combined variant (XRCC1 Atr/Trp+Trp/Trp, Arg/Gln+Gln/Gln;XRCC3 Trp/Met+Met/Met) genotypes. However, these results were not significantly different. Therefore, the data suggest that the genotypes had no effect, under our experimental conditions, on the minisatellite mutation frequencies. The lack of association may be caused by the small sample size of exposed and control groups.

EM050

GASOLINE EXPOSURE EFFECT ON PROFESSIONALS IN EL-MINYA GOVERNORATE IN UPPER EGYPT (2007-2008)

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El-Minya City lies in the middle of Egypt between the Nile River and the Ibrahimiya Canal and considered as a market and financial center for its region. There were no previous studies on pollution caused by Gasoline exposure in El-Minya Governorate in Upper Egypt . So we tried in this study to concern on the effect of air pollution from automobile exhaust in El-Minya, Unleaded gasoline was introduced in the greater area of El-Minya, 10% of the total sales of gasoline was unleaded. In this study,blood lead levels, liver function, kidney function, and hematological parameters were measured in groups of professionals exposed occupationally to polluted air with heavy traffic or gasoline fumes. According to the present study, we found a significant difference in blood lead levels in each of the exposed groups and an increase in hemoglobin level in all tested groups. On the other hand, ceratinine level shows decrease significant in all tested groups, increase SGOT and SGPT levels in gas station employees and taxi derivers and increase SGPT in bus drivers. On the other hand, there is no significant in SGOT level in bus driver. Perhaps the elevated transaminases resulted from exposure of gas station employees to hepatotoxic constituents of gasoline.

EM051

GENOTOXIC EFFECT OF OCTYLPHENOL ON MALE MICE

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The aim of the present study is to investigate the genotoxic effect of 4-tert-octylphenol (OP) in mice using cytogenetic and molecular methods. Male mice were orally treated with different doses of OP at the level of 50, 150 and 450 mg/kg b. w. for a single day, or for 5 and 7 consecutive daily doses. Cytogenetic parameters were investigated in somatic and germ cells. The results show that after 24 h. OP induced

different types of structural chromosomal aberrations in bone-marrow cells which increased significantly with increasing the dose. In germ cells chromosomal aberration was observed in mice spermatocytes after treatment with OP with a dose-dependent relationship. The repeated dose treatment induced a more pronounced effect than a single dose-treatment. Also, morphological sperm abnormalities increased significantly with increasing the dose. The RAPD fingerprints were investigated in control and treated mice using five arbitrary primers to detect the alterations in DNA produced by OP. The results indicated that the total percentage of polymorphism is 66.07, which means that the majority of patterns of the detected markers are polymorphic between the tested groups. A relatedness dendogram clustered the three treated groups together independently from the control group, which confirm the ability of RAPD technique to detect DNA alterations induced by chemicals.

EM052

ANALYSIS OF CHROMOSOMAL ABERRATION FREQUENCIES AND GLUTATHIONE-S-TRANSFERASE GENES IN WORKERS OCCUPATIONALLY EXPOSED TO URANIUM IN NORTHERN KAZAKHSTAN

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The Republic of Kazakhstan has one of the largest deposits of uranium in the world. Therefore, uranium mining activities have existed for many years. Although mine workers have been exposed, their health risk has not been investigated. In the present study, uranium mine-workers in the Stepnogorsk mining-milling complex in Northern Kazakhstan were investigated for the expression of chromosome aberrations and for genetic factors that can modify the exposure-related expression of chromosome damage. From our interview of volunteers, 100 most qualified workers and 56 controls were selected. Controls were frequency-matched to the workers with respect to age (+/- 5 years), gender and ethnic origin. The workers were subdivided into 3 groups according to the duration of exposure: group I - 1-10 years, group II - 11-20 years, group III - 21-25 years. The controls were healthy volunteers who had not been exposed to radiation or other hazardous agents. Our data shows that workers in all three exposure groups had higher frequencies of chromosome aberrations than the control group. Uranium exposed workers who had inherited the null GSTM1 and/or GSTT1 genotypes had a significant increase in the frequency of chromosome aberrations compared with those who had the intact GSTM1 and GSTT1 genes for the different group of workers. Our study, therefore, indicates that uranium mine workers in Northern Kazakhstan have excessive exposure that can cause them to have increased risk for health consequences such as cancer. In addition, GSTM1 and/or GSTT1 null genetically susceptible individuals may have higher health risk.

EM053

BIOLOGICAL ACTIVITIES OF ENDOGENOUS MUTAGENS/CARCINOGENS, AMINOPHENYLNORHARTMAN AND N-NITROSO BILE ACID CONJUGATES.

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Aminophenylnorharman (APNH), being a reaction product of norharman and aniline, is a strong mutagen/carcinogen, as the case of other heterocyclic amines. APNH formed the DNA adduct, dG-C8-APNH, and showed mutagenicity in S.typhimurium strains and clastogenicity in Chinese hamster lung cells. In a long-term carcinogenicity experiment of APNH, hepatocellular carcinomas and colon adenocarcinomas were developed in both sexes of F344 rats fed 40 ppm of APNH. PCR-SSCP analysis revealed β-catenin gene mutations in 24% of HCCs, and K-ras, β-catenin and Apc gene mutations were found in 22, 44 and 33% of colon cancers, respectively. APNH was detected in eighteen human urine samples, at levels of 21 to 594 pg per 24 hr urine. To clarify chronic exposure of humans to APNH, amounts of APNH in human hair samples were also measured. In the hair samples collected from nine healthy volunteers, APNH was detected in all the samples at levels of 222-532 pg per g hair. From the values of APNH in human hair and urine samples, the daily exposure level of APNH was estimated to be 0.02 – 0.2 μg/person. N-Nitroso bile acid conjugates, N-nitrosoglycocholic acid (NO-GCA) and N-nitrosotaurocholic acid (NO-TCA), have been demonstrated to exert mutagenic and carcinogenic activity. The major DNA adducts derived from NO-GCA and NO-TCA are shown to be O6-carboxymethyl-deoxyguanosine (O6-CM-dG) and 3-ethanesulfonic acid-deoxycytidine (3-ESA-dC), respectively, in in vitro experiments. To examine the formation of these DNA adducts in vivo, O6-CM-dG and 3-ESA-dC were analyzed using HPLC and the 32P-postlabeling method in the glandular stomach of rats subjected to duodenal content reflux surgery. The levels of O6-CM-dG and 3-ESA-dC in the duodenal reflux group after 8 weeks of surgery were 56.3±3.2 and 8.9±1.0 per 108 nucleotides, respectively, whereas the values were 5.9±0.5 and 2.0±1.0 per 108 nucleotides for the sham operation group. When the duodenal reflux animals were treated with a nitrite trapping agent, thioproline, the levels of O6-CM-dG and 3-ESA-dC were reduced to almost the same levels as those of the sham operation animals. Thus, both NO-TCA and NO-GCA were formed in the stomach of a rat duodenal reflux model, and might be involved in stomach cancer development.

EM054

UPTAKE, TRANSLOCATION AND CELLULAR EFFECTS OF NANOPARTICLES IN HUMAN CELLS: COMPARISON OF FE(III) AND TIO2

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Titanium dioxide (TiO2), also known as titanium (IV) oxide or anatase, is the naturally occurring oxide of titanium. It is also one of the most commercially used form. Previously conducted studies had established these nanoparticles to be mainly non-cyto- and –genotoxic, although they had been found to generate free radicals both acellularly (specially through photocatalytic activity) and intracellularly. The present study determines the role of TiO2-NP (anatase, < 100 nm) using several parameters such as cyto- and genotoxicity, DNA-adduct formation and generation of free radicals following its uptake by human lung cells in vitro. For comparison, iron containing nanoparticles (hematite, Fe2O3, < 100 nm) were used. The results of this study showed that both types of NP were located in the cytosol near the nucleus. No particles were found inside the nucleus, in mitochondria or ribosomes. Human lung fibroblasts (IMR-90) were more sensitive regarding cyto- and genotoxic effects caused by the NP than human bronchial epithelial cells (BEAS-2B). In contrast to hematite NP, TiO2-NP did not induce DNA-breakage measured by the Comet-assay in both cell types. Generation of reactive oxygen species (ROS) was measured acellularly (without any photocatalytic activity) as well as intracellularly for both types of particles, however, the iron-containing NP needed special reducing conditions before pronounced radical generation. A high level of DNA adduct formation (8-OHdG) was observed in IMR-90 cells exposed to TiO2-NP, but not in cells exposed to hematite NP. Our study demonstrates different modes of action for TiO2- and Fe2O3-NP. Whereas TiO2-NP were able to generate elevated amounts of free radicals, which induced indirect genotoxicity mainly by DNA-adduct formation, Fe2O3-NP were clastogenic (induction of DNA-breakage) and required reducing conditions for radical formation.

EM055

“GENOMICS BIOMARKERS OF ENVIRONMENTAL HEALTH” (ENVIROGENOMARKERS): A NEW FP7 MOLECULAR EPIDEMIOLOGY PROJECT

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EnviroGenomarkers is a new FP7 (Theme: Environment; and Health) medium-scale collaborative project, which started in March 2009 with a duration of 4 years. It is coordinated by NHRF and has a total of 11 partners. The project concerns the large-scale application of full range –omics technologies in a population study aiming at a) the discovery and validation of novel biomarkers, predictive of increased risks of a number of chronic diseases, b) the exploration of the association of such biomarkers with environmental exposures, including high-priority pollutants and emerging exposures and c) the discovery and validation of biomarkers of exposure to the above and other high-priority environmental exposures. The project utilises three prospective cohorts:

- Cancer related biomarkers will be developed using a case-control study nested within 2 cohorts (EPIC Italy and the Northern Sweden Health and Disease Study), with samples collected prior to disease diagnosis, as well as exposure data and follow-up health information. Biomarkers will be compared in 600 breast cancer cases, 300 B-cell lymphoma cases and equal numbers of matched controls, to evaluate their risk predictivity.
- Chronic disease biomarkers established during early childhood that persist into adult life will be evaluated using the Rhea mother-child cohort, being set up in the context of NewGeneris, an FP6 integrated project on genotoxic exposure risks in newborns. 600 Rhea children will be analysed at birth, 2yrs and 4yrs. Results will be compared with relevant clinical indices, to evaluate the intra-individual biomarker variation and its relationship with disease progression. Biomarker search will utilize state-of-the-art metabonomics, epigenomics, proteomics and transcriptomics technologies, in combination with advanced bioinformatics and systems biology tools. Exposure assessment will exploit exposure biomarkers, questionnaires, modelling and GIS technology. Specifically targeted exposures include PCBs, PAHs, cadmium phthalates and PBDEs. Additional data available through other projects will also be utilised, thus generating substantial added value.

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EM056

USING OF ALLIUM-TEST FOR TOXICITY ASSESSMENT OF WATER AND SOIL SAMPLED FROM RADIOACTIVELY CONTAMINATED SITES

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The main aim of our research is to improve Allium-test method for bioindication of radioactive and chemical pollution and apply this to an assessment and forecast of the environment state within the Semipalatinsk Test Site (STS). Radioactive contamination of the STS territory was formed as a result of nuclear weapon testing. Up to now, within the STS there are areas with a very high level of radioactive contamination. Doses in these areas would be enough to induce biological effects in plants and animals. For our bioindication studies we chose sites where the overwhelming majority of nuclear tests were completed. γ-radiation dose rate at some locations within STS exceeds 20-60 μGy/hour. Collected samples of soil and water from experimental area were analyzed in laboratory to measure radionuclides activity and heavy metals content. We applied the Allium-test to assess cytotoxicity and genotoxicity of the soil and water sampled from the STS in 2006 and 2007. For that, onion bulbs are plunged into water or soil under test for germination and, then, root meristem cells are used as test-system to indicate geno- and cytotoxicity of water/soil contamination. Results from cytotoxicity of samples testing show the values of mitotic index is lower than in control. Cytogenetic disturbances frequency at the bulbs germination in sampled from the affected sites significantly exceeds the control. Frequency of the severest genetic disturbances (chromosome bridges as well as lagging chromosomes, C-mitoses and three-polar mitoses) is significantly higher in the nuclear tests’ epicenter. The extent of cytogenetic indices does not show any correlation with γ-dose rate. Possible reason is that γ-dose rate measured at the sampling points is mainly formed by radionuclides deposited not in water itself but at banks and bottom sediment. Moreover, γ-dose rate does not take into account contribution from β- and α-emitting isotopes. The experiment with an additional exposure of onion bulbs germinating on water samples at doses calculated to compensate this difference between natural and laboratory conditions shows that such an Allium-test modification allows us to describe ecological situation at the sampling points with high levels of radioactive contaminations more correctly.

EM057

SOME APPROACHES TO EVALUATION OF THE ENVIRONMENTAL GENOTOXICITY.

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This study is dedicated to the development of efficient, informative and inexpensive methods of estimation of the mutagenic effect of various pollutants on the environment. We detected soil genotoxicity using plant test-system *Glycine max.* (L.) Merill., which was proposed by B.K.Vig (1975). The soils were contaminated by heavy metals (the vicinity of tungsten and molybdene factory) and products of combustion and domestic cottage processing of oil (the areas of oil mini-factories location). Seeds were germinated in contaminated soils. It was found that the polluted soils increased the mutation level by a factor of 2-8 with dose-response effect. This plant test system turned out to be more sensitive than Ames test in the case of oil products contamination. For assessment environmental genotoxicity *in situ* we studied mutation rates in seeds of native plants growing on the same territories using seedling’s root tip anaphase aberration test. 8 plant species were studied (*Anisantha tectorum*, *Koeleria cristata*, *Hyoscyanus niger, Yurinea ciscaucasica, Taraxacum officinale, Matricaria recutita, Rumex confertus,* and *Plantago major.* All of them were quit sensitive. We found that mutation rates in seeds from plants growing on contaminated soils exceeded the background levels by factors of 2—4.5 (heavy metals) and 4-7 (oil processing products). Increasing the height and decreasing the number of flowering and seeds and germination capacity were observed in the plants which seeds have high mutation level. We propose to use dandelion (*Taraxacum officinale Wigg. s.l.*) for testing genotoxicity of environment. This widespread species is sensitive to various types of pollutants, simple in using and inexpensive object for genetic monitoring. For assessment of health risk some indexes of reproductive health of population in the district of tungsten and molybdene factory were analyzed. Increased frequency of miscarriages was detected. The rate of congenital malformations and hereditary diseases were not differ from the corresponding indexes of the control regions. The increasing of cancer was not detected. We suppose that our toolkit for evaluation of the genetic effect of industrial enterprises, including various index plants, is sufficient and evidentiary.

EM058

OXIDATIVE STRESS OF SILVER NANOPARTICLES CAENOHABDITIS ELEGANS : FUNCTIONAL GENOMICS APPROACH

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The toxicity of silver nanoparticles (AgNPs) was investigated in Caenorhabditis elegans using functional toxicogenomics approach. Whole genome microarray was used to screen for global changes in C. elegans transcription profiles after AgNPs exposure, followed by quantitative analysis of selected genes. The integration of gene expression profile with organism and population level endpoints was investigated using C. elegans functional genomics tools, to test the toxicological relevance of AgNPs-induced gene expression. AgNPs exerted considerable toxicity in C. elegans, most clearly as dramatically decreased reproduction potential and increased expression of the superoxide dismutases-3 (sod-3) occurred concurrently with significant decreases in reproduction ability. The results of functional genomic studies using mutant analyses suggested that the sod-3 gene expressions may have been related to the AgNPs-induced reproductive failure in C. elegans and that oxidative stress may have been an important mechanism in AgNPs toxicity. To understand the observed molecular mechanism of AgNPs-induced oxidative stress, a mechanism study was conducted focusing on the involvement of the oxidative stress responding signal transduction pathway and transcription factors in the toxicity of AgNPs. The upstream signaling mechanism responsible for regulating oxidative stress was studied focusing on the mitogen-activated protein (MAP) kinase cascades, as well as, transcription factor, SKN-1. Acknowledgment: This work was accomplished through the supports of the Ministry of Environment as “The Eco-technopia 21 project”.

EM059
COMPARATIVE TOXICITY OF SILVER NANOPARTICLE IN HUMAN CELL LINES

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Toxicity of silver nanoparticles (AgNPs) and silver ions (Ag ions) were compared in human cell lines. Among 6 tested cell lines, cell viability of Jurkat cells decreased significantly by AgNPs, whereas, not by Ag ion. Similarly, apoptosis was observed by AgNPs, whereas, not by Ag ions. To investigate underlying mechanism on this different sensitivity of Jurkat cells to AgNPs and Ag ion exposure, an in vitro toxicity assay was conducted focusing on the involvement of the oxidative stress responding signal transduction pathway and transcription factors in the toxicity of AgNPs. The upstream signaling mechanism responsible for regulating oxidative stress was studied focusing on the mitogen-activated protein (MAP) kinase cascades. Three groups of well-characterized MAP kinase cascades, extracellular signal-regulating kinase (ERK), p38 and c-Jun N-terminal kinase (JNK), were investigated. Redox-sensitive transcription factors, such as nuclear factor-kappaB (NF-□B) and nuclear factor-E2-related factor-2 (Nrf-2), were investigated as target transcription factors of AgNPs toxicities. Antioxidant enzyme activities were also investigated. Acknowledgment: This work was accomplished through the supports of the Ministry of Environment as “The Eco-technopia 21 project”.

EM060
URINARY FUMONISIN B1 AS A BIOMARKER OF FUMONISIN EXPOSURE AND ITS APPLICATION IN INTERVENTION STUDIES

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Fumonisins (FB), produced by Fusarium Spp that contaminates maize worldwide, are possible human carcinogens and exhibit nephrotoxicity and hepatotoxicity in animals. We developed a urinary FB1 exposure biomarker measured by a liquid chromatography - mass spectrometry (LC-MS) method following a solid phase extraction using deuterium labeled FB1 as an internal standard. The method can quantitate as low as 20pg FB1 per ml urine. Previously in 75 Mexican women regularly consuming maize based tortillas, urinary FB1 level was compared amongst individuals from high, medium and low maize intake groups. 75% of the women had detectable urinary FB1. The level was strongly correlated with maize based tortillas consumption [1]. This biomarker was further applied to evaluate the effectiveness of handsorting and washing maize to reduce dietary FB exposure in 22 women from the rural Eastern Cape, South Africa. Morning urine was collected at baseline. The participants were then trained in how to hand-sort and wash maize to reduce fumonisins. Subsequently they performed hand-sorting and washing before the maize was cooked for their own consumption during the following two days when morning urine was also collected. Urinary FB1 was detected in 95% of the women. The urinary FB1 geometric mean level was 224 pg/ml urine (95% CI 144-349 pg/ml) before the intervention, and 107 pg/ml (95% CI 83-138 pg/ml, P = 0.002) after. There was a strong correlation between urinary FB1 and food FB levels (p=0.027). Conclusion: Urinary FB1 level is significantly correlated with maize consumption, as well as with FB contamination thus representing a valid and sensitive biomarker of dietary FB exposure. Simple interventions such as hand-sorting and washing maize before cooking are feasible in rural populations and resulted in a mean 52% reduction in the urinary FB1 level in a rural South African population. This work was supported by grants from the NIEHS, USA and Sir Halley Stewart Trust

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EM061
GENOTOXICITY OF NANOPARTICLES IN IN VITRO MICRONUCLEI, IN VIVO COMET AND MUTATION ASSAY SYSTEMS

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Manufactured nanoparticles such as fullerenes (C60), carbon black (CB) and ceramic fiber are widely used because of their desirable properties in industrial, medical and cosmetic fields. Accordingly, these particles can be released into the human environment and then can be inhaled. Most exposure to airborne nanomaterials occurs in the work place. However, there are few data on these nanoparticles in mammalian mutagenesis and carcinogenesis. To examine genotoxic effects by C60, CB and kaolin, an in vitro micronuclei (MN) test was conducted with human lung cancer A549 cells. These three nanoparticles increased MN frequencies in A549 cells in a dose-dependent manner at doses of 0.02 – 2 μg/mL. In addition, C60, CB and kaolin induced DNA damage in the lungs of C57BL/6J measured by comet assay, intratracheally instilled with single dose of 0.2 mg per animal of nanoparticles. Moreover, single (0.2 mg per animal) or multiple (four consecutive doses of 0.2 mg per animal per week) instillations of C60 and kaolin, increased either or both of gpt and Spi- mutant frequencies in the lungs of gpt delta transgenic mice. Mutation spectra analysis showed transversions were predominant, and among these, the G:C to C:G was commonly increased by these nanoparticle intratracheal instillations. Thus, CB, C60 and kaolin were shown to be genotoxic in in vitro and in vivo assay systems.

EM062

COMPARISONS OF THE SENSITIVITIES OF ONION AND BROAD BEAN TO GENOTOXINS IN THE ROOT TIP CHROMOSOME ABERRATION ASSAY

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Plant genotoxicity assays are relatively inexpensive, chemicals which cause chromosomal aberration (CA) in plant cells frequently produce identical CA in cultured animal cells too and there is growing ethical concern for the use of animals in toxicology research and testing. Plant species however, differ in their sensitivities. In this study, onion and broad bean were compared for sensitivity to Chlorpyrifos (CHL), Mercury Chloride (HgCL2), Ethyl methanesulphonate (EMS) and Garden Ripcord (GR) (20% cypermethrin) using the root tip CA assay. Three doses of each chemical representing the EC50, ½ EC50 and ¼ EC50 determined in preliminary germination inhibition tests were assessed for cytotoxicity and genotoxicity. Seeds were germinated on moistened filter paper in Petri dishes at room temperature until radicles appeared and then exposed to solutions for 24 hours. About 1-2mm length of root tips from five seeds/dose were cut, fixed in acetic alcohol at 4-6oC overnight, washed with ice cold water, hydrolyzed in 1M hydrochloric acid (60 oC), stained with aceto-carmine and squashed on glass microscope slides. Cytotoxicity was determined by the mitotic index (MI). A dose of chemical was adjudged toxic if the MI of treated cells was ≤ ½ of control. To onion, all the chemicals were toxic at one or more doses and GR also stimulated cell division at low concentrations. To broad bean, EMS and HgCL2 were toxic, CHL was not toxic and GR stimulated cell division at low concentrations. Genotoxicity was determined by analyzing 100 anaphase and telophase cells for chromosome fragments, bridges, vagrant chromosome, c-anaphase, multipolarity and stick chromosomes. The CA values at each dose were compared with the control values using the Chi-squared test. All four chemicals were genotoxic (P<0.05 and/or 0.01) to both plants. Sensitivities were compared using the EC50 (in Moles), CA/mole at 50% MI and the concentration that induced the highest CA. Onion was more sensitive to CHL and EMS when all three parameters were considered, HgCL2, when EC50 and concentration that induced the highest CA were considered and GR in CA/mole. Broad bean was more sensitive to GR when EC50 and concentration that induced the highest CA were considered and to HgCL2 when CA/mole was considered.

EM063

SUMMARY OF COLLABORATIVE STUDIES OF LIVER MICRONUCLEUS ASSAY IN YOUNG RATS -JEMS•MMS COLLABORATIVE STUDY GROUP-

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Rodent erythrocyte in vivo micronucleus (MN) assays have been widely conducted using bone marrow (BM) cells or peripheral blood (PB) to assess the clastogenic potential of chemicals. Positive responses in the BM/PB MN assays correlate highly with carcinogenicity in multiple organs. However, it is well known that some chemicals showing genotoxicity following metabolic activation in the liver tended to give negative responses in the BM/PB MN assays. The MN-inducing potential of such chemicals can be detected in the liver MN assays. To evaluate the suitability and utility of the liver MN assay, we have been investigating the assay using young (up to 4 weeks old) rats in the JEMS•MMS collaborative studies for several years. This assay system utilizes natural proliferation of hepatocytes in the young rats and gets an advantage over the partial hepatectomy method, co-treatment method with mitogens and in vivo/in vitro testing method. In our liver MN assay system, test chemicals including undetectable in the BM/PB MN assays were administered to male rats at 4 weeks of age and hepatocytes were isolated by liver in situ perfusion with collagenase solution. In the standard (STD) method, rats were administered by single dosing and hepatocytes were collected 3, 4 and 5 days after the treatment. Specimens of the hepatocytes were stained with acridine orange (AO) -4',6-diamidino-2-phenylindole dihydrochloride (DAPI) solution and observed under a fluorescent microscope. The outcomes of our studies gave a high correlation between genotoxic hepatocarcinogenicity and MN induction in hepatocytes by the STD method. To make the liver MN assay system simpler and more convenient, we introduced new method using double treatments and single sampling of hepatocytes. Some chemicals that gave positive responses in the STD method were objected to the new method. As a result, all of the chemicals used in the new method gave positive responses as well as by the STD method. It is concluded that the new simpler liver MN assay is a useful tool to detect in vivo clastogenicity of chemicals that give negative or unclear responses in the BM/PB MN assays.

EM064

GENOTOXICITY TESTING OF ACLONIFEN IN VIVO AND IN VITRO

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Aclonifen is a potent herbicide belonging to the phenoxy-aniline (diphenylether) group. For years it has been used in several types of cultures in many countries. However toxicity information of Aclonifen is not clear. In this study, genotoxic effects of Aclonifen was investigated using both in vivo chromosomal aberrations (CA) test in mouse bone marrow cells and in vitro chromosomal aberrations, sister chromatid exchanges (SCE) and comet assay in human peripheral lymphocytes. Effects of this herbicide on the mitotic index (MI) and replication index (RI) were also investigated. Different concentrations of Aclonifen (3.125, 6.25, 12.5, 25, 50 mg/kg bw) were treated to mouse intraperitonally for 24 h and same interval of concentrations (3.125, 6.25, 12.5, 25, 50 μg/ml) were treated to human lymphocytes in vitro for both 24 h and 48 h. Aclonifen significantly increased the frequency of CA's in almost all concentrations and treatment times in a dose dependent manner both in vivo and in vitro. Aclonifen also increased SCE/cell frequencies dose dependently in all treatment times and concentrations. It decreased mitotic index dose dependently, however replication index was not effected. In comet assay, the primary DNA damage significantly increased by all concentrations compared to the control. In this study significant differences in CA, SCE, MI frequencies and comet assay results between treatment and control groups indicated that Aclonifen is clastogenic, mutagenic, cytotoxic and caused DNA damage in mammalian cells.

EM065

EVALUATION OF A SPECIFIC METHOD FOR INTEGRATING GENOTOXICITY TEST INTO GENERAL TOXICITY TEST

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In a proposed revision of an ICH guideline, reduction in the number of animals and integration of genotoxicity tests into general toxicity tests are of the most important concerns. A specific method for integrating a genotoxicity test into a general toxicity test was evaluated. We reported that the integration of a bone marrow micronucleus test into a 28-day repeated dose toxicity test gave lower sensitivity when only evaluating bone marrow samples (collected at the end of the test), but provided higher sensitivity when peripheral blood (collected 2-4 days after the initial treatment) was also evaluated. As hemosiderin pigmentation in the spleen is a good marker for micronucleus induction in the early phase of administration, we have confirmed its usefulness as an index for determining the necessity of an additional micronucleus test. Contrarily, it is difficult to integrate a comet assay into a general toxicity test as the final treatment has to be made 3 hours prior to sampling. In this regard, TK animals are considered extremely useful for integrating an additional micronucleus test using peripheral blood and a comet assay into the 28-day repeated dose toxicity test. We also evaluated the advantages and disadvantages over the use of TK animals, especially for the effects of TK blood sampling on the hematopoietic function and induction of micronucleated cells. In the bone marrow micronucleus test, TK blood sampling induced accelerated hematopoietic function and a tendency for a slightly higher sensitivity to genotoxic agents; however, it was considered to have no effect on micronucleus induction as there was no effect on the micronucleus induction in the negative control group. No effect on TK blood sampling was noted in the comet assay. We also evaluated the advantage of a liver micronucleus test for its cumulative effect when the test was integrated into a repeated dose general toxicity test.

EM066
GENOTOXIC EFFECTS OF SODIUM BENZOATE IN HUMAN LYMPHOCYTES IN VITRO

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Sodium benzoate is a preservative which is added to foods, medicines and cosmetics to prevent fermentation and mold. In this study, genotoxic effects of sodium benzoate on human peripheral blood lymphocytes were investigated by using chromosome aberrations (CAs), sister chromatid exchanges (SCEs) and comet assay. Lymphocytes were treated with various concentrations (6.25, 12.5, 25, 50 and 100 □g/ml) of sodium benzoate for 24h and 48 h. This additive significantly increased the frequency of SCE and CA at all concentrations and treatment periods compared with the control in a dose-dependent manner (for SCE; r=0,95 and r=0,94; for CA; r=0,99 and r=0,97, at 24h and 48h, respectively). Most common abnormalities were chromatid and chromosome breaks. The primary DNA damage in comet assay significantly increased by almost all concentrations compared to the control. However, this increase was not dose-dependent. In addition, sodium benzoate decreased the mitotic index at all concentrations and treatment periods, dose-dependently (r=0,97 at 24 h, r=0.92 at 48 h). There was no effect on the replication index (RI). It is concluded that sodium benzoate is clastogenic and cytotoxic and caused DNA damage in human lymphocytes in vitro.

EM067
INDIVIDUAL SUSCEPTIBILITY TO METAL-INDUCED OXIDATIVE DAMAGE

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Transition metals in fine and ultrafine combustion-generated particulate matter increases the oxidative and inflammogenic reactivity of this ubiquitous airborne pollutant by the in vivo ROS generation. Oxidative changes in bio molecules are implicated in almost all pathogenic mechanisms of chronic-degenerative diseases. Endogenous free radical scavengers are exploited to limit damages. To the variability of this mechanism exogenous scavengers as vitamins contribute. Moreover, susceptibility to the ROS-induced genotoxicity is related to genomic stability, synthesis and DNA repair mechanisms. Consequently cellular damages are the outcomes of interactions between environment and host-related factors producing a wide range of susceptibility also due to genes polymorphisms. To assess interindividual variability, lymphocytes of 47 Caucasian health subjects were exposed to an water metals solution obtained from oil fly ash and formed by V(IV), Fe(III) and Ni (II) (68.8, 110.4 and 18.0 □M respectively). Oxidative damage was evaluated by cell viability, intracellular ROS and 8-OH-dG analysis and, as repair-capacity marker, by Halo-Comet. Other than the polymorphic genes GSTM1, GSTT1, hOGG1, and MTHFR, demographic and behavioural factors were considered. ROS values ranging by ~20-fold both basal and exposure conditions while 8-OH-dG values ranging by 6 and 7-fold respectively. DNA repair capacity was moderate($p<0.05$ in comparison to background values) and ranged by 25-fold. The results showed a significantly increased limphocytic DNA damage in delete GSTT1 subjects. Increases in cytoplasmic ROS and decreases in DNA repair capacity($p<0.05$) were observed in the variants C677T and A1298C of MTHFR and imputable to increased homocysteine, uracil misincorporation and DNA hypomethylation. However multivariate analyses highlighted a marked($p<0.01$) protective effects due to vegetable intake, in both cytoplasmic and nuclear compartment, and a harmful effect of meat intake on DNA repair capacity, probably due to the highly bioavailable iron. Smoking habit also decreased repair capacity while the age increased DNA damage during exposure. On the whole it is underlined as lifestyle factors modify the genes-environment interactions, contributing to the observed variability.

EM068
IN VITRO ASSESSMENT OF MITOCHONDRIAL IMPAIRMENT METALS- INDUCED IN HUMAN EPITHELIAL ALVEOLAR CELLS

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Bioavailable transition metals in inhalable particulate matter(PM) trigger a dose–time–dependent induction of reactive oxygen species(ROS) production in epithelial cells of respiratory tract. As previously observed, other than DNA damages, ROS cause a marked lipid peroxidation in cytoplasmic compartment of pneumocytes. Due to the high content of PUFA mitochondria are susceptible targets to ROS, producing changes in their metabolism. Since mitochondria are the major natural cellular source of ROS, we tested the hypothesis that an impairment of oxidative phosphorylation, metals-induced, causes an increase in endogenous ROS production, prolonging oxidative damage both in exposed cells and in their progeny. In synchronized A549 pneumocytes cultures, exposed to subtoxic dose of a water metals solution obtained from oil fly ash, and in their 3 following subcultures intracellular ROS levels, mitochondrial mass and membrane potential($\Delta\Psi$) were measured by cytofluorimetric analysis. Single synthetic metal solution formed by V(IV) and Fe(III), at the same molarity present in OFA solution, were also tested. The results in exposed cells showed that the increased ROS level ($p=0.002$ and n.s. in G0-G1 and G2-S respectively), due to Fenton-like reactions metals catalyzed, caused a decreases in mitochondrial mass and, especially, in $\Delta\Psi$($p= 0.018$ and 0.04 in the 2 cell cycle phases respectively). This metabolic impairment was maintained in the obtained subcultures that showed, in comparison to con-

trol, significant increases in endogenous ROS and mitochondrial mass ($p=0.045$). The latter is imputable to the homeostatic mechanism of biogenesis. Moreover the values of ΔΨ decreased significantly ($p=0.009$). The results obtained in cells exposed to single metal solutions showed that the functionality changes were almost all imputable to the vanadium presence in OFA.The study underlines how, despite the absence of exposure to environmental sample, the marked mitochondrial dysfunction transmitted to progeny makes chronic oxidative stress in respiratory epithelium. Other than COPDs pathogenesis, the observed effects, causing a quick cellular turn-over due to mitochondrial-mediated apoptosis pathways, could trigger the multi-step cancerogenic process in the less damaged cells.

EM069

DNA DAMAGE INDUCED BY TOBACCO SMOKE AND ENVIRONMENTAL POLLUTANTS IN MOTHERS AND ITS TRANSPLACENTAL TRANSFER TO THE FOETUS

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32P-postlabelling and PAH-ELISA using the antiserum #29 were employed to analyze DNA adducts in venous and umbilical cord blood and the placenta of 79 mothers giving birth to 80 living babies in Prague (Czech Republic). Ambient air exposure was measured by stationary measurements of basic air pollutants (PM2.5, c-PAHs) during the entire pregnancy. Tobacco smoke exposure was assessed by questionnaire data and by plasma cotinine levels. The total DNA adduct levels in the lymphocytes of mothers and newborns were elevated by 30–40% ($p<0.001$) compared with the placenta. B[a]P-like DNA adduct (adduct with the identical chromatographic mobility on TLC as major BPDE derived DNA adduct) levels were elevated in the blood of mothers compared with the placenta and the blood of newborns ($p<0.05$ and $p<0.01$). In tobacco smoke-exposed mothers, higher DNA adduct levels in the blood of mothers and newborns compared with the placenta were found ($p<0.001$), whereas the total and B[a]Plike adduct levels were comparable in the blood of mothers and newborns. B[a]P-like adducts were elevated in the blood of mothers unexposed to tobacco smoke compared with that of corresponding newborns and the placenta ($p<0.01$). Total and B[a]P-like DNAadducts were increased in the placenta of tobacco smoke-exposed compared with unexposed mothers ($p<0.001$ and $p<0.01$). In lymphocytes of tobacco smoke exposed mothers, the comparison of total adduct levels (1.18±0.67 vs. 0.92±0.28) and B[a]P-like DNA adducts (0.22±0.12 vs. 0.15±0.06 adducts/108 nucleotides) with newbornsindicated a 40–50% increase of adducts in mothers. Almost equal PAH-DNA adduct levels were detected by anti-BPDE-DNA ELISA in the placenta of tobacco smoke-exposed and -unexposed mothers. Our results suggest a protective effect of the placental barrier against the genotoxic effect of some tobacco-smoke components between the 3 circulation of mother and child. We found a correlation between adduct levels in the blood of mothers and newborns.The study was supported by the Czech Ministry of the Education (grant number 2B06088) and by the Academy of Sciences of the Czech Republic (grant number AVOZ50390512).

EM070

EVALUATION OF SODIUM SORBATE GENOTOXICITY IN HUMAN LYMPHOCYTES IN VITRO

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In this study, genotoxic effects of antimicrobial food additive sodium sorbate was investigated in human lymphocytes using chromosome aberrations (CA), sister chromatid exchanges (SCE), micronucleus (MN) and comet assays in vitro. Cells were treated with 100, 200, 400 and 800 µg/ml concentrations of sodium sorbate for 24 h and 48 h. Sodium sorbate significantly increased the CA frequency at only 800µg/ml concentration at both treatment periods. Sodium sorbate caused seven types of chromosomal aberrations. Structural chromosomal aberrations were chromatid break, chromosome break, fragment, sister union, chromatid exchange and dicentric chromosomes. Polyploidy was recorded as numerical chromosomal aberration. The most common chromosomal aberration was chromatid breaks. On the other hand, sodium sorbate increased SCE frequency and MN formation at 400 and 800 µg/ml concentrations at 24 h and 48 h treatment periods in a dose-dependent manner (For SCE r=0,90 and r=0,95 respectively, for MN r=0,97). However, sodium sorbate did not effect replicative index (RI) and cytokinesis-block proliferation index (CBPI) in all treatment concentrations. In contrary, it significantly decreased the mitotic index (MI) at 200 µg/ml concentration for 48h treatment period (r=-0,54). Comet assay was used to evaluate the DNA damaging effects of sodium sorbate in human cells. The results of the present study showed statistically significant increase in mean comet tail intensity and tail length at all concentrations (except 400 g/ml for tail length) compared with control. However, this increase was not dose-dependent. The results of these experiments showed that sodium sorbate have clastogenic and mutagenic effects especially at heights concentrations in human lymphocytes in vitro.

EM071

THE AMES MPF™ PENTA I ASSAY: MUTAGENICITY TESTING IN LIQUID MICROPLATE FORMAT USING OECD GUIDELINE 471 COMPLIANT STRAINS S. TYPHIMURIUM TA98, TA100, TA1535, TA1537 AND E.COLI WP2 UVRA PLUS E.COLI WP2 [PKM101]

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The necessity of testing compounds for genotoxic liabilities is constantly increasing. In drug discovery, genotoxic substances should be removed from further development as early as possible, often at stages where very limited quantities are available. But also the testing of environmental samples, or new regulatory requirements for re-testing of existing chemicals increase the need for higher throughput mutagenicity assays. We have earlier introduced the liquid Ames II and Ames MPF (microplate format) assays, which have the advantage of requiring less test compound, consumables and hands-on-time. We are now able to offer in this format all strains required by the OECD guideline 471 for Testing of Chemicals. The complete bacterial reverse mutation test includes at least five tester strains. S. typhimurium TA98, TA100, TA1535 and TA1537 are already successfully used in the microplate format. These 4 tester strains have GC base pairs at the primary reversion site and may therefore not detect certain classes of chemicals. A tester strain with an AT base pair at its primary reversion site was until now not available in the microplate format. The mutagenic response to 13 reference compounds, including streptonigrin, mitomycin C, aldehydes oxidizing agents and hydrazines, was examined in E.coli wp2 uvrA and E.coli wp2 [pKM101]. The two strains had different sensitivities towards different mutagens. When combined during exposure as "E.coli Combo", it was the more sensitive strain that prevailed in the E.coli Combo test. When compared with published plate incorporation data, the results and were found to be identical. The new Ames MPF E.Coli Combo assay was combined with the Ames MPF 98/100/1535/1537 assay to create the Ames MPF PENTA I test which meets the strain requirements of the OECD guideline 471. The new Ames MPF PENTA I test is based on the fluctuation method using a preincubation procedure of 90 minutes. The use of a liquid format and 384-well microplates offers a time- and cost effective alternative to the plate incorporation test. As both formats use the same tester strains, results can be compared with existing data sets. The new test includes ready-to-use media and quality controlled bacteria, and allows for high throughput testing.

EM072

EXPOSURE TO VARIABLE AIR LEVELS OF BENZENE AS A FUNCTION OF DIFFERENT TRANSPORTATION MEANS IN FLORENCE, ITALY

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Benzene is a mutagenic and carcinogenic pollutant present ubiquitously in the air of cities. The most important sources of outdoor benzene are of motor vehicle exhaust and evaporation from petrol tanks and filling stations. Airborne exposure to benzene is though to cause an increased risk of cancer and international guidelines recommend the reduction of benzene concentration in city air. Outdoor benzene is inhaled by all city inhabitants. However, the level of exposure might be dependent of the amount of time spent in city traffic and on the choice of transportation means. We developed a sensitive method for determining airborne concentrations of benzene in city air by means of adsorption of benzene to solid phase micro extraction (SPME) fibres and later automatic desorption with a robotic device and quantitative measurement in GC-MS. Using this method we could measure the mean air concentration of benzene during a relative short time spent in city traffic, while commuting along an itinerary from a peripheral site to the central train station (approximate distance: 4 km). The parallel measures with different transportation means were repeated everyday of the week for a week. The transportation means used were: foot, train, bicycle, motorcycle, auto and bus. The measures were carried out during the winter season (January 2006 and March 2009). The mean transportation time varied from 9.8 to 16.4 min. In 2006 the average benzene concentration was the maximum using a motorcycle and minimum with a train (56.3 ± 17.3 and 11.1±5.6 µg/m3 respectively, means ±SD). In 2009 the average exposure with the same transportation means was reduced to 14.2.1±4.8 and 1.6 ± 0.5 µg/m3, respectively. New rules for pollution control and the modernization of motor vehicles are the likely cause of this decrease. Contrary to common opinion using a bicycle in congested traffic did not expose to benzene concentration considerably higher than using other transportation means (8.1±2.9 µg/m3 in 2009). A decreased use of polluting motor vehicles seem to offer the best strategy for reducing exposure to low level of airborne mutagenic and carcinogen chemicals like benzene in city air.

EM073

ASSESSMENT OF DNA DAMAGE IN LEAVES OF A WEEDY PLANT SPECIES, CASSIA OCCIDENTALIS, GROWING WILD ON FLY ASH BASIN OF A COAL-FIRED THERMAL POWER PLANT

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Fly ash is a coal combustion byproduct generated by coal fired thermal power plants. It consists environmental contaminants such as trace elements (metals and metalloids), polycyclic aromatic hydrocarbons, and radionuclides. Open dumping of fly ash in ash disposal areas (fly ash basins or dumps) has significant adverse environmental impacts due to its elevated trace element content. Fly ash has been shown to be genotoxic and mutagenic in experimental model organisms but genotoxicity of fly ash has not yet been demonstrated in natural populations of plants inhabiting fly ash disposal areas. In situ biomonitoring of genotoxicity is of practical value in realistic hazard identification of fly ash. Leaves from natural populations of C. occidentalis growing at two contrasting sites - one having weathered fly ash (fly ash basin) and the other having soil (reference site) as plant growth substrates - were used for the estimation of As, Ni, Cr, Co and Cu by atomic absorption spectrophotometry and DNA damage by comet assay. Correlation analyses were carried out to find out any association between DNA damage and foliar concentrations of trace elements. The foliar concentrations of As, Ni and Cr were 2 to 8 fold higher in plants growing on fly ash as compared to the plants growing on soil, whereas foliar concentrations of Cu and Co were similar. We report, for the first time, based upon comet assay results, higher levels of DNA damage in leaf tissues of Cassia occidentalis growing wild on fly ash basin compared to C. occidentalis growing on soil. Correlation analysis between foliar DNA damage and foliar concentrations of trace elements suggests that DNA damage may perhaps be associated with foliar concentrations of As and Ni. Our observations suggest that (i) fly ash triggers genotoxic responses in plants growing naturally on fly ash basins; and (ii) plant comet assay is useful for in situ biomonitoring of genotoxicity of fly ash.

EM074

POLYPLOIDY INDUCTION BY FIVE SIZE-DIFFERENT POLYSTYRENE PARTICLES IN A CHINESE HAMSTER CELL LINE CHL

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We have been developing an in vitro safety evaluation system for nanomaterials (NMs) to properly manage the growing toxicological concerns of them. In this study, to understand the relationship between the particle size and the cytotoxicity or genotoxicity of aggregates of NMs, we performed the cytotoxicity and the chromosome aberration test with eleven size-different polystyrene (PS) particles in a Chinese hamster cell line CHL. [Materials and methods] PS spheres with defined diameters ranging from 0.1 to 9.2 µm were purchased from Spherotec Inc. (IL, USA). Their sizes were 0.10 ± 0.0024, 0.20 ± 0.005, 0.51 ± 0.012, 0.92 ± 0.023, 1.09 ± 0.027, 2.07 ± 0.05, 3.17, 4.45, 5.26, 6.8 and 9.2 □m in mean diameter ± SD, respectively. They were suspended in deionized water containing 0.02% sodium azide. Their cytotoxicity was investigated in the colony formation assay. The chromosome aberrations were investigated with cells treated with PS particles for 24 or 48 h. [Results] We have already reported that the 4.45-µm PS particles were most cytotoxic while sizes 0.1 and 0.2 µm showed no cytotoxicity up to 1000 µg/ml. There was a big difference in cytotoxicity between the 4.45- and 5.26-µm particles, although both precipitated and covered cells to the same extent in the culture. Surface analysis by FT-IR/ATR and chemical analysis of both particles did not show any difference between them. PS particles with the mean diameter of 0.92 to 4.45 µm induced polyploidy and the intensity was correlated well with their cytotoxicity. We observed CHL cells that incorporated PS particles a lot under the scanning electron microscope (SEM) and found that the uptake of particles into cells can be measured by the flow cytometric analysis semi-quantitatively. [Conclusions] The findings suggest that polyploidy may be induced by cytokinesis block due to incorporated PS particles observed by SEM. It is interesting to find that spheric materials induced polyploidy in the chromosome aberration test. As the size-specific toxicity may be induced by NM aggregates as well, we may need to provide data on the aggregate size or some parameters indicating dispersion condition in the NM suspension tested to precisely evaluate the safety of NMs.

EM075

INTERMEDIATE FREQUENCY MAGNETIC FIELDS DID NOT HAVE GENOTOXIC POTENTIALS IN IN VITRO GENOTOXICITY TESTS

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BACKGROUND: In contrast to extremely low frequency and radio

frequency electromagnetic fields (EMFs), the biological effects of intermediate frequency (IF; 300 Hz to 10 MHz) EMFs have not been studied extensively. The WHO Environmental Health Criteria No.238, which published for health assessment of EMFs (0Hz <, <100kHz) in 2007, states the needs of further biological research on IF-EMFs. AIMS: The aim of this study is to evaluate the effects of intermediate frequency magnetic fields (MFs) on genotoxicity. METHODS: We used bacterial mutation tests, yeast genotoxicity tests, *in vitro* micronucleus tests and mouse lymphoma assay. For the *in vitro* research, we constructed a Helmholtz type exposure system which can generate vertical and sinusoidal MFs, such as 0.91mTrms (146 times greater than ICNIRP guidelines for public) at 2kHz, 1.1mTrms (176 times) at 20kHz and 0.11mTrms (18 times) at 60kHz. The assays of genotoxicity were carried out for each MF exposure condition. For the mutagenicity tests, four strains of *S. typhimurium* (TA98, TA100, TA1535 and TA1537) and two strains of *E. coli* (WP2 uvrA and WP2 uvrA/pKM101) were selected to cover a wide spectrum of point mutation. For the gene conversion tests, we used the yeast test strain, *S. cereviciae* XD83. In both tests, the revertant colonies were counted and analyzed statistically to estimate the genotoxicity potentials of the IF MFs. For *in vitro* micronucleus tests, we used the Chinese hamster V79 cells. Rates of micronucleus formation were determined as the proportion of binucleus cells with micronucleus to the total number of binucleus cells. For the mouse lymphoma assay, which can detect both point mutation (as large colony) and chromosomal aberration such as deletion (as small colony), a mouse lymphoma cell line, L5178Y *tk*+/- 3.7.2c, was used. RESULTS AND CONCLUSIONS: In statistical analysis for all above genotoxicity tests, neither significant nor reproducible differences were found between exposed and control groups. These results indicate that the 2 kHz, 20 kHz or 60 kHz MFs did not have genotoxic potentials, which are related with the mechanisms of point mutation, gene conversion, micronucleus formation and chromosomal aberrations, in the *in vitro* experimental conditions.

EM076

OXIDATIVE MODIFICOMICS ANALYSIS OF CYSTEINE THIOLS.

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Reactive oxygen species (ROS) are thought to mediate the toxicity of oxidative stress because of their greater chemical reactivity such as covalent modification of lipids, DNA, and protein. Recently, they have also known as intracellular signaling molecules. In order to identify target proteins of ROS and their signaling cascades, we have developed an unbiased quantitative proteomic analysis for cysteine oxidation which was named as DLIAA (Double Labeling using Isotope Amino Acids and Affinity Tags). The key technologies of this method are two-step labeling of reduced and oxidized cysteines by Isotope Coded Affinity Tags (ICAT) and unbiased quantification by Stable Isotopic Labeling using Amino Acids in Cell Culture (SILAC). These technologies give four different masses for a peptide containing cysteine(s) which enabled us to quantitate relative amount of reduced and oxidized cysteine in control and exposed cells. Our preliminary results showed that Peroxiredoxin6 and Glyceraldehyde 3 phosphate dehydrogenase are early response proteins which oxidized after 15 min of exposure of H_2O_2 in HepG2 cells. After 90min of exposure, eight proteins were oxidized and expression levels of 12 proteins were elevated. Furthermore, we identified sulfonic acid modification as oxidative form of Peroxiredoxin6 by immnoprepicitation-SILAC quantitation method. Our results suggest DLIAA is a useful method to detect oxidative stress marker proteins and quantitate oxidative modification(s) of marker proteins.

EM077

ALDH2 GENOTYPE IS CRITICAL FOR DNA ADDUCTS FORMATION IN MICE TREATED WITH ALCOHOL AND ACETALDEHYDE.

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Ingested ethanol is oxidized to acetaldehyde, which is subsequently oxidized by mitochondrial aldehyde dehydrogenase 2 (ALDH2) to produce acetate. ALDH2*2, being a genetic polymorphism observed in oriental countries, is positively related to oral and esophageal cancer. The provable routes of acetaldehyde exposure are not only via alcohol ingestion but also via inhalation. Acetaldehyde is generally containing in room air (WHO guideline is 0.17ppm), automotive emission gas and tobacco smoke (0.8-1.4mg/cigarette). In this study, we analyzed acetaldehyde-derived DNA adducts (N2-Eti-dG; N2-ethylidene-2-deoxyguanosine) in DNA of Aldh2 knockout (Aldh2-/-) mice which were exposed by ethanol or acetaldehyde to assess the significance of Aldh2 genotype on DNA adducts formation in both exposure scenarios. [Materials and Methods] Male C57BL/6 (Aldh2 +/+) mice and Aldh2 -/- mice were exposed to ethanol intrapetitoneally, or atmospheres containing acetaldehyde at levels of 0, 125, and 500 ppm for 24 h/day during 14 days. The mice were sacrificed after exposure and N2-Eti-dG in DNA of various organs was measured using LC/MS/MS. [Results] In case of ethanol exposure, although no significant increase of N2-Eti-dG was observed in Aldh2 +/+ mice, the increase of N2-Eti-dG level was observed in almost all organs of Aldh2 -/- mice after 1hr or 3hr of ethanol administration. In case of inhalation scenario, the average N2-Eti-dG levels in lung, nasal epithelium, and dossal skin DNA from Aldh2 -/- mice in exposure group was significantly higher than that from Aldh2 +/+ mice, while N2-Eti-dG levels in liver DNA was not significantly different between Aldh2 -/- and +/+ mice. These results indicate that ALDH2 genotype is critical for DNA adducts formation in both exposure scenarios.

EM078

MITOCHONDRIAL-MEDIATED APOPTOSIS PATHWAY IN PNEUMOCYTES EXPOSED TO WATER OFA SOLUTION

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In the recent years we have acquired a consistent knowledge on the biological effects of bioavailable transition metals absorbed on oil fly ash(OFA). All these effects, included DNA base modifications, strand breaks and DNA-protein cross links, have been attributed to redox imbalance, due to generation of reactive oxygen species(ROS). Previously we had observed mitochondrial dysfunction in OFA exposed pneumocytes and in their progeny. Such dysfunction, causing chronic oxidative stress in respiratory epithelium, has prompted us to study the proapoptotic potential of these airborne pollutant components. As well known mitochondria play an important role in intrinsic apoptosis pathway and defects in the apoptosis regulation can be a mechanism of cancer induction. Apoptosis detection was performed in A549 pneumocytes cultures, exposed to subtoxic dose of a water OFA solution and in their 3 following subcultures, by flow cytometry. The use of AnnexinV-FITC, in addition to propidium iodide, allowed contemporaneously the identification of cell surface changes occuring early during the apoptotic process and cytoplasmic membrane damages in the late stage of apoptosis. Apoptotic cells were assayed also by cytometric measures of hypoploid peak in cell cycle analyses. Single synthetic metal solution at the same molarity present in OFA solution, were also tested. To check the role played by mitochondria in apoptosis pathway, we used the caspase 9 inhibitor (Z-LEHD-FMK). The results showed increases of apoptotic cells(P=0.002) in cultures

exposed to OFA solution (formed by 68.8, 110.4 and 18 μM for V, Fe and Ni respectively) and in their progeny. In comparison to control, apoptotic cells were on average more three times. Analogously, they were 2.5, 2.1 and 1.9 in cells exposed to V, Fe and Ni respectively. The values of phosphatidylserine analyses and hypoploid cells were almost superimposable(P=0.02 to Spearmann test). The ester used as mitochondrial-mediated apoptosis inhibitor on average halved apoptotic cells, showing the intrinsic pathway triggered by the examined metals. Like some carcinogenic metals as Cr and Cd, in the major metals of OFA genotoxic and pro-apoptotic properties coexist. The latter could induce cancer mechanism, causing impaired cell turn over.

EM079

ASSESSMENT OF GENOTOXICITY IN ENVIRONMENTALLY BORON EXPOSURE BY USING ALKALINE COMET AND CBMN ASSAY

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Boron which is a naturally occurring substance found in the environment mostly in the sediments, is used in a variety of products. The aim of the present study was to investigate the genotoxic effects in environmentally boron exposed population. Accordingly blood samples were taken from the voluntary individuals (n=38) living in the boron rich area (Balıkesir-Bigadiç) of Turkey in order to evaluate the cytogenetic effects of boron exposure. The increase of sister chromatid exchange (SCE) and micronuclues (MN) frequencies in peripheral blood lymphocytes were used as a cytogenetic biomarker of boron exposure. Additionally DNA damage in the peripheral blood lymphocytes of the residents environmentally exposed to boron was investigated by the alkaline COMET assay combined with bacterial formamido pyrimidine glycosylase (Fpg) protein. Statistically significant decrease in tail intensity and also non-significant decrease in tail moment were observed in the exposed group compared to their controls suggesting that boron exposure did not induce additional DNA damage in peripheral blood lymphocytes. SCE frequencies in boron exposed individuals (7,28 ± 1,60) were significantly (Mann Whitney U- test, p<0,05) lower than the controls (9,50 ± 1,83). The same decrement was also observed for the mean MN values in boron exposed individuals. Nevertheless the difference was statistically not significant (□2 testi, p>0,05). MN values in boron exposed and control group were 9,81 ± 1,94 and 20,32 ± 3,98 respectively. Consequently, the results of this study suggest that boron exposure does not induce DNA damage in the peripheral lymphocytes. When the results of SCE frequencies and MN values were taken into consideration the lower levels of SCE and MN values in environmentally boron exposed population might be a result of a protective effect of boron exposure. However this suggestion surely needs further investigation.

EM080

EXTRACTS OF CYANOBACTERIA OR PURE MICROCYSTIN-LR ARE INACTIVE IN MICRONUCLEUS ASSAYS.

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Cyanobacteria are sometimes common in lakes and can produce potent toxins, which can be dangerous for animals drinking the water, e.g. wildstock cattles and dogs. If the toxins are taken up by fish and other seafood they might pose a problem also for humans. Furthermore, many people obtained their drinking water from lakes. Microcystin-LR, a hepatotoxic cyclic peptide, is one of the most frequently found cyanobacterial toxin. Results on the genotoxic potential of microcystin LR and other cyanobacterial toxins are contradictory. Here we report results from test using the micronucleus assay in vivo and in vitro. To increase the sensitivity we have used the flow cytomer-based micronucleus assay in mouse. In this study both pure microcystin-LR and extracts from four different samples from different lakes in Sweden have been analysed. Although doses almost up to lethality were used and an average of 200 000 young erythrocytes, polychromatic erythrocytes, analysed from each animal, no genotoxic effect occurred. Neither in the in vitro study, using human lymphocytes, a clastgenic effect was disclosed.

Conclusion: The cyanobacterial toxin microcystin-LR or extracts from cyanobacteria are inactive in micronuleus assays.

EM081

BIOMARKERS OF OXIDATIVE STRESS IN MOTHERS AND NEWBORNS – THE EFFECT OF TOBACCO SMOKE EXPOSURE

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Oxidative damage to macromolecules may have numerous negative health consequences. We measured oxidative damage to DNA, proteins and lipids in 80 newborns and 79 mothers, analyzed the effect of mother's tobacco smoke exposure on oxidative stress and assessed correlations between oxidative stress markers and bulky and polycyclic aromatic hydrocarbon (PAH) DNA adducts. Mean levels (±SD) of 8-oxodeoxyguanosine (8-oxodG) per 105 dG in the placenta were 2.85±0.78; we did not see a difference between 8-oxodG levels in newborns born to mothers exposed and unexposed to tobacco smoke. Protein carbonyl levels, a marker of protein oxidation, were comparable in umbilical cord and in maternal venous blood plasma (17.4±3.2 and 17.6±4.2 nmol/ml in newborns and mothers, respectively, p=0.66). Lipid peroxidation measured as levels of 15-F2t-isoprostane (15-F2t-IsoP) in plasma was significantly higher in newborns than in mothers (mean±SD of 15-F2t-IsoP (pg/ml plasma): 362±129 and 252±130 in newborns and mothers, respectively, p<0.001). We did not find any effect of tobacco smoke exposure on either biomarker in any group. Levels of both protein carbonyls and 15-F2t-IsoP in cord blood significantly correlated with those in maternal plasma (p<0.001). 8-oxodG levels positively correlated with plasma carbonyls in cord and maternal plasma as well as with cotinine levels (a marker of tobacco smoke exposure) in maternal plasma. 8-oxodG levels also correlated with bulky and PAH-DNA adducts in lymphocyte DNA of both newborns and mothers. Our results showed higher lipid peroxidation in newborns than in mothers, close correlation of analyzed oxidative stress markers between newborns and mothers, and a relationship between oxidative stress and induction of DNA adducts. Supported by Czech Ministry of the Education (2B06088) and by Academy of Sciences of the Czech Republic (AVOZ50390512).

EM082

CELLULAR STRESS RESPONSES OF NON-GENOTOXIC CARCINOGENS IN MOUSE EMBRYONIC STEM CELLS

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Non-genotoxic carcinogens (NGTX) cause tumorigenesis without generating direct DNA damage. There is lack of predictive models to identify potential non-genotoxic carcinogen hazard of novel chemical entities. Moreover, the signaling pathways of non-genotoxic carcinogens are not well understood. We attempt to assemble biomarker-based cell reporter systems to detect chemicals with potential NGTX features.

Gene expression biomarkers were selected by evaluating the cellular stress responses of mouse embryonic stem (mES) cells to 3 concentrations of 4 established NGTXs, including cyclosporine A (CsA), diethylstilbestrol (DES), 17β-estradiol (E2), and wyeth-14643 (WY). Affymetrics micro-array analysis indicated that mES cells were highly responsive to CsA, DES and WY. Several probe sets were identified showing expression changes in response to 2 or 3 NGTXs, indicating that the respective genes may be valuable biomarkers. However no probe sets were identified that predict all 4 NGTX responses, since mES cells presumably were relatively non-responsive to E2. Next, we compared the gene expression responses of mES cells to the 4 NGTXs with the response to the genotoxic (GTX) agent cisplatin (CDDP). Approximately 50 percent of the probe sets that detected expression changes in response to cisplatin were not significantly affected after exposure to the 4 non-genotoxic compounds. The remaining biomarkers significantly changed after NGTX exposures, but expression changes were considerably lower compared to cisplatin. Thus the DNA damaging biomarkers are qualified to discriminate 3 NGTXs from cisplatin.

EM083

ANEUGENIC AND CLASTOGENIC EFFECTS OF AMORPHOUS SILICA NANOPARTICLES IN A549 HUMAN LUNG CARCINOMA CELLS: DOES SIZE MATTER?

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Background/Aims: In contrast to crystalline silica nanoparticles (SNPs), no evidence of genotoxic effects induced by amorphous SNPs has been demonstrated up to now. This study aimed at re-assessing their genotoxic potential with emphasis on size-effects. Furthermore the dosimetry relevant for genotoxicity studies and the mechanisms by which the SNPs induce genotoxicity were investigated. Methods: The in vitro cytochalasin-B micronucleus assay (CBMN assay) was applied to determine the genotoxic potential of SNPs with three different diameters (16, 60 and 104 nm) in A549 human lung carcinoma cells. The cellular dose was determined by inductively –coupled plasma mass spectroscopy. Mechanisms of SNP-induced genotoxicity were investigated by the alkaline comet assay with and without FPG (formamidopyrimidine DNA glycosylase) enzymatic treatment. Combination of the CBMN assay with fluorescence in situ hybridisation (FISH) with pancentromeric probes, was used to make a distinction between the formation of micronuclei containing chromosome fragments and micronuclei containing entire chromosomes after treatment with 40 and 60 µg/ml of 16 and 60 nm SNPs. Mitotic indices and induction of mitotic slippage were investigated in parallel with the CBMN assay. Results and conclusions: The in vitro CBMN assay showed an induction of MN frequencies after treatment with the 16 and 60 nm SNPs, with a higher fold induction after treatment with the smallest SNPs. No clear dose-response was observed. When considering the cellular dose, expressed as number of cell-associated particles or as cell-associated surface area, a quasi-linear relationship with the fold MN induction can be observed, indicating that these dose metrics are determinants for genotoxic effects. The alkaline comet assay showed no DNA breaks after treatment with 16 or 60 nm SNPs. However addition of FPG revealed the induction of oxidative DNA damage by both the 16 and 60 nm SNPs. Besides oxidative damage DNA damage, chromosome breakage and chromosome loss was induced by 16 and 60 nm SNPs as detected by the CBMN combined with FISH. Besides oxidative damage, 16 and 60 nm SNPs induce other genotoxic effects, such as chromosome loss, metaphase block and mitotic slippage, suggesting interference with the mitotic spindle.

EM084

TOXIC RESPONSES TO TiO_2 NANOPARTICLES IN HUMAN HEPATOMA CELLS HepG2 AND IN ZEBRAFISH (*Danio rerio*) EMBRYO

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Titanium dioxide (TiO_2) is, because of its low acute toxicity, widely used in the production of cosmetics, paints, paper, plastics and etc. However, recent studies have shown that in mice and rats nano-sized TiO_2 (<100 nm) can cause pulmonary inflammation, fibrosis and tumors, and also genotoxic effects, which generates a concern about the possible adverse effects of nano-sized TiO_2. We evaluated toxic and genotoxic responses to two types of nano-sized TiO_2 (5 nm anatase and 10x40 nm rutile) with two models, human hepatoma cells HepG2 and zebrafish (*Danio rerio)* embryos. The nanostructure of tested particles was determined with Scanning electron microscopy (SEM). The viability of HepG2 cells exposed to TiO_2 particles was determined with the MTT assay, the intracellular reactive oxygen species (ROS) formation was determined with DCFH-DA fluoroprobe, genotoxicity was measured with classical and with modified comet assay with Fpg and EndoIII enzymes that allow for detection of oxidative DNA damage. The genotoxic response was confirmed by measuring the mRNA expression of the DNA damage responsive genes (p53, p21, Gadd45 and MDM2). In the model with zebrafish embryos acute toxicity and sublethal effects of TiO_2 particles were determined with the standardized fish embryo test (FET), and genotoxicity with the comet assay. The SEM analysis showed that both sizes of TiO_2 are spherical and irregular shapes, quite agglomerated with sizes at nanometer scale (around 25 nm for anatase TiO_2 and under 100 nm for rutile TiO_2). In the model with HepG2 cells TiO_2 particles were not cytotoxic. Only 5 nm TiO_2 particles induced significant increase of intracellular ROS formation. The 5 nm particles, but not 10x40 nm TiO_2, induced slight increase in % tail DNA and significant oxidative DNA damage. In the model with zebrafish embryos TiO_2 particles did not cause lethal and sublethal toxic effects, whereas both types induced significant DNA damage at concentrations as low as 0.01 and 0.1 µg/mL, by 5 nm and 10x40 nm TiO_2 particles, respectively. Our results indicate that in both test models the genotoxic effects of TiO_2 particles are size and/or type dependent and they present good basis for future toxicity studies of nano-sized TiO_2 and other nanoparticles.

EM085

VALIDATION OF A NEW IMMUNOCHEMICAL ASSAY WITH HIGH-THROUGHPUT POTENTIAL FOR PAH- DNA ADDUCT DETECTION

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Highly sensitive, high-throughput methods to measure human exposure to polycyclic aromatic hydrocarbons (PAHs) are required for large-scale molecular epidemiology studies. A highly sensitive benzo[a]pyrene diol-epoxide (BPDE)-DNA sandwich enzyme-linked immunosorbent assay (ELISA) using a rabbit anti-BPDE-DNA antiserum has been developed in NHRF, Athens to detect PAH-DNA adducts. The aim of the present study was to further validate the new assay. The test DNA samples were from MCF 7 cells treated with benzo[a]pyrene (BaP) in culture, from liver of mice treated in vivo with several doses of BaP, benzo[b]fluoranthene (BbF) and dibenzo[a,h]anthracene (DBahA), and from lung tissue from smoking lung cancer patients. DNA isolations were done with phenol extraction, salting-out and a Qiagen kit. DNA adduct measurements were performed by the new immunoassay and by the ^{32}P-postlabelling method. BaP-DNA adduct levels from the MCF 7 cells were in the order of 1 in 10^5

normal nucleotides, the ratio between the adduct values measured by ELISA and ^{32}P-postlabelling was about 0.5. There was a very strong, highly significant positive correlation between the DNA adduct measurements of the PAH dose-response curves by ELISA and ^{32}P-postlabelling in the animal samples (r between 0.87 and 0.99).However, the adduct levels were significantly lower by ELISA than by ^{32}P-postlabelling, i.e. 1:5 for BaP, 1:30 for BbF and 1:5 for DBahA, which also indicates the different affinity of the anti-BPDE-DNA antiserum to the different PAH-DNA structures. The adduct levels in 20 human DNA samples were under the detection limit of the immunoassay of about 1 adduct/10^8 normal nucleotides. Quality of the DNA substantially affected the performance of the immunoassay. Further comparative DNA adduct measurements are underway for additional validation of the new immunoassay. Acknowledgements. Treated MCF 7 cells were obtained from Dr. D.H. Phillips (ICR, Sutton). The anti-BPDE-DNA antiserum was provided by Dr. M.C. Poirier (NCI, Bethesda, USA). The BPDE-DNA standard was provided by Dr. F.A. Beland (NCTR, Arkansas, USA). K. Kovács was recipient of an ECNIS exchange fellowship to NHRF in 2008. This work has been supported by ECNIS NoE No 513943.

EM086

TELOMERE DYSFUNCTION AND DNA REPAIR DEFICIENCY: MARKERS OF SENSITIVITY TO MUTAGENS AND CARCINOGENS?

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Genetic susceptibility to environmental disease is believed to play an important role in determining individual differences in the development of cancer. Genetic alterations in critical regulatory pathways such as control of genomic stability may predispose cells to carcinogenesis . Previously, we have demonstrated that cells lacking key DNA repair factor, PARP-1, displayed enhanced sensitivity to arsenite. Here, we used telomerase deficient (mTERC-/-) mouse embryonic fibroblasts with long and short telomeres to investigate the extent of oxidative damage by comparing the differences in telomere loss and chromosome aberrations at 24 and 48 hours of exposure to arsenite. Increasing doses of arsenite augmented the chromosome aberrations, which contributes to genomic instability and lead to possibly apoptotic cell death and cell cycle arrest. Elevated DNA damage points to an impaired repair ability of arsenite induced DNA lesions in mTERC-/- mouse cells with short telomeres. By using micro-array analysis, we have identified few genes which are differentially expressed upon arsenite treatment and we are elucidating the signal transduction pathways involved in arsenic-induced oxidative damage. The genes involved in telomere length regulation and maintenance (such as ATM, Adprt1, NBS1, TRF1 and 2 etc) are differentially expressed in mTERC-/- cells with short telomeres. Similar results were obtained for both PARP-1-/- and mTERC-/- cells following exposure to different doses of radiation. In a separate study, we have also observed that deficiency of functional nucleotide excision repair paradoxically renders cells more susceptibility to oxidative stress-induced while reducing cytotoxicity. Taken together, data from the present study and our ongoing studies on different DNA repair deficient mammalian cells imply that short dysfunctional telomeres impair the repair of oxidative damage caused by arsenite and ionising radiation and point to the increased sensitivity of cells which are deficient in PARP-1 and telomerase to carcinogens and mutagens. *This study is supported by AcRF, National University of Singapore, National Medical Research Council, Singapore and Defence Innovative Research Programme, Defence Science and Technology Agency, Singapore.

EM087

USE OF MICRONUCLEUS TEST TO IDENTIFY HEALTH RISK FROM EXPOSURE TO “X” RAY

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The following study was designed to examine possible DNA damage levels in exfoliated bucal cells, using the micronucleus test (MN-test). The aim of this study was to evaluate the genotoxic effect of “x” radiation on exfoliated bucall cells at patients which use the “x” ray for dental diagnostic purposes, and to compare the genotoxic effect of “x” ray after five , ten and twenty days after skiagram. For this purpose 26 patient were studied. First bucal cell samples were taken before skiagram. After skiagram, the samples were taken : 5, 10 20 days after skiagram. Samples taken before the skiagram use as control. Founded high frequency of MN in exfoliated bucal cells after skiagram compared with exfoliated bucal cells before skiagram , but not significant.

EM088

MUTAGENESIS AND EMBRYOTOXICITY IN ZEBRAFISH IN AN AREA OF THE PATOS LAGOON ESTUARY, RIO GRANDE DO SUL, BRAZIL, UNDER INDUSTRIAL AND PETROCHEMICAL INFLUENCE

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Research has shown that anthropogenic activities of different origins modify the ecosystems, releasing many different pollutants that can cause mutagenic effects to the biota as well as be potentially carcinogen and embryotoxic. During this study we analyzed three areas under petrochemical influence (Saco da Mangueira) and a reference point (Saco do Justino), all located at the southern extremity of the Lagoa dos Patos estuary. Between March 2005 and July 2006 sediment samples were collected, the interstitial water extracted by centrifugation and the organic compounds by sonication, using dichloromethane as solvent. As biomarkers we selected the mutagenic and cytotoxic events using the Salmonella/microsome test and embryotoxic modifications analyzed in bioassays using zebrafish (Danio rerio). Organic compounds (aliphatic hydrocarbons and polyaromatic HPAs) were also characterized. The results revealed the presence of direct and indirect mutagenic activity in frameshift and base pair substitution types in the organic extracts and in the interstitial water. Cytotoxicity was constant, even at the control point. Pollution was defined as being of mixed origin, hydrocarbonates of biogenic and pyrolitic origins prevailing, including petrochemical, with medium and high molecular weight (4-5 rings) HPAs predominating in some areas. During the embryotoxicity assessment more than 12,000 embryos were analyzed, exposed to dry sediments and interstitial water at five different concentrations/dose-response. At the 100% and 75% concentrations of the sample, effects were more lethal than at lower concentrations. More significant anomalies were observed in embryos exposed to areas under higher petrochemical influence, with a predominance of edemas and morphological deformations. The biomarkers used showed a correlation with the most aggressive forms of the organic compounds identified. Funding: Alßan- Programme European Comission andCNPq.

EM089

USE OF STRAINS OF SALMONELLA TYPHIMURIUM WITH HIGH LEVELS OF ACETYLTRANSFERASE AND NITROREDUCTASE FOR THE DETECTION OF NITROCOMPOUNDS IN SOIL SAMPLES

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The Salmonella typhimurium reverse mutation assay, Ames test, is the major assay used for the early diagnostic of the presence of mutagens in environmental samples. The basic protocols use specific strains to access different DNA damages and, more recently, strains with high

sensitivity to detect some groups of compounds, such as alkylating agents, heavy metals, polycyclic aromatic hydrocarbons (PAHs), nitroarenes and aromatic amines. The group of the nitrocompounds, including the nitro-PAHs, is important in ecotoxicological studies due to their mutagenic and carcinogenic effects. The strains YG1041 and YG1042 are derived from the basic strains (TA98 and TA100). These strains have received the plasmid pYG233, which carry the genes of two enzymes that increase the sensitivity to detect nitrocompounds: acetyltransferase and nitroreductase. Since their use impart a higher resolution for the detection of mutagens in environmental samples, these strains are being incorporated in the studies with soil samples in the southern Brazil. The research applied the Salmonella/microsome assay and its methodological variants as biomarkers to assess the presence of hazardous compounds in soil samples. These strains have been used for detection of nitrocompounds in organic extracts of different types of soil: coal bottom ash deposit, riparian forest and suburban and agricultural areas, besides in fractionated extracts of nitro-PAHs from a contaminated area with wood preservatives. The results showed an increase in the mutagenic induction when compared to the parental strains, varying between 1.7 and 90 times for the strain YG1041 and between 1.9 and 6680 for the strain YG1042. The chemical analysis of the extracts confirms the presence of nitrocompounds in the organic extracts that induced the highest mutagenicities in the strains sensitive to them. Thus, associating the use of strains with high sensitivity for specific classes of compounds and chemical profiling proved to be effective for the forecast of biological damages caused by soil samples, constituting an important step towards the incorporation of this strategy in environmental legislation. Financial Suppor CNPq; CAPES

EM090

PHOTOPROTECTIVE EFFECT AND GENOTOXIC POTENTIAL BY EXTRACTS FROM THE ANTARTIC MOSS *Sanionia uncinata*

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Cyclo-butane dimers have not been detected in *Sanionia uncinata* DNA (order Hypnales, family Amblystegiaceae) exposed to UV radiation. In the present work, photoprotective effects against UV-C irradiation, mutagenesis induction and genotoxicity of aqueous and ethanol extracts from the Antarctic *S. uncinata* (Ecology Glacier, King George Island) have been investigated. Data were analyzed by one-way ANOVA and Student *t*-test. Only significant differences were considered at $P < 0.05$. In the presence of each extract (1 mg/mL), survivals curves of NER-proficient (AB1157) and NER-deficient (AB1884, AB1885 and AB1886) *Escherichia coli* strains at exponential growth phase were compared after UV-C exposure. Photoprotective activity of both extracts was observed at exposures higher than 374 J/m^2 for the proficient strain and higher than 5.8 J/m^2 for the deficient ones. Mutagenesis induction was evaluated through spot test procedure of *Salmonella*/microsome assay using *Salmonella typhimurium* TA97, TA98, TA100, TA102 and TA104 strains with and without exogenous metabolic activation (S9 mix). Aqueous and ethanol extracts induced a positive mutagenic response in the TA102 ($uvrB^+$) in the presence of S9 whereas only ethanol extract induced without S9. A decrease in the number of revertants colonies in the TA98 was induced by aqueous extract in the absence of S9 and by ethanol extract in the presence of S9. Significant induction was not observed for other strains, including TA104 ($uvrB^-$), for both conditions. Genotoxicity was evaluated by incubation of each extract (20-900 microg/mL) with *p*UC18 plasmid (140 mg/mL) at 37ºC for 1 h under aerobic conditions followed by agarose gel electrophoresis (60 V, 1.5 h, 1.5%, Tris-acetate-EDTA buffer, pH 7.8) and DNA cleavage was not detected. Our results suggest that the photoprotective effect against UV-irradiation by *S. uncinata* extracts can be due to an intense absorption of UV light. The two highest maximum absorptions occurred below 280 nm (UV-C range). The mutagenesis induction can be associated to the presence of crosslinking compounds, which are detected only when NER system is intact. Support: CNPq, Faperj and SR2-Uerj.

EM091

MUTAGENICITY OF WEAR PARTICLES FROM AUTOMOTIVE FRICTION COMPOSITES

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Potential genotoxic effect of model friction composite material, its wear debris generated during friction process, and two commercial brake pad samples is addressed in this study. It was demonstrated that volatile chemicals and solid wear particles are being emitted to the environment during friction between brake pad and rotating wheel discs. The properties of debris can significantly differ from properties of the initial components present in the composite bulk material. Previous research indicated considerable amounts of fine and ultrafine biologically available wear particles are produced during friction. They contain carbon, phenolics and their derivatives, as well as metals and metal oxides (mainly of Fe and Cu type). Model friction material was prepared in laboratories, wear debris samples were obtained from full-scale automotive brake dynamometer simulations and the milled samples representing the bulk material were generated in vibrating mill. Both the ball-milled pad and wear debris samples, respectively, were extracted in a Soxhlet extractor with dichloromethane and re-dissolved in dimethylsulfoxide. The bacterial tests, SOS Chromotest and Ames test, were used to detect mutagenicity. The mutagenicity was detected in three of a total of five samples using the tests with an in vitro metabolic activation. The model milled pad composite increased significantly the induction activity in the SOS Chromotest +S9 in doses of 50-300□g/ml and two commercial pad samples were mutagenic when using the strain TA98 in the Ames test +S9 (100-450□g/plate). At higher concentrations, all milled pad samples were toxic. Generated debris and initial resin used for model friction composite preparation were toxic. However, a significant increase of the number of revertants was not proven. The exact mechanisms responsible for the mutagenic and toxic responses are not clear. Nevertheless, the results suggest that screening of the potential genotoxic risk is highly desirable when developing new friction composites. In this way, the environmental load with mutagens can be considerably reduced.

The study was supported by the grant GACR 106/07/1436 „Friction materials on the basis of polymers with a metal content and their impact on the environment“.

EM092

ASSOCIATION OF MULTIPLE GENETIC POLYMORPHISMS WITH 8-OXODEOXYGUANOSINE (8-OXOD G) IN CHILDREN

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The purpose of this study was to assess the role of genetic factors on pregnancy outcomes and to establish the functional relationship between genotype and phenotype with interactions to the air pollution. Oxidative stress has been hypothesized as a common link underlying associations between adverse fetal growth and preterm birth and elevated risks of certain chronic diseases. In the present study we analyzed the association between oxidative DNA damage (8-oxodG levels) measured in placenta DNA and a custom-made panel of 768 single nucleotide polymorphisms (SNPs) containing most important representatives of 4 groups of genes involved in metabolism of xenobiotics, DNA repair, immune response and oxidative damage. DNA samples were extracted from 1200 Caucasian women placenta tissues, residing in two districts in the Czech Republic: Teplice and Prachatice. Genetic polymorphisms were assessed using the Illumina BeadStation 500 system. The data were analyzed using the Illumina BeadStudio software. After exclusion of 39 SNPs that did not cluster well, the statistical analysis was performed to find possible associations between 729

SNPs and level of 8-oxodG as a marker of oxidative damage in placenta DNA. Ten genes involved in the metabolism of xenobiotics, including CCNH, CYP1A1, CYP1A2, CYP1B1, CYP2C9, CYP2D6 CYP2E1 , CYP3A4 ,GSTP1, SULT1A1; 7 DNA repair genes (ERCC2, ERCC4, LIG1, MGMT, TP53, XPC, XPC-02),16 immune and inflammatory genes (C5-08,CCL24 ,CCL26, CTLA4, IL4, IL8,IL10,IL 13,IL1B, IL1RB, LTA ,MBL2, NOS2A,STAT4, TOLLIP) and one gene involved in oxidative stress response (HMOX1) were significantly correlated with 8-oxodG levels (P<0.05). Further analyses of associations between pregnancy outcomes and oxidative DNA damage in placenta and their modulations by genetic polymorphisms, as well as lifestyle factors are under way. Supported by the Czech Ministry of Environment SP/1b3/50/07and Czech Academy of Sciences AVOZ50390512.

EM093

ACETALDEHYDE, AFLATOXIN B1 AND BENZO[A]PYRENE IN VITRO-INDUCED TP53 MUTATIONAL PATTERN IN NORMAL HUMAN FIBROBLASTS AG1521 USING THE FASAY (FUNCTIONAL ANALYSIS OF SEPARATED ALLELES IN YEAST).

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Background: TP53 mutations are the most common genetic alterations involved in cancers. TP53 mutational pattern can be sometimes linked to carcinogen exposure. In hepatocellular carcinoma (HCC), a specific G>T transversion in codon 249 is classically described as a fingerprint of aflatoxin B1 (AFB1) exposure. Likewise particular patterns of mutations have been described in human lung cancers related to tobacco exposure. Methods: by using the FASAY, the present study depicts the mutational pattern of TP53 in normal human fibroblasts after in vitro exposure to well-known carcinogens: AFB1, benzo[a]pyrene (B[a]P) and acetaldehyde. These in vitro patterns of mutation were then compared to those found in human and experimental tumours. Results and conclusion: AFB1 mutational pattern reveals that codon 245 is the main hot spot, whereas no mutations are found in codon 249. The locations of mutations within GG and GC/CG sequences are well in accordance with AFB1-adducts location data. In our assay, AFB1 mainly induces G>A transitions, followed by G>T and A>G mutations. This suggests that G>T transversion at codon 249 is likely the result of a selection bias in human HCC rather than a true fingerprint of AFB1 adducts. Indeed, a comparison of the mutation pattern with that found in human HCC excluding codon 249 reveals that the two spectra are quite similar. Furthermore, the similarity between our in vitro spectrum with that identified in AFB1-induced mice lung tumours suggests that AFB1 may be a potent lung carcinogen in humans. B[a]P mutational pattern shows more G>A transitions than G>T transversions. This result is a discrepancy with the literature data reporting G>T transversions as predominant mutations after experimental B[a]P exposure. However, our pattern is still close to the one found in human lung squamous cell carcinoma (SCC). Furthermore the main hot spot in the B[a]P-induced pattern is codon 248, one major hot spot in human lung SCC. Acetaldehyde mutational pattern is very different to the B[a]P and AFB1 ones. We compared this pattern of mutations with those found for upper aerodigestive tract cancers in human. The results support the role of acetaldehyde, the first metabolite of ethanol, in TP53 mutations in oesophageal and head and neck cancers.

EM094

MOUSE SPERMATOZOA QUANTITY AND QUALITY FOLLOWING BISPHENOL A OR COMBINED X-RAYS-BISPHENOL A EXPOSURE

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Bisphenol A (BPA) is ubiquitous used in many products including the interior coating of food and beverages containers, plastic bottles and dental sealants. The aim of the study was to estimate the effects of bisphenol A (BPA) or combined X-rays-BPA exposure on the sperm production, motility and morphology as well as on the induction of DNA damage in male gametes. Male mice were exposed during 2 weeks to BPA (5 mg/kg bw, 10 mg/kg bw, 15 mg/kg bw, 20 mg/kg bw, 40 mg/kg bw), X-rays (0.05 Gy, 0.10 Gy) or to a combination of both (0.05 Gy + 5 mg/kg bw BPA, 0.10 Gy + 10 mg/kg bw BPA). Groups of animals were sacrificed 24 h after the end of exposure (effects induced in spermatozoa and in late spermatids) and 5 weeks later (effects induced in spermatogonia). Treatment of male mice gametes with BPA alone or irradiation alone caused deterioration of sperm quantity and decreased sperm motility. Combined exposure of spermatogonia, late spermatids and spermatozoa to low doses of X-rays and BPA and exposure of spermatogonia to higher doses of both agents decreased sperm count compared to effects of BPA alone, whereas exposure of spermatozoa and late spermatids to higher doses increased sperm count compared to results of each agent alone. Combined exposure to X-rays and BPA improved the motility of spermatozoa compared to irradiation alone. Treatment with BPA caused not dose-dependent increase in the percent of abnormal spermatozoa. Irradiation alone enhanced the frequency of malformed spermatozoa especially after exposure of spermatogonia. Combined exposure of spermatozoa and late spermatids to low doses of X-rays and BPA decreased the percent of abnormal spermatozoa compared to effect of BPA alone. Combined exposure of gametes to higher doses and combined exposure of spermatogonia to lower doses of both agents increased the percent of abnormal spermatozoa. Treatments of male gametes with BPA induced enhanced level of DNA damage. Combined exposure to X-rays and BPA in lower as well as higher doses decreased level of DNA damage compared to effects induced by BPA alone. Bisphenol A caused deterioration of male mice spermatozoa quantity and quality. Combined X-rays-BPA exposure modulated the effects induced by BPA alone. This work was funded by Polish Ministry of Higher Education and Science (2008-2011), Project no N N404 029 535

EM095

DNA DAMAGE CAUSED BY LIPOPHILIC TOXINS IN THE MUSSEL MYTILUS GALLOPROVINCIALIS EVALUATED BY COMET ASSAY

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Okadaic acid-group lipophilic toxins, comprising okadaic acid (OA) and its analogues, are substances of paramount interest because of their tumoral promoter features, and the fact that they accumulate in the digestive organs of filter-feeding shellfish causing diarrheic shellfish poisoning (DSP) in humans. Bivalve molluscs are among the most useful organisms to monitor the presence of toxic compounds due to their specific characteristics: wide distribution, abundance, size and mainly their filter feeding activity, which induces the bioaccumulation of contaminants. Comet Assay is recognised to be one of the most sensitive methods for measuring DNA strand breaks and estimate genetic damage at the level of single cells in a fast and simple way. In this study, DNA damage in gills and haemocytes of *Mytilus galloprovincialis* was evaluated by Comet Assay in individuals from a mussel-culture raft closed due to the presence of lipophilic toxins, as well as after 72h and 16days of depuration in aerated fresh water. Unexposed mussels were used as control. OA body burden analyses were also carried out. Comet Assay was basically performed according to Pérez-Cadahía et al. (2004), with slight modifications. TL (Comet Tail Length) was the parameter chosen to explain the obtained results. At the arrival of the mussels, TL value in both tissues was higher than in control, being the difference significant only in haemolymph. In haemolymph, after 72h

of depuration, TL increased significantly compared either with control or exposed mussels, but decreased after 16days. In gills, TL decreased significantly when comparing 72h depurated mussels both with control and lipophilic toxins-exposed mussels. No significant effect was found in gills after 16days. These results may reflect the different capacities of response against a pollutant that exist between the two tested tissues, which would be confirmed by the fact that both in control and exposed groups, haemolymph had significantly higher TL than gills.

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EM096

INFLUENCE OF HIF1 ON CARCINOGEN METABOLISM AS A MECHANISM FOR GENETIC INSTABILITY

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Background: Hypoxia has been suggested to promote genetic instability as exposure of cultured cells to low oxygen levels results in an elevated mutation frequency. This contribution to genetic instability may provide an explanation for clinical data that associates hypoxia with more aggressive disease and poor outcome. The hypoxia responsive transcription factor HIF1a is regarded as the primary molecular switch to alter gene expression in response to reduced oxygen tension. The activation of HIF1a requires its heterodimerization with the dually named cofactor HIF1b or aryl hydrocarbon receptor nuclear translocator (ARNT). Interestingly, the aryl hydrocarbon receptor (AhR), a receptor for a number of potent environmental chemical carcinogens including polycyclic aromatic hydrocarbons (PAHs), also dimerizes with ARNT leading to the induction of xenobiotic metabolizing enzymes including the cytochrome P450 family members, CYP1A1 and CYP1B1. These enzymes are critically involved in the metabolic activation/detoxification of carcinogenic PAHs. It is our hypothesis that HIF1a activation attenuates the AhR mediated protective gene expression, which may lead to increased genetic instability and malignant progression. Methods: The human lung carcinoma cell line A549 were dually stimulated with the hypoxic mimetic CoCl2, and varying concentrations of the prototypical carcinogenic PAH benzo[a]pyrene (B[a]P) for 18 hours. Both quantitative PCR (QPCR) and Western blot analysis were used to determine changes in expression at the gene and protein levels respectively. To further investigate the cellular consequences, we quantified both the amount of B[a]P induced DNA adduct formation using the 32P-postlabelling technique as well as the mutation frequency by HPRT-mutation analysis. Results and conclusions: QPCR and immunoblotting analysis indicated that the induction of the hypoxic response pathway significantly reduced the transcription of AhR downstream targets CYP1A1 and CYP1B1. We further demonstrated that although the levels of DNA adducts are only marginally increased, the observed mutation frequency is markedly amplified. These data indicate a novel mechanism by which hypoxia can induce genetic instability by altering AhR downstream processes.

EM097

PRENATAL EXPOSURE TO FLAVONOIDS: IMPLICATION FOR DEVELOPMENT OF LEUKEMIA

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Background: Flavonoids, the polyphenolic compounds present in plant foods are most commonly known for their antioxidant activity and are therefore freely available on the market as high dose dietary supplements. Recently we have demonstrated that in vitro exposure of human haematopoietic stem cells to 2 widely consumed dietary flavonoids, genistein (G) and quercetin (Q), can lead to DNA double-strand breaks and rearrangements in the mixed-lineage leukaemia (Mll) gene. Mll is involved in normal haematopoiesis and is frequently rearranged in childhood and adult leukaemia. Since the concentrations used in vitro can also be achieved in vivo, it is crucial to examine the safety of flavonoids in the foetus. Methods: To examine the safety of dietary exposure to flavonoids, heterozygous Atm-ΔSRI mice were placed on a normal diet (flavonoid-poor), G- or Q-enriched diet, from 3 days before conception until the end of the pregnancy. These mice carried a mutation in ataxia-telangiectasia-mutated (Atm) protein kinase, which mediates early cellular responses to double-strand breaks. After birth all mice were placed on a normal diet. At 12 weeks of age the pups were sacrificed, checked for tumour development and the blood was analysed using the ADVIA 1200 Haematology to look for leukaemia onset. In addition, mice bone marrow cells were incubated for 24 hours with G (50 □M), Q (50 □M) or etoposide (50 □M). The isolated DNA was subsequently used to set up an inverse PCR method to detect murine Mll rearrangements, which was then used for screening mice prenatal exposed to flavonoids. Results: Our results demonstrate that in vitro exposure of mice bone marrow cells to both flavonoids induces several Mll rearrangements as detected by our inverse PCR methodology. Prenatal exposure to G is also associated with an increased number of malignancies. Ten percent (4/41) of mice in this group suffered from different malignancies at 12 weeks of age in comparison with 2 percent (1/41) in the control group. Conclusions: Our preliminary results indicate that prenatal exposure to G can increase the risk of leukaemia. This has implications for the public health and therefore strict guidelines should be set for marketing flavonoid supplements to reduce the risk of leukaemia.

EM098

MATURATION OF EGYPTIAN SHEEP OOCYTES.

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Present study was carried out to illustrate the role of hormone supplements (PMSG+hcG+E2) in combinations with or without FBS to the culture media (TCM-199 or RPMI-1640) on the in vitro maturation (IVM) of sheep oocytes.The effects of culture media and oocyte quality were also investigated. Sheep ovaries were collected form local slaughterhouse. COCs and denuded oocytes were recovered by aspiration method. The collected oocytes were matured in culture media for 26-29h at 39□C under 5% CO2 in air and 95% humidity. The results indicted that the addition of hormones combined with FBS to the basic medium (TCM-199) significantly ($P<0.05$) improve the IVM of COCs as compared to the control group (41.25 vs. 3.50, respectively). Supplementing TCM – 199 with hormones alone (PMSG+HCG+E2) insignificantly increased the IVM of COCs compared to the control group (14.75 vs. 3.50, respectively). However, supplementing TCM – 199 medium with hormones alone or hormones combined with FBS did't improve the IVM of denuded oocytes. On the other hand, the addition of hormones combined with FBS to RPMI medium significantly ($P<0.05$) improved the IVM of COCs compared to the control group (7.22 vs. 4.69, respectively). However, the addition of hormones alone to RPMI medium did't improve the IVM of COCs. Supplementing RPMI medium with hormones alone significantly ($P<0.05$) improved the IVM of denuded oocytes compared to the control group (3.30 vs. 0.00, respectively). In contrast,the addition of hormones plus FBS did not improve the IVM of denuded oocytes. Concerning, the effect of type of culture media, the results showed that the proportion of oocytes (COCs or denuded oocytes) reaching MII significantly increased ($P<0.05$) in the group that were matured in TCM-199 medium than that matured in RPMI medium (19.84 vs. 3.98; or 2.50 vs. 1.07, respectively).Regarding the oocye quality,the present results revealed that, COCs recorded higher maturation rates ($P<0.05$) compared to denuded oocytes in either TCM-199 Supplemented groups (15.30 vs. 1.40, respectively) or in RPMI supplemented groups (5.92 vs. 0.53, respectively).

EM099

ENVIRONMENTAL DIAGNOSIS BASED ON GENOTOXIC BIOMARKERS IN FISH

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Aquatic environments contamination by industrial and urban wastes, resulting in the mortality or reduction of aquatic fauna, increased interest in studies directed to evaluation of polluted water genotoxicity. Genetic biomarkers such as micronuclei and comet assay have been used in different aquatic organisms to detect the effects of environmental contaminants, allowing genotoxic diagnosis of contaminated areas. Special attention has been given to fish as possible biological monitors due to its role in throphic chain. Several areas have been studied in our State through in situ genotoxic analysis of exposed native fish populations. The assays used were micronucleus and nuclear abnormalities in fish erythrocytes and comet assay using blood, gills and liver. The area of Sinos river receives urban, agricultural and industrial discharges with major influence of metals. Another area, Taquari river, receives the influence of soil contaminated with wood preservatives. Two sites at Sinos (SI 048 and SI 028, sites heavily influenced by anthropogenic activities), and three in Taquari river (Ta032- upstream, Ta010- in front of contaminated area and Ta006- downstream) were studied and compared to external reference areas, Quadros and Pinguela lagoons. The results obtained by the biomarkers used in fish from Sinos river identified the two sites located in the area mostly impacted in this river. No significant MN and NA induction were verified in fishes from Taquari sites. Index of damages (DI) measured by comet assay were higher in fish blood of from Ta006 and Ta010; in gills from Ta010 and Ta032, and in liver from TA032. Results for fish from different Taquari river places, showing different responses for the three tissues analyzed, suggest multiple contributions in the studied sites. Therefore, we can not consider results only as a consequence of the contaminated site, once they appear also upstream. Our data pointed out the importance of using different assays in water monitoring. This information is important for environmental agency's management of polluted areas, basing decisions and back grounding regulations.
Support. CNPq

EM100

ANALYSIS OF MICRONUCLEI AND COMET ASSAY IN V79 CELLS TO ASSES GENOTOXICITY OF SURFACE WATER.

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Cytogenetic damage evaluation is an important step in the tier of different approaches used in genetic toxicology. Micronucleus assay (MN) and Comet assay (CA) have been used as fast and sensitive indicators for structural DNA alterations. In vitro tests using mammals cells are effective to detect genotoxic agents. In the present study we used the Chinese hamster cell line V79 to evaluate the genotoxic potential of samples from Taquari river (Triunfo, RS, Brazil), in area influenced by soil disposal of wood preservatives and the sensitivity of these assays for surface water quality. Superficial and interstitial waters from three river sites were sampled, A- upstream, B- in front of contaminated area and C- two kilometers downstream. Water samples (D, E, F) from contaminated internal area, adjacent to the river were also analyzed. For MN, 5 x 104 cells were inoculated with 5ml of medium, and 200µl of sterilized raw water samples, incubated for 24 hours at 37ºC, 5% of CO2, in parallel to negative controls (distilled sterile water) and positive ones (Bleomycin 2µg/ml). 2000 cells/sample were analyzed. Cytotoxicity was determined by observing the effect on cloning efficiency. Alkaline comet assay (pH > 13), was performed applying electric current of 300 mA and 25 V (0.90 V/cm) for 15 min. The slides were stained with ethidium bromide. Images of 100 cells/culture were scored visually according to tail size into five classes ranging from undamaged (0), to maximally damaged (4). The damage index (DI) can range from 0 (undamaged, 100 cells x 0) to 400 (with maximum damage, 100 x 4). The damage frequency (D%) was also calculated. Citotoxicity was detected both in the river and in internal area being higher in C, D and F. All samples induced positive responses in CA, both for DI as D%. Summer samples of internal area sites (D, E) and summer and winter superficial samples of site B, presented highest DIs. Only sites B and D induced positive micronuclei response. These results showed the differential sensitivity of applied assays to detect genotoxicity of this pollutants and the importance of using several assays to environmental quality diagnoses allowing better effects characterization. Support. CNPq

EM101

GENOTOXIC EVALUATION OF SURFACE WATER INFLUENCED BY WOOD PRESERVATIVES THROUGH MICRONUCLEI AND COMET ASSAY IN HUMAN LYMPHOCYTES

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Human lymphocytes cultures are sensitive biological systems for genotoxicity testing of chemicals. Comet assay (CA), or single cell gel electrophoresis, is a rapid, simple, and sensitive technique for measuring DNA breakage in individual cells. Micronucleus test (MN) detects also DNA strand breakage besides chromosome loses (i.e. spindle problems). The MN test measures a small subset of unrepaired DNA strand breaks, whereas the alkaline Comet assay measures strand breaks and labile sites that may be removed subsequently by the DNA repair system. This study presents the results of micronuclei and comet assay performed in human lymphocytes cultures exposed to superficial and sediment interstitial waters from Taquari river, aiming to evaluate water quality. The study area in Taquari river is influenced by wood preservatives composed by metals (CCA) and pentachlorophenol, which were disposed in the soil. Three river sites were sampled: A– upstream, B- in front of the contaminated area and C- two kilometers downstream. Heparinized peripheral whole blood from three healthy donors was used for MN assay. Raw water samples (0.2 ml) sterilized using a Sartorius filter with a 0.22 micra pore membrane were added to the cultures and in parallel to negative (distilled water) and positive (2mg/ml Bleomycin) controls. 2000 binucleated cells obtained by the cytokinesis blocking method with cytochalasin-B were analyzed per donor/sample (three donors/sample). For CA, alkaline assay (pH > 13) was performed after blood exposition to water samples and applying to slides an electric current of 300 mA and 25 V (0.90 V/cm) for 15 min. No citotoxicity was observed. Positive responses were verified for MN induction both for surface and interstitial waters of site B and for site C interstitial water. CA analysis showed positive responses for damages index (DI) and damage frequency (D%) for all sites. Both assays showed sensitivity to detect genotoxic effects in the evaluated area. MN assay was more sensitive to chemicals of contaminated site while CA detected a broader pollutants spectrum as could be seen by the upstream site responses also detected by this assay. Support. CNPq

EM102

3,3'-DINTRITRO-BISPHENOL A SIGNIFICANTLY DISTURBS MATURATION AND SPINDLE FORMATION, AND CHROMATIN INTEGRITY IN MOUSE OOCYTES

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Introduction: Bisphenol a (BPA), an endocrine-disrupting chemical present in the environment and food, has been suspected to induce aneuploidy and adversely affect fertility in female mammals. Although we

could not detect increased aneuploidy in mouse oocytes to low BPA, we and others suggested that components in diet could synergistically affect response to BPA. Nitrit in BPA-contaminated food might cause formation of 3,3'-dintritro-bisphenol A (dn-BPA). We analysed meiotic maturation in mouse oocytes exposed to dn-BPA. Methods: Spindle formation and chromosome congression were analysed by anti-tubulin, anti-pericentrin and anti-Mad2 immunofluorescence, and chromosomal constitution by C-banding. Anaphase I progression was assessed by non-invasive polarisation microscopy. Numbers of γH2AX foci were determined in oocytes exposed to 1-10μM dn-BPA during 5h arrest in GV-stage by cilostamide, a specific PDE3-inhibitor. Results: 1-10μM dn-BPA significantly increased numbers of meiosis I-arrested oocytes ($p < 0.001$) and extended expression of Mad2 checkpoint protein at centromeres of homologous chromosomes. Prolongation of the spindle-assembly checkpoint (SAC) was suggested by delayed anaphase I progression in 5-10μM dn-BPA. 10μM dn-BPA caused significant increases in spindle aberrations and chromosome congression failure ($P < 0.001$) while there was no increase in hyperploidy in any of the dn-BPA-exposed meiosis II oocytes. However, in the highest concentration, dn-BPA parthenogenetically activated some oocytes. Numbers of γH2AX foci increased significantly in GV-arrested dn.BPA-exposed oocytes. Conclusions: Low concentrations of dn-BPA do not induce spindle aberrations or aneuploidy in maturing mouse oocytes. However, exposure to dn-BPA prior to resumption of maturation might be critical for causing DNA breaks with unknown consequences for chromosomal integrity and developmental potential. Disturbances in imprinting are currently investigated. Higher chronic dn-BPA concentrations at maturation interfere with spindle function and maturation kinetics in oocytes. Interactions between environmental chemicals in diet may synergistically affect mammalian oogenesis, fertility and health of offspring.

EM103

TELOMERE AND CENTROMERE CONTENTS OF MICRONUCLEI IN HUMAN LYMPHOCYTE OF WORKERS EXPOSED TO LOW LEVELS OF IONIZING RADIATION.

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The genotoxic effects of high doses of ionizing radiation (IR) in humans are well known, but the risk associated with exposure to low doses is still a matter of debate. Many studies have indicated the importance of cytogenetic biomarkers such as chromosomal aberrations (CAs) and micronuclei (MN) in radiobiology. In the latter type of assay, fluorescence *in situ* hybridization (FISH) with pan-centromeric DNA probes has been applied to distinguish MN harbouring chromosomal fragments (C^- MN) from those containing whole chromosomes (C^+ MN). In principle, FISH characterization of MN is expected to improve the sensitivity of the MN assay in detecting the clastogenic effect of IR. Conflicting results has been obtained regarding to increased frequency of C^- MN in populations exposed to low levels of IR. Thus, it is presently unclear if FISH characterization of MN can really help in detecting the genotoxic effects of low-dose exposure to IR. In the present study, in 21 radiology workers and 21 matched controls we applied centromeric (C^+) and telomeric (T^+) FISH to examine the contents of the MN induced, to specifically characterize MN containing chromosomal fragments which IR preferentially induce. MN with only telomere label (C^-T^+ MN), probably representing acentric terminal fragments, were higher in radiation-exposed individuals (2.15±0.97) than unexposed subjects (0.91±0.78; $P=0.011$). The proportion of chromosome-type fragments MN (C^- $2T^+$ MN of all C^-T^+ MN) seemed to increase in the exposed (39%) than the controls (26%), while chromatid-type fragments (C^-1T^+) were more prevalent in the controls (71%) than the exposed (59%), although these differences were not statistically significant ($P=0.068$, Fisher's exact test, two-tailed). When MN with whole chromosomes were defined as C^+T^+ MN, there were no statistically significant differences in their frequency between the exposed and controls. Our findings support the idea that exposure to low levels of IR can have detectable genotoxic effects and that centromeric and telomeric FISH, providing more specific MN characterization, could be a useful biomarker in biomonitoring of radiation exposure. The exposure to low levels of IR increase the MN frequency harboring terminal fragments and in particular those chromosome-type.

EM104

RECOMBINAGENIC ACTIVITY OF WATER AND SEDIMENT FROM SINOS RIVER AND ARAÇÁ AND GARÇAS STREAMS (CANOAS, BRAZIL), IN THE DROSOPHILA WING-SPOT TEST.

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This study characterizes the likely interaction of surface water and sediment samples with DNA to quantitatively and qualitatively establish their mutagenic, clastogenic, and/or recombinagenic activity. Samples were collected at 5 different sites within the area of Araçá Stream and 2 different sites within the Sinos River mouth and Garças Stream in the municipality of Canoas, RS, Brazil. The area is impacted by untreated urban discharges (sites 1-7), agricultural pesticides (sites 5 and 7), hospital waste (site 3), animal dejects (site 5), small industries (sites 4, 5 and 6) and vehicular discharges (sites 2, 4, 5 and 6). The somatic mutation and recombination test (SMART) is based on the loss of heterozygosity (LOH) induction, which may occur through various mechanisms, such as point mutation and certain types of chromosome mutations, as well as mitotic recombination. This versatile short-term in vivo assay simultaneously detects mutational and mitotic recombination events, and is able to quantify the recombinagenic activity of a compound in a genotoxicity screening. We applied the SMART in its standard version with normal bioactivation (ST) and in its variant with increased cytochrome P450-dependent biotransformation capacity (HB). Mutant spot frequencies found in treatments with unprocessed water and sediment samples from the test sites were compared with the frequencies observed in negative controls. In both ST and HB crosses all the water samples from the seven sites displayed a massive recombinagenic response, but no mutagenic activity was ascribed for any of the sites investigated. This is the first study with unprocessed water and sediment samples attributing a massive and exclusive recombinagenic action associated to the induction of homologous recombination — a genetic phenomenon involved in the loss of heterozygosity. The SMART wing test in *Drosophila melanogaster* was shown to be highly sensitive to detect genotoxic agents present in the aquatic environment, and should be better brought to use for monitoring areas under anthropogenic discharges.

EM105

EVALUATION OF BIOMARKER RESPONSES IN A RAT HEPATOMA CELL LINE (H4IIE) AFTER EXPOSURE TO EXTRACTS OF URBAN SOIL

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Urban soil represents a complex mixture of chemical pollutants of both organic and inorganic character. Gaining information about the toxic potential of city soils is important in order to assess the risk of organisms exposed to the soil, and to make use of the information in area planning and development of the city. Surface soils from the city of Trondheim has been analysed for chemicals including heavy metals, dioxins, polycyclic aromatic hydrocarbons (PAHs) and brominated flame retardants (BFRs). The purpose of the following work is to assess the cytochrome P450 1A (CYP1A)-inducing- and genotoxic potential of the soil samples in the H4IIE rat hepatoma cell line, and to use mul-

tivariate data analysis to examine possible links between responses and the contaminant patterns measured in the samples. Extracts of soil were made by ultrasonic treatment using dichlormethane (DCM) as a solvent. Cytotoxic effects of the soil extracts were evaluated using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. CYP1A induction was measured as ethoxyresorufin-o-deethylase (EROD) activity. DNA strand breaks and oxidative DNA damage was assessed using the Comet assay combined with formamidopyrimidine DNA glycosylase (FPG) enzyme treatment. The results will be presented and discussed.

EM106

HUMAN BIOMONITORING - OCCUPATIONAL AND ENVIRONMENTAL EXPOSURE TO PAH IN SILESIAN POPULATION

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Background: HBM is one of two methods for protection of human health in case of exposure to chemical substances, which is based on controlling the amounts taken up by human body. Now HBM of exposure to PAH is based on the measurement of some PAH metabolites in urine, which turned out to be reliable and specific e.g. 1-hydroxypyrene. Urine mutagenicity reflects the extent of recent exposure to mutagens and it has been used in biomonitoring of population exposed to complex mixtures. Aim: the aim of the present study was to demonstrate usefulness of simultaneous determination of 1-hydroxypyrene in urine (1-OHP) and urinary mutagenicity in the assessment of occupational and environmental human exposure to PAH. Methods: the occupationally exposed group included workers from two Silesian coke-plants. The environmentally exposed group was composed of men, women and children from the Upper Silesia. The control group included women from Bia□ystok and men, women and children living in Masovian Province. The determination of 1-OHP in urine was carried out using method developed by Jongeneelen et al. (1985). Mutagenic activity was assayed with the Salmonella/microsome test, using strains TA98 and YG1024. Results: results for 415 persons, including 144 men, 183 women and 88 children, were obtained. The highest concentration of 1-OHP and urinary mutagenicity (YG1024+S9) were observed among coking-plant workers. It was also noted that the concentrations of 1-OHP and mutagenicity in urine were significantly higher among people from the Silesian Region than in the control group. Both the urinary mutagenic effect and the concentration of 1-OHP were higher among smokers. There were no significant differences in the 1-OHP concentration among adults and children, however, the mutagenicity effect was much higher among children, both in the environmentally exposed group and the control one. Conclusions: the determination of 1-OHP concentration in urine allows only to assess exposure to non-carcinogenic pyrene, which occurs in different proportions in mixtures of PAH. Simultaneous determination of the mutagenic effect in the same urine sample, shows which part of the substances is metabolized to mutagenic derivatives and enables better assessment of the exposure.

EM107

GENOTOXIC EFFECT IN ROOT-TIPS OF RYEGRASS (LOLIUM MULTIFLORUM) GROWN IN SOIL EXPOSED TO ORGANIC WASTE AND HEAVY METALS

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Although the regular application of organic waste to Norwegian farm land is relatively small, its continual use over extended periods can result in accumulation of toxic compounds to levels detrimental to plants. The cytogenetic effects of 2 and 20 ton per hectare organic waste (sewage sludge, manure and compost) and different levels of Cd/Zn/Cu-mixture on root-tips of ryegrass (Lolium multiflorum) were investigated. Three concentrations of the heavy metals-mixture were used, 2, 20 and 200 times the maximum permitted concentration in sewage sludge in Norway. The treatments with organic waste and industrial sludges did not show any detectable genotoxic or toxic effects to roots of ryegrass compared with the control plants. There was no reduction of MI or an increased amount of chromosome aberrations. The middle concentration of heavy metal-mixture showed the highest detectable genotoxic effect (not significant), but of the highest concentration the roots could not be spread for cytogenetic analyses. Gross anatomical change in root-tip growth was observed at this high concentration. DNA dsbs analysis, by the method of agarose gel electrophoresis of high molecular weight DNA embedded in low melt agarose plugs, showed a significant dose response for the heavy metals mixture over all concentrations, and also a significant increase in genotoxicity with increased exposure time. The results indicate that the use of Norwegian organic waste in these levels are probably not genotoxic to ryegrass, but that heavy metals level above twice the permitted concentration in soils are, especially for chronic exposures.

EM108

ASSESEMENT OF MICROCYSTIN-LR GENOTOXICITY IN LIVER- AND KIDNEY-DERIVED CELL LINES

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The increasing use of surface freshwater reservoirs as a source of drinking water is raising the problem of chronic human exposure to cyanobacterial toxins. Microcystin-LR (MCLR) is a cyanotoxin that causes severe human and animal acute hepatotoxicity and, to a lower extent, intestine and kidney toxicity. Moreover, it is classified by IARC as a possible human carcinogen (class 2B), mostly based on its tumour promoter activity. However, the question whether genotoxic mechanisms additionally contribute to its carcinogenicity is still open to debate. The present work aimed at characterizing the genotoxic effects of MCLR using the alkaline version of the single cell gel electrophoresis (comet assay) and the micronucleus (MN) assay in a monkey kidney- and a human liver-derived cell line (Vero-E6 and HepG2 cells, respectively). The results revealed that, at sub-cytotoxic concentrations (5 and 20 μM), MCLR is able to induce a dose-related significant increase in the frequency of MN in both cell lines, although it does not increase the level of DNA strand breaks, detectable by the comet assay. These findings corroborate our previous micronuclei data from an extract of a MCLR-M. aeruginosa producer strain tested in Vero cells. Despite inducing micronuclei, MCLR is not able to induce sister chromatid exchanges in Vero cells and, according to other authors, it fails to produce chromosome aberrations in lymphocytes, suggesting that it may act by an aneugenic rather than by a clastogenic mechanism. Aneuploidy induction might result from MCLR interference with the mitotic spindle, as part of a more general effect on cell cytoskeleton, due to its recognized inhibitory activity of protein phosphatases PP1/PP2A. In addition, our studies have shown that low MCLR concentrations affect cell signalling and stimulate cell proliferation. We hypothesize that the observed genotoxic and mitogenic effects might cooperate for the MCLR carcinogenicity. Furthermore, considering that cyanobacteria blooms are often composed by several species/strains, the impact of long-term exposure to mixtures of microcystins variants and other cyanotoxins in human health should be further investigated. Work partially supported by CIGMH, Portuguese Foundation for Science and Technology

EM109

GENOTOXICITY STUDIES WITH DISINFECTION BYPRODUCTS (DBPs)

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Background/Aims. Drinking water contains compounds designed as disinfection byproducts (DBPs), generated by the interaction of chlorine (or other disinfecting chemicals) with organic matter, anthropogenic contaminants, and with bromide/iodide naturally present in most source waters. Several epidemiological studies indicate that there is a cancer risk associated to DBPSs exposure. Thus, it is necessary to clarify if DBPs act as genotoxic carcinogens and which type of genetic damage they produce. Different chemical groups of DBPs have been described such as halogenated acetaldehydes, halonitromethanes, hydroxyfuranones and nitrosamines, among others. For most of them little information exists on their genotoxic potentials. To fill this gap, two representatives of each group have been tested using the comet and the micronucleus assays in human cultured cells. Methods. The comet assay has been used to detect: primary genetic damage, oxidative damage induction (by using FPG and EndoIII enzymes) and repair kinetics of the induced damage. The micronucleus assay (Cyt-B method) was used to detect fixed damage as consequence of chromosome breaks and/or aneuploidy. The human lymphoblastoid TK6 cell line was used for both tests. In addition, human peripheral blood lymphocytes were also used for the micronucleus assay. Results. The obtained results indicated that, with the exception of nitrosamines, all the tested compounds were clearly genotoxic in the comet assay, inducing a high proportion of oxidative damage. All this damage was repaired after 24 hours, although the repair kinetic depends of the particular chemical. All brominated compounds were more genotoxic than the chlorinated counterparts. On the contrary, with the exception of one hydroxyfuranone compound (mucochloric acid), all tested compounds were unable to increase the frequency of micronucleus in binucleated cells. Conclusions.The results found are of interest when dealing with the potential risk associated with the consumption of water containing DBPs. Thus, although a high genotoxicity is observed in the comet assay for most of the DBPs, the general negative findings obtained in the micronucleus assay would minimize the potential mutagenic risk.

EM110

ANTIOXIDANT AND ANTIMUTAGENIC ACTIVITIES OF ETHANOLIC EXTRACTS OF Nectandra cissiflora (LAURACEAE)

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In this study, the antioxidant activity of ethanolic extracts (EE) obtained from the leaves or stem of *Nectandra cissiflora* Ness was evaluated *in vitro* using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) test. The DPPH test data demonstrated the antiradical activity of these plant extracts. The wing somatic mutation and recombination test (SMART) using *Drosophila melanogaster* was used to evaluate the genotoxicity and antigenotoxicity of EE from the leaves or stem of *N. cissiflora.* Third stage larvae obtained from a standard (ST) cross and high bioactivation (HB) cross were treated with: 1] a solution containing a final concentration of 0.625 mg mL^{-1} or 1.25 mg mL^{-1} or 2.50 mg mL^{-1} of EE from the leaves of *N. cissiflora* alone or combined with the genotoxic and antineoplastic agent doxorubicin (DXR) (0.125 mg mL^{-1}); and 2] a solution containing a final concentration of 0.625 mg mL^{-1} or 1.25 mg mL^{-1} or 2.50 mg mL^{-1} of EE from the stem of *N. cissiflora* alone or combined with DXR (0.125 mg.mL^{-1}). No statistically significant differences in spot frequencies were observed for the ST cross or HB cross in treatments with EE alone, indicating that under these experimental conditions the extracts have no mutagenic effects on spontaneous DNA lesions. When EE were combined with DXR, the results indicated a dose-related antigenotoxic effect. However, EE from leaves of *N. cissiflora* showed a more pronounced effect than that obtained with EE from the stem. These results suggest that these different protective effects may be attributed to different secondary metabolites found in each type of extract, which probably operate through different mechanisms of action. Further investigations are needed to identify, by HPLC analysis, the secondary metabolites involved in the protective effects of these extracts. Supported by Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES); and Universidade Federal de Uberlândia (UFU), Brazil.

EM111

MICRONUCLEI FREQUENCY MEASURED BY AUTOMATED IMAGE ANALYSIS AS A MARKER OF AIR POLLUTION EXPOSURE

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The analysis of chromosomal damage as a result of air pollution exposure is one of major aims of biomonitoring studies. The investigation in the Czech Republic is focused on the regions with high concentrations of carcinogenic polycyclic aromatic hydrocarbons (c-PAHs), like the capital city of Prague. In the present study, we compared the frequency of micronuclei (MN) per 1000 binucleated cells (BNC) measured by automated image analysis from two studies. The effect of c-PAHs on the frequency of MN was assessed in the group of 50 bus drivers, 50 administrative workers and 56 city policemen living and working in Prague. The average age of the participants was 50 years for bus drivers and administrative workers and 34 years for city policemen. The concentrations of air pollutants were obtained from stationary and personal monitoring. Both two studies were conducted during the season with high levels of air pollutants (bus drivers and administrative workers: November 2006; city policemen: February 2007); furthermore the group of city policemen was followed repeatedly in May 2007, when the concentration of c-PAHs was significantly lower. The average levels of c-PAHs and B[a]P from personal monitoring in city policemen in February vs. May 2007 were 6.15±4.22 ng/m^3 and 1.04±0.76 ng/m^3 vs. 2.17±3.66 ng/m^3 and 0.24±0.54 ng/m^3, respectively, and in bus drivers vs. administrative workers in November 2006 5.48±4.19 ng/m^3 and 1.04±0.76 ng/m^3 vs.3.99±1.77 ng/m^3 and 0.75±0.36 ng/m^3, respectively. The frequency of MN was determined by the automated image scoring (MetaSystems Metafer 4, version 3.2.1) of DAPI-stained slides. The results showed following differences in frequencies of MN/1000 BNC (mean±SD): 8.48±3.17 (bus drivers in November 2006) vs. 5.92±2.83 (administrative workers in November 2006), $p<0.001$, and 7.32±3.42 (city policemen in February 2007) vs. 4.67±2.92 (city policemen in May 2007), $p<0.001$. Our data show the impact of air pollution on the frequency of micronuclei in the monitored groups. We suggest this method that uses the automated image analysis as a highly sensitive for evaluating the effect of c-PAHs. Supported by the grant # SP/1b3/8/08.

EM112

GENOTOXIC EFFECTS OF CARBAMATE INSECTICIDES IN Vicia faba

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Environmental mutagens can be detected by higher plant assay systems. *Vicia faba* is one of the most used species to evaluate the genotoxic effects of pesticides. It is a sensitive method considered as an effective biological test system for studying the response to short treatments because it allows the standardization of methods for comparing reports among several laboratories in the world. Methomyl, propoxur and carbofuran are carbamate insecticides applied in agriculture and used for domestic purposes as well as for the control of vector insects.

A large part of the human population is occupationally exposed to these compounds, but contact with these chemical products or their derivatives occur through the food that they eat as well. The aim of this research was to evaluate the genotoxic effect of the three carbamate pesticides mentioned above, by studying the root tip meristems of *Vicia faba* through chromosomal aberrations in metaphase and anaphase cells and with the micronucleus test. The growing roots were treated for 4 hours using several concentrations of the carbamates, followed by 18 hours of recovery period. Thereafter, the roots were fixed in a methanol-acetic acid (3:1), hydrolyzed in HCL 5N at 60 ^{0}C and stained by the Feulgen technique; a squash was made under a cover glass in a drop of 45% acetic acid. The results showed that carbofuran, methomyl and propoxur produced significant differences in chromosomal aberrations (metaphase and anaphase cells); they also exhibited a concentration-effect response (except aberrations in metaphase cells with propoxur). The aberrations observed with the three insecticides were of chromatid type and they appeared delayed, indicating that an S-dependent effect was involved. Furthermore, these insecticides can be considered as promutagens activated by the plant metabolism, which in *Vicia* constitute the S10 fraction. Micronuclei were also significantly different from the control. The mitotic index decreased with the increase in concentration. As human beings are constantly exposed to environmental pollutants, the use of sensitive bioindicators of damage at the genotoxic and cytotoxic level is advisable.

EM113

EVALUATION OF GENETIC DAMAGE IN WORKERS EXPOSED TO PESTICIDES IN GUERRERO STATE, MEXICO, UTILIZING COMET ASSAY AND MICRONUCLEUS TEST IN EXFOLIATED BUCCAL CELLS

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Pesticides are toxic substances widely used throughout the world to control pests and organisms noxious to human beings because as they are indiscriminately used polluted soil, air and water, and constitute a health risk to exposed workers and to the population in general. Because of the relevance of these compounds in agriculture, the aim of this study was to evaluate the genotoxic effect produced by pesticides using the comet assay and the micronucleus test in exfoliated buccal cells of workers occupationally exposed in the area of Arcelia, Ajuchitan and Tlapehuala located in a region called Tierra Caliente, Guerrero in the Mexican Republic. The study was made in 111 agricultural workers. The consent of the donors was obtained and an extensive questionnaire was applied to gather essential data related to age, time of exposure, habits as smoking and alcohol consumption, drugs and diets, in addition to medications and recent illness, type of work and protective measures used. Their exposure ranged from 1 to 57 years, and ages extended from 13 to 83 years; the non-exposed group constituted 50 individuals whose ages went from 15 to 66 years. All the participants were males. Cells of the buccal ephitelium were sampled and the comet assay was used as biomarker to know the DNA damage; the average of the comet tail was screened in 100 cells of each individual and the micronuclei test was carried out in 3 000 epithelial cells for each participant; other nuclear anomalies as broken eggs, karyolysis, karyorrhexis and binucleate cells were also evaluated. The results revealed that in the exposed group of the three areas studied the frequency of cells with comet increased significantly in relation with the non-exposed group. The same behavior was observed in the tail migration of DNA. Micronuclei exhibited significant differences as well between the exposed and the non-exposed groups, and they showed nuclear anomalies associated with a cytotoxic or genotoxic effect. A positive correlation was noted between exposure time and genotoxic effect, whereas no significant effect on genetic damage was observed as a result of smoking and alcohol consumption. This study afforded valuable data for establishing the possible risk to human health associated with pesticide exposure.

EM114

NUCLEAR IMPACT GENERATED BY ELEMENTS DERIVED FROM BIOMASS BURNING IN TWO BRAZILIAN REGIONS.

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Atmospheric air quality is influenced by several factors and affects directly, the health of organisms. Among atmospherics pollutants, particulate matter from biomass burning represents the most important pollutant. In Brazil, several occupational activities are done employing biomass burning. So, the aim of this study was to analyze the mutagenicity of particulate matter obtained from biomass burning by micronucleus assay of *Tradescantia pallida*. Two areas were analyzed: one is located in a central region, MT, known as Brazilian Legal Amazon suffering influence of pollutants from jungle and sugarcane burning. The second area is located in the northeast, RN, and is influenced by pollutants emitted during the cashew nuts processing. The Tradescantia micronuclei assay (Trad-MCN) were done in two experimental system: i) *in situ*, by direct exposition of plants to agents presents on atmospheric of both areas and ii) *ex situ*, by the direct exposition of particulate matter extracted from Teflon filters exposed in Legal Amazon region, in a hydroponic system. The results were expressed in MCN per 100 tetrads analyzed. The *in situ* analysis of *Tradescantia pallida* in Legal Amazon region showed a MCN frequency of 8.9 ± 3.85 in contrast with 3.9 ± 1.77 from the reference area. For the area exposed to cashew nut burning the MCN frequency was 5.5 ± 1.5 in contrast with 1.2 ± 0.6 for the negative control area. For *ex situ* analysis, three concentration of particulate matter was tested (0.1 mg/l, 0.5 mg/l and 1mg/l) and have induced a dose-related increase in MCN frequencies. All these analysis showed an increase of MCN formation in the tested area or in the presence of particulate matter of approximately 5 fold. So the results indicated that the exposition to agents produced from biomass burning is able to increase the micronuclei formation in DNA of Tradescantia exposed.

EM115

ANTIGENOTOXIC EFFECT OF GRAPEFRUIT JUICE AGAINST BENZO[a]PYRENE AND ITS POSSIBLE RELATIONSHIP WITH CYTOCHROME P450 INHIBITION.

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Inhibitory properties of grapefruit juice (GFJ) on Cytochrome P450 3A (CYP3A) activity and its interaction with the pharmacokinetics of some drugs used in human therapy have been well documented. GFJ has also been shown to alter the metabolism of other drugs that are not substrates for CYP3A suggesting that additional CYP molecules may also be affected. Some of the GFJ components, bergamottin (BG), 6',7'-dihidroxybergamottin (DHB) and naringin (NG) have shown potent inhibitory activity on certain CYP isoforms. The aim of this study was to determine the inhibitory effect of GFJ, BG, DHB and NG on the activity of CYP 1A and 2B subfamilies. We examined: 1) in vitro inhibition of microsomal CYP1A1 ethoxyresorufin-O-deethylase (EROD) activity by GFJ; 2) in vitro inhibition of microsomal CYP1A1 EROD, CYP1A2 methoxyresorufin-O-deemethylase (MROD), CYP2B1 penthoxyresorufin-O-dealkylase (PROD) and CYP2B2 benzyloxyresorufin-O-dealkylase (BROD) activities by GFJ components and 3) GFJ antigenotoxic potential on the damage produced by bezo[a]pyrene (BP) using the micronucleus test in mice peripheral blood. Results demonstrated that GFJ inhibits in a concentration-dependent manner, the in vitro EROD activity of mice hepatic microsomes obtained from animals treated with BP. Maximal GFJ concentration used (5% v/v) inhibits 85% of the EROD activity. The ingestion of

GFJ before BP administration diminished by 29% the rate of BP-induced micronucleated polychromatic erythrocytes (MNCE) at 48 h and 57% at 72 h. Microsomal hepatic and intestinal EROD activity from mice treated with GFJ and BP showed a 20% and 44% reduction, respectively, of that found in BP treated animal tissues. Concerning the GFJ components, CYP1A1 activity was inhibited by BG (IC50 0.19 μM) and by DHB (IC50 3.15 μM). CYP2B2 was also inhibited by BG (IC50 4.5 μM) and DHB (IC50 48.2 μM) but not at the same extent than CYP1A1. BG inhibited CYP1A2 more than DHB (IC50 5.1 and 54.5 μM, respectively) and CYP2B1 was the less inhibited by both components (IC50 9.5 and 55 μM, respectively). NG did not show an inhibitory effect over the CYP isoforms evaluated, suggesting that BG could be one of the major GFJ active ingredients with potential antigenotoxic effect for its ability to suppress CYP1A1.

EM116

DNA FRAGMENTATION EVALUATION IN HUMAN SPERM CELLS EXPOSED TO ENVIRONMENTAL POLLUTANTS

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Environmental pollution of waters caused by the release of chemical substances, and in particular active agents, is becoming a threat for both man and the environment. The active pharmacological agents discharged into the superficial waters but also into waste waters can interact with the cell DNA and induce genetic damage such as breakage of the double strand of DNA or point mutations (insertions, deletions, chemical modifications of the bases, chromosome alterations). The studies carried out on the waste waters of both Italian and European treatment plants have shown that among the chemical compounds most commonly found in the waste waters of these treatment plants, and thus responsible for contamination, there are many active pharmacological agents. The aim of the present work was to evaluate the potential genotoxic effects of nine pharmacological agents, Atorvastatin, Sildenafil citrate, Gemfibrozil, Ibuprofen, Atenolol, Ofloxacin, Carbamazepine, Bezafibrate and Diclofenac present in the waste waters of some Italian treatment plants. Genotoxic effects were evaluated by using three tests, the Comet Assay, the Diffusion assay and the TUNEL test, and were evidenced as % of DNA damage as well as DNA fragmentation index (DFI) in human sperm cells. We previously performed a standard analysis of semen parameters according to WHO (2001) indications, then the cells were incubated at the mean concentrations found into the waste water of treatment plants for 15, 30 and 45 minutes for each pharmacological compounds. All drugs tested induce a statistically significant reduction in the integrity of sperm DNA and high DFI values, above the 27%, which is generally considered the threshold value. The concentrations of pharmaceuticals found in the wastewater are then able to induce damage to sperm DNA, that can be highlighted in a very early stage of cell suffering (damage to the nucleus), even before it is capable to induce other metabolic disorders. In fact DNA fragmentation is the first sign of cell suffering detectable in spermatozoa caused by different stressful conditions that lead them to apoptosis.

EM117

PROTECTIVE EFFECTS OF FOOD ANTIOXIDANTS ON ARSENIC TOXICITY

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A critical component of arsenic toxicity is intracellular oxidative stress, produced in part by activation of NADPH oxidase and the subsequent production of superoxide. Numerous studies have explored the effect of arsenic on oxidative stress response and the effect of, often high doses, of As on intracellular signalling pathways. Other studies have shown that food antioxidants can protect against arsenic toxicity both in vivo and in vitro. The aim of this study is to explore the mechanism by which low doses of inorganic arsenic and a variety of food antioxidants, together and separately, interact with intracellular signalling pathways. Neutral red toxicity assays using HaCaT and HeLa cells grown in DMEM show a clear additive protection against arsenic toxicity by antioxidants that interact with the Nrf2-Keap1 antioxidant stress pathway, such as caffeic acid phenethyl ester (CAPE), sulforaphane and N-acetylcysteine. In contrast, inhibitors of the p38 and MEK1/2 signalling pathways have no apparent effect on cellular viability at low doses of arsenic but cause an increase in toxicity at higher doses of arsenic (20 μM or above). This increased toxicity appears to be synergistic with high doses (> 10 μM) of sulforaphane. We are currently looking at whether these changes in cellular viability are associated with changes in the concentration or phosphorylation status of key signalling factors such as the Src family of proteins and Rac-1. Low doses of arsenic have been shown to trigger the p38 and ERK signal pathways, whereas higher doses induce toxicity by activating JNK and NF-κB-dependent apoptosis. The goal of this research is to shed further light on the initial events of arsenic-induced signalling using food antioxidants and defined enzyme inhibitors as probes.

EM118

DETERMINATION OF 3,6-DINITROBENZO[E]PYRENE IN SURFACE SOIL AND AIRBORNE PARTICLES, AND ITS POSSIBLE SOURCES, DIESEL PARTICLES AND INCINERATOR DUSTS

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We recently identified a novel mutagen, 3,6-dinitrobenzo[*e*]pyrene (DNBeP), as a major contaminant in surface soil in residential areas in Kyoto, Osaka, and Aichi prefectures, Japan. 3,6-DNBeP is highly mutagenic toward *S. typhimurium* and showed genotoxicity *in vitro* to mammalian cells, such as mutagenicity in *hprt* gene and induction of sister chromatid exchange and micronucleus. Furthermore, 3,6-DNBeP produced DNA damage in the cells of several organs in mice in the comet assay. The purpose of this study is to reveal the distribution of 3,6-DNBeP in the environment. We developed a highly sensitive analytical method of 3,6-DNBeP and analyzed 3,6-DNBeP in surface soil and airborne particles. Moreover, 3,6-DNBeP in diesel particles and incinerator dusts, which were thought to be the sources of 3,6-DNBeP, was analyzed. Surface soils and airborne particles were collected in three metropolitan areas, the Kinki, Chukyo and Kanto regions, Japan. Diesel particles collected from an engine used for general automobiles and SRM1975, which is extract of diesel particles collected from an engine used for industrial forklifts, were analyzed. The bottom ash and fly ash of Incinerator dusts were collected from five different incinerators. After purification with a silica gel column and 2 reversed phase columns on HPLC, 3,6-DNBeP was injected to a reducer column connected to a stationary phase continually with a fluorescence detector. 3,6-DNBeP was detected as 3,6-diaminobenzo[*e*]pyrene. 3,6-DNBeP was detected in all surface soils and airborne particles in the ranges of 8–5,004 pg/g of soil and 19–1,238 fg/m^3, respectively, and the contribution ratios of 3,6-DNBeP to the mutagenicities of surface soils were 5–75%. The strong correlation was observed between the mutagenicity of surface soil and the amount of 3,6-DNBeP in surface soil. These results suggest that 3,6-DNBeP is largely distributed in surface soil and airborne in Japan and is a major mutagen in surface soil. 3,6-DNBeP was detected in all diesel particles and incinerator dusts. The result indicated that the combustion in diesel engines and incinerators are potential sources of 3,6-DNBeP detected in ambient air and surface soil.

EM119

CHILD-ADULT DIFFERENCES IN EVALUATION OF *IN VIVO* GENOTOXICITY OF ACRYLAMIDE

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[Background/Aims] The recent discovery of acrylamide (AA), a potent carcinogen, in the frying or baking a variety of foods raises human health concerns, in particular, for children, because AA is relatively highly contained in snacks, cereals, and baby foods. AA is known to be metabolized by CYP2E1 to glycidamide (GA) which is responsible for AA-inducing genotoxicity and carcinogenicity. The activity of CYP2E1 varies during postnatal development implying that the genotoxic and carcinogenic risk of AA may be different between adults and children. [Methods] To elucidate *in vivo* genotoxicity of AA and its child-adult differences, we treated adult or young male rats (*gpt*-delta transgenic F344 rats 3w,11w or SD rats 3w,11w) with 0, 20, 40, 80 ppm or 0, 50, 100, 200 ppm of AA in drinking water for 28 days, and examined the genotoxicity in the blood, liver and testis. We also analyzed DNA adducts (*N7*-GA-Gua) derived from GA in the liver, testis, mammary gland and thyroid gland. [Results] We observed the dose-related increases of micronuclei in peripheral blood. In liver, alkaline Comet assays were positive in the middle and high doses, but the *gpt* mutations were not induced. On the other hand, testis, especially, in young rats, showed significant genotoxic response in the micronuclei, Comet and the *gpt* mutations. DNA adduct analysis revealed that *N7*-GA-Gau was significantly formed in testis and mammary gland in a dose-dependent manner. The adduct level in testis in the high dose was 8-folds higher in young rats than in adult rats. [Conclusions] The genotoxicity of AA in peripheral blood and liver were not severe in these experiments, and did not show significant difference between the adult and young rats, In contrast, AA caused significant genotoxicity in testis, especially, in young rats, and it corresponded to the adduct level. We may be more concerned about germinal mutagenicity and reproductive toxicity in children exposed with AA through ordinary foods.

EM120

FORMATION OF FORMALDEHYDE FROM UV-IRRADIATED MALTOL AND ETHYLMALTOL SOLUTION

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Maltol (3-hydroxy-2-methyl-4H-pyran-4-one) and its derivative ethylmaltol have caramel odor and are used as a flavor enhancer in breads and cakes. We previously reported that they became mutagenic to *Salmonella typhimurium* TA100 and TA97, when their solutions were irradiated with either UVA (a black light, 320-400 nm) or UVC (a germicidal lamp) for 2-10 min prior to the exposure of bacterial cells. The photo-activated (or decomposed) products seemed to be relatively stable so that about 80% mutagenic activity remained at 60 min after irradiation. UVA-irradiated maltol induced predominantly G:C->T:A transversions and G:C->A:T transitions. Addition of thiol compounds such as cysteine or glutathione to the UVA-irradiated maltol diminished the mutagenicity. Contribution of 8-OHdG to the photomutagenicity of maltol was found to be very small. DNA strand breakage measured by relaxation of supercoiled (Form I) plasmid DNA to open circular (Form II) DNA was observed by the addition of UVC-irradiated maltol solution. A crude fraction containing mutagenic photoproduct from UVC-irradiated maltol (or ethylmaltol) solution was isolated by HPLC with an ODS column. LC-TOF/MS analysis has not been successful. The crude mutagenic fraction was further analyzed existence of aldehyde compounds using 2,4-dinitrophenylhydrazine-impregnated silica cartridge and HPCL. A considerable amount of formaldehyde and small amount of acetaldehyde, and possible dialdehyde compound were detected. Attempts are now in progress to determine dialdehyde compounds. (Ref. M Watanabe-Akanuma, Y, Inaba, and T. Ohta, Mutagenicity of UV-irradiated maltol in *Salmonella typhimurium*. Mutagenesis, 22, 43-47, 2007)

EM121

CAENORHABDITIS ELEGANS AS A MODEL ANIMAL FOR ASSESMENT OF THE NEUROTOXICITY INDUCED BY MICROCYSTIN-LR

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Background: Among more than 80 variants of MCs isolated so far, microcystin-LR is the most toxic and commonly encountered one. Exposure to microcystin-LR mainly represents adverse effects of acute, subchronic and chronic to the liver and kidney. On the other hand, there are only a few studies indicated that brain is also one of the targets of MCs. Nematode Caenorhabditis elegans has been explored as a model to study the neurotoxicity of microcystin-LR. Methods: The chemotaxis and thermotaxis defects of Caenorhabditis elegans caused by MC-LR exposure were measured. The morphological changes in sensory neurons and expression patterns of genes controlling the chemotaxis to NaCl and thermotaxis behaviors were monitored. Results: The results showed that the fold of tissue-specific hsp16-2-gfp expression in the nervous systems were found to be greater than 1.0 in adult animals, and caused significantly defects of chemotaxis to NaCl and thermotaxis. Morever, the chemotaxis to NaCl and thermotaxis were more significantly reduced in microcystin-LR ($\geq$1 μg/L) exposed mutant animals of che-1(p674), odr-7(ky4), ttx-1(p767), and ttx-3(ks5) than those in exposed wild-type N2 animals at the same concentrations. In addition, the expression levels of che-1 and odr-7 were significantly decreased in animals when exposed to microcystin-LR with the concentration lower than 10 μg/L, whereas the expression levels of ttx-1 and ttx-3 could be lowered in animals even exposed to 1 μg/L of microcystin-LR. Conclusion: Microcystin-LR exposure can induce severe defects of chemotaxis to NaCl and thermotaxis. The neurotoxicity may be largely mediated by the damages on functions of ASE, AWA, AFD, and AIY neurons, which indicates that the nervous system is primarily affected by MC-LR in nematodes. (This work was sponsored by the Important National Science & Technology Specific Projects of China (2008ZX07101-011), the Jiangsu 333 Project Foundation (07056), and the Natural Science Foundation of Jiangsu Province (BK2008320)).

EM122

EVALUATION OF GENETIC DAMAGE IN TOBACCO FARMERS

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The damaging health and environmental impacts of tobacco begin long before cigarette consumption. From the moment the tobacco seed is planted to the time it is harvested and cured, a health of those who cultivate the crop is constantly exposed at the large amount of pesticides as well as at nicotine present in raw tobacco leaves. Southern Brazil is a major tobacco-leave producing area, employing over 950,000 workers. The present study aimed at assessing if prolonged exposure to this mixture leads to an increase in DNA damage and at mutagenicity in tobacco farmers. The study involved a total number of 161 individuals: 106 were farmers from Venâncio Aires (State of Rio Grande do Sul, south of Brazil) and 55 were controls. They were sampled during at the tobacco harvest in 2008. The DNA damage was evaluated by comet

assay in blood cells and the mutagenicity by micronuclei test in blood and exfoliated buccal cells. The analysis of comet assay values indicated a significant increase in damage index(DI) and damage frequency(DF) ($P<0.001$) for the exposed group as compared to the control group (Mann–Whitney *U*-test). Binucleated lymphocytes and exfoliated buccal cells showed micronucleus frequency ($P<0.001$) increase in relation at control. Difference between men and women was found in the DF ($P<0.05$). In conclusion, our results indicate that the tobacco farmers studied have experienced genotoxic and mutagenic exposure, which is manifested as an increase in repairable DNA damage detected by comet assay and an increase in micronucleated cells. This effect can be due to heavy and repeated use the pesticide as well as the nicotine absorption from contact with wet tobacco leaves. In tobacco cultivation, men, in the majority, are involved with pesticide application and leaves harvest and the women, with leaves harvest. Probably this explains the difference in DF males and females. Although the significance of increased genotoxic effects is difficult to predict for individual subjects, the positive findings suggest a genotoxic and mutagenic hazard at the group level. Therefore, more studies are needed to know and to prevent the possible occupational diseases in tobacco farmer's associates.

EM123
APPROACH TO TOXICITY EVALUATION OF C60 FULLERENE USING SVERAL IN VITRO METHODS

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C60 fullerene is an attractive nano-material in the various fields such as chemistry, electronic engineering, and pharmacy due to its unique chemical and physical properties. Therefore, the release of C60 into the environment by its expanded use has been suggested to be a potential risk with possible ecological implications. However, its potential toxicity is not completely understood. This study was aimed to add more knowledge concerned in C60 potential toxicity, particularly mutagenicity. We evaluated several toxicity of colloidal C60 in water, which is the most possible state in the environment. The arlhydrocarbon receptor (AhR) activity of colloidal C60 was evaluated using AhR yeast reporter gene assay and the cytotoxicity was evaluated by Hepa1c1c7, mouse hepatocarcinoma cell line using MTS assay. The mutageniciy was evaluated using two different bacterial methods, umu test and Rec assay, and we quantified several DNA adducts using LC/MS/MS analysis and 32P post labeling method by exposure of HepG2, the human hepatocarcinoma cell line, to colloidal C60. Though AhR yeast reporter gene assay did not detect AhR activity of colloidal C60, MTS assay showed that colloidal C60 inhibited cell growth of Hepa1c1c7. Umu test using S-9, the fraction of microsome in rat liver, in common with Rec assay indicated that colloidal C60 induced dose-dependent DNA damages which induce SOS response. 32P post labeling method indicated that exposure of HepG2 to colloidal C60 did not caused bulky DNA adducts like C60 core body-binding DNA adducts, but LC/MS/MS analysis indicated that colloidal C60 tended to cause oxidative DNA adducts such as 8-oxo-deoxyguanosine. These data indicate that one of the reasons that colloidal C60 inhibits cell growth may be due to its mutagenicity, possibly caused by reactive oxygen species (ROS) derived from colloidal C60.

EM124
FREQUENT INCORPORATION OF FORMALDEHYDE DERIVED N2-METHYL-2'-DEOXYGUANOSINE TRIPHOSPHATE INTO DNA DURING DNA SYNTHESIS CATALYZED BY BACTERIAL AND MAMMALIAN DNA POLYMERASE.

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Formaldehyde is present in many foods, automotive exhaust gases and cigarette smoke, and is also produced in living cells during the metabolism of serine, methionine and choline. Therefore, humans are endogenously and exogenously exposed to formaldehyde. Several studies in which formaldehyde was administered to rats by inhalation showed carcinogenicity, particularly induction of squamous-cell carcinomas of the nasal cavities. Evidence that formaldehyde causes cancer in experimental animals indicates that it may be a carcinogenic to humans. This chemical reacts with 2'-deoxyguanosine-triphosphate (dGTP) in the nucleotides pool of cells, resulting in the formation of N2-methyl-2'-deoxyguanosine triphosphate (N2-Me-dGTP). The concentration dependent formation of N2-Me-dG DNA adducts was observed in formaldehyde treated cultured human cells by using LC/MS/MS. In the living cell, damaged deoxynucleoside 5'-triphosphate plays an important role in mutagenesis. In this study, the utilization of N2-Me-dGTP during DNA synthesis was determined by steady-state kinetic studies using the Klenow fragment of E. coli DNA polymerase I (with or without the 3'→5' exonuclease activity; exo- KF, exo+ KF), and calf thymus DNA polymerase α(pol α). N2-Me-dGTP was incorporated opposite template dC at an extremely high frequency in reactions catalyzed by all the DNA polymerases tested. Small amount of N2-MedGTP was inserted opposite dT and dG when Klenow fragment was used. The insertion frequency (Fins) of N2-Me-dGTP opposite dC was slightly affected by the 3'→5' exonuclease activity of Klenow fragment. The overall translesion synthesis (Fins×Fext) past the dC:N2-Me-dGMP pair was 0.3 for exo- KF, 0.21 for exo+ KF, and 0.15 for pol α. The Fins of N2-Me-dGTP was several orders of magnitude higher than that of 7,8-dihydro-8-oxo-2'-deoxyguanosine triphosphate (8-oxo-dGTP) and 2-hydroxy-2'-deoxyadenosine triphosphate (2-OH-dATP). These results suggest that N2-Me-dGTP can be readily incorporated into DNA during DNA synthesis and may cause mutation when replicated.

EM125
CAENORHABDITIS ELEGANS AS A MODEL ANIMAL FOR ASSESEMENT OF THE NEUROTOXICITY INDUCED BY MICROCYSTIN-LR

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Background: Among more than 80 variants of MCs isolated so far, microcystin-LR is the most toxic and commonly encountered one. Exposure to microcystin-LR mainly represents adverse effects of acute, subchronic and chronic to the liver and kidney. On the other hand, there are only a few studies indicated that brain is also one of the targets of MCs. Nematode Caenorhabditis elegans has been explored as a model to study the neurotoxicity of microcystin-LR. Methods: The chemotaxis and thermotaxis defects of Caenorhabditis elegans caused by MC-LR exposure were measured. The morphological changes in sensory neurons and expression patterns of genes controlling the chemotaxis to NaCl and thermotaxis behaviors were monitored. Results: The results showed that the fold of tissue-specific hsp16-2-gfp expression in the nervous systems were found to be greater than 1.0 in adult animals, and caused significantly defects of chemotaxis to NaCl and thermotaxis. Morever, the chemotaxis to NaCl and thermotaxis were more significantly reduced in microcystin-LR ($\geq$1 μg/L) exposed mutant animals of che-1(p674), odr-7(ky4), ttx-1(p767), and ttx-3(ks5) than those in exposed wild-type N2 animals at the same concentrations. In addition, the expression levels of che-1 and odr-7 were significantly decreased in animals when exposed to microcystin-LR with the concentration lower than 10 μg/L, whereas the expression levels of ttx-1 and ttx-3 could be lowered in animals even exposed to 1 μg/L of microcystin-LR. Conclusion: Microcystin-LR exposure can induce severe defects of chemotaxis to NaCl and thermotaxis. The neurotoxicity may be largely mediated by the damages on functions of ASE, AWA, AFD, and AIY neurons, which indicates that the nervous system is primarily affected by MC-LR in nematodes. (This work was sponsored by the Important National Science & Technology Specific Projects of China (2008ZX07101-011), the Jiangsu 333 Project Foundation (07056), and the Natural Science Foundation of Jiangsu Province (BK2008320)).

EM126

MOLECULAR DOSIMETRY OF URINARY N7-(2-HYDROXYETHYL) GUANINE AMONG ETHYLENE OXIDE -EXPOSED WORKERS

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Ethylene oxide (EO), a direct alkylating agent and a carcinogen, can attack the nucleophilic sites of DNA bases to form a variety of DNA adducts. The most abundant adduct, N7-hydroxyethylguanine (N7-HEG), has been well studied in animals. Although not promutagenic, N7-HEG can be depurinated spontaneously or enzymatically from DNA backbone. The excised N7-HEG can be excreted through urine so that analysis of urinary N7-HEG can serve as an EO exposure and potential risk-associated biomarker.This is the first systematic study of N7-HEG in urine collected from 89 EO-exposed and 48 non-exposed hospital workers and 20 exposed and 10 non-exposed factory workers by using our new-developed on-line extraction isotope-dilution LC-MS/MS method. Analysis of data shows that exposed factory workers excreted significantly greater concentrations of N7-HEG than both the nonexposed factory workers and hospital workers. Further data analysis using multiple linear regression show that EO-exposed factory workers had a significantly greater post-shift urinary N7-HEG than their non-exposed coworkers and hospital workers. These results demonstrate that analysis of urinary N7-HEG can serve as an EO exposure biomarker for future molecular epidemiology studies to better understand the role of the EO-induced DNA adduct formation in the process of human carcinogenesis and certainly for routine surveillance of occupational EO exposure for the health impacts on workers.

EM127

SIMULTANEOUS ANALYSIS OF HEMOGLOBIN ADDUCTS OF ACRYLAMIDE AND GLYCIDAMIDE BY ISOTOPE DILUTION LC-ESI-MS/MS IN ACRYLAMIDE EXPOSED WORKERS

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Acrylamide (AA) is an industrial chemical widely used in the production of polyacrylamide and also an environmental chemical found in tobacco smoke and food. It is classified as a probable human carcinogen (Group 2A) by the IARC. Workers are at risk of AA exposures. AA undergoes metabolism to glycidamide (GA). Both AA and GA react with hemoglobin (Hb) to form adducts (AA-Hb and GA-Hb). Analysis of both adducts are considered as biomarkers for long-term AA exposures. The purpose of this study was to assess long-term AA exposures by analysis of AA-Hb and GA-Hb in AA-exposed workers. Forty-nine AA-exposed workers and 35 controls were recruited and provided a post-shift blood sample for this study. AA-Hb and GA-Hb were determined simultaneously using isotope-dilution liquid chromatography-electronspray ionization/tandem mass spectrometry (LC-ESI-MS/MS). The detection limits for AA-Hb and GA-Hb are 5.5 and 5.2 pmol/g globin, respectively. The results showed the detection rate was100% and 44.9% for AA-Hb and GA-Hb, respectively. The median (range) level of AA-Hb and GA-Hb was 193.6 (68.2-6540.6) (pmol/g globin) and 438.1 (29.5-1957.5) (pmol/g globin), respectively. There was a significantly positive correlation between the Hb adducts of AA and GA (spearman rank coefficient r=0.942, *p*<0.0001). Using multiple linger regression analysis, air concentrations were predictive of AA-Hb levels after adjusting for smoking status (*p*<0.0001). However, smoking status did not significantly predict AA-Hb concentrations (*p*=0.955). Air concentrations were marginally associated with GA-Hb levels after adjusting for smoking status (*p*=0.07). The ratio GA-Hb/AA-Hb, reflecting the rate of oxidative metabolism, was determined with a median of 0.27 and in the range of 0.13-0.45. Besides, the AA-Hb concentrations for the AA-exposed workers were significantly higher than those for the controls. These results suggest that AA-Hb can be utilized as a biomarker for long-term AA exposures, while GA-Hb can be used as a biomarker for the genotoxic impact.

EM128

QUANTITATIVE ANALYSIS OF N7 GUANINE ADDUCT DERIVED FROM SAFROLE 2',3'-OXIDE TREATED HepG2 CELL USING LIQUID CHROMATOGRAPHY ELECTROSPRAY TANDEM MASS SPECTROMETY

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Safrole is a wildly present nature product and has been reported as a risk factor of oral cnacer in Taiwan. Safrole 2',3'-oxide (SFO), an active metabolite of safrole can attack DNA bases to form DNA adducts which may cause mutation. Among the DNA adducts, N7-Guanine adduct is predominant, but highly liable to depurination and to formation of apurinic sites, which can lead to mutation and ultimately cancer development if not repaired prior to cell proliferation. The objective of this study was to develop a liquid chromatography electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS) method to quantitate this adduct. N7γ-SFO-Gua was synthesized and characterized by by UV absorbance, ^{1}H and ^{13}C NMR spectroscopic, and mass spectrometric features. HepG2 cells were treated with SFO for 24 hrs, and cell culture medium was collected. Solid phase extraction (SPE) was employed for cell culture medium to purify and concentrate DNA adduct. HPLC-ESI-MS/MS-MRM was used for quantitation by spiking a $[^{15}N_5]$-N7γ-SFO-Gua internal standard. The fragmentation of the $[M+H]^+$ ion corresponding to the protonated nucleobase under collision-induced dissociation was monitored. The methods developed in this work provided the means to study dose-response relationships of SFO-DNA adduct to validate that N7γ-SFO-Gua can serve as biomarker to study the association between areca quid chewer and oral cancer.

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EM129

EVALUATION OF THE ANTIMUTAGENIC EFFECT OF VITAMIN C AGAINST DNA DAMAGE AND CYTOTOXICITY INDUCED BY TRIMETHYLTIN IN MICE

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TMT is one of the organotin compounds which are widely used as polyvinyl chloride heat stabilizers and marine biocides. The objective

of this study is to investigate the utility of comet assay and chromosome aberrations analysis for detecting the possible antimutagenic activity of vitamin C to reduce the genotoxic effect of Trimethyltin (TMT). In this study, male Swiss mice were treated interapretoneally (i.p.) with three tested doses 0.25, 0.50 and 1.0mg TMT/kg b.wt. for 1, 2 and 3 days. Alkaline comet assay in nucleated bone-marrow cells and chromosome analysis in spermatocytes were performed 24h after the last treatment. The amount of DNA damage in cells was estimated from comet tail length as the extent of migration of the genetic material. A significant increase in comet tail length indicating DNA damage was observed at all concentrations compared with control ($p<0.05$). The mean comet tail length showed a concentration- related and time-dependent increase. Also, the percentage of chromosome aberrations in spermatocytes was statistically significant ($p<0.05$) and showed dose and time dependent manner. Concurrent administration of vitamin C (VC) orally at 20mg/kg b.wt. with the highest dose of TMT for 1, 2 and 3 days reduced DNA damage in somatic and germ cells to a significant extent. In conclusion, our results indicated that vitamin C ameliorated DNA damage and genotoxicity induced by trimethyltin in mice somatic and germ cells *in vivo*.

EM130
A NEW PARADIGM FOR MOLECULAR TOXICOLOGY: INHIBITION OF P53-MEDIATED DNA REPAIR BY HEAVY METAL NICKEL.

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The regulation of DNA repair by p53 tumor suppressor is an emerging important topic in the field of molecular toxicology and oncology, largely distinct and separable from more-studied cell cycle arrest and apoptosis response regulated by p53. One of a number of tumor suppressor protein p53-regulated genes, Gadd45a has been shown to delay carcinogenesis and decrease mutation frequency. Here, we report an emerging role for Gadd45a in BER using the pure alkylator methyl methanesulfonate (MMS) repaired entirely by BER. Gadd45a-null mouse embryo fibroblast (MEFs) and human colon cells exhibited slow BER after treatment with MMS. In addition, the localization of apurinic/apyrimidinic endonuclease 1/redox factor 1 (APE1/Ref1) and PCNA known as rate-limiting component of BER pathway within the nucleus were observed in gadd45a wild-type cells, not in gadd45-/- cells. Inasmuch as p53 has been shown to regulate BER as well as NER DNA repair pathways, our data suggest that p53-regulated gene Gadd45a contribute to the BER response by affecting the localization of APE1/Ref1 with PCNA on nucleus. Indeed, APE1/Ref1 and PCNA were interacted with Gadd45a indicating that the APE1/Ref1, PCNA and Gadd45a might play an important role as complex in the MMS-induced BER. Furthermore, we found that heavy metal nickel was able to suppress UV-induced NER with the disruption of gadd45a-PCNA interactions as well as to reduce functional activity of p53 tumor suppressor at the sublethal dose of nickel chloride. Our data suggest that the inhibition of p53-mediated DNA repair might be one of potent mechanisms on carcinogenicity of metal carcinogen nickel. In conclusion, p53-mediated DNA repair is an emerging field in molecular toxicology that would impact on the study on carcinogenesis and genotoxicity. (This research was partially supported by a grant of Eco-topia 21 project (2009-09001-0083-0) from Ministry of Environment).

EM131
ANTIMUTAGENIC POTENTIAL OF AQUEOUS AND ORGANIC EXTRACT OF CURCUMA AROMATICA

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Curcuma aromatica, a wild turmeric rhizome known as "Van Nang Kam", is used as folk medicine in Thailand for anti-inflammation as well as food additive. The aim of study is to investigate for antimutagenicity of aqueous and organic extracts of the rhizome. Fresh rhizomes were harvested, washed, oven dried at 50°C and pulverized into powder. Crude aqueous was prepared by refluxing with distilled water for 2 h and filtered. The organic extracts were soxhlet extracted with n-hexane and residual was subsequently extracted with chloroform and methanol, respectively; however, the ethanol extract was prepared by macerating rhizome powder in 80% methanol for 3 d and filtrate was rotary evaporated to dryness. All extracts were evaluated for their antimutagenicity by Ames' test. All organic extracts at their highest dose of 10 and 50 ug/plate in the absence and presence of metabolic activation showed no mutagenic activity toward *S. typhimurium* TA98 and TA100, respectively. The aqueous extract at the highest dose of 2 mg/plate showed no mutagenic activity. All crude extracts failed to exhibit antimutagenic activity against NDPA-induced mutation in the absence of metabolic activation. On the contrary, chloroforn extract showed a weak antimutagenic potential with 4NQO-induced mutation in TA98 and TA100, methanol extract exhibited a weak antimutagenic activity against 2-AF_2- and 4NQO-induced mutation in both TA98 and TA100 and a strong antimutagenic activity against 4NQO-induced mutation in TA100. Crude ethanol extract showed slightly weak inhibition when all mutagens were induced. Aqueous crude extract exhibited weak antimutagenicity and showed more antimutagenicity against AF_2- and 4NQO-induced mutation in TA100 than TA98. When metabolic activation was included, crude organic and aqueous extracts failed to establish antimutagenicity. In addition, Pre-treatment of aqueous and organic extracts prior to AF_2- and 4NQO-induced mutation exhibited more antimutagenicity in TA100 than TA98. The study revealed that pre-treatment of all extracts prior to occurred mutation was much more effective than post-treatment of extracts applied to existed mutation. NDPA = 4-nitro-o-phenylenediamine (NDPA); 4NQO = 4-nitroquinoline-1-oxide; AF_2 = 2-(2-furyl-3,5-nitro-2-furyl) acrylamide.

EM132
EXPERIMENTAL DESIGN AND ENVIRONMENTAL MONITORING BY THE COMET ASSAY

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Ecological indicators can provide an early warning signal of changes in the environment, or diagnose the cause of an environmental problem. Thus, they need to capture the complexities of the ecosystem, yet remaining simply enough to be easily and routinely monitored. They have to be sensitive, be anticipatory, and estimate effects of stressors on endpoint attributes to support decision making. The recent proliferation of studies performed by the comet assay, a very sensitive method for DNA damage detection, to describe the impact of genotoxins (single or in mixtures) on the environment didn't go with a severe effort to harmonize the protocols and to adopt stringent experimental designs, taking into account the nature of the biomarker by-itself and the environmental complexity. In our experience, different factors/variables can affect comet assay results. The schedule of sampling time is a crucial step to avoid false negative results since the "DNA damage" detected by the Comet assay is a balance between damaging activity and cellular response to the toxic/genotoxic insult (detoxification, repair, death). A lot of biotic (e.g. age, lifestages, sex, reproductive stage, inter-individual variability) and abiotic factors can result as confounders in in field studies due to the extreme assay sensitivity, giving rise to conflicting results. Stresses related to specimen transportation/ adaptation/ manipulation could be a source of great variability. The identification of convincing "reference" population greatly depends from pollutant's diffusion/containment and multiple exposure sources: only stringent comparisons could define the real significance of the results for both human and non-human populations subjected to airborne genotoxic stress. Higher plants could represent a stable sensor in an ecosystem and hence allow to follow the evolution of the genotoxic impact. The measurement of DNA damage in the nuclei of higher plant tissues is

still a debated area of study. Our studies stated that some plant species are sensitive bioindicators if the experimental approach takes in consideration them as a complex multicellular environment where the efficiency of different protection or repair mechanisms can be modulated by cellular homeostasis.

EM133

GENOTOXICITY OF AIR POLLUTION PARTICULATE-MATTER (PM2.5): DETERMINATION OF THE TUMOUR SUPPRESSOR GENE TP53 MUTATION PATTERN, USING THE FASAY

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Background/Aims: In the past decades, many studies highlighted the role of ambient airborne Particulate Matter (PM) as an important environmental factor for many different cardiopulmonary diseases and lung cancer. The consistency between the findings from epidemiological studies argues for a causal association, but it remains difficult to attribute acute health effects to concentration levels in the light of the current knowledge. The mechanisms underlying these adverse effects are not fully understood, and major questions still remain concerning specific size fraction, chemical composition and causative toxicological mechanisms leading to the observed health effects. Methods: To contribute to complete the knowledge of these mechanisms, a fine fraction of airborne aerosol ($PM_{2.5}$) was collected in Dunkerque, a French seaside city heavily industrialised. Since mutations in the tumour suppressor gene *TP53* are the most common genetic alterations involved in human cancer, especially in lung cancer, our objective was to establish the first mutational pattern induced by PM on the *TP53* gene in human type II-like alveolar epithelial A549 cells by using the Functional Analysis of Separated Alleles in Yeast (FASAY). Results and Conclusions: Sixteen mutations linked to PM exposure were found: 8 one- or two-base deletions, and 8 single nucleotide substitutions, leading to missense mutations. A>G and G>A transitions were the most prevalent (19% for both cases). Other mutations included A>T and G>T transversions (6% for both of them). Since G>A transitions are usually correlated to endogenous mechanisms implied in indirect mutagenic effects, such as oxidative stress, a specific study of the *TP53* mutational pattern induced by the inorganic fraction of collected PM was carried out. Such inorganic components are often considered as a prominent source of reactive oxygen species. A fraction of collected PM was also submitted to a thermal desorption to extract the organic fraction. The desorbed PM-induced *TP53* mutational pattern showed the predominance of G>A transitions (44%). The key role played by oxidative stress in the occurrence of indirect mutagenic effect was confirmed by formation of the well known oxidative injury 8-hydroxy-deoxyguanosine adduct in PM-exposed cells.

EM134

COMET ASSAY IN GILL ERYTHROCYTES (OREOCHOMIUS NILOTICUS) FOR EVALUATION OF GENOTOXICITY IN LAKE OF LUCRÉCIA, RIO GRANDE OF NORTH – BRAZIL

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The Lake of Lucrécia is one of the main superficial water reservoirs of supplying in volume of this region of the State of the Rio Grande do Norte, that possess two distinct rain stations (dry and humid). The fishes (*Oreochromius niloticus*) are sensible biomarker for genetic ecotoxicology studies in freshwater and Comet test (SCGE) is a method for rapid screening of chemical contaminant dangerous and presence of pollutants with genotoxicity potential in environmental aquatic. SCGE is currently the most widely employed method to detect DNA lesions in eco-genotoxicology. The impact of genotoxic chemicals on the integrity of cellular DNA is one of the first events in organisms exposed to contaminants (Frenzilli *et al*., 2009). Two point water samples (P1 and P2) were collected in Lake of Lucrécia to physic-chemicals, microbiologic (cyanobacterium), metals and radiation analysis. This trench belongs to one of the most important hydrograph basin of this region of Rio Grande do Norte Brazil with seasonal disparate in rain distribution. These superficial water samples established significant increased (p£0.05) of cyanobacterium density (cells/mL) and metal (mg/L) concentration than related from to the negative control (distilled water) by Dunnett's T test. Twenty five (N=25) fishes (*O. niloticus*) known popularly as tilapia do Nilo (exotic) were seasonality collected in study area between October of 2006 (dry season) to march of 2008 (rainy season) and ten (N=10) in negative control group maintained by 120 days in aquarium with distilled water. The results of Comet test demonstrated significance difference (p£0.05) and increased in frequencies breaks on strand of nuclear DNA (Kobayashi *et al*., 1995) of gills blood erythrocytes cells of the fish averaged in Lake of Lucrécia when related from to the negative control group (aquarium fishes) by Dunnett's T test. The obtained results showed that the water samples collected in reservoir represent toxic metals and cyanobacterium that can more elevate frequencies de DNA damage in exposed fishes. This data suggest the presence significant of anything genotoxins in these superficial water samples from Lake of Lucrécia and indicating poor water quality and risk health by human and ecosystems.

EM135

AMES II AND HIGH-THOUGHPUT COMET ASSAY FOR EFFICIENT SCREENING OF DRINKING WATER (SOURCES) FOR GENOTOXIC CONTAMINANTS

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To safeguard its good quality and safety, Dutch drinking water and it sources are continuously screened for the presence of contaminants. The present chemical analysis needs a complementary toxicity analysis to enable detection of unknown hazardous contaminants and to measure the total effect of the mixture. Because of the relatively low levels of contaminants in these waters, only low-dose toxic effects, such as genotoxicity, are of relevance. To enable detection of all types of genotoxicants, it is broadly advised to perform both a mutagenicity and a chromosome damage test, including a test with mammalian cells. We have investigated whether indeed such tests are complementary in the screening of drinking water sources for genotoxic compounds. We have therefore applied both the Ames II and the high-throughput comet assay to analyze a set of diverse, well-known genotoxic compounds as well as several samples of drinking water sources and wastewaters in the Netherlands. Both assays are relatively new versions of the classic tests, with the advantage of being more efficient to perform, needing less compound/water extract and enabling the analysis of fractionated water samples for the toxicity identification and evaluation (TIE). The water samples were also analyzed with a micronucleus assay to compare with the comet assay. The conditions in both assays were kept as similar as possible to enable fair comparison. We have found that the Ames II and high-throughput comet assay had different sensitivities for the various compounds tested, indicating they are indeed complementary. Additionally, the comet assay detected genotoxicity in some water samples, where the Ames II and micronucleus assay did not. This again shows that the Ames II and comet assay complement each other, and that the comet assay is more sensitive than the micronucleus. These results show that the Ames II and high-throughput comet assay togeth-

er are a suitable and efficient genotoxicity screening system for the quality guarding of (drinking) water.

EM136

MUTAGENIC EVALUATION OF THE PYROLYSIS LIQUIDS OBTAINED FROM DIFFERENT SEWAGE SLUDGE SAMPLES

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Sewage sludge is the waste produced in wastewater treatment plants. Various thermal treatments such as gasification and pyrolysis are currently being studied to valorize this waste. A biological testing protocol was employed to evaluate the mutagenic potential of the pyrolysis liquids extracted from two sewage sludge samples at 530ºC. The analytical procedures used included bioassay with *Salmonella typhimurium* for the detection of point mutation. The pyrolysis liquids of all two samples exhibited mutagenic responses in the bioassay. At a dose level of 25% per plate, the pyrolysis liquid of the Madrid sewage sludge sample induced 490 net revertants; while at the same dose level the pyrolysis liquid of the Valladolid sewage sludge sample induced 215 net revertants, in the Salmonella assay without metabolic activation. The results of this research indicate that the pyrolysis liquids tested had mutagenic activity. This activity is associated with different compounds formed during the pyrolysis process.

EM137

ULTRAFINE SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES INTERACT WITH FLUOROMETRIC AND COLORIMETRIC DYES

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The reactivity of nanoparticles is often an unknown entity. Consequently the possibility of direct interactions between nanoparticles and experimental assay components cannot be ignored, particularly when these tests are reporting on the nanoparticles' ability to induce cellular damage. Such interactions have the potential to result in false or misleading information. This study investigates such interactions between dextran coated ultrafine superparamagnetic iron oxide nanoparticles (DUSPION) and fluorometric and colorimetric dyes. In the present study the fluorometric dyes dichloroflorescein (DCF) and 3'-(p-Aminophenyl) fluorescein (APF) were used to quantify the oxidative stress response induced by DUSPION (Fe2O3 and Fe3O4). In a cell free system increasing concentrations of DUSPION (Fe3O4) (100ng/ml 1µg/ml, 10µg/ml, and 100µg/ml) induced a dose dependent decrease in DCF (2µM and 4µM) signal as compared to control levels. In contrast, the same concentrations of DUSPION (Fe2O3) induced a dose dependent increase in DCF signalling. When the fluorogenic probe APF was used as an alternative to DCF, both Fe2O3 and Fe3O4 DUSPION induced a dose dependent decrease in fluorescence. The present study also suggests that DUSPION may directly interact with colorimetric probes. For example, interactions between MTS and DUSPION (Fe2O3 and Fe3O4) have also been observed in a cell free system, typified by an increase in the colorimetric signal when exposed to 100µg/ml DUSPION as compared to control. This study emphasises where colorimetric or fluorometric dyes are to be relied on for experimental test systems, potential interactions with nanoparticles need to be considered and controlled for if the investigators wish to reliably use these assays in a quantitative manner, or alternative methods for the measurement of oxidative stress should be tested such as the 8-OHdG assay. Interestingly, the present study also draws attention to the importance of the oxidative state of iron oxide nanopartices in relation to their interactions with the fluorometric dyes as distinct differences were observed.

EM138

BIOTRANSFORMATION AN GENOTOXIC EFFECTS OF MONOMETHYLARSONOUS ACID [MMA(III)] IN METHYLATING AND NON-METHYLATING HUMAN CELLS

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According to WHO arsenic is currently considered the most harmful toxin in drinking-water worldwide. In contrast to the temporary administration of high doses of arsenic in cancer therapy, chronic exposure to only small amounts causes i.a. bladder cancer. Especially the trivalent arsenic species MMA(III) (monomethylarsonous acid) and DMA(III) (dimethylarsinous acid) exhibit cyto- and genotoxic properties. In the present study we analysed the biotransformation products of MMA(III) in methylating HepG2 cells (hepatocytes) and non-methylating UROtsa cells (urothelial cells) after one hour of exposure. Using an HPLC-ICP/MS technique we detected MMA(V) as an oxidation product of MMA(III), and, to a minor degree, DMA(V) as a methylation and oxidation product of MMA(III) in HepG2 cells. In contrast, only traces of MMA(V) but no DMA(V) were detected in UROtsa cells. The LDH-test used for measuring the cytotoxic activity of MMA(III) in both cell lines at a concentration of 10 µM showed no effects within the chosen exposure time. The genotoxicity of MMA(III), DMA(III) and inorganic As(III) in UROtsa cells was assessed by the Alkaline Comet Assay which detects single strand breaks as well as double strand breaks. All three trivalent arsenicals induced significant DNA-damage already after 30 min exposure in UROtsa cells. DMA(III) was the most genotoxic arsenic species followed by MMA(III) and inorganic As(III). In summary, MMA(III) is intracellularly biotransformed to a pentavalent arsenic compound in methylating and non-methylating cells within one hour. Cytotoxic effects were not detectable during this time period whereas genotoxicity was already observed after 30 min without any significant changes after 60 min of exposure. We conclude from our study that MMA(III) rapidly induces genotoxic effects before it is biotransformed to a less toxic pentavalent arsenic compound.

EM139

CYTOGENETIC BIOMONITORING OF A GROUP OF PETROLEUM REFINERY WORKERS

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Petroleum refinery workers are exposed to a wide range of potentially carcinogen compounds (benzene, aliphatic and polycyclic aromatics hydrocarbons and heavy metals), as assessed by IARC. In this study we evaluated the potential genotoxic effects induced by occupational exposure in a petroleum refinery, by using chromosomal aberrations and cytokinesis-block micronucleus assays on human peripheral blood lymphocytes. Out of 500 workers enrolled in the study, we selected 79 male exposed subjects (46 non-smokers, 33 smokers; mean age 38,6±10,7; mean working activity 13,4±11,7; mean benzene exposure 0,093±0,11 mg/m3). A group of 50 control subjects were enrolled from usual blood donors (34 non-smokers, 16 smokers; mean age 37,1±7) and adequately matched. Subjects were divided into sub-groups in respect to smoking habit to investigate the possible effects (synergistic/additive/antagonist) of smoke intake on chromosome damage. Despite recent improvements of the security measures adopted, the exposed group showed a significantly higher frequency of genetic damage (BNMN‰ 6,7±4,7; CA% 4±2,5) when compared to the control group (BNMN‰ 3,8±2,5; CA% 1,7±1,3) ($p<0.001$). These results are indicative of a potential genotoxic risk and corroborate the need to increase safety measures to avoid exposure. Surprisingly, the frequencies of chromosome damage were higher in exposed non-smokers than in exposed smokers (BNMN‰ 8,5±5 vs 4±2,6; $p<0.001$) (CA% 4,2±2,9 vs 3,7±1,9) suggesting a possible protective role of smoke.

EM140

DNA DAMAGE ASSESSMENT OF HUMAN POPULATIONS EXPOSED TO AIRBORNE POLLUTANTS FROM INDUSTRIAL AND URBAN SOURCES

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Urban populations nearby industrial area are exposed to increased adverse health effects, mainly air pollutants. Genetic biomarkers are useful tools for the assessment of exposure to occupational and environmental pollution. The aim of the present study was to investigate genotoxic effects on people residing and/or working downwind from an oil refinery (group 1) and an urban population living in area with limited traffic and industrial influence (group 2) in southern Brazil. Also, mutagenicity of airborne particulate matter from both studied areas was investigated: PM10 in group 1 city; and PTS in group 2 city. Organic matter from particulate airborne samples was extracted with dichloromethane and assessed for mutagenic activity in the Salmonella/microsome assay (TA98, YG1021 and YG1024 strains). Samples of peripheral blood and buccal mucosa cells from 37 individuals for each group were evaluated using the comet assay and the micronucleus (MN) assay, respectively. All PM10 organic extracts (group 1 city) showed positive mutagenic responses (reaching 11.7rev/µg) and the effect decreased in the presence of S9 mix (9.1rev/µg). The results for PTS (group 2) were lower than those observed for PM10 (3.3rev/µg highest value in S9 mix absence and 5.1rev/µg, in the presence). The strains identifying nitrocompounds indicated the prevalence of nitro and amino derivatives compounds, identified by strain YG1024, 40.4rev/µg being the highest value in group 1 city and 13.2rev/µg in group 2 city. Despite YG1024 strain being more sensitive, YG1021 strain also showed relevant responses mainly in city 2. The group in the area under influence of the oil refinery (group 1) showed significantly higher DNA damage in lymphocytes than individuals from group 2. The MN frequencies in buccal mucosa were very low for both groups and no difference between groups was observed. No association was found between age and tobacco smoking habit and level of DNA damages measured by the comet assay. Atmospheric environment from urban and industrial areas showed marked genotoxic activity, strongly influenced by the presence of nitroarenes and/or amine aromatics and the comet assay was a sensitive tool to detect DNA damage in subjects under the influence of an oil refinery. (CNPq/CAPES)

EM141

EXPRESSION OF DNA DAMAGE RESPONSE GENES IN THE A MONOGONONT ROTIFER *BRACHIONUS ROTUNDIFORMIS* EXPOSED TO UVB

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Esuarine monogonont *Bracgionus rotundiformis* is frequently exposed to UVB. UVB may induce detrimental effects such as lower survival, reproduction and development rate of rotifer. To evaluate the effect of UVB on *B. rotundiformis*, we conducted the acute toxicity test with extensive UVB dose range (0-20 or 0-36 kJ/m^2) in the condition of two UVB intensities (50 and 100 µW/cm^2) and also examined the expression pattern of DNA repair related genes. To investigate the gene expression pattern induced by UVB, we conducted real time RT-PCR with the rotifer which was irradiated to UVB (24.6 kJ/m^2 at 50 µW/cm^2 intensity). When the copepod was exposed to UVB with the intensity of 50 µW/cm^2, levels of LD_{50} were 24.6 kJ/m^2. Expression patterns of DNA repair related genes were shown significant modulation according to time course. Also we checked activated form of p38 kinase and Jun N-terminal kinase (JUNK) to see what kind of signal transduction pathway would be involved in DNA damage by UVB. Upon DNA damage by UVB, those kinases were up-regulated with up-regulated expression pattern of relevant genes. Therefore, *B. rotundiformis* can be considered as a promising estuarine species for ecotoxicity testing and risk assessment of UVB.

E142

GENOTOXIC EFFECTS OF DICLOFOP METHYL ON MAMMALIAN CELLS IN VIVO AND IN VITRO

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Diclofop-methyl (DM) is a chlorophenoxy derivative used in large quantities for the control of annual grasses in grain and vegetable crops. The aim of the present study was to investigate the ability of DM to induce chromosomal aberrations in human lymphocytes and mouse bone marrow cells and, of nuclear DNA damage in isolated human lymphocytes. 15.63, 31.25, 62.5 and 125 mg/kg concentrations of DM were used for in vivo assay and 15.63, 31.25, 62.5, 125 and 250 µg/ml concentrations were used for in vitro assay. In both test systems a negative and a positive control (MMC) were also conducted. In in vivo treatments, all of the DM doses significantly increased the total chromosome aberrations compared with both negative and solvent controls. In in vitro treatments, all the concentrations of DM (except the lowest concentration; 15.63 µg/ml) significantly increased the frequency of chromosome aberrations. In human lymphocytes, 250 µg/ml concentration at 48h treatment was toxic. Cell proliferation was significantly effected by DM applications in in vivo (except 125 mg/kg) compared to solvent control. In in vitro treatments, DM significantly decreased the mitotic index (MI) only at the highest concentration for 24h, however, at 48h treatment, DM significantly decreased the MI at 62.5 and 125 µg/ml concentrations and, was toxic at the highest concentration. A significant increase in tail intensity was observed at 62.5, 125 and 250 µg/ml concentrations compared to negative and solvent controls. The mean comet tail length significantly increased at all concentrations. Our results demonstrate that DM is genotoxic in mammalian cells in vivo and in vitro.

EM143

DNA DAMAGE ON Crocodylus moreletti FROM AN URBAN LAKE AT SOUTHERN GULF OF MEXICO

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Some crocodile populations are decreasing their number due to anthropogenic pressure to their natural habitats at southern Gulf of México. “Laguna de las Ilusiones”, is an urban lake and there is a continuous input of pollutants into their aquatic ecosystems, like solid wastes, municipal wastes waters, pluvial waters carrying combustion particles and oil deposited by auto motors. This situation exposes crocodiles to a variety of pollutants, some of them with risk to health and reproduction capabilities. In the present study we determined genotoxic effect on DNA from a wild population of *Crocodylus moreletii* from an urban lake. Simultaneously Lead was measured in blood cells and dermal scale samples from organisms from the same lake, in order to establish correlation. Analytical method was the Mexican-199-SSA1-2000 Standard method. Blood and dermal plates were digested in nitric acid. Samples, their respective blanks and standards were analyzed by triplicate with an A.A. spectrometer (Perkin Elmer 2380) with graphite fur-

nace. DNA strands brakes were evaluated with the Single cell electrophoresis assay (Comet Assay) on peripheral blood cells of wild individuals taken from the lake and compared to a control group from a breeding farm. DNA migration from the nucleus measured in µm ranged from 19.5 up to 49.88 and from 0 up to 35 in the wild and control groups respectively. DNA migration, reported as the Tail/head ratio, was 1.6 ± 0.10 (mean ± S.E.) for wild group, and 0.4 ± 0.16 for control group. Difference was statistically significant ($p<0.05$). Lead concentration in wild animals blood samples ranged from 6.55 up to 15.34 µg/dl with a mean of 12.01±1.6 µg/dl, and it was slightly higher than captive organisms (8.15 ± 1.7µg/dl), however this was not statistically significant ($p<0.05$). Lead concentration in dermal plates was higher than in blood samples, and ranged from 4.2 up to 55.29 ppm with a mean value of 22.62 ± 3.2 ppm for wild animals. DNA damage evaluated on wild organisms was medium to high, meanwhile it was medium to low at control group. Crocodylus DNA damage founded in wild organisms is probably due to pollutants, like lead derived from urban activities, posing them at risk to mutagenic or reproductive effects.

EM144

INDUCTION OF OXIDATIVE DNA DAMAGE IN RAW 264.7 CELLS BY CARBON NANOTUBES.

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The induction of DNA and chromosome damage following *in vitro* exposure to Carbon Nanotubes (CNTs) was assessed on the murine macrophage cell line RAW 264.7 by means of the micronucleus and the comet assays, the latter performed in its modified version, for the detection of oxidised purines and pyrimidines. The experiments were performed by using increasing mass concentrations (from 0.01 µg/ml to 100 g/ml) of two types of commercially available CNTs: single-walled (SWCNTs > 50%), and a MWCNTs preparation (> 90%). A statistically significant increase of binucleated micronucleated cells in function of the concentrations for both types of CNTs ($r= 0.84$, P=0.03 and $r=0.99$, P=0,0002 for SWCNTs and MWCNTs respectively) was found. The comet assay revealed increasing levels of DNA strand-breaks in a dose-dependent amount. The effects of the treatments with SWCNTs were detectable at all concentrations tested (1-100 µg/ml): oxidised purines increased significatively as compared to baseline values, whereas pyrimidines showed a significant increase ($P< 0.001$) at the highest mass concentration. In cells treated with MWCNTs an increase in DNA migration, due to oxidative damage to purines was observed at the concentration of 1 and 10 µg/ml, whereas again pyrimidines showed a significant increase only at the highest mass concentration used (100 µg/ml). These findings suggest that both the assays can be reliably used to detect small amount of damaged DNA at chromosome and nuclear levels in the murine RAW 264.7 cell line. The modified version of the comet assay allows to specifically detect the induction of oxidative damage to DNA, which is the underlying mechanism involved in the CNTs-associated genotoxicity. Supported by PRIN grant No. 2006069554 from the Italian Ministry of University and Scientific Research.

EM145

PHOTOGENOTOXICITY EVALUATION IN THE IN VITRO MICRONUCLEUS ASSAY USING HUMAN PERIPHERAL BLOOD LYMPHOCYTES

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Recent results demonstrating the occurrence of pseudophotoclastogenicity (clastogenicity induced by irradiation of chemicals that do not absorb visible or near UV light) have cast doubt on the specificity and ultimate utility of current test systems. Since studies largely have been done in transformed cell lines that are inherently genetically unstable, the use of primary cells should avoid this issue. Previous studies have demonstrated the utility of normal human keratinocytes for this purpose. In this study, we have adapted the *in vitro* micronucleus (MN) assay in human peripheral blood lymphocytes (HPBL) for the evaluation of photogenotoxicity. Venous blood was collected from normal human volunteers and isolated lymphocytes were obtained and stimulated to grow (at t = 0) in mass culture in phenol red-free media under standard conditions. Individual 5-mL cultures containing 2.5 x 10^6 lymphocytes were prepared in 60-mm dishes (at t = 48 hr) and treated with 8-methoxypsoralen (8-MOP), 10% (v/v) saline (vehicle control), or 0.4 mg/mL mitomycin C (MMC; positive control without photoactivation). Cultures were incubated in the dark for ~10 minutes, and half of the cultures then were exposed to a Xenon arc solar simulator lamp with a UV filter (290 nm cut off). All cultures were re-incubated until the end of treatment and then washed (at t = 51 hr) by centrifugation and transferred to media containing cytochalasin B. Cultures were harvested (at t = 72 hr) and slides were prepared and stained with Giemsa and May-Grunwald. For each culture, 200 total cells were scored for cytotoxicity (cytochalasin B blocked proliferation index, CBPI), and 400 binucleated cells were scored for MN frequency (%MN-BN). Initial results indicate 8-MOP induced statistically significant increases in %MN-BN, to approximately 5-fold control values in the presence of light exposure. No increases in %MN-BN frequency were observed for 8-MOP in the absence of light exposure. Thus, these results demonstrate the feasibility of detecting micronuclei induced by photogenotoxic compounds in normal HPBL. Validation with additional compounds is in progress.

EM146

AIR POLLUTION AND APOPTOSIS: THE EFFECT OF PARTICULATE MATTER DERIVED FROM DIESEL EXHAUSTS ON ALVEOLAR TYPE II CELLS IN CULTURE

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Air pollution is a serious health problem in large cities, being responsible for numerous respiratory infections, worsening allergic and cardiovascular diseases, besides being associated to lung cancer. Despite the efforts of Brazilian environmental agencies in establishing regulations for pollutant emissions by motor vehicles through PROCONVE (Program for Control of Air Pollution from Motor Vehicles), which contributed to lowering vehicular emissions, the increasing number of vehicles in cities as São Paulo is still responsible for high levels of air pollution. Among the air pollutants, particulate matter is of particular interest due to its major contribution to the health effects abovementioned. The objective of this study was to evaluate the effects of particulate matter (PM), derived from diesel exhausts from São Paulo buses, on apoptosis in immortalized human alveolar type II cells (A549). PM induced dose and time-dependent apoptosis, as measured by FACS, using quantification of sub-diploid nuclei (sub-G1 events) after cell cycle analysis of propidium iodide stained cells. A549 cells pre-treated with the ATM/ATR kinases inhibitor caffeine showed increased apoptosis levels in response to PM. These kinases are known to phosphorylate and activate p53 in response to some types of stress. However, cells treated with diesel-derived PM apparently did not phosphorylate or activate p53 at the times tested, as shown by Western Blot. UV irradiation of A549 cells was used as a positive control for p53 activation. We are currently studying other apoptotic pathways that may be involved on PM-induced apoptosis.

EM147

BOLDO LEAVES INFUSION, BOLDINE AND CATECHINE INCREASE ANTIOXIDANTE CAPACITY AND PREVENTS GENETIC DAMAGE INDUCED BY CISPLATIN IN MICE

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Peumus boldus Molina (boldo) a native shrub to Chile has long been part of the folk medicine tradition of the chilean people. The chemical characterization of boldo leaves infusion has revealed the existence of the alkaloid boldine and the flavonoid catechin as the principal plant metabolites with a significant antioxidant capacity (AC). This study evaluates the effect of a complete boldo leaf infusion on lipoperoxidation (MDA determination at 532 nm) induced by cisplatin (cis-DDP) in mice liver and its effect on the genetic damage (DNA migration) induced by this antitumoral drug in bone marrow cells measured by the comet assay (Tail Moment, TM). To determine if the observed effects on selected biomarkers (AC and TM) can be explained by the action of boldine or catechin, each compound was studied separately. Animals were separated in 8 groups (n=8) i) (control⁻ water), ii) cis-DDP 6mg/kg.bw., iii) cyclophosphamide 20 mg/kg.bw. (control +), iv) boldine 50 mg/kg.bw., v) catechine 50 mg/kg.bw., vi) pretreated with infusion and postreated with cis-DDP vii) pretreated with boldine and postreated with cis-DDP viii) pretreated with catechine and postreated with cis-DDP. The AC was evaluated in liver by the malondialdehyde (MDA) determination by thiobarbituric acid reaction. The genetic damage was evaluated in bone marrow cells by the comet assay. Experimental data on AC were statistically analyzed using U – t test of graph pad software. The values of TM were analyzed by ANOVA. P values ≤ 0.05 were considered as significant. Our results show that: 1) Cis DDP increased ($p<0.01$) lipoperoxidation as well as TM values in comparison to control, 2) Pretreatment with boldo leaves infusion significantly ($p<0.05$) disminished lipoperoxidation and DNA damage compared to the animals treated only with cis-DDP, 3) Pretreatment with boldine or catechin significantly ($p<0.05$) disminished both the lipoperoxidation as well as the genetic damage induced by cis-DDP. It can be concluded that boldo leaves infusion and its contituents boldine and catechine increase antioxidant capacity in mice preventing the oxidative damage on DNA. This work was supported by grant 055109 3R - DIUB – UBB – CHILE. Part of the results were published in Phytotherapy Research, DOI: 10.1002/ptr.2746, 2009

EM148

GENOTOXIC, CYTOTOXIC AND CARCINOGENIC EFFECTS OF 4-AMINOBIPHENYL IN LIVERS OF MALE AND FEMALE N-ACETYLTRANSFERASE NULL MICE

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Several aromatic amines such as 4-aminobiphenyl (ABP) are known or strongly suspected to be carcinogenic in humans and animal models. These chemicals require metabolic activation to DNA-damaging electrophiles for their carcinogenicity to be observed. It has been suggested that cytotoxicity caused by protein binding of bioactivated metabolites may also play a role in establishing a tumor-promoting chronic inflammatory environment. Among the pathways of aromatic amine biotansformation that may impact bioactivation, acetylation by the genetically variable arylamine *N*-acetyltransferases NAT1 and NAT2 has been linked to variation in aromatic amine-induced toxicity and cancer risk. Since NAT1 and NAT2 are potentially capable of either detoxifying aromatic amines by *N*-acetylation or activating their hydroxylamine metabolites by *O*-acetylation, it is unclear how these enzymes may either enhance or prevent the toxicity of particular agents. To more precisely define the role of acetylation reactions in modulation of aromatic amine toxicity, we created a strain of null mice, Nat1/2$^{-/-}$, which lack both mouse Nat1 and Nat2 and are devoid of any detectable acetylating activity. We tested the *in vivo* carcinogenicity of ABP in Nat1/2$^{-/-}$ mice using the neonatal assay. Compared to wild-type (C57BL/6) mice, male Nat1/2$^{-/-}$ mice showed a 50% reduction in the incidence and multiplicity of liver tumors observed one year after the administration of ABP at postnatal days 8 and 15. Both wild-type and Nat1/2$^{-/-}$ female mice were much less susceptible to ABP-induced liver tumors than their male counterparts. Since the *in vivo* clearance of ABP was unchanged in Nat1/2$^{-/-}$ mice and did not differ between genders, the protection may be due to deficient hydroxylamine *O*-acetylation. However, preliminary results suggest that ABP-DNA adduct levels do not differ between strains, and may be higher rather than lower in females. Although the protection of female mice may relate to gender differences in inflammatory responses to ABP-induced cytotoxicity, we also observed less acute liver damage from ABP than has been observed for other liver carcinogens such as diethylnitrosamine. Thus the mechanisms underlying the strain and gender differences in ABP-induced liver tumors remain to be established.

EM149

ECOLOGICAL DECREASE, BIOLOGICAL CONCENTRATION AND GENOME STRESS OF RADIONUCLIDES IN PLANTS AND ANIMALS AFTER CHERNOBYL CATASTROPHE

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South districts of Belarus are still highly radiocontaminated even after 23 years from the Chernobyl catastrophe in 1986, and consequent environmental changes are stored in the soil, plants and animals. The major radionuclides in the contaminated areas are ^{137}Cs and ^{90}Sr, and their physical half-lives are 30.2 and 28.9 years, respectively. It is easily predicted that the radionuclides are concentrated by the food chain into the living organisms in the contaminated area, and radionuclides remain in the irradiated organisms not only externally but also internally for long periods. The evidences of radiation effects on the organisms living in the contaminated area have been reported by many scientists. But most reports have not shown the exact radioactivity and period of exposure. The exact radioactivity in organisms should have been known to assess the long term low dose rate and low dose internal and external radiation effect. We measured the ^{137}Cs radioactivity and its distribution in the plants (trees, berries, mushrooms) and animals (insects, frogs, moles, mice) in the highly contaminated area (Masani) and the middle contaminated area (Babchin). The ^{137}Cs radioactivities in the organisms were proportional to those in the soil, and ^{137}Cs was highly concentrated in the vessel of plants and in the muscle of higher animals. The ^{137}Cs radioactivities of mice in 2005 were compared with those in 1997. The remaining amounts of ^{137}Cs in organs was about 2% of those in 1997. To simulate the radiocontamination in middle contaminated areas of Belarus (1997), mice were maintained for 8 months in the radioisotope facility with free access to drinking water containing ^{137}CsCl (10Bq/ml and 100Bq/ml). Mice were assessed the long term low dose rate and low dose internal and external radiation effect by the quantitative measurement of the contaminated radionuclides-induced DNA double-strand breaks by g-H2AX foci in the organs. The g-H2AX foci were observed even in mice drinking 10 Bq/ml of ^{137}Cs water. It was suggested that the genome damage caused internal low dose ^{137}Cs radiation were occurring chronically even in low dose contaminated area. (Supported by MEXT, JSPS and Heiwa-Nakajima Foundation).

EM150

ASSESSMENT OF THE ANTIOXIDANT, GENOTOXIC AND ANTIGENOTOXIC ACTIVITIES OF ETHANOLIC EXTRACTS OF *Aiouea trinervis* IN THE WING SPOT TEST OF *Drosophila melanogaster*

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Aiouea trinervis Meisn. is a shrub that grows in the "cerrados" (a savanna ecosystem) of Brazil. In the present study, fractionation of ethanolic extracts (EE) from the leaves of *A. trinervis* led to the isolation of the butanolide (g-lactone) namely isoobtusilactone A, as well as the lignans namely sesamin, methylpiperitol and polyprenol-12. Their structures were determined by spectroscopic analyses. The anti-oxidant effects of the EE obtained from leaves of *A. trinervis* were examined using the 1,1-diphenyl-2-picrylhydrasyl (DPPH) assay. The data obtained revealed antioxidant activity. The lack of information relating to the genotoxic and antigenotoxic properties of EE from the leaves of *A. trinervis* prompts us to study this EE alone and in association with the chemotherapeutic free-radical generator doxorubicin (DXR), used as reference mutagen. The genotoxic properties were evaluated for mutagenic and recombinagenic effects using the wing spot test of *Drosophila melanogaster* (Somatic Mutation And Recombination Test – SMART). The standard and the high bioactivation crosses were used. The latter cross is characterized by a high sensitivity to promutagens and procarcinogens. The results observed in both crosses were similar and indicated that EE from the leaves of *A. trinervis* did not show genotoxicity at the doses used and suppressed the DNA damage induced by DXR in a dose-response manner. The combined treatments demonstrated that EE have anti-mutagenic activity. The results indicate that, under these experimental conditions, EE from the leaves of *A. trinervis* protects against the genotoxic effects of the DXR, probably due to the *A. trinervis* anti-oxidant activity.

EM151

UNICELLULAR ORGANISMS IN THE COMET ASSAY

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As the comet assay is not yet standardized to be used with specific cells many scientists have tried to develop the assay to be used with different cells. When working with ecogenotoxicology it is a challenge to find species that are good representatives for the ecosystem and also have a good performance in the comet assay. In principle the comet assay can be used on all kinds of eukaryotic cells. As unicellular organisms are ecotoxicological relevant it was decided to use yeast cells, Saccharomyces cerevisiae, in this comet assay study. The first problem with the yeast cells was to open the cells and isolate the nuclei because yeast cells have a thick and strong cell wall. Different methods were used and the best results were obtained by using 0.4mg/ml Zymolase 100T incubated for 30 min at 35o C. However, the comets were very weak because the haploid cells of S. cerevisiae have very little DNA (13 Mbp) in their nuclei. We tried to solve this problem by using a tetraploid strain of S. cerevisiae. This resulted in much better comets than shown with the haploid strains. We found significant higher DNA damage for cells exposed to 20, 50 and 100 μM H2O2 and to 200 mg/l of acrylamide. However, we do not recommend using yeast cells in the comet assay because we think that even using tetraploid cells the amount of DNA (52 Mbp) is not high enough. On this background we tried to use the ciliate, Tetrahymena, in the comet assay knowing that this specie had a higher DNA content than yeast. With these cells we experienced other kinds of problems. The nuclei could not be isolated because the cells of Tetrahymena have a cytoskeleton which we were not able to remove with traditional lysis and enzymes. Therefore, it was not possible to carry out the comet assay. After having used a lot of time trying to open the cells of T. thermophila and T .pyriformis we can conclude that these cells are, just as the yeast cells, not suited for the comet assay. The work was supported by the Danish Society for Protection of Laboratory Animals and the Alternative Fund.

EM152

NONINVASIVE POLYORGAN KARYOLOGIC TEST FOR ESTIMATION OF CYTOGENETIC, CYTOTOXIC AND POTENTIAL CARCINOGENIC EFFECTS OF ENVIRONMENTAL FACTORS IN HUMAN STUDY

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Polyorgan karyologic test is founded on the microscopic analysis and quantitative account of karyologic end points in buccal, nasal, urothelial and bronchial epithelyocytes. It is designed categorization of end points on cytogenetic, proliferative and early and late nucleus destruction indexes. Integral cytogenetic index as amount of micronuclei, protrusion, nuclear bridges in 1000 cells of tissue is offered. Also integral index of proliferation (amount of bi-, polynucleated cells and double-nucleated cells) and apoptosis (amount of cells with nucleus destruction) is offered. Criteria of the determination and table diagnostic sign full spectrum of karyologic end points are designed. They are determined approximate normative levels of karyologic end points of buccal, nasal, bronchial and urothelial epithelyocytes of children and adults («Medical genetics» in Russian N° 11, 2007). It is revealed dependency of some indexes of proliferation and nucleus destruction from age, sex, smoking. Relationship the amount of congenital morphogenetic variants on one child with cytogenetic end point was revealed. It was shown dependency of karyologic indexes of buccal epithelium from local immunity. Polyorgan karyologic test was applied in Institute for estimation of the influence on the human populations (about 700 surveyed people) of the atmospheric air pollution in Tula; the working conditions of the large office centre; pollution of cellulose-paper plant; dioxin-contaminated regions of South Vietnam. Following laws have been revealed: air pollution induces significant (sometimes 10-times) increasing of the frequency of the exfoliated cells with cytogenetic damages and decreasing of apoptotic index. This trend is extremely disadvantage for human population, and promotes the accumulation genetic changed cells that can conduct to development of the cancer. It was revealed increasing of the rate of the cells with nuclear protrusions, atypical nucleus and in all studies integral cytogenetic indexes; indexes of proliferation; basically apoptotic index in patients with bronchial and lung pathology. It was shown possibility to correction of cytogenetic disturbances by means of acceptance vitamin A and C in recommended daily dose.

EM153

PERSISTENT DYSREGULATION OF DNA METHYLATION IN CELLS WITH ARSENIC-INDUCED GENOMIC INSTABILITY

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The mechanisms by which arsenic-induced genomic instability is initiated and maintained are poorly understood. In our previous studies, the long-term progression of chromosomal instability was typified by increasing aneuploidy in V79 Chinese hamster cells and human HaCaT keratinocytes treated with low doses arsenite for two cell cycles and maintained in arsenic-free medium up to 120 and 40 cell generations, respectively. In the current study, we evaluated DNA methylation levels in these cell cultures at several time points during the expanded growth. We have found altered genomic methylation patterns in cells that were briefly exposed to arsenic with evidence for widespread dysregulation of CpG methylation that persists for many population dou-

blings after the treatment. In V79 cells increasing genomic instability characterized by aneuploidy, dicentric chromosomes and/or telomeric associations, complex chromosome rearrangements, and mutator and transformed phenotype correlated with modifications of global DNA methylation pattern evaluated by immunofluorescence with anti-5-methylcytosine antibody and MeSAP-PCR. The results show that short-term exposure to arsenite induced an apparent genome hypomethylating effect within a short time after exposure. In human HaCaT keratinocytes, genomewide methylation levels were measured by LINE1 pyrosequencing and gene-specific methylation status was assessed by Methylation-Specific-PCR. Global demethylation seen after treatment was followed by a renewal of DNA methylation. Moreover, the results from MS-PCR and determination of expression levels by RT-PCR of several genes (p16, hMLH1, hMSH2, DNMT1, DNMT3a and DNMT3b) demonstrated that hMSH2 gene was epigenetically regulated and that down regulation of DNMT3a and DNMT3b genes occurred in an arsenite dose-dependent manner. The results reported here demonstrate that acute arsenic exposure promptly induces genomewide DNA hypomethylation and support the hypothesis that the cells undergo epigenetic reprogramming both at gene and genome level, in the absence of further arsenite treatment; these DNA methylation changes are likely contributing to long-lasting genomic instability that manifests as aberrant chromosomal effects and mutator and transformed phenotypes.

EM154

POTENTIAL GENOTOXIC EFFECT OF DIFFERENT SUPERFICIAL MORPHOLOGY OF AMORPHOUS SILICA EVALUATED IN MURINE ALVEOLAR MACROPHAGES (RAW 264.7) CELL LINES BY COMET ASSAY AND MICRONUCLEUS TEST

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Exposure to crystalline silica may cause lung fibrosis, carcinomas and autoimmune diseases. Instead amorphous silica micrometer-sized is accepted as having low toxicity and is used as a food additive. No sufficient evidence exists about the health hazards caused by amorphous (non-crystalline) silica of lower dimension, nor as regard its superficial conformity. The aim of the study is to investigate the potential genotoxic effects of amorphous silica nanosized particles (250-500 nm diameter) with different superficial morphology, by *in vitro* experimental models. Murine alveolar machrophages (Raw 264.7) cell lines have been used as representative of occupational and environmental exposure. Genotoxicity was evaluated by Comet Assay and Micronucleus Test. Cytotoxicity was tested using both Trypan Blue and Crystal Violet methods. Cell lines have been treated with 5-10-20-40-80 μg/cm^2 of MCM-41 mesoporous silica particles (250, 500 nm) and SiO_2 dense spheres (250, 500 nm). Powders were suspended in complete MEM solution, and sonicated for 30 minutes at 37°C (35 KHz) to prevent aggregation. Comet assay was used to evaluate genotoxicity at 4 and 24 hours exposure. The same doses of exposure were tested by micronucleus test. Hydrogen peroxide was used as positive control for DNA primary damage in Comet assay, Mytomicin C for micronucleated cells. Preliminary Comet assay results only seem to indicate a weak genotoxic effetct except for the highest dose (80 μg/cm^2) of MCM-41 mesoporous silica particles, where a statistically significant effect was found at the highest time exposure (24 hrs) and dimension (500 nm). For the dense sphere of the same dimension of MCM-41 a statistically significant increase of DNA damage was also observed at the lowest dose after 24 hrs exposure. A different trend was showed by the smaller size compunds which displayed a statistical significant increase of DNA migration at the lowest time exposure (4 hrs) and dose. Generally, dense silica spheres appear to be more genotoxic at lower doses (5-10 μg/cm^2). Smaller ones (250 nm) induce an increase of DNA damage at 4h, while the bigger (500 nm) at 24h. Further studies are needed to better investigate silica particles genotoxic effects even after long lasting exposures.

EM155

COMPARATIVE GENOTOXICITY OF AIRBORNE PARTICULATE MATTER (PM2,5) ISSUED FROM URBANO-INDUSTRIALIZED AREA ON DIFFERENT LUNG CELLS MODELS.

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Background Air pollution plays a role in lung cancer, which is a major cause of death worldwide. Particulate matter smaller than 2,5 μm (PM2,5) are of particular interest since they coated numerous toxic substances (metals, PAH, VOC, PCB, bacteria, fungi) and penetrate deeply in the respiratory tract. Then, these substances can be distributed and/or bioactivated to reactive species that could interact with lung DNA. We have studied the genotoxic potency of PM2,5 collected in Dunkerque, a French seaside city characterised by a lot of industrial activities associated with a heavy vehicle traffic. Both total particles (PM) and desorbed particles (dPM) were comparatively studied for their capabilities to form bulky-DNA adducts. Methods DNA adduct formation capabilities of PM2,5 were analysed by 32P-postlabeling method, after in vitro exposure of L132 cell line, of human alveolar macrophages (MA) or a co-culture (AM + L132). Cells were exposed 72 hours to PM and dPM at their respective LC50, and to BaP (1μM) as a positive control. Results After exposure to BaP, DNA adducts were observed on MA but not on L132, according to their respective EROD activities measured elsewhere. The association (MA+L132) in a co-culture model led to the formation of bulky adducts on both cell types, and validates this co-culture model. After exposure to PM, DNA adducts were similarly observed on MA and on L132, even if the level of total PAH carried on these particles is relatively low (BaP is for example 250 fold lower than in the positive control). More surprisingly, faint DNA adducts were also observed after exposure to dPM, indicating that outgassing method used to obtain dPM did not probably remove completely the PAHs. Conclusions We chose a protocol of direct exposure to particles, which is closer to environmental conditions but for which the quantities of bioavailable genotoxins are probably strongly reduced, compared with more classical studies using organic extracts. The formation of bulky DNA-adducts was nevertheless observed on this model of co-cultures (MA+L132), indicating that human macrophages can bioactivate genotoxins like PAHs, allowing the formation of DNA adducts on a second cellular type, even if this later is not competent from a metabolic point of view.

EM156

GENOTOXICITY OF AIR PARTICULATE ORGANIC EXTRACT IN HUMAN EPITHELIAL LUNG CELLS (A549)

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Particulate air pollution is an important environmental health risk and PM10 airborne particulate matter is associated with a range of health effects including lung cancer. In the present study, we have investigated the ability of chemically characterized organic-soluble extracts of PM10 from an high vehicular traffic site of the town of Montesilvano (Pescara, Italy) to induce genotoxic response by micronuclei production in the human epithelial lung cell line A549. We also evaluated the association between the chemical characteristics of the PM and its genotoxicity. A Hi-Vol air sampler was used to collect PM10 on glass fiber filters. PM mass was determined by gravimetric analysis of the filters. One section of each PM10 filter was processed in a Soxhlet apparatus with acetone to extract the organic-soluble compounds. A549 were exposed to different concentrations of PM10 extracts and the cytokinesis blocked micronucleus assay was performed to measure DNA damage. Extracts induced a significant concentration-related

increase in the micronuclei frequency. The viability of A549 was assessed by the Trypan Blue method and by measuring MTS dye absorbance of living cells. Cell death by apoptosis (PCD) was determined by a TUNEL test after A549 cells treatment. Organic extracts corresponding to PM concentration of 200, 100, 50 and 25 µg/ml induced micronuclei in a dose-dependent manner but not a significant apoptotic response. The intracellular reactive oxygen species (ROS) formation and the level of oxidative DNA damage were estimated by using 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate, acetyl ester (CM-H2DCFDA) as a fluorescent probe. Data indicate that the organic-soluble fraction of PM10 is both important in the production of micronuclei and ROS response by A549 cells which present interestingly capability of xenobiotics activation as type II lung epithelial cells (Hukkanen J. et al., 2000). Effects observed, point to the risk of PM organic extracts exposure in the urban high vehicular traffic areas and shows the need of integrative studies. This work was supported by Environmental Sciences Center, Consorzio Mario Negri Sud, S. Maria Imbaro (CH) and by RIA grants of University of L'Aquila to T. Pagliani and A. Poma.

EM157

BIOMONITORING OF GENOTOXIC RISK IN AGRICULTURAL WORKERS FROM FIVE COLOMBIAN REGIONS: ASSOCIATION TO OCCUPATIONAL EXPOSURE TO GLYPHOSATE

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In order to assess possible human effects associated with glyphosate formulations used in the Colombian aerial spray program for control of illicit crops, a cytogenetic biomonitoring study was carried out in subjects from five Colombian regions, characterized by different exposure to glyphosate and other pesticides. Women of reproductive age (137 persons 15-49 y) and their spouses (137 persons) were interviewed to obtain data on current health status, history, lifestyle, including past and current occupational exposure to pesticides and factors including those known to be associated with increased frequency of micronuclei (MN). In regions where glyphosate was being sprayed, blood samples were taken prior to spraying (indicative of baseline exposure), five days after spraying, and four months after spraying. Lymphocytes were cultured and cytokinesis-block micronucleus cytome assay was applied to evaluate chromosomal damage and cytotoxicity. Compared with Santa Marta, where organic coffee is grown without pesticides, the baseline frequency of binucleated cells with micronuclei (BNMN) was significantly greater in subjects from the other four regions. The highest frequency of BNMN was in Boyacá where no aerial eradication spraying of glyphosate was carried out and in Valle del Cauca where glyphosate was used for maturation of sugar cane. Region, gender, and older age (≥ 35 years) were the only variables associated with the frequency of BNMN measured before spraying. A significant increase in frequency of BNMN between first and second sampling was observed in Nariño (<0.001), Putumayo (p=0.009), and Valle (p<0.001) immediately (<5 days) after spraying. Four months after spraying, a statistically significant decrease in the mean frequency of BNMN compared with the second sampling (p<0.001) was observed in Nariño, but not Putumayo and Valle del Cauca. We conclude that the genotoxic risk potentially associated with exposure to glyphosate in the areas where the herbicide is applied for coca and poppy eradication is low and appears to be transient. Further studies are needed to better understand our findings of the potential genotoxic risk associated with the application of glyphosate for sugar cane maturation.

EM158

DO NANOPARTICLES HAVE AN EFFECT ON INTESTINAL CELL PERMEABILITY?

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Nanotoxicology is one of the fascinating research areas of the chemically engineered nanoparticles. A wide range of nanomaterial applications are already on the market and the use of nanomaterials is and will increase strongly also in the food science area. Nanomaterials may enter to the GI-track through various routes in which inhalation or via food are the major routes. Nanomaterials may facilitate the penetration of chemical compounds from intestine to the systemic circulation and hence promote the harmful effects of the contaminants or compounds present in food. Some nanoparticles themselves have been show to have mutagenic properties. We studied the effects of nanoparticles on cell permeability of Caco-2 cells in respect to MeIQx. Cells were grown on a membrane as a monolayer and the effects of nanomaterials on permabilility were studied by using the highly mutagenic MeIQx as an indicator of the possible change in permeability. Visual quality of cell layer was monitored using microscope and the chemical integrity was monitored by using mannitol as an indicator compound. Cells were exposed to silver nanoparticles with 50 and 19 micrograms/mL for 2.5 hours. MeIQx (40 µM) was added to the apical side of the cells. Samples (100µl) were taken at 15minutes min intervals from the basolateral side, and analysed with HPLC/TOF-MS for the presence of MeIQx and mannitol with 13C-mannitol as an internal standard. Microscopic observations demonstrated that cells exposed to silver nanoparticles were ruptured if compared to control cells. The analysis of mannitol and MeIQx with HPLC/TOF-MS indicated that MeIQx easily penetrated Caco-2 cells, but the preliminary analysis of the data did not demonstrate a major difference between the cells exposed to silver nanoparticles and MeIQx or MeIQx only.

EM159

SUCCESSFUL TRANSFER OF CYTOTOXICITY ASSESSMENT BY FLOW CYTOMETRY (FCM) CONFIRMS SUPERIOR REPRODUCIBILITY OF MITOTIC INDEX (MI) MEASUREMENTS

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Prevention of non-relevant positive results for the in vitro chromosome aberration assay depends, at least in part, upon the accuracy of cytotoxicity measurements. A FCM procedure for determining MI, developed and utilized routinely at Pfizer, has been adopted successfully by Covance. This method, using antibodies against phosphorylated H3 (S10) in the presence of nucleic acid cross-staining, has been evaluated at the two independent test sites and compared to manual scoring. Primary human lymphocytes were treated with cyclophosphamide, mitomycin C, benzo(a)pyrene and etoposide at concentrations inducing dose-dependent cytotoxicity. Deming's regression analysis indicates that the results generated via FCM were more consistent between sites than those generated manually. Further analysis using the Bland-Altman modification of the Tukey mean difference method supports this finding, as the standard deviations of differences in MI generated by FCM were less than half of those generated manually. Decreases in scoring variability due to the objective nature of FCM and the greater number of cells analyzed makes FCM a superior method for MI measurements. Additionally, the FCM platform has proven to be transferable and easily integrated into standard genetic toxicology laboratory operations.

EM160
MICRONUCLEI AND BULKY DNA ADDUCTS IN CORD BLOOD IN RELATION TO MATERNAL EXPOSURES TO TRAFFIC-RELATED AIR POLLUTION

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Exposure to traffic-related air pollution in urban environment is common and has been associated with adverse human health effects. In utero exposures that result in DNA damage may affect health later in life. This poster summarizes the results of a cross-sectional biomonitoring study with healthy pregnant women living in the Greater Copenhagen area, Denmark. The overall aim was to improve the complex assessment of environmental in utero exposures through measurements of validated biomarkers in comparable samples from healthy mother-newborn pairs together with folate and vitamin B12 measurements. Modeled residential traffic density, a proxy measure of traffic-related air pollution exposures, was verified by indoor levels of nitrogen dioxide and polycyclic aromatic hydrocarbons in 42 non-smoking homes. Bulky DNA adducts and micronuclei (MN) were measured in blood from 75 women and 69 umbilical cords, concurrently collected (<1 - 5 hours) at the time of planned section. To our knowledge this is the first study that investigated the associations between traffic-related air pollution exposures and bulky DNA adduct levels as well as MN frequencies in mother-newborn pairs.

EM161
MUTAGENICITY OF RIVER SEDIMENT UNDER INFLUENCE OF CONTAMINATED SOILS WITH WOODEN PRESERVATIVES

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The Ames test is an important tool to detect the mutagenicity in a wide variety of samples, with an important correlation in the carcinogenicity. A mutagenicity and cytotoxicity study were development at interstitial water sediment of the Taquari River. The study area is located in the Triunfo municipality, Rio Grande do Sul State, Brazil and understands a small farm with soil contamination specific for wooden preservatives, as pentachlorophenol, creosote and CCA hydrosalt (copper-chromium-arsenic). The place is covered by water bodies associated in direction to the main draining, forming sub – basins. Through the Salmonella microsuspension bioassay, the objective of study was to relate the genotoxicity activity with routes of pollutants dispersion to the main river and diagnosis the sediment quality of the Taquari River (km of the estuary), in front (010), upstream (032) and downstream (006) of the small farm contaminated. Two samplings were carried through in December of 2007, September or November of 2008. In the assays had been used interstitial water sediment fractions and diverse strains that allow to evaluate different DNA damages, like frameshift (TA98 and TA97a) and base pair substitution (TA100) in the absence (-S9) and presence (+S9) of metabolic activation. For comparison a place in the same river without influence of the cited pollutants was chosen, as control site (491) as well as the Lagoon of Pinguela (PI) in the Osório municipality. Indications of mutagenicity, like frameshift were found in the site 006 of December sampling (-S9), in the sites 491 (-S9 and +S9) and PI (-S9) of September and November sampling respectively. Base pair substitution were found in the site 032 (+S9) of September sampling, being that the site 010 of sampling 2007 (-S9) showed citotoxicity. Organic extracts of sediment samples are still being evaluated as for mutagenicity and citotoxicity; hepatic oxidative stress is evaluated in the Gymnogeophagus gymnogenys deriving by the study area. Support: FEPAM/CNPq (n°555187/2006-3).

EM162
HAZARD ASSESSMENT OF NANOMATERIALS: WHAT IS DIFFERENT?

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Development of nanomaterials with new functionalities is increasing and facilitates a wide range of innovative applications. Along with the design of nanomaterials, the safety of these materials must be addressed. In order to improve testing efficiency with a minimal need for animal testing, our research focus on relevant *in vitro* screening assays. To evaluate the difference between nano and micro-sized materials, Silica (SiO_2) and Cerium oxide (CeO_2), two commercially available and widely used materials were evaluated in four different assays. RAW264.7 macrophages were exposed to micro and nano-sized SiO_2 and CeO_2 for 4 and 24h. Dose levels ranged from 2.6-333 μg/cm^2.
(1) Cytotoxicity. Clear dose-related responses (MTT conversion and LDH leakage) were induced by both materials. However, SiO_2 was more cytotoxic compared to CeO_2 and nano-sized materials induced the highest responses: $IC50_{24h}$ (μg/cm^2) SiO_{2nano} 6, SiO_{2micro} 24, CeO_{2nano} 23, CeO_{2micro} > 333). LDH response was less sensitive compared to the MTT assay. Based on these results dose levels were selected to measure the other parameters.
(2) Inflammatory response. Micro-sized CeO_2 and SiO_2 did not induce TNFα release, while nano-sized SiO_2 (but not CeO_2) induced a response at both 4 and 24h.
(3) Oxidative stress. A clear dose-related induction of HO-1 was measured by micro-sized SiO_2. The response was higher after exposure to nano-sized SiO_2. Nano-sized CeO_2 induced only a response at the highest dose, while micro-sized CeO_2 did not induce HO-1.
(4) Genotoxicity. The Comet assay was used to evaluate the potential for inducing DNA strand breaks. SiO_2 did not induce DNA damage, whereas micro-sized CeO_2 increased the %DNA in the tail two-fold. Nano-sized CeO_2 increased the %DNA with a maximum of 7-fold compared to the untreated controls. The results obtained with SiO_2 were consistent with microarray analysis previously performed and indicated that nano-sized SiO_2 mainly induced genes involved in cell membrane integrity and oxidative stress (van Vugt 2009). In conclusion, different mechanisms of (geno)toxicity were observed between the two materials and size of the materials. CeO_2 appeared less toxic than SiO_2, but induced DNA damage, particularly in nano-sized form.

EM163
EX VIVO HUMAN SKIN MODEL FOR PHOTOGENOTOXICITY TESTING

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Photogenotoxicity testing aims at the early detection of the potential of compounds to induce tumors upon activation with UV (photocarcinogenicity). In contrast to standard genotoxicity testing, there are no equivalent *in vivo* photogenotoxicity assays for additional evaluation of a positive (or equivocal) result *in vitro,* or *in vivo* tests can not be used, as for personal care products, due to EU legislation. Moreover, oversensitivity and the occurrence of pseudo-effects with *in vitro* assays have become a major problem. Consequently, the number of false positives and unnecessary *in vivo* photocarcinogenicity studies will increase. For these reasons, a relevant *in vitro* assay for evaluation of a photogenotoxic potential in skin, was developed using *ex vivo* human skin. Advantages of the human skin model are:
best mimicking the human situation
target organ for photocarcinogenicity
relevant barrier properties of the stratum corneum
metabolic capacity
Human skin is obtained from surgery. Circular membranes are cultured in an air-liquid interface and exposed to test chemicals for 1 h, either via the medium or the topical site of the skin, followed by UV exposure (5 J.cm^{-2} UVA, 0.19 J.cm^{-2} UVB) and preparation of cell suspen-

sions for comet analysis. Predictivity of the photo-comet assay was evaluated using 6 compounds (fluoroquinolones) with varying degrees of photosafety liability. Sparfloxacin, lomefloxacin, and ciprofloxacin (known photocarcinogenic potential) clearly demonstrated a dose-related increase in %tail DNA in the presence of UV, while the non-photocarcinogenic compounds levofloxacin, gemifloxacin and gatifloxacin did not. The %tail DNA was not increased in the absence of UV. In parallel, the same compounds were evaluated in the *in vitro* photo-comet with L5178Y cells and an *in vivo* skin photo-micronucleus assay with rats, and compared with results of *ex vivo* human skin to develop a tiered testing strategy for photogenotoxicity assessment. The results with human and rat skin were comparable, while the *in vitro* photo-comet appeared to be more sensitive. Results thus far indicate that the human skin model can be a relevant alternative for photogenotoxicity evaluation of compounds that reach the skin, such as personal care products and pharmaceuticals.

EM164

LINKING NICKEL-INDUCED GENOTOXICITY WITH CELLULAR AND PHYSIOLOGICAL EFFECTS IN MARINE MUSSELS, MYTILUS EDULIS

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Nickel, a known human carcinogen, is regarded as a priority hazardous substance for the aquatic environment. There is however, insufficient scientific information available as to the potential detrimental impact of nickel on the aquatic biota, which could have short- and long-term implications for their survival. An integrated study was conducted to evaluate the biological responses to nickel contamination at different levels of biological organisation in the marine mussel, *Mytilus edulis.* Replicate samples of mussels (n=5) were exposed to a range of concentrations of dissolved nickel (18 to 180 µg l^{-1}) for 5 days. During the exposure period, mussels were not fed. The genotoxic effect was quantified in terms of DNA single-strand breaks using alkaline single-cell gel electrophoresis (Comet assay), the cellular toxicity was determined by neutral red retention (NRR) assay and the physiological effect at the whole organism level was determined using the feeding rate. The concentrations of dissolved nickel in the water and its tissue-specific accumulation were determined quantitatively using ICP-OES and ICP-MS, respectively. The study revealed a concentration dependent increase in the response for the induction of DNA strand breaks in the haemocytes. The cytotoxicity as measured by NRR assay did not show significant differences among the doses, however, the multiple range test showed a significant difference compared to control. The physiological effect as measured by feeding rate showed a statistically significant difference between higher doses with that of control. The correlation of the genetic damage with that of cellular toxicity and physiological effects revealed that the concentration range used affects the organism at genetic and cellular level. Over all, the impact of nickel warrants further studies in order to protect the health of marine biota and its potential impact on human health.

EM165

LINKING COMET ASSAY DATA AND MODELLED AIR QUALITY DATA: A CASE STUDY ON ADOLESCENTS PARTICIPATING IN THE FLEMISH ENVIRONMENT AND HEALTH SURVEY (FLEHS)

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Flanders, the northern part of Belgium, is a highly urbanized, densily populated region characterized by intense industrial and agricultural activities as well as a dense road network. A five-year Flemish Environment and Health Survey (FLEHS) (2002-2006) was carried out by the Flemish Centre of Expertise on Environment and Health to assess the concentrations and health impact of environmental pollutants. The campaign was commissioned, financed and steered by the Flemish Government. Air pollutant concentrations were calculated for 4x4 km grids using the AURORA air quality model. Emission data used by the model were derived from the EMEP- and MSC-E- databases for Europe and from recent emission inventories for Flanders. The youngsters' residence and school was allocated to the grids. The concentrations of the air pollutants were averaged over the 2 days preceding blood collection. Blood was taken by venipuncture from 390 non-smoking youngsters. The alkaline comet assay was assessed on fresh whole blood within 24h after blood collection. The relationship between modelled air quality parameters and endogenous DNA damage measured with the comet assay, was examined. The multiple regression models were run for each of the pollutants seperately, since the air pollutant concentrations were inter-correlated (single-pollutant models). The comet assay results were influenced by sex (boys higher damage than girls) en educational level of the parents (less damage if higher educational level). Ambient NO2 levels were not associated with the measured DNA damage. However, DNA migration during the comet assay, was positively associated with benzene ($r2 = 0.06$, $p<0.001$), benzo(a)pyrene ($r2 = 0.09$, $p<0.001$), PM10_prim ($r2 = 0.04$, $p<0.001$), and PM2.5_prim ($r2 = 0.06$, $p<0.001$) levels. This means DNA damage reflected the exposure to several environmental pollutants, of which particulate matter was in the range of EU guidelines. It was concluded that the comet assay was a good biomarker to reflect a combined pollution pressure, Flemish youngsters were exposed to in their daily life.

EM166

EVALUATION OF CYTOTOXICITY AND GENOTOXICITY OF SOME ORGANIC AIR POLLUTANTS COLLECTED FROM URBAN AND RURAL SITES IN THE PHILIPPINES

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Air pollution continues to be a health hazard to human health. Using PUF passive air samplers we collected samples from three rural and three urban sites in the Philippines for six weeks with two sampling periods (dry season as sampling period 1 and wet season as sampling period 2). Gas chromatography/mass spectrometry analysis showed the presence of persistent organic air compounds at varying concentrations. Low molecular weight polyaromatic hydrocarbons (LMW PAHs) were found to have the highest concentration in all sites. Also detected were high molecular weight polyaromatic hydrocarbons (HMW PAHs), organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs). In this study, the cytotoxicity and genotoxicity of these organic air pollutants were evaluated using MTT assay and *Allium cepa* test. Cell cultures of MCF10a normal breast and MCF7 breast carcinoma cell lines were exposed to air samples from the six sites, five chemical standards (LMW PAHs, HMW PAHs, Functionated PAHs, OCPs and PCBs) in 10 nM, 100 nM and 1000 nM concentrations with 0.2% DMSO and method blanks as controls. Results showed that organic air pollutants, whether from rural or urban area, showed cytotoxicity to MCF10a normal cells in sampling period 1 and proliferative effects in sampling period 2. The effect on MCF7 breast carcinoma cell line showed cytotoxicity for both sampling periods. Cytotoxicity may be attributed to the LMW and HMW PAHs while increase in cell number may be due to the OCPs and PCBs. For cells exposed to the five chemical standards, LMW and HMW PAHs had cytotoxic effects to both cell lines at all concentrations. The OCP standard was cytotoxic to both cell lines at 10 nM but had proliferative effect at higher concentrations. For the PCB standard it was cytotoxic to normal cells but proliferative to cancerous cells at higher concentrations. Cytotoxicity of the air samples and chemical standards was also

manifested in the *Allium cepa* cells as shown by the lower mitotic indices than the control. Chromosomal aberrations were also observed indicating genotoxic potential of the different treatments.

EM167

TRANSCRIPTOMIC PROFILES INDICATIVE FOR EXPOSURE TO GENOTOXIC AND NON-GENOTOXIC CARCINOGENS; IN VITRO STUDIES IN PERIPHERAL BLOOD MONONUCLEAR CELLS

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Background/aim: During past decades, there has been an increase in the incidence of different types of cancer, especially among children. There may be an association between the onsets of these diseases and exposure to environmental or dietary carcinogenic chemicals. Gene expression profiling has recently been shown to be a promising tool to study such associations at the whole transcriptome level. The objective of the current research was to investigate whole genome transcriptomics in human peripheral blood mononucleated cells (PBMC) in response to exposure to a range of environmental and/or dietary carcinogenic compounds in vitro. Furthermore, it was aimed to retrieve gene expression profiles that are indicative for exposure to genotoxic and non-genotoxic carcinogens. Methods: Per chemical, PBMC of five independent, healthy donors were exposed for 20h to the highest non-cytotoxic concentration, a ten-fold dilution and a hundred-fold dilution, in the presence of a S9 metabolic system to best mimic the in vivo situation. Using Agilent oligonucleotide microarrays, whole genome transcriptomic profiles were generated. Data were analyzed by different approaches. This lead to a set of transcripts indicative for exposure to genotoxic and a separate set for non-genotoxic carcinogens. Pathway analysis by means of a Fisher Exact test was performed to investigate overrepresentation of biological pathways in the lists of genes, and assess biological relevance of the retrieved profiles. Results/Conclusion: The analysis delivered transcriptomic a profile indicative of genotoxic exposure and a profile indicative of non-genotoxic carcinogic exposure. Whether similar responses can be witnessed in the human population, must and will be investigated in in vivo studies.

EM168

DEAD ZONES, HYPOXIA AND FISH HEALTH: AN INTEGRATED STUDY TO DETERMINE IMPACT OF OXIDATIVE STRESS IN FISH

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Anthropogenic activities (human foot print) have led to exponential increase in oxygen-starved locations around the world, the so called 'Dead Zones' with potential serious consequences for ecosystem functioning. While hyperoxia is known to have detrimental biological impact, there has not been any study where the responses of these two conditions have been compared simultaneously at different levels of biological organisation in natural biota. An integrated approach was adopted in which mirror carp, (*Cyprinus carpio* L.) were exposed chronically (30 days) to hypoxic (1.8 ± 1.15 mg O_2/l) and hyperoxic (12.3 ± 1.8 mg O_2/l) conditions and the resultant biological responses were compared with fish held under normoxic conditions (7.1 ± 1.04 mg O_2/l). The biochemical, physiological responses included the activities of glutathione peroxidase (GPx) (in the plasma and liver samples), measurement of oxidative DNA damage (using modified Comet assay using bacterial enzymes: FPG and Endo III). The study suggested that while the levels of GPx were unaffected in the liver samples, there was a significant difference in activity in the blood plasma under different exposure conditions. Oxidative DNA damage was significantly higher in both hypoxic and hyperoxic conditions compared to normoxia. FPG treatment showed enhanced level of damage compared to the Endo III treatment, suggesting that purine bases to be more susceptible. Haematocrit %, RBC counts and haemoglobin level were significantly different between hypoxic and hyperoxic conditions. Transmission electron microscopic studies also showed damage to liver and gill tissues in both the conditions. Specific growth rate (SGR) of fish was significantly lowered in hypoxic compared to normoxic or hyperoxic conditions. Taken together, these results show that prolonged exposure to both hypoxic and hyperoxic conditions induce oxidative stress responses at different levels of biological organisation, and hypoxic can also result in compensatory changes in growth. Results from this integrated study have implications not only for intensive culture of carp but also to wild fish populations subjected to environmental stress.

EM169

LINKING GENOTOXIC RESPONSES WITH PHYSIOLOGICAL/ BEHAVIOURIAL RESPONSES IN MARINE INVERTEBRATES FOLLOWING EXPOSURE TO CHEMOTHERAPEUTIC AGENT

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Neurotoxicity is considered to be one of the most frequent side-effects of chemotherapy. Despite concern over the presence of pharmaceuticals in the hydrosphere, there has not been enough study to evaluate their impact on the aquatic organisms. In addition, there has been no serious attempt to explore linkages between genotoxic responses with responses at higher levels of biological organisation, the key objective of ecotoxicological studies. Adopting non-invasive sampling, the study compared multiple biomarker responses in the sea star (Asterias rubens), a representative of important group of marine organisms, close to chordates and having homologus nervous system in the deuterostome line of development. The sea stars and the blue mussel (Mytilus edulis), a commonly used bioindicator species, were exposed to a range of concentrations of cyclophosphamide (CP), an anti-cancer drug, to probe the hypothesis that genotoxic responses are expressed at higher levels of biological organisation. Methyl methane sulfonate (MMS) was also used for reference. Biomarkers of cytotoxicity (neutral red retention assay), induction of micronuclei (Mn) and DNA strand breaks (Comet assay) were measured concurrently with behavioural ('righting time' for sea stars) and physiological ('clearance rate' for mussels) responses. Prior to evaluation of these sub-lethal responses, determination of maximum tolerated concentrations (MTC) suggested A. rubens to be more sensitive than M. edulis. For each species, cytotoxicity did not differ significantly from the controls. Apart from the MMS exposure to A. rubens (which showed high levels of mortality), clear dose-response relationships were observed for both genotoxicity endpoints, showing good correlations between DNA breaks and Mn induction. Following exposure to CP, correlations were also found between the behavioural response and genetic damage in both species. This integrated approach to simultaneously determine the responses at different levels of biological organisation indicates the potential usefulness of behavioural and physiologic measures in marine organisms and adds to information pertaining to potential use of adult echinoderms as a sensitive group of organisms for the protection of human and environmental health.

EM170

AN INTEGRATED APPROACH TO DETERMINE EFFECTS OF COPPER IN BIVALVE MOLLUSC, Mytilus edulis

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While copper is considered to be an essential trace element, its overexposure induces a wide spectrum of effects including DNA damage, which could impact short and long term survival of organisms. Given that copper is a highly relevant contaminant in the marine environment, we aimed to evaluate the induction of DNA strand breaks (using the comet assay) in haemocytes, and concurrently determined biological responses at higher levels of biological organisation in bivalve molluscs, *Mytilus edulis*, following exposure to a range of environmentally realistic levels of copper concentration (18-100 μg l^{-1}) for 5 days. Prior to evaluation of genetic damage, the maximum tolerated concentration (MTC) was determined; complete mortality of the exposed animals was observed at the highest concentration level (100 μg l^{-1}), but no mortality was observed at 56 μg l^{-1}. Levels of glutathione were determined in the posterior adductor muscle, and levels of copper were determined in different tissues using ICP-MS. The higher level biological responses included histopathological examination of organs and the '*feeding*' or '*clearance*' rate of the individuals. Copper levels in adductor muscle (P = 0.012) and gills (P = 0.029) were significantly higher, whereas digestive gland showed no significant difference compared to unexposed individuals (P = 0.400). While the exposure showed a strong concentration-dependent induction of DNA damage, the total glutathione level in adductor muscle increased by 1.83- fold at 56 μg l^{-1} copper. Interestingly, histopathological examination of selected tissues (i.e. adductor muscle, digestive gland and gills) showed abnormalities for the exposure period and concentrations used. The *feeding* or *clearance rate* also showed significant differences compared to controls even at the lowest concentration used (18 μg l^{-1}; P = 0.003). This study suggests that copper exposure is capable of inducing DNA damage which may manifest itself at the higher levels of biological organisation.

EM171

THE MODULATION OF DNA DAMAGE DUE TO PESTICIDES EXPOSURE BY REPAIR POLYMORPHISMS

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Grape production is an important economic activity in northeast region of Rio Grande do Sul State. In this activity, pesticides complex mixtures are constantly utilized and represent a human health potential risk to exposes people. The metabolizing and repair systems are important exposure effects modulators. The present study had as objective to evaluate the repair gene polymorphisms (OGG1*326, XRCC1*194, XPD*312, *751, Rad51*135, XRCC4*401) influences in DNA damage levels of exposed pesticides workers. In addition verify the relation with PON1 genotype. The genotyping of these polymorphisms was performed by PCR/RFLP. The DNA damage was evaluated by Comet assay (damage index – DI and damage frequency – DF) and Micronuclei test (MN) in 107 exposed workers and 65 non-exposed to pesticides. Differences in ID, DF, MN and between the different repair genotypes individually or combined to PON1 genotype, of which protein are involved in pesticides metabolizing, having a significant deviation from normality, were tested by the non-parametric Mann-Whitney U test. The XRCC1*194 and Rad51*135 polymorphisms, individually and combined to PON1*192 genotype, not demonstrated a statistical differences in biomarkers levels, even though both polymorphisms present a protection tendency to pesticides exposure (XRCC1*Trp/– and Rad51*G/G). The OGG1*326 genotype showed influence in DI and DF levels into exposed group, individually (P=0.032 and P=0.009), and when combined to PON1*Gln192Arg, once that the wild type genotype (OGG1*Ser/–) presents lower DNA damage levels. In exposed individuals, the wilt type genotype XRCC4*Ile/ – presents a protector effect to MN frequencies (P=0.024). The XPD polymorphisms in non-exposed individuals presents influence in DI and DF in individually analyses, when the wild type genotype correspond to lower biomarkers levels (312: P=0.028 and P=0.035 and 751: P=0.002 and P=0.007). Our results demonstrated that damage detected in Comet Assay are repaired principally by BER pathway, that are described as responsible for repair of small lesions as oxidized and reduced bases. While in the repair of damage detected in MN test are the RH and NHEJ that act in double strand break, as the NER pathway also has a influence.

EM172

EVALUATION OF DNA DAMAGE IN COAL MINERS

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Coal is one of the most used raw materials as an energy source, making its characterization and estimation of their risks of extreme importance to the security of who handles and the environment. Mineral coal in Rio Grande do Sul (RS), the southern state of Brazil, exceeds 30X109 tons, i.e., about 87% of the Brazilian reserves. The coal of this state is typically obtained by stripping operations. It is primarily used at the Presidente Médici power plant, located in Candiota/ RS (Brazil). This region produces 38% of the country's coal. The coal is low quality and generally contains substances with potential genotoxic, as PAHs and heavy metals. The aim of this study was evaluate DNA damage in coal miners from Candiota using Comet assay. The study includes 31 coal miners (mean age: 44.2 ± 6.8) and 65 individuals non-exposed to coal (mean age: 37.8 ± 10.6). Blood samples were collected in March and April - 2009. DNA damage was evaluated by Comet Assay (Damage Index – DI and Damage Frequency – FD). Coal miners' cells presented significant higher DI and DF (17.39 ± 9.9 and 13.7 ± 7.6, respectively) compared with non-exposed individuals (4.4 ± 5.8 and 1.91 ± 2.1, respectively – both P < 0.001). No differences were observed considering mask use, in coal miners. Comparing Coal miner's smokers and non-smokers differences in DI and DF were observed. In conclusion, our results suggest a genotoxic effect by coal exposure and agree with previous data on coal and derivatives, mainly PAHs, in relation to different organisms. Others biomarkers are been used with these blood samples, like as micronuclei in binucleated lymphocyte and in exfoliated buccals cells, as also as been asses the influence of metabolizing and repair polymorphisms in effects of coal exposure.

EM173

TOXICOGENOMIC ANALYSIS OF ARISTOLOCHIC ACID IN NORMAL HUMAN KIDNEY CELLS

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Herbs containing Aristolochic acid (AA) have been used worldwide for centuries as folk medicine. AA is found in *Aristolochia fangchi* that causes urothelial carcinomas in patients with AA Nephropathy. To further understand the molecular mechanism underlying the pharmacological and toxicological effects of AA, we used microarray analysis to investigate the global changes in gene expression induced by AA in normal human kidney (HK-2) cells. A 24 hr exposure to 10, 30, and 90 μM of AA exhibited a dose-dependent decrease in kidney cell growth with IC_{50} value of 30 μM. AA treatment of 30 μM for 1, 3, 6, 12, and 24 hours inhibited cell growth in a time-dependent manner. The AA-treated cells from the dose-dependent and time-dependent studies were collected for microarray analyses. All microarray experiments were performed in triplicate. Using Limma and Ingenuity Pathway Analysis software, we found that AA at doses of 10, 30, and 90 μM down-regulated several biological pathways in HK-2 cells. AA at a subtoxic dose of 10 μM (about 80% relative survival) effectively down-regulated the genes responsible for infectious and immune diseases. Network analysis revealed that nuclear factor-kappa B (NF-κB) played a central role

in the network topology. The inhibition of NF-κ B by AA was further confirmed by confocal microscopy in HK-2 cells and by NF-κ B luciferase reporter assay in HK-2/NF-κB transgenic cells. Using differentially expressed genes analysis software, we found that DNA repair genes were most significant related with AA treatment at doses of 30 and 90 μM in HK-2 cells. AA effectively down-regulated genes involved DNA repair pathway. The inhibition of DNA repair genes by AA was further confirmed by real time RT-PCR. These data are correlated with the 5-fold increase in the frequency of micronuclei formation in binucleated HK-2 cells after AA treatment. In conclusion, our data show that AA at a subtoxic dose of 10μM could affect gene expression profiles by suppressing NF-κB activity, suggesting its anti-inflammatory effects. Moreover, our results provide insight into the involvement of down-regulation of DNA repair gene expression as a possible mechanism for AA-induced mutagenesis and carcinogenesis.

EM174

COMPARATIVE STUDY ON IN VIVO GENOTOXICITY OF OCHRATOXIN A AND ARISTOLOCHIC ACID AS A CAUSATIVE FOR THE BALKAN ENDEMIC NEPHROPHASY

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Balkan Endemic Nephropathy (BEN) is a chronic renal disorder which is seen among rural area along the Danube River. BEN often progress to urothelial cancer and its aetiology seems to be associated with environmental mutagens. There have been several hypotheses on the aetiology. Ochratoxin A (OTA), a mycotoxin found in contaminated food in this area, has been a great concern, but recently, another key hypothesis became more feasible with molecular evidence. Aristolochic acid (AA) was known as Chinese herb nephropathy that resembles to BEN. AA was found in birthwort plant (*Aristolochia clematitis*) growing in this region, which contaminated into flour harvest. In order to evaluate genotoxic potential of OTA and AA, we have tested in vivo mutagenicity of two compounds using transgenic mouse (Muta™Mouse) assay.

Method: AA (20 mg/kg) or OTA (15 mg/kg) was administered intragastically to male Muta™Mouse weekly for 4 weeks. 48 h after the final treatment mice were sacrificed and various organs were collected and subjected for mutation assay with *lacZ* and *cII* genes. Micronucleus assay was simultaneously performed with peripheral blood collected from tail after the first treatment. Results and Discussion: AA induced extremely strong mutagenicity in kidney and bladder. It also induced similarly strong mutagenicity in forestomach and colon while other organs showed lower but significant increase in mutagenicity. Therefore unique organ-specific mutagenicity was observed. In contrast, OTA induced almost no increase in mutagenicity for all organs. No increase of micronucleated reticulocytes was observed with OTA and AA. These results demonstrated potent mutagenicity of AA in urinary organ, suggesting its preferential involvement for BEN aetiology. Sequence analysis of AA-induced *cII* mutants revealed preferential induction of AT to TA transversion mutations, probably derived from the reported AA-adenine adduct. This characteristic mutation spectrum matched with p53 mutations found in tumours from BEN patients. With this molecular evidence, together with its strong mutagenicity, we confirmed AA as a causative mutagen for BEN.

EM175

INVOLVEMENT OF AIR POLLUTANTS/ALLERGENS MEDIATED OXIDATIVE STRESS IN THE EXACERBATION OF BRONCHIAL ASTHMA

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Exposure to noxious mutagenic pollutants and allergens can activate neutrophils and macrophages to produce reactive oxygen species (ROS) and reactive nitrogen species (RNOS). The subsequent oxidative stress induced by this way may increase the risk of developing exacerbated conditions of bronchial asthma through the promotion of T cell response. The purpose of this study is to investigate the implication of pollutants and allergens in the exacerbation of asthmatic conditions and the involvement of oxidative stress. Lung function test was evaluated using spirometric measurements in 60 asthmatic children (6 – 14 years old) with clinical history of asthma. Total equivalent antioxidant capacity (TEAC), Superoxide dismutase (SOD) and Glutathione peroxidase (GPx) were evaluated using kits commercially available from Randox-Company. Malondialdehyde (MDA) was estimated by thiobarbituric acid reaction. A general lung obstruction profile was detected in all our patients (from mild to severe) corresponding to the clinical score ranging from 3 to 8 and confirmed by FEV (Forced Expiratory Volume) % ranging from 71 to 75 % and by the measured ratio FEV/FVC (Forced Expiratory Volume/Forced Volume Calculated) ranging from -12 to -38 %. Hematologic results displayed a high level of eosinophils (2.8 to 20 %) for the majority of patients corresponding to the IgE values exceeding 500 KU/L. Positive *Phadiatop* has been observed in these patients who also displayed low values of TEAC and GPx (<1.42 mmol/L and <700 U/mL respectively and high values of 8 isoprostane (>7.5 pg/ml) in contrast with high level of MDA (>3.5 mmol/L). However, exacerbated conditions of bronchial asthma were clinically confirmed only in 18 patients (clinical score: 6 – 8) with FEV/FVC ratio ranging from -16 % to -38 %. Exacerbated conditions of bronchial asthma are a consequence of hyperproduction of eosinophils and IgE by oxidative stress generated by the combustion products inducing a slight deficiency in antioxidant defense system and acting as adjuvants in the immune system of those patients sensitized to the observed allergens.

EM176

MOLECULAR EVIDENCE OF SYNERGYSTIC EFFECT OF CITRININ, FUMONISIN AND OCHRATOXIN A: ROLE IN HUMAN NEPHROPATHY AND UROTHELIAL TRACT TUMOURS

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Analyses of several food and feed items show the simultaneous presence of several mycotoxins, notably fumonisins (FB), citrinin (CIT) and ochratoxin A (OTA). All the three toxins induce nephrotoxicity. The aim of this study was to determine the cytotoxic and genotoxic combined effects of either CIT and OTA or FB and OTA. The studies were conducted (i) in cells culture and (ii) in vivo on rat fed with ground wheat enriched with OTA and/or CIT; and/or FB. When OTA and CIT, or OTA and FB are simultaneously present, the decrease of viability (measured by MTT test) of human kidney cells (HK2) induced by OTA is considerably enhanced. Expression of biotransformation enzymes (CYP, COX, LIPOX) in HK2 is differently modulated depending of the treatments: CIT alone, OTA alone or both together. Induction of COX2 and LIPOX by CIT increased genotoxicity of OTA measured by DNA adducts formation detected by P32 post-labeling. DNA adducts patterns of rat kidney after a 3-weeks feeding by either CIT alone or OTA alone or both together, are similar to those obtained on cell cultures. The main OTA DNA-adduct, found in human tumours, identified as C8 dG-OTA is increased by simultaneous presence of CIT and OTA. In the same way, in in vivo studies on rat and pig fed simultaneously by OT and FB in feed we observed increased OTA specific DNA adducts including C-C8dG OTA adduct and the both OTHQ related adduct. Both toxins (OTA and FB) modulate the arachidonic acid cascade and induce c-jun. These specific adducts are found in human urothelial tumours, notably in Balkan regions. This data is particularly interesting as we previously shown that families suffering BEN eat food more frequently contaminated by OTA and CIT. In cell as in blood and kidney of human and rat we isolated OTA and several

OTA derivatives identified by HPLC ms/ms, including quinone OTA (OTHQ), GSH-OTA, GSH-OTHQ, DC-OTHQ. The data indicate clearly that exposure to low concentration of mycotoxin which is considered as safe when they are present together can lead to dramatic effect. Until now, regulation does not take into account co-contamination. Granted by the 'Région Midi-Pyrénées, program Food safety 2003-2008', ARC 2005-2007; Ligue National Française contre le cancer, 2006; EU 'Ota risk assessment' 2002-2005.

EM177

CASPASE: KEY PROTEIN BIOMARKER FOR SILICOSIS

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Background/Aim: Silica is the potent occupational fibrogenic agent capable of inducing lung fibrosis and other occupational diseases. In India, there are three million people, exposed to silica in mines and industries like stone cutting, silica mining, slate pencil, foundry work, rock grinding and tunneling etc. Apoptosis is a distinct form of cell death characterized by cell shrinkage, plasma membrane blebbing, nuclear chromatin condensation, and DNA fragmentation. Silica induced apoptosis in the alveolar macrophages could potentially favors a proinflammatory state occurring in the lungs of silicotic patients resulting in the activation of caspase prior to induction of the intrinsic and extrinsic apoptosis pathway. Apoptosis plays central role in oxidative stress and DNA adduction in silicosis by the activation of caspases. Deposited silica particle in the lung of silicotic patient, damage macrophages and epithelial cells. This consequence may release a wide variety of enzymes and inflammatory cytokines such as Interleukin-1, tumor necrosis factor (TNF- α), neuclear transcription factor-κB (NF-κB), platelet- derived growth factors and inducing the late fibrogenic reaction. Recent studies indicated that apoptosis may involved in pulmonary disorders, such as acute lung injury, diffuse alveolar damage, idiopathic pulmonary fibrosis, and other lung disorders caused by bleomycin, silica, endotoxin, and the deposition of immune complexes

Method: Measurement the caspase and Fas/FasL activity in silicotic individual may provide beneficial tool for the prognosis of silicosis. It might be open new area for the diagnosis of silicosis. Result: Fas and Fas related molecules in patients with silicosis indicate a dysregulation of autoimmunity after long-term exposure to silica. Major effector caspases play a critical role in the characteristic apoptotic changes including chromatin condensation, DNA fragmentation, and formation of apoptotic bodies. It could be used as an effective biomarker for the study of occupational disease. Conclusion: It could be a key protein which can be used as an effective biomarker for the study of occupational diseases. It may provide an important link in understanding the molecular mechanisms of silica-induced lung pathogenesis. Caspase and Fas/FasL mediated apoptosis in silicosis not only provide clues for the pathogenesis and treatment of immunological disorders but also aid in predicting the pre-clinical status of complicated autoimmune diseases found in occupational disease.

EM178

EVALUATION OF THE GENOTOXIC POTENTIAL OF THE COAL PYRITE TAILINGS THROUGH THE HELIX ASPERSA BY THE COMET ASSAY (MÜLLER, 1774)

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Introduction: The Santa Catarina Coal Basin located in the southern Brazil is a traditional mining area in the country. Over the past 120 years, coal production has been vital to regional economic development and it has also had a major impact on the environment when surface mining was intensified to increase domestic coal production as a replacement for imported oil. Coal, as a sedimentary rock, is a complex heterogeneous mixture of organic and inorganic constituents containing intimately mixed solid, liquid, and gaseous phases from allothigenic or authigenic origin. When coal is separated from its impurities by cleaning processes are formed coal pyrite tailings that are deposited usually close to the mining area. This material presents elements as carbon, hydrogen, nitrogen and sulphur, besides a variety of heavy metals. The coal pyrite tailings from coal improvements constitute one of the major environment problems. So, the aim of the present study was to evaluate the genotoxic potential of coal pyrite tailings from the beneficiation of coal mining in southern Santa Catarina by the Comet assay, using the land mollusk Helix aspersa (Müller, 1774) as a bioindicator. Material an Methods: Adult H. aspersa land mollusks (n=144) were divided in 3 groups of exposition, clustered in plexiglass cages, during 1 month: control (animals fed with organic lettuce, n= 48), coal tailings (animals living in a layer of pyrite tailings from the beneficiation of coal and fed with organic lettuce, n=48) and lettuce mine (animals fed with lettuce grown in an area located on deposit of coal tailings, n=48). The mollusks hemolymph was collected at the time of exposure: 24 h, 48 h, 72 h, 96 h, 1 week, 2 weeks, 3 weeks and 1 month, for comet assay analyzes (n=6/time/group). Results and Conclusions: Our results showed that the animals of the coal tailings and mine lettuce groups presented higher levels of DNA damage in relation to the control group at all hours of exposure ($P<0.01$, t-Student test). After the period of 48 hours, there were a decrease in DNA damage in coal tailings and mine lettuce groups, but still significantly higher than for the control group. These results demonstrate that the coal pyrite tailings coming from extraction and beneficiation of coal are potentially genotoxic for snails H. aspersa and probably to different animals in other trophic levels. In addition, the animals exposed to the lettuce growing next to environments impacted by coal mining showed genotoxicity. Our study provides biological data of this land snail exposed to the coal tailings, as well as, fed by lettuce grown in the coal mine and confirms the sensitivity of the Comet Assay and the snail H. aspersa in the assessment of environmental complex mixtures.

EM179

ASSESSMENT OF POLLUTION OF FISH IN SUEZ CANAL WITH PETROCHEMICALS AS HEALTH HAZARD INDICATOR

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Polycyclic aromatic hydrocarbons (PAH) were detected in Suez. Canal fish (yellow eyed mullet) by gas liquid chromatography as indicators of pollution with petrochemicals, which reflect the hazard effect to marine organisms and human health. PAH were evident in all parts of fish(muscle, skin and digestive tract). The most frequent compound of individual PAH detected (more than three benzene rings were benzo (a) anthracene, benzo (b) flouranthine, benzo(k) flouranthine, benzo(g, h, i) anthracene (1, 2, 3 cd) pyrene, which are known as potent carcinogen. The highest individual PAH concentration were benzo (g, h, i) pyrelene and indeno (1, 2, 3 cd) pyrene. The low molecular weight of individual PHA (less than four benzene rings) as acenaphthylene was detected in high concentration in some fish samples. In all samples PAHs were detected with no significant differences before and after cooking. Indeno (1, 2, 3 cd) pyrene residue were higher after cooking, whereas concentration of benzo (g, h, i) pyrelene were reduced. The highest concentration of individual PAH was detected in digestive tract sample and muscle respectively. The presence of PAHs of low molecular weight (les than four benzene rings) in some samples may indicate the direct crude oil pollution.

Mutagenesis and health effects

MH001
THE PROTECTIVE ROLE OF ROYAL JELLY AGAINST MUTAGENIC EFFECT OF ADRIAMYCIN AND GAMMA RADIATION SEPARATELY AND IN COMBINATION
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The present study investigated the protective role of royal jelly against mutagenic effect of adriamycin and/or cobalt gamma radiation in rats. The pretreatment of royal jelly for ten days before adriamycin treatment or/and gamma exposure showed a decrease in their mutagenic effect. Royal jelly administration decreased the different types of chromosomal aberrations induced by adriamycin without significant levels in 2nd, 4th, 7th and 14th days after adriamycin treatment, except the total structural aberrations with gaps in 7th day decreased significantly at $p<0.05$. Also the royal jelly pretreatment induced decreases of DNA fragmentation induced by adriamycin with a highly significant level ($P<0.001$) in 4th day after adriamycin treatment and this significant decrease continued for two weeks after adriamycin treatment. The result showed that there was no significant decrease in the number of aberrations in 2nd and 4th days after irradiation, while the total number of structural aberrations decreased significantly at $p<0.01$ in 7th day and at $p<0.05$ in 14th days after gamma exposure. Also, the royal jelly pretreatment induced a significant decrease of the DNA fragmentation in 2nd day after gamma exposure at a level $P<0.01$ and this significant increase reached to $P<0.001$ level in 4th, 7th and 14th after exposure. The statistical analysis showed that the royal jelly pretreatment decreased the numbers of all aberrations induced in combined treatment (adriamycin plus gamma radiation) group without any significant level, except the total number of structural aberrations in 2nd day where it decreased at $p<0.05$. The royal jelly pretreatment induced a decrease of DNA fragmentation without significant level in 2nd and 4th days after combined and this decrease reached to the significant level at $p<0.001$ in 7th day and at $p<0.05$ in 14th day.

MH002
GENOTOXICITY AND CYTOTOXICITY IN MULTIPLE ORGANS INDUCED BY RENOVASCULAR HYPERTENSION IN WISTAR RATS
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Hypertension is a major public health problem of the world and it is one of the major risk factors contributing to cardiovascular disease. The aim of this study was to investigate whether blood, liver, heart and brain are particularly sensitive organs for DNA damaging and cellular death during renovascular hypertension by the single cell gel (comet) assay. A total of 15 male Wistar rats were divided into two groups: negative control (n=5) and experimental (n=10), in which was submitted to the partial obstruction of the left renal artery with a silver clip during 6 weeks for inducing hypertension. The results showed that hypertension was able to induce genetic damage in blood, heart, liver and brain cells as depicted by the mean tail moment. Furthermore, an increase of cytotoxicity was noticed to heart and brain cells. In addition, this study confirms significantly the hypothesis of a relationship between increased blood pressure and DNA damage in leukocytes ($p<0.05$, $r>0.5$). In conclusion, our results suggest that hypertension could contribute to the damage of DNA at all organs evaluated. High cytotoxicity was noticed in heart and brain cells. Since DNA damage and cellular death are an important step in events leading to carcinogenesis, this study represents a relevant contribution to the correct evaluation of the potential health risk associated with hypertension.

MH003
CITOTOXICITY OF *Sassafras albidum* AND *Rosmarinus officinalis* IN DIFFERENT CELL LINES.
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Many extracts of plants have been extensively used in medicine and are very well known for their therapeutical values. It has been clear that many people around the world use this extracts to treat some diseases. *Sassafras albidum* belongs to the *Lauraceae* family, and its infusions are used by some populations in Colombia, giving its diuretic, digestive and antigenotoxic properties. On the other hand, *Rosmarinus officinalis, Laminaceae* family, has been used with different purposes such as analgesic, antimicrobial, diuretic and antioxidant activities. The aim of the study was to determine the citotoxicity of the ethylic extract, obtained from the leaves, of these two plants in the search of new substances with clinical importance. For each extract, the following concentrations were used: 0.13, 0.64, 3.2, and 16.0 µg/ml. Chronic citotoxicity was measured with MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide), and acute citotoxicity was measured with Trypan Blue assay in two cell lines: cervix cancer cells (HeLa), and fibroblasts (CHO-K1). The results were analysed using the test of Pearson in order to study the correlation between cell viability and the replicas for each concentration used of the extract of both plants. For chronic citotoxicity, after 48 hours of exposure, a reduction of cell viability was observed for the two extracts, and in both cell lines, cell viability was less than the 80% at the concentration of 3.2 µg/ml. For the acute citotoxicity assay, the results, after 4 hours of exposure showed no effect in cell viability for the concentrations used. There was no difference in the results between HeLa or CHOK1 cells. These results suggest that the therapeutical use of *Sassafras albidum* and *Rosmarinus officinalis* organic extracts do not have a cytotoxic effect in concentrations lower than 3.2 µg/ml. These concentrations should be used for further studies with each plant extract to determine the active compound and its clinical potential.

MH004
GENOTOXICOLOGICAL, BIOCHEMICAL AND HISTOLOGICAL EVALUATIONS FOR THE TRICYCLIC ANTIDEPRESSANT: AMITRYPTLINE ON MALE ALBINO MICE.
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The tricyclic antidepressant drug amitryptline which is widely used in Egypt was evaluated on laboratory mice after oral treatments with the three therapeutic doses described by the physicians and manufacture instructions.The results showed the treatment with amitryptline induced increase in the incidence of chromosomal abnormalities in both somatic (bone marrow) and germ cells (spermatocytes) and this increase was significantly over the control in the high dose only. The high dose also induced significant increases in sperm head and tail abnormalities accompanied by a significant decrease in sperm count. Liver enzymes (AST; ALT and γ GT) were found to be significantly increased over the control after the high dose treatment. Blood testosterone showed significant decrease which explains the sperm count decreasing; in contrast prolactin did not show any significant changes. Histological preparations showed that the drug high dose treatment induced remarkable necrosis and degeneration in the testicles of the treated males. The result in general highlights the danger of overusing the drug by the Egyptian men and the normal dose is safe according to our results.

MH005

GENOTOXICITY EFFECTS OF AGRIMONA PILOSA LEAF METHANOLIC EXTRACT IN SALMONELLA TYPHIMURIUM AND CHINESE HAMSTER OVARIAN CELLS.

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ABSTRACT: The present study was conducted to evaluate the efficacy of the Agrimonia pilosa leaf methanolic extract on genotoxicity in Salmonella typhimurium, in the Chinese hamster ovary (CHO) cell line and in the mouse micronucleus test. In the reverse mutation assay test using Salmonella typhimurium TA98, TA100, TA1535, TA1537 and Escherichia coli WP2urvA-, the methanolic extract of the dose range at 5, 2.5, 1.25, 0.62, 0.312 mg/plate was not induced reverse mutation in the presence or absence of S9 mix metabolic activation. In the chromosome aberration test using CHO cells, methanolic extract (1.25, 2.5 and 5 μg/Ml) was revealed a little incidence of structural and numerical aberrations, but in the comparison with positive group, in both of absence or presence of S9 mix metabolic activation, those incidence was not significantly enhanced. In the mice micronucleus test, no significant increase in the occurrence of micronucleated polychromatic erythrocytes was observed in ICR male mice orally administrating methanolic extract at the dose of 2.0, 1.0, 0.5 g/kg. From these results, It is proposed that the methanol extracts of Agrimonia pilosa leaf is not induced any harmful effects on the gene in bacteria, mammalian cell system and in the bone marrow cell of mice used in this experiments.

MH006

COMPOUNDS UTILIZED IN THE CLONING OF MAMMALS ARE MUTAGENIC AND ALTER REPRODUCTIVE PERFORMANCE AND INTRAUTERINE DEVELOPMENT IN MICE.

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The compounds 6-dimethylaminopurine (6-DMAP) and cyclohexamide (CHX) are utilized for the development of embryos produced by nuclear transfer in the production of cloned mammals with currently much success. This study investigated the effects of 6-DMAP and CHX in vivo, by means of biological assays for the evaluation of reproductive performance of females, teratogenesis and mutagenesis. The results of this study demonstrate that the activating agents of oocyte cytoplasm, 6-DMAP and CHX, have the capacity to alter the reproductive performance of experimental animals as well as cause an increase in the rate of malformations. Besides these adverse effects on reproductive performance in mice, the administration of these compounds in pregnant females demonstrated genotoxic and mutagenic toxicity, determined by the comet and micronucleus assays in peripheral blood, respectively. Based on these findings and considering that alterations in DNA are of importance based on studies in mutagenesis, morpho-functional teratogenesis and diminished embryonic viability, the present study suggests that 6-DMAP and CHX, utilized in the process of cloning mammals, is responsible for the fact that embryos produced by nuclear transfer have a low viability after their implantation in utero or after birth due to congenital malformations and/or alterations in their DNA. Supported by CNPq and CAPES.

MH007

INDIVIDUAL GENOMIC INSTABILITY IN THE ORGANISM OF CHILDREN, BORN AFTER THE ACCIDENT AT THE ChNPP

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Study of hildren from territories contaminated with radionuclides and children of fathers-liquidators born after the accident at the Chernobyl NPP revealed increased levels of aberrant genomes and aberrations of chromosomal type in blood lymphocytes (n=180, p<0.05). This is accompanied increased morbidity. This is indicative of the radiation-induced in low doses genomic instability and its transgenerational phenomenon in their organism. To elucidate this phenomenon, experiments were undertaken to model genomic instability by using fractionated in vitro γ-irradiation (137Cs) of peripheral blood lymphocytes samples of the children (n=9) and their parents (n=6) at doses of 10, 20 and 30 cGy. The spectrum and frequency of chromosomes aberrations were studied in the 1st and 2nd cell generations. Single doses were 10, 20, 30 cGy. During of fractional irradiation in doses 10cGy +10cGy and 10cGy+10cGy+10cGy blood samples were irradiated at 24h intervals. Cultivation of lymphocytes was carried out for 48 h and 72 h. Increased levels of aberrations of chromosomal type as compared to the control (p<0.05) are observed in 3 subgroups these children with different initial individual aberrant genomes frequency (IAGF): children with a high IAGF (≥3%) (n=60), children with a middle IAGF (2%) (n=68) and children with a low IAGF (≤1%) (n=52). After fractionated and single γ-irradiation (137Cs) of blood in vitro at a dose from 10cGy to 30 cGy, similar patterns of increase in the average chromosome aberrations frequencies are observed in 1st and 2nd mitosis, depending on the initial IAGFs in children and parents. Significantly increased levels of chromosome aberrations is revealed in children with a high IAGF (≥3%) as compared to the control (p<0.05). This is indicative of individual radiosensitivity probably depending on genotypic peculiarities, initial state (sensitivity) of the genome, pathophysiological processes in the organism of children. Amplification of cells with single-break chromosome aberrations in the mitosis 2, as compared to mitosis 1 suggests the replication mechanism of realization of potential damage in DNA and the occurrence of genomic instability in succeeding cell generations.

MH008

MICRONUCLEUS IN EXFOLIATED UROTHELIAL CELL FROM PATIENTS WITH HISTORY OF TRANSICIONAL CELL CARCINOMA

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One of the main features of the transitional cell carcinoma (TCC) is it high recurrence rate (70% to 80%). Therefore, the use of sensible techniques for detecting early disease in subjects with high risk for tumor development, and recurrent tumors in patients with history of TCC are extremely relevant. In this context, we used the micronucleus assay to evaluate whether cytogenetic disorders could be a good biomarker for monitoring TCC development and recurrences. The frequency of micronucleus was established in urinary bladder exfoliated cells, obtained by intravesical instillation of 0.9% saline solution, from 105 patients with (52) and without (53) history of TCC, either smokers and non-smokers, but with current diagnosis of “negative for neoplasia”. Conventional MN staining (Giemsa) and fluorescence in situ hybridization (FISH) with α-satellite probes for centromeres of all human chromosomes techniques were performed, in order to evaluate the frequency of micronucleated exfoliated cells, and also the main mechanism involved in the micronucleus formation. The results showed a significant increase (P<0.01) of MNC in patients with history of TCC, with no difference between smokers and non-smokers. Data

also showed higher number of micronucleus resulted from chromosome fragments than from whole chromosome in those patients with history of TCC (P<0.01). In conclusion, the results demonstrated that chromosome damage is an import early step in urothelial carcinogenesis and independent on cigarette smoking habit. Furthermore, it seems that these genetic abnormalities occur mainly by chromosome breakage than by aneuploid, and could be related to urothelial genomic instability. Thus, MN test could be an auxiliary tool for monitoring subjects at risk or history of TCC, but with a macroscopic diagnosis of normal urothelium. This study was supported by FAPESP and CNPq.

MH009

ANTICANCER DRUG ONCONASE REVEALS MULTIPLE CONFORMATIONAL CHANGES AT THE INTERACTION WITH RNA SUBSTRATES

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Onconase (ONC), otherwise known as ranpirnase or P-30 protein, which was initially purified from extracts of Rana pipiens (Northern leopard frog) oocytes and early embryos, exhibits anticancer and antiviral activity both in vitro and in vivo and is in phase III clinical trials for tumor therapy. ONC is the smallest member of the pancreatic ribonuclease A superfamily, consisting of 104 amino acids. ONC is an unusually stable protein and shows high resistance to the action of proteases at physiological salt concentrations. The active site of ONC is located in the cleft its kidney shape. This active site contains the catalytic triad (His10, Lys31, and His97) that is characteristics of the RNase A superfamily. In this work we have applied stopped-flow kinetics to analyze the conformational dynamics of specific (r(CUGGAG), dArUdGdA) and nonspecific (dArUdAdA) substrates during processing of recombinant onconase at pH 7.4 and 6.0. These substrates were labeled with FRET dye pair fluorescein/rhodamine fluorescence quenching.In substrate fluorescein was held in proximity to rhodamine residue and its fluorescence was quenched. When the substrate was cleaved, the fluorescence was increased 180-fold. Monitoring fluorescence in stopped-flow experiments reveals multiple conformational transitions in the enzyme-substrate complex during the catalytic cycle. At least three conformational transitions occur in enzyme/substrate complex during the interaction of ONC with ribooligonucleotide duplex within 50 ms - 100 s time ranges. These transitions reflect the stages of enzyme binding to RNA and mutual adjustment of RNA and enzyme structures to achieve catalytically competent conformation. Only a single conformational change (at 70 s) is connected with nucleic acid cleavage. The data obtained provide evidence that several fast sequential conformational changes occur in ONC after binding to its substrate, converting the protein into a catalytically active conformation. This work was supported by grants from the Russian Foundation of Basic Research (07-04-00191, 08-04-00334, 08-04-12211), Russian Ministry of Education and Science (NSch-652.2008.4.), and the Siberian Branch of the Russian Academy of Sciences (28, 48, 90, 21.22).

MH010

DESIGNING OF SMALL INTERFERING RNA (SIRNA): A UNILATERAL 2-NT 3'-OVERHANG ON THE ANTISENSE STRAND IS MORE IMPORTANT THAN THE RELATIVE THERMODYNAMIC STABILITIES AT THE ENDS OF SIRNAS

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Short interfering RNAs (siRNAs) are valuable reagents for sequence-specific inhibition of gene expression via the RNA interference (RNAi) pathway. Although it has been proposed and largely accepted that the relative thermodynamic stability at the both ends of siRNAs plays a crucial role, the terminal structure of siRNA seems to be the predominant determinant of which strand participates in the RNAi pathway. To address this issue, we designed basically four types of siRNAs targeted against genes: (1) symmetric blunt ends (b-b), (2) symmetric 2-nt overhangs (o-o), (3) asymmetric 2-nt antisense strand overhang (o-b), and (4) asymmetric 2-nt sense strand overhang (b-o). A large variety of variants were also synthesized and tested. HeLa S3 cells were cotransfected, typically, with siRNA, firefly luciferase-DNA (target, forward or reverse orientation) andβ-galactosidase-DNA (control). Relative luciferase activities were determined 24 h after the transfection. When the endogenous GAPDH or Lamin A/C gene was targeted, Western blot analysis was conducted. Results indicated that siRNAs with a unilateral 2-nt 3'-overhang on the antisense strand (o-b) were more effective than other siRNAs (b-b, o-o, and b-o), due to preferential loading of the antisense strand into the RNA-induced silencing complex (RISC), regardless of the relative thermodynamic stabilities at siRNA ends. This finding was confirmed by another model experiments targeting SARS-corona virus genes. Two model target sequences (forward and reverse orientations) were connected to the firefly luciferase gene, and activities of siRNA, 2-nt overhang of which on the sense or antisense strand was either match or unmatch to the target strand, were tested. Of all nine possible combinations, a match 2-nt overhang at the 3'-end of the antisense strand (o-b) showed the highest activity, irrespective of the thermodynamic stabilities at terminal ends. Moreover, we showed that sense strand modifications, such as deletions or DNA substitutions, of siRNAs with unilateral overhang on the antisense strand have no negative effect on the antisense strand selection. Our findings provide useful guidelines for the design of potent siRNAs and contribute to understanding the crucial factors in determining strand selection in mammals.

MH011

p73 G4C14-to-A4T14 GENE POLYMORPHISM AND INTERACTION WITH p53 EXON 4 Arg72Pro ON CANCER SUSCEPTIBILITY: A META-ANALYSIS OF THE LITERATURE

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P73 gene (1p36-33) is involved in cancer development through cell growth inhibition by inducing apoptosis in a p53-like manner. p73 G4C14-to-A4T14 dinucleotide polymorphism has been extensively studied in association with cancer over the past years, with conflicting results. Our meta-analysis aimed to assess the overall effect of p73 G4C14-to-A4T14 polymorphism on cancer risk and its interaction with demographic data, lifestyle habits and p53 exon4 Arg72Pro polymorphism. We searched for studies investigating the association between p73 G4C14-to-A4T14 polymorphism and cancer on Medline and Embase up to June 2008. In addition individual level data were collected from the published studies. From the meta-analysis including data from 16 case-control studies (5145 cancer cases and 5135 controls), an increased risk for cancer in any site was found to be associated with the inheritance of the p73 homozygous variant genotype (AT/AT) [Odds Ratio (OR)=1.28 (95%CI, 0.98-1.67)]. By stratifying the effect of p73 AT/AT on cancer according to p53 exon 4 Arg72Pro status, a 2.15-fold increased risk (95%CI, 1.27-3.64) was observed for individuals both p73 variant homozygotes and p53 exon 4 heterozygotes if compared with wild type p73 allele carriers, suggesting biological synergism among the two polymorphisms. No evidence of an effect modification of p73 AT/AT by age, gender, ethnic group or smoking status resulted from the subgroup meta-analyses. This quantitative review showed that the p73 G4C14-to-A4T14 homozygous variant genotype might be a

risk factor for cancer, especially in combination with p53 exon 4 Arg72Pro polymorphism. Further studies looking at p73 G4C14-to-A4T14 and p53 exon4 Arg72Pro interaction together with functional reports on the interaction among p73 and p53 proteins are required to confirm our findings.

MH012

ASSESSMENT OF MUTAGENIC BACKGROUND IN THE URBAN ENVIRONMENT BY MICRONUCLEUS TEST IN BUCCAL HUMAN CELLS

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Background. One of the important tests for cytogenetic monitoring used for estimating the general mutagenic background of the urban environment is a micronucleus (MN) test in buccal human cells. The test involves a non-invasive, screening express method. Methods. The MN test was used for estimating the influence of harmful environmental factors on the organisms of about 600 pre-school age children living in different conditions, in terms of levels and types of technogenic loadings, in Ukrainian cities. The MN test was also used for estimating the effectiveness of rehabilitation measures based on the application of natural adaptogenes in their natural conditions, i.e. physiologically active substances with antitoxic and antimutagen properties, Through special questioning among the group of children for cytogenetic investigation, a selection was made of healthy, and practically healthy, children in the age range 5-7 years (1st and 2nd health groups) because such children are the most sensitive to the unfavourable influences of external factors. Results. The level of microkernels in the cells of children living in industrial regions of Ukraine with a high technogenic loading is 2.2-10.9 times higher than those in a “conditionally clean” control area. The dynamics of the analysed index change are negative. In the cities investigated there were defined groups with an increased genetic risk, and the high risk group ranged from 0% (control) to 61 % (in Zhovti Vody which is the centre of uranium production). The elevated mutagen background levels created the need for carrying out rehabilitation measures directed at increasing the protective functions of organisms. It was established that after complex rehabilitation treatment, including humic preparations for human organisms, there was a positive reduction in the level of cells with genetic disorders by 1.93 - 3.0 times. Conclusions. Under the influence of harmful ecological factors in human organism there was a measurable increase in the frequency of occurrence of cells with microkernels. However, the cytogenetic condition of one’s organism can be improved by using special rehabilitation courses of natural adaptogenes, or their combinations, under the favorable ecological conditions of sanatorium complexes.

MH013

POSTGENOMIC ALTERATIONS IN AICARDI-GOUTIÈRES SYNDROME, A RARE NEURODEGENERATIVE DISEASE OF MUTATIONAL ORIGIN

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Aicardi-Goutières syndrome (AGS) is a mutation-related neurodegenerative disease, arising during the first year of life, which is characterized by progressive cerebral atrophy, leukodystrophy, and raised levels of interferon-alpha in the cerebrospinal fluid (CSF) in the absence of any infection. Microarray analysis, examining the expression of 18,880 human genes, has been applied to CSF lymphocytes of 20 AGS cases (age 4.5 ± 4.4 years, mean ± SD), all of them bearing high levels of interferon-alpha in CSF, and 20 matched controls (age 4.4 ± 4.3 years). AGS cases displayed an upregulation of genes involved in interferon-dependent pathways and lymphocyte activation, paralleled by down-regulation of genes encoding for angiopoietic activities. Gene expression patterns were consistent with a progressive attenuation of clinical symptoms with age. The results obtained provide evidence that lymphocytes in AGS patients produce proteolytic enzymes, such as cathepsin D, with an established ability to damage the neurological tissue. This alteration is accompanied by a severe damage in blood vessel development. Identification of the genes whose altered expression is responsible for AGS provides a novel insight into interferon-dependent neurodegenerative mechanisms.

MH014

MUTATIONS OF GENES ENCODING FOR NUCLEASES AND AICARDI-GOUTIÈRES SYNDROME

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Aicardi-Goutières Syndrome (AGS) is a rare neurodegenerative disease of mutational origin arising in healthy newborns. AGS is characterized by raised levels of interferon alpha in cerebro-spinal fluid (CSF) resulting in brain atrophy. Molecular mechanisms relating interferon alpha and brain damage have recently been depicted by microarray analysis performed in CSF lymphocytes demonstrating that inhibition of angiogenesis and activation of neurotoxic lymphocytes are major pathogenic mechanisms involved (Izzotti et al., Neurology 2008; Brain Pathol. 2008). Thereafter, we explored the influence of different AGS mutations on AGS clinical course in 21 AGS patients, who were characterized by detecting the occurrence of AGS1 biallelic mutation, AGS2 bi- and mono-allelic mutations, AGS4 bi-allelic mutation, no mutation in AGS1-4 genes. AGS2 bi allelic mutation was the most frequent mutation, being detected in 11 patients. The negative results obtained in 4 patients indicate that at least one still unidentified AGS locus exists. Mutational status affected clinical feature mainly as related to age at diseases onset, interferon alpha levels, brain atrophy, and occurrence of epileptic symptoms. Different mutations differently dysregulated gene expression profile as analyzed by microarray in the CSF lymphocytes. The specific effect of AGS mutation is related to the specific functions of their targeted genes. AGS1 mutation targets TREX gene, a pivotal intracellular exonuclease. AGS2 and AGS4 mutations target genes encoding for RNase H subunits, which is a pivotal endogenous RNase involved in the silencing of microRNA. The increased half life of intracellular nucleic acid consequent to AGS mutations triggers interferon alpha production through activation of TLR receptor pathways. To attenuate the effect of AGS mutations, lymphocytes bearing AGS multiple mutations were co-cultured with endothelial vascular cells and neural cells, and their ability to counteract angiogenesis on matrigel and to induce nuerotoxic effects was determined. Gene-silencing of genes dysregulated by AGS mutations, i.e. cathepsin D and brain angiogenesis inhibitors, was performed to attenuate the adverse effects observed.

MH015

CANCER CHEMOPREVENTIVE PROPERTIES OF EXCOECARIA PHILIPPINENSIS MERRILL

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Cancer chemoprevention has gained widespread attention among biomedical scientists because it revolutionized the current cancer management and proven effective in lowering cancer incidence worldwide. To find possible drug candidates in cancer prevention, this study evaluated the potential of the extracts and an isolate from an endemic plant, Excoecaria philippinensis Merrill based on their ability to scavenge the free radical DPPH (2,2’-diphenyl-1-picrylhydrazyl), inhibit the transformation of the pre initiated murine epidermal JB 6 cells, and induce differentiation of the human promyelocytic HL 60 cells. Crude extracts of the plant, which showed consistent bioactivities in the 3 assays, were

fractionated using a bio-activity directed purification scheme yielding the active isolate. The isolate was identified using spectroscopic techniques such as NMR and MS. Bioassay of the isolate resulted in a dose-dependent DPPH free radical inhibition with IC50 of 4.11 µg/mL comparable to the positive control gallic acid with IC50 of 3.03 µg/mL. It effectively blocked the transformation of the pre initiated murine epidermal JB 6 cells into a malignant phenotype at 4 µg/mL with 75.28% inhibition while the positive control retinoic acid and the negative control DMSO have 87.25% and 0.89% inhibitions, respectively. Furthermore, the isolate induced differentiation of HL 60 leukemia cells determined by the NSE/SE assay with 57.18% differentiation at 4 µg/mL and exhibited 43.43% NBT reduction at the same concentration. The positive control Vit. D3 exhibited 71.4% and 51.95% HL 60 differentiation determined by the NSE/SE and NBT assays, respectively. The non cytotoxic activity of the isolate as judged by the trypan blue assay suggests that terminally differentiated cells stop dividing probably due to modulation of certain signal transduction pathways leading to cell cycle arrest during the induction of cell differentiation. These results confirm the correlation observed among antioxidants, the ability to inhibit pre neoplastic JB 6 cell transformation and induction of differentiation in leukemic cells. The results also suggest that the endemic plant, Excoecaria philippnensis could be a potential source of compounds for cancer prevention.

MH016

MITOCHONDRIAL DAMAGE IN THE TRABECULAR MESHWORK OF GLAUCOMATOUS PATIENTS

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Oxidative damage to the trabecular meshwork, the tissue regulating aqueous humour outflow in the eye, is a pathogenic mechanism contributing to glaucoma, the main cause of irreversible blindness worldwide. The source of this oxidative stress still remains to be identified. Since no environmental risk factors for glaucoma is recognised, we focused our attention on mitochondria, the main endogenous source of reactive oxygen species. Mitochondrial damage was evaluated analysing the common mitochondrial DNA (mtDNA) deletion by real-time PCR in the trabecular meshwork of 79 primary open-angle glaucomatous patients and 156 unaffected controls collected at surgery. In the same samples, polymorphisms of genes encoding for antioxidant defences (GSTM1), repair of oxidative DNA-damage (OGG1), and apoptosis (FAS) were tested. MtDNA deletion was dramatically increased in trabecular meshwork of glaucomatous patients versus controls. This finding was paralleled by a decrease in the number of mitochondria per cell and by cell loss. Glaucomatous patients bearing the GSTM1 null genotype showed increased amounts of mtDNA deletion and a decreased number of mitochondria per cell as compared to GSTM1 positive subjects. Patients bearing Fas homozygous mutation showed a decreased number of mitochondria per cell only. The results obtained indicate that the mitochondrion is a target for the glaucomatous degenerative processes involved in the development of primary open-angle glaucoma. Some subjects bearing adverse genetic assets may be more susceptible.

MH017

INTERACTION OF NUCLEOTIDE EXCISION REPAIR FACTORS XPC-HR23B, XPA AND RPA WITH DAMAGED DNA

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Topic(s): DNA damage responses

One of the most versatile DNA repair mechanisms in eucaryotes is nucleotide excision repair (NER), which can remove a wide range of lesions from DNA. In particular, in mammalian cells NER is the major repair pathway to eliminate bulky DNA adducts, such as pyrimidine dimers, arising upon UV irradiation, and chemical adducts, arising upon exposure to carcinogens and some chemotherapeutic drugs. NER defects lead to severe diseases, including some forms of cancer. The interaction of NER factors XPC-HR23B, XPA, and RPA with 48-mer DNA duplexes imitating damaged DNA was investigated using photoaffinity labeling technique, footprinting and electrophoretic mobility shift assay. It was demonstrated that proteins under investigation exhibited low specificity of binding to damaged DNA compared to undamaged DNA duplex. RPA stimulated binding and crosslinking of XPC-HR23B to DNA containing photoreactive nucleotide residue, 5I-dUMP, in one strand and fluoresceine substituted analog of dUMP as a bulky lesion in the opposite strand of DNA duplex. Stimulating effect was shown to base on protein–protein interactions between RPA and XPC-HR23B. The data on localization of XPC-HR23B on damaged DNA obtained by affinity modification were in accordance with the X-ray data for Rad4 (Min and Pavletich, 2007, Nature, 449, 570-575). Using DNA duplexes containing photoreactive nucleotide residues in the certain positions either in damaged or in undamaged strands, direct evidence of preferential RPA contacts with the 5'-side of undamaged strand was provided, however efficient crosslink with damaged strand was also detected when 5I-dUMP was localized to the 5'-side from a DNA damage. The same position yielded maximum level for the XPA modification; in addition two contacts of this protein with undamaged strand were detected. When these proteins were added simultaneously we detected triple complex formation, different modification levels and better protection from nuclease digestion that could reflect some conformational changes during the complex formation. These data permit to create a scheme of RPA and XPA subunits localization on damaged DNA during damage recognition process. This work was supported by the Russian Foundation for Basic Research, project nos. 08-04-91202 and 09-04-00479.

MH018

DNA OXIDATION BY O2 AND H2O2 IN THE PRESENCE OF Co(II)- OR Fe(II)-PHTHALOCYANINE-OLIGONUCLEOTIDE CONJUGATES

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Some metal-phthalocyanine complexes are extensively used over the last decades in the photodynamic and catalytic therapies of cancer. The antitumor action of these complexes is connected with the production of ROS, which induce oxidative degradation of DNA and others molecules and lead to death of the cancer cells. The present work was aimed at the investigation of single-stranded oxidation of DNA by O2 and H2O2 in complementary complexes with Co(II)- or Fe(II)-phthalocyanine-oligonucleotide conjugates (MePcneg). In addition, the ssDNA oxidation by O2 and H2O2 in the presence of dimeric complexes of negatively and positively charged Fe(II)- and Co(II)-phthalocyanines (MePcpos•MePcneg) was investigated. These complexes were formed directly on ssDNA due to complex formation between negatively charged phthalocyanine MePcneg consisting of conjugate and positively charged Co(II)- or Fe(II)-phthalocyanine (MePcpos) in solution. We have shown that ssDNA is efficiently damaged in the presence of H2O2 and complementary conjugate MePcneg-oligonucleotide. The dimeric complexes MePcpos•MePcneg showed significant increase of catalytic activity compared with MePcneg. MePcpos•MePcneg catalyzed the DNA oxidation by O2 with high efficacy and led to direct DNA strand cleavage. In all cases the guanine residues located close to the source of the oxidizing species in the complementary complex were the most susceptible to modification. We investigated the dGMP oxidation as a model compound to provide molecular insight into the structure of guanine lesions generated by metallophthalocyanine-oligonucleotide conjugates. The dGMP oxidation by O2 catalyzed by MePc led to formation of 1,N2-glyoxal-adduct of dGMP and 7,8-dihydro-8-oxo-dGMP. The formation of 8-oxoguanine derivatives, as well as the products of sugar oxidation, could be evidence of several oxidizing species

in the reaction mixture: hydroxyl radicals and high valent metal-oxo species. The obtained results show that MePc as a reactive group within oligonucleotide conjugates are very effective towards DNA oxidation O2 and H2O2. Supported by a Grant from RFBR (08-04-00334-a), President Grant (652.2008.4), U.M.N.I.K. 8775, Grant from Russian Ministry of Education and Sciences (2.1.1/1499).

MH019

SODIUM ARSENITE ENHANCES THE EXPRESSION OF β2 INTEGRIN

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Arsenic is a ubiquitous element on earth shown genotoxic to human. Long-term exposure to arsenic is associated with an increased risk of various kinds of cancer in epidemiological studies. Besides, arsenic is identified as one of the major risk factors for an endemic peripheral vascular disease, blackfoot disease, in Taiwan. Residents in endemic areas of this disease were reported to drink high-arsenic–tainted artesian well water for more than 50 years. In this country, the long-term ingestion of high-arsenic drinking water also was linked to cardiovascular disease, such as ischemic heart disease, coronary heart disease. Arsenic-associated atherogenesis has been proposed as one of the possible mechanisms involved. Events of endothelium damage, mononoyte adhesion to endothelium and smooth muscle cell proliferation characterize the initiation of the atherosclerotic lesion. Arsenic has been demonstrated to cause damage to endothelial cells and to stimulate smooth muscle cell proliferation. Whether the arsenic may induce atherosclerosis through its interference with monocyte adhesion, however, has not been documented. We thus aimed to investigate change of the monocytic adherence under arsenic treatment to elucidate the pathogenesis of vascular effect associated with arsenic pollution. A human myeloblastic leukemia cell with promyelocytic features was used as a model here. Regulation of monocytic adhesion involves expression of β2 integrins, which share a common β subunit, CD18, and have distinct β chains, CD11a, 11b and 11c. Influence of their expression by arsenic treatment was examined. Since increasing in oxidative stress has been proposed as one of the possible mechanisms for arsenic-induced atherosclerosis, antioxidants were used to investigate the association between oxidative stress and adhesion molecule expression induced by arsenic. The enhancement of adhesion molecule expression is likely due to the activation of various signaling pathway. Members of MAPK family that are activated by dual phosphorylation on both tyrosine and threonine residues have been implicated in the transduction of a wide variety of extracellular signals. Investigations on the influence of MAPK pathways on arsenic-induced adhesion molecules expression therefore were included.

MH020

INFLUENCE OF SEX ON SUBCHRONIC TOXICITY OF ERYTHROPHLEUM SUAVEOLENS PLANT EXTRACTS IN RABBITS

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Background/Aims: Erythrophleum suaveolens is an expensive medicinal plant of African origin. It is known to be toxic to humans and other animals. Nonetheless, knowledge on its sub-chronic toxicity is limited. The research sought to investigate sub chronic toxicity of Erythrophleum suaveolens plant extract in rabbits at 2.5 and 5.0mg/kg body weight/day dose levels, and find out the influence of gender on the toxicity. Methods: The extracts were daily administered orally by gavage to male and female rabbits for 8 weeks at dose levels of 2.5 and 5.0mg/kg, while control male and female groups took water. During the test period, mortality, clinical signs and changes in body weight were recorded. Hematological and serum biochemical parameters were determined. Furthermore, necropsy and histopathological examinations were done. Results: Neither mortality nor clinical signs were observed. A general significant ($P < 0.05$) increase in body weight was noted in all groups. Hematological parameters reported significant differences ($P < 0.05$) in rabbits in high-dose groups, irrespective of sex. Biochemical parameters showed no significant differences ($P > 0.05$). However, both hematological and biochemical parameters prior and post experiment, were within reference ranges. Necropsy revealed lesions in lung, liver, kidney and brain. Histopathological examination confirmed the observed lesions and further revealed mild cellular hepatocyte infiltration in both control and test rabbits, suggesting that the lesions are probably not-extract-related. Conclusion: Based on the aforementioned findings, it is probable that 5.0mg/kg body weight/day of aqueous extract of fresh leaves of Erythrophleum suaveolens has no observable toxicity effect in rabbits irrespective of sex. Key words: sex, sub-chronic toxicity, rabbits, NOAEL, Erythrophleum suaveolens

MH021

EFFECTS OF OPIUM ADDICTION ON SOME SERUM FACTORS OF ALLOXAN-INFUCED DIABETIC RATS.

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This study was carried out to determine the effect of opium on biochemical parameters in addicted rats. Traditional opium was given orally (10mg / kg body weight) to all experimental rats except the control negative (normal health) group for 30 days. Diabetes mellitus was induced in adult male albino rats, using intra- peritoneal injection of 120 mg / kg BW. Blood glucose, serum insulin, total protein, urea, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglycerides (TGs) and total cholesterol were measured in the serum of rats. Serum total protein, ALT and AST were lower compared to non-addicted diabetic rats. Cholesterol and triglycerides tend to be lower in addicted diabetic rats. Creatinine and urea were higher in addicted diabetic rats compared to non-addicted diabetic rats. According to our results, opium increases serum insulin and decreases serum glucose but non-significantly, and thus adds to metabolic disorders in diabetic rats. These results suggest that opium reduces blood glucose in diabetic rats and the mechanism of this effect is unclear.

MH022

TOXICITY OF CHLORINATED BENZO[A]PYRENE

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Many kinds of polycyclic aromatic hydrocarbons (PAHs) are discharged from various emission sources in air environment and in aqueous environment. PAHs discharged in the atmosphere are flowed into aqueous environment through natural falling or rain water. It seems PAHs are received the changes (oxidation, reduction, decomposition, substitution, conjugation etc.) by chemical reaction in the aqueous environment, photoreaction, metabolism of the aquatic organism and various treatment processes. Especially, in the case of raw water for drinking water source, these compounds are exposed chlorine in the process for the disinfection in drinking water treatment plant and then are reacted oxidation and/or chlorine displacement. Among disinfection by products, there are many compounds causing the adverse effect to the human. It is guessed that PAHs are also similarly changed to various reaction products by chlorination in the process for the disinfection. Additionally, it is afraid the reaction products affect on human health by drinking. However, there is not much information on the reaction products of PAHs by chlorination in drinking water treatment plant. Among PAHs, benzo[a]pyrene (B[a]P) is most commonly detect-

ed in various environmental samples and might play harmful effect on human and wild lives. We have examined focusing on B[a]P. The chlorinated forms of B[a]P, mono-chlorinated B[a]P and dichlorinated B[a]P, were identified by gas chromatography with mass spectrometric detector in selected ion monitoring (SIM) mode in water sample containing B[a]P after chlorination. Now, we have not obtained the high purity standard materials of chlorinated B[a]P as a commercial product. So, mono-chlorinated B[a]P and dichlorinated B[a]P were synthesized and were identified the purity by quantitative NMR method as the standard for the evaluation of toxicity. We have examined the cytotoxicity using cultured cells for 24 hours, HepG2 cells and mouse undifferentiated embryonic stem cells. Chlorinated B[a]Ps showed the significant cytotoxicity highest in comparison with B[a]P in both cells. Both chlorinated B[a]P and dichlorinated B[a]P showed the mutagenicity at less concentration than original B[a]P both without and with metabolic activation by the umu test.

MH023

INHIBITORY EFFECTS OF CATECHINS AGAINST THE WEAK MUTAGENICITY OF (–)-EPIGALLOCATECHIN IN CHL/IU CELLS

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Anticancer and anti-mutagenic effects of tea have been receiving great attention. (–)-Epigallocatechin-3-O-gallate (EGCG), which is one of green tea catechins, plays a major role in these beneficial effects. Recently, we reported (–)-epigallocatechin (EGC) had a weak mutagenic activity in vitro. Research on an interaction between the weak mutagenic catechin (EGC) and the other tea catechins ((–)-Epicatechin-3-O-gallate (ECG), EGCG, (–)-Epicatechin (EC)) is important for risk assessments of green tea. In this study, we investigated such interaction by the chromosome aberration test and the comet assay using Chinese hamster CHL/IU cells. As a result, ECG completely prevented chromosome aberrations and DNA damage by EGC, which suggested a strong anti-mutagenic effect. EGCG, which is known for its high activity of anti-mutagenic effect, prevented DNA damage but showed no preventive effects against chromosome aberrations. EC showed no anti-mutagenic effects. Hydrogen peroxide (H2O2) was responsible for the mutagenicity of EGC because catalase, which decomposes H2O2 into water and oxygen, inhibited chromosome aberrations caused by EGC. Therefore, we mixed ECG and EGCG each with H2O2 to confirm that their mode of action is similar to catalase. ECG prevented chromosome aberrations and DNA damage by H2O2 while EGCG did not prevent chromosome aberrations. From these results we conclude that the mutagenic mechanism of EGC is a production of hydrogen peroxide of which mutagenicity is prevented by the catalase-like activity of ECG. The fact that EGCG did not show catalase-like activities indicated possible different anti-mutagenic mechanisms against the weak mutagenicity of EGC.

MH024

OXIME REACTIVATORS OF AChE INDUCE DNA DAMAGE IN HepG2 CELLS

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Oximes are nucleophilic substances that reactivate the organophosphorus ester inhibited acetylcholinesterase (AChE) by removing the organo-phosphoryl moiety and thus restoring AChE activity. They are used for treatment of poisoning with organophosphorus AChE inhibitors and are currently the only clinically available enzyme reactivators. The antidote properties of oxime reactivators are well explored, whereas the information on their toxic and genotoxic properties is very limited. Acute toxicity for rodents is relatively high (LD50 for pralidoxime (2-PAM) is 263,6 mg/kg and LD50 for obidoxime is 188,4 mg/kg), and seems to be due to their own AChE-inhibiting potency. There has been also concern regarding possible hepatotoxicity of certain oximes. We examined cytotoxicity and genotoxicity of pralidoxime and obidoxime in human hepatoma HepG2 cells. The cells were exposed to graded doses of reactivators (0.01 – 3 mM) for 24 h, and then the viability was determined with the MTT assay, and genotoxicity with the comet assay. Pralidoxime was not cytotoxic, while obidoxime reduced viability of HepG2 cells for 32 % only at highest tested concentration (3 mM). Both reactivators, at doses 0.11 – 3 mM, induced strong, dose dependent increase in DNA damage, comparable to that of benzo(a)pyrene, which was used as the positive control. In the literature survey we found no genotoxicity studies of oxime-reactivators, and we are the first who showed their genotoxic potential. This result indicates that further studies are needed to explore the mechanisms of genotoxicity of oxime-reactivators in order to assess their risk for human health particularly when they are used for prophylaxis.

MH025

EFFECT OF STATIC MAGNETIC FIELDS ON THE INDUCTION OF MICRONUCLEI IN MICE.-A POSSIBLE MECHANISM-

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We have previously found that strong static magnetic fields (SMF) itself induce micronuclei and increase the frequency of micronuclei induced by several mutagens (Mutagenesis. 2001 and Environ. Health and Prev. Med. 2006). In the present study, we investigated the effects of antioxidant chemical (ascorbic acid) on co-mutagenic effect of SMF and radical-generating chemicals and X-ray using in vivo mouse bone marrow micronucleus test. Moreover, 8-hydroxy-deoxyguanosine (8-OHdG) in bone marrow cells was determined to examine the status of oxidative stress. BALB/c mice were treated with ascorbic acid at a dose of 200 mg/kg body weight for 20 minutes before injection of doxorubicin (DOX: 6 mg/kg), mitomycin C (MMC: 0.5 mg/kg) or X-ray (0.5-6 Gy). Mice were then immediately exposed to a 5 T (tesla) SMF for 24 hours. After exposure to the SMF, bone marrow smears were stained with May-Grünwald Giemsa. The number of micronucleated polychromatic erythrocytes in 1000 polychromatic erythrocytes was counted in each animal under a light microscope. DNA from bone marrow cells was extracted by NaI method. Extracted DNA was hydrolyzed by nuclease P1 and alkaline phosphatase, and then did ultrafiltration of the hydrolyzate with Microcon YM-10 filter. 8-OHdG concentration was measured by an ELISA Kit. Ascorbic acid itself did not induce micronuclei. The frequency of micronuclei induced by DOX, MMC or X-ray was increased by co-exposure to SMFs, but these increases were inhibited by pretreatment with ascorbic acid. Moreover, 8-OHdG of bone marrow cells was increased significantly after 24 hours exposure to 5 T SMF. 8-OHdG concentration in DNA by irradiation to X-ray (0.5 and 1 Gy) increased significantly after 24 hours co-exposure to 5 T SMF. These increases of 8-OHdG concentration were suppressed by pretreatment of ascorbic acid. From these results, it is suggested that SMF exposure would have a potential to enhance micronuclei in mouse bone marrow cells by affecting on behavior of free radical species produced within cells.

MH026

UREA, THE MOST ABUNDANT COMPONENT IN URINE, CROSS-REACTS WITH A COMMERCIAL 8-HYDROXYDEOXYGUANOSINE ELISA KIT

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8-Hydroxy-2'-deoxyguanosine (8-OH-dG, also called 7,8-dihydro-8-oxo-dG) is one of the major types of oxidative DNA damage and is often analyzed as a marker of physiological and pathological oxidative stress. 8-OH-dG has been analyzed by ELISA and LC-MS-MS methods, in addition to the HPLC-ECD method. Among them, the ELISA method is most often used for urinary 8-OH-dG analysis. However, discrepancies have been observed between the data obtained by the ELISA and HPLC methods. It suggests that the monoclonal antibody for 8-OH-dG (N45.1) is not sufficiently specific for urinary 8-OH-dG detection, and may cross-react with other urinary components. If the ELISA kit recognizes urine components other than 8-OH-dG, it may yield misleading results, and interrupt scientific progress in the field of free radical biology. As an approach to clarify above problems, we fractionated a human urine sample by HPLC and examined the urine components that cause competitive inhibition in the ELISA. [Results and Discussion] In addition to the 8-OH-dG fraction, a positive reaction was observed in the first eluted fraction. The components in this fraction were examined by the ELISA. As a result, urea was found to be the responsible component in this fraction. Urea is present in high concentrations in the urine of mice, rats and humans, and its level is influenced by many factors. Therefore, certain improvements, such as a correction based on urea content or urease treatment, are required for the accurate analysis of urinary 8-OH-dG by the ELISA method. Furthermore, we found that performing the ELISA at 4 ˚C reduced the recognition of urea and improved the 8-OH-dG analysis by ELISA. However, at 4 ˚C, the ribonucleoside 8-OH-G, which has a different source from that of 8-OH-dG, cross-reacted with the ELISA in addition to urea. Therefore, further improvements are required for the accurate analysis of urinary 8-OH-dG by the ELISA method.

MH027

ANEUPLOIDY INVOLVING CHROMOSOME 1 MAY BE AN EARLY PREDICTIVE MARKER OF INTESTINAL TYPE GASTRIC CANCER.

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Intestinal type gastric cancer is still a significant cause of mortality, therefore a better understanding of its molecular basis is required. In early gastric carcinogenesis high levels of oxygen free radicals are known to drive mutation and signalling abnormalities. We assessed if either aneuploidy or NF-kB activity increased incrementally during pre-malignant gastric histological progression, if they could be useful Biomarkers and also if they correlated with each other in patient samples, as they are both induced by oxygen free radicals. In a prospective study of 54 (aneuploidy) and 59 (NF-kB) consecutive patients, aneuploidy was assessed by interphase FISH for chromosome 1 using exfoliated cells collected by brush cytology during endoscopy. NF-kB was assessed in gastric biopsies through expression of IL-8 mRNA, and in a subset, by immunohistochemistry for active NF-kB (p65 subunit). Aneuploidy levels increased incrementally across the histological series: 2.76% of exfoliated gastric cells with normal histology (95% CI, 2.14% - 3.38%) showed aneuploidy for chromosome 1, this increased to averages of 3.78% (95% CI, 3.21% – 4.35%), 5.89% (95% CI, 3.72% – 8.06%) and 7.29% (95% CI, 4.73% - 9.85%) of cells from patients with gastritis, H. pylori positive gastritis and atrophy/intestinal metaplasia respectively. In contrast, IL-8 expression was only increased in patients with current H. pylori infection. NF-kB analysis showed some increased p65 activity in all inflamed tissue types. IL-8 expression and aneuploidy level were not linked in individual patients. Aneuploidy levels increased incrementally during histological progression; were significantly elevated at very early stages of gastric neoplastic progression and could well be linked to cancer development and hence used to assess cancer risk. Oxygen free radicals induced in early gastric cancer are presumably responsible for the step wise accumulation of this particular mutation. Hence, aneuploidy measured by FISH using cells collected by brush cytology, would be worthy of consideration as a predictive marker in gastric cancer and could be clinically useful in pre-malignant disease to stratify patients by their cancer risk.

MH028

EVALUATION OF OXIDATIVE DAMAGE INDUCED BY ENVIRONMENTAL CIGARETTE SMOKE (ECS) IN DROSOPHILA

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Epidemiological evidence indicates that tobacco smoke is harmful for our health. Although many investigations document the damage induced by mainstream cigarette smoke, there is less experimental data regarding the biological effects induced by environmental cigarette smoke (ECS). In this study, we examined the effect of exposure to ECS on the viability of Drosophila using 3 wild-type strains (Oregon-R, Hikone-R and Canton-S) and two mutant strains (y v ma-l and ry1) that are sensitive to oxidative stress. Viability was expressed as the ratio of the number of adult flies coming from exposed larvae to that from unexposed larvae. When larvae were exposed to ECS for 4 h, the viabilities of mutant strains (y v ma-l and ry1) were significantly lower than those of Oregon-R and Hikone-R, and at 6 h of exposure their survivals were 35%, 47%, 73% and 78%, respectively. These mutants lack xanthine oxidase activity and are therefore urate-null. As uric acid is thought to function as an important antioxidant in Drosophila, these results suggest that ECS induces oxidative damage in larvae. We observed that oxidative damage should also occur in germ cells. When we measured the amount of uric acid in larval body fluid, the amount of uric acid in Oregon-R increased significantly and unexpectedly after 6 h of exposure. In y v ma-l and ry1 the amount of precursors of uric acid, such as xanthine and hypoxanthine, increased according to the exposure time. These results suggest that the synthesis of uric acid is stimulated during exposure to protect the larva from oxidative stress. However, the survival of Canton-S, a wild-type strain, decreased to a level similar to the urate-null mutant, despite an increased amount of uric acid. This suggests that larvae have other systems against oxidative stress that might be deficient in Canton-S. The level of glutathione is considered important for regulation of oxido-redox status in a cell. The amount of glutathione decreased by over 90% and 45% in y v ma-l and the wild-type, respectively, compared to amounts measured before exposure to ECS. This suggests that glutathione is also one of the important antioxidants in Drosophila, especially in urate-null strains.

MH029

ARSENIC LEVEL IN PROSTATE CANCER PATIENTS AND THE ATTENUATION OF ACCUMULATING MUTATIONS BY NUTRITIONALLY ESSENTIAL ELEMENTS IN PROSTATE CARCINOGENESIS

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Prostate cancer (PCa) ranks amongst the most common cancers in men. Arsenic has been implicated in experimental prostate carcinogenesis. Environmental exposure to arsenic (As) is prevalent in many industrialized and industrializing nations. Arsenic is currently one of the highest priority hazardous substances globally and well recognized for its toxicity and carcinogenicity. As with other solid tumors, accumulation of mutations and decline in DNA repair during aging may lead to PCa .The extent of involvement and mechanisms of nutritionally essential

elements is uncertain. Sixty clinically diagnosed PCa patients, mean age 65.0 ±13.0 with elevated levels of prostate specific antigen (PSA) and forty apparently healthy men with benign prostate hyperplasia (BPH), mean age 63.0 ± 10.0 exhibiting PSA levels in the normal range (0-4µg/l) were selected for the study. Plasma arsenic level (for stoichiometry) and those of zinc (Zn), copper (Cu), phosphate and the endogenous antioxidant, uric acid were determined in both patients and controls. Arsenic level in PCa patients and controls were similar ($P>0.05$). Inorganic phosphate, an antagonist of As was also similar in both patients and controls. Zinc level was significantly lower in PCa patients than in controls ($P<0.05$). Copper levels were very similar in both patients and controls. Uric acid level though lower in PCa patients was not significant. Expectedly PSA was significantly higher in the patients than in controls ($P<0.001$); over 60-fold level in controls and was not significantly correlated with As. Interestingly, Zn that was significantly lower in PCa patients was significantly positively correlated with As ($r = 0.21$; $P<0.05$) and inversely correlated with PSA but not significantly. Other correlations were not significantly different. These complex data contribute to and extend the on going debate on the involvement of arsenic in human prostate carcinogenesis. Zinc is a co-factor in proteins involved in antioxidant defenses, DNA repair and p53 protein expression. These findings may suggest an attempt by the nutritionally essential elements particularly Zn to enhance DNA repair, reduce high error rates in DNA repair and attenuate the accumulating mutations characteristic of prostate carcinogenesis.

MH030

COMPARATIVE STUDY OF BETA-CAROTENE AND BETA-CAROTENE MICROENCAPSULATED. EVALUATION OF THEIR CYTOTOXIC, MUTAGENIC AND ANTIMUTAGENIC EFFECTS

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The great number of food colorants available, both natural and synthetic, has led researches to assess the mutagenic and antimutagenic of these compounds. Many natural colorants have antimutagenic potential in at least one test system, but this does not mean that natural colorants are innocuous. In this context, beta-carotene microencapsuled (BCM) became a very useful colorant, due to its antioxidant functions. Therefore, the present study was undertaken to investigate the cytotoxicity, mutagenicity, and antimutagenicity of beta-carotene pure (BC) and BCM by a spray drier in Wistar rat bone marrow cells, evaluated by micronuclei test. The treatment was performed with two doses of carotenoid (2.5 or 5 mg/kg b.w.) pure or microencapsulated, by gavage for 14 days; followed by application of saline or the antitumoral doxorubicin (DXR, 16 mg/Kg b.w.), used as a mutagenic agent, just after the last gavage, intraperitoneally. The animals were divided in 14 groups (n=6/group), and were sacrificed 24 h after the intraperitoneal injection. The results obtained showed lack of cytotoxic or mutagenic effects in the animals treated with this carotenoid, except BC that induced micronucleus in bone marrow cells at the highest dose, in comparison with negative control ($p<0.05$). Animals treated with a single dose of DXR presented a statistically significant increase in the frequency of micronucleus when compared to negative control ($p<0.05$). The results indicate that the association between BC or BCM with DXR could be effective in protecting against DXR-induced micronucleus formation in animals that received the carotenoid. Furthermore, the association between BCM and DXR was no more effective than BC and DXR in the inhibition against chromosome damage induced by DXR. The decrease in the micronucleus formation can be explained by the antioxidant properties of this carotenoid. Nevertheless, when a higher dose is administrated, the cleavage products of beta-carotene could increase the reactive oxygen species, potentializing the micronucleus formation. Due to insufficient published data concerning the effects of microencapsulated carotenoids on the cellular mechanism, more studies must be done to evaluate the kinetics of this compound.
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MH031

GENETIC MARKERS OF SUSCEPTIBILITY INVOLVED IN PROSTATE CANCER

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The prostate cancer is the second cause of death in Brazilian men. One relevant factor for this disease is the presence of allelic variants in genes involved with prostate cancer development**.** Polymorphisms in the genes belonging to the androgen pathway, as the Androgen Receptor (*AR*) and Prostate Specific Antigen (*PSA*), DNA repair pathway (*APEX1* and *XRCC1*) and metabolism of drugs (*CYP1A1, CYP1B1, CYP3A4, GSTM1* and *GSTT1*) are particularly important because they may contribute to a higher risk of cancer and tumor progression. The aim of this study was to analyze the impact of polymorphisms in these genes in relation to prostate cancer risk. Blood samples from 177 patients and 177 matched controls were obtained for DNA analysis and all genotypes were evaluated by based PCR methods. Beyond the blood sample, tumors from 60 patients were genotyped by a fragment analysis on an automated DNA sequencer to verify the length of the CAG repeats of *AR* gene. The polymorphisms were correlated with clinical and pathological parameters to examine its relation with aggressiveness. The statistical tests used were OR (Odds Ratio CI = 95%), Chi-square and ANOVA. The variants of the genes *APEX1 (G/G)* (OR=1.68, CI95% 1.1-2.58) and *PSA(G/G)* (OR=1.75, CI95% 1.05-2.92) increased the risk for the development of prostate cancer; the same was found for the short CAG of *AR* gene (OR=1.89, CI95% 1.21-2.96). The results obtained to the variants of *CYP1B1(T/T)* (OR=1.55, IC 95% 0.98-2.47) and *CYP3A4(G/G)* (OR=1.51, IC95% 0.97-2.37) were in the limit of the statistical significance. The combined analysis of the genes *PSA* and *AR* showed a double risk for the neoplasia (OR=2.02, CI 95% 1.07-3.82), as well as the combined genotypes of variants *CYP1A1 (G/G)*, *CYP1B1(T/T)* and *CYP3A4(G/G)* (OR=2.55, IC95% 1.08-5.99). Furthermore, the short repeats of CAG (OR=8.8, IC95% 1.06-73.05), the genotype *G/G* of *PSA* gene (OR=2.54, IC95% 1.27-5.07) and the variant *CYP1A1(G/G)* (p=0.038) were related with high tumoral aggressiveness. The tumoral analysis showed that 38.3% of the samples had instability of microsatellites. In conclusion, our results shown that polymorphisms in the genes *AR, PSA, APEX1, CYP1B1, CYP3A4,* and *CYP1A1* may be indicated as potential markers of susceptibility and prognostic for prostate cancer.
Financial Support: Fundação Araucária, CAPES-DS and CNPq-PQ

MH032

N – NITROSODIETHYLAMINE (NDEA) GENOTOXICITY EVALUATION: A CYTOCHROME P450 INDUCTION STUDY IN RAT HEPATOCYTES

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Hepatocytes are the most suitable model to investigate CYP induction and their relation with drug metabolism. In rats N-nitrosodiethylamine (NDEA) induces tumours mainly in the liver, due to different enzymes responsible for its metabolic activation. Our goal was to correlate the NDEA genotoxicity (micronuclei and mitotic index) and cytotoxicity approaches (survival, apoptosis and necrosis rates) in primary cultures

of female rat hepatocytes with different CYPs mRNA expression. DNA fragmentation is a marker for genotoxic effects confirmed by micronuclei assay and chromosomal aberration. Single incubation with NaCl 0.9% used as negative control resulted in apoptotic cells of 0.2 % and twice in the presence of NDEA, at 105 µg/mL. The number of necrosis was twice of the control. Beside the cytotoxic effects, a decrease (4 times) on mitotic index and percentage of micronuclei cells (3 times) was observed. A significant increase on MN cells was detected when hepatocytes were incubated in the presence of NDEA at 2.1 µg/mL. Chromosomal aberrations revealed no significant differences on DNA damage induced by NDEA, although it was observed a discrete dose-response curve. The NDEA treatment induced an alteration on CYPs mRNA: an increase on CYP2B2 (1.8 times) and CYP2E1 (1.6 times) for no cytotoxic NDEA concentrations (0.21-21 µg/mL); for CYP2B1 it was detected an increase on mRNA levels only at 0.21 µg/mL (2.5 times) and 1.3 times for CYP4A3; and an increase of mRNA CYP3A1 (1.9 times), at 2.1 µg/mL. Discrimination of various cumulative effects in target cells during carcinogenesis is crucial to understand the mode of action of potential carcinogens and develop comprehensive chemicals toxicity profiles. The metabolic activation can lead to the local formation of DNA adducts, impairment of bases and DNA breaks . The increase in MN induction became close to the mitotic index suggesting that the DNA repair can be inactive. NDEA besides being metabolized to reactive electrophiles, can generate reactive oxygen species. The uncoupling of electron transfer and oxygen reduction from monooxygenation by CYP2B1, CYP2B2 and CYP2E1 could result in the release of O2•– and H2O2 and consequently the increase of DNA damage. Support: OEAD, CAPES, CNPq, Faperj, SR2-Uerj.

MH033

N – NITROSODIETHYLAMINE (NDEA) GENOTOXICITY EVALUATION: PHENOBARBITAL CYTOCHROME P450 INDUCTION STUDY IN RAT HEPATOCYTES

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Nitrosamines are a versatile group of carcinogens that require metabolic activation through P450-catalyzed a-hydroxylation, generating unstable metabolites. Hepatocytes are the most suitable model to investigate CYP induction and their relation with drug metabolism. Our goal was to correlate the *N*-nitrosodietylamine (NDEA) genotoxicity (micronuclei and mitotic index) and citotoxicity approaches (survival, apoptosis and necroses rates) in primary cultures of female rat hepatocytes in the presence of CYPs inducers, as phenobarbital. The effect of PB in the presence of NDEA increase the number of cell death about 2 times for necrosis and 8 times for apoptosis induction beside a decrease in the number of micronuclei cells and the mitotic index. The levels of chromosomal breakage of NaCl 0.9% W/o and PB groups were similar but when NDEA were administered after PB, however, the level of chromosomal breakage was elevated. The NDEA treatment, without inducers (W/o), induced an alteration on CYPs mRNA as an increase on CYP2B2 (1.8 times) and CYP2E1 (1.6 times) for no cytotoxic NDEA concentrations (0.21-21 µg/mL). For CYP2B1 it was detected an increase on mRNA levels only at 0.21 µg/mL (2.5 times) and 1.3 times for CYP4A3. NDEA treatment induces the increase of mRNA CYP3A1 1.9 times, at 2.1 µg/mL. Only PB treatment induces an increase on CYP2B1 (8 times) and CYP2B2 (2.3 times) mRNA. NDEA concentration in the presence of PB does not interfere on CYPs RNAm analysed. Our data demonstrated that the hepatocytes treated with NDEA and PB do not present xenobiotic-induced suppression of apoptosis suggesting an intrinsic mechanism of cells removal with DNA damage. Support: OEAD, CAPES, CNPq, Faperj, SR2-Uerj.

MH034

EVALUATION OF GENOTOXICITY OF TWO EXTRACTS OF THE PLANTS FROM INDIGOFERA GENERA AND OF THE ISATIN, A COMPONENT OF THESE EXTRACTS IN MICE CELLS

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Among the several species of Indigofera, the I. suffruticosa Mill. and the I. truxillensis Kunth have a wide distribution and their isolated compounds showed remarkable biological activities. Isatin, one of the substances that can be isolated from these two species, exhibits different activities, such as anticholinesterasic, antiinflammatory, antihypertensive and antimicrobial. The objective of this study was to evaluate in vivo the genotoxic and mutagenic effects of the methanolic and chloroformic extract from I. suffruticosa and I. truxillensis and also the alcaloid bis-indolic isatin, precursor of others alcaloids in these plants. The extracts were investigated in three concentrations: 200, 400 and 540mg/kg b.w. For the isatin, only the mutagenic test was performed and the doses used were: 50, 100 and 150 mg/kg b.w. It were used as negative and positive controls animals that received, respectively, water and cyclophosphamide (40mg/kg bw) or doxorubicin (16mg/kg b.w.). The blood was collected from the animals 4 and 24 hours after the treatment for the comet assay and 30 hours for the micronucleus assay. For the cytological analysis, 100 nucleoids were scored in comet assay and 1000 reticulocytes were analyzed for the micronucleus assay. The ANOVA and Tukey-Kramer test were performed to compare the results obtained between the different groups. The average values of the scores obtained after 4 hours from the treatment only with the methanolic extract of I. suffruticosa (200 and 400 mg/kg b.w.) were statistically different from the negative control, indicating genotoxic activity. For the micronucleus test it was found negative results for all groups with I. suffruticosa and I. truxillensis. Negative results also were found for the micronucleus test with animals treated with isatin, precursor of different compounds in the species studied. The results of this study indicated a moderate genotoxicity to the methanolic extract of I. suffruticosa indicating that whereas the extracts of I. truxillenses and isatin at the concentration assessed showed to be safe for human use, the methanolic extract of I. suffrutticosa needs more studies before the use as phytomedicine. Financial Support: Programa Biota-FAPESP; CNPq/PIBIC-PQ; PROIC/UEL; CAPES/DS

MH035

POLYMORPHISM OF THE ANTIOXIDANT GENE CAT IN LARYNGEAL CANCER

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Laryngeal cancer (LC) is strongly associated with tobacco smoke, alcohol and other possible exogenous risk factors. Cigarette smoke is one of the major risk sources of free radicals and oxidative stress (OS). OS is believed to play an important role in the pathogenesis of considerable number of complex diseases, including LC. The antioxidant enzymes catalase (CAT) is one of the prominent components of cell defense against OS, and polymorphisms in the genes which regulate their expression may contribute to differences in susceptibility of individuals to oxidative damage caused by reactive oxygen species. CAT reacts very efficiently with H_2O_2 and converts it to water and molecular oxygen. There is a common polymorphism in the promoter region of the *CAT* gene consists of C to T substitution.

Because of OS is one of the important mechanisms in carcinogenesis and importance of biological role of the CAT enzyme against ROS, we have investigated association of this gene polymorphism and laryngeal cancer development. *CAT* C(-262)T polymorphism was analysed in 97 larynx cancer cases and 104 healthy individuals using PCR-RFLP technique. The CC genotype was found in 63 (60.5%), the heterozygous CT genotype in 35 (33.7%), and there were 6 (5.8%,) individuals homozygous for mutant T-allele in the control group. The frequencies of the *CAT* CC, CT, and TT genotypes were 56.7, 39.2, and 4.1% respectively, in the cases. There was not a significant association in variant alleles of *CAT* (OR= 1.173, 95% CI 0.670-2.054, p= 0.577). Our findings suggest that the *CAT* C(-262)T variant allele may not play a major role in the etiology of laryngeal cancer.

MH036

ACTIVATION OF LCK AND JNK IS CRITICAL FOR THE IONIZING RADIATION-INDUCED BRAIN CANCER STEM-LIKE CELL POPULATION

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Ionizing radiation is the most effective treatment for malignant brain cancer. Brain cancer presents frequently recur or progress after radiation treatment as focal masses, indicating that a crucial subcellular population of radiation resistant tumor cells with potent tumorigenic activity is responsible for re-growth. However, the mechanisms underlying repopulation of tumor cells after radiation have remained unclear. In this study, we show that ionizing radiation enhances cancer stem-like cell (CSLC) populations in human brain cancer cells. Positive populations for CD133, Nestin and Musashi-1 possessing tumor-initiating potential were expanded after radiation in human brain tumor cells. Ionizing radiation also induced a marked activation of LCK tyrosine kinase and JNK activation. Inhibition of LCK or JNK with specific siRNA resulted in suppression of tumorigenic potential in cells treated with radiation. Moreover, inhibition of LCK or JNK led to a decrease in expressions of CD133, Nestin and Musashi. The data we elucidated in this study indicate that radiation enhances CSLC populations in human brain tumor cells through activation of LCK and JNK signaling pathway, and may provide pivotal points for therapeutic intervention in brain cancer treatment with ionizing radiation.

MH037

INDUCTION OF CHROMOSOMAL ABERRATIONS BY CARBON NANOTUBES AND TITANIUM DIOXIDE NANOPARTICLES IN HUMAN LYMPHOCYTES IN VITRO

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Interest in nanotechnology has recently grown tremendously, and the number of applications and marketed products utilizing nanomaterials has greatly increased. Although some information exists on the genotoxicity of nanomaterials, few data are available about their effects on chromosomal aberrations (CAs). The CA assay is important in regulatory genotoxicity testing, but it is unclear how well the existing test guidelines are applicable to insoluble nanomaterials. The aim of the present study was to examine if three commercially available nanomaterials - singlewall carbon nanotubes (SWCNTs; length 1-5 um, outer diameter <2 nm; SES Research), multiwall carbon nanotubes (MWCNTs; length 1-2 um, outer diameter 10-30 nm; SES Research), and nanosized titanium (IV) oxide anatase (99.7%, <25 nm) - can induce CAs in cultures of isolated human lymphocytes. To find a suitable sampling time, the cells were treated with various doses (6.25-300 ug/ml) of the nanomaterials for 24, 48 and 72 h, starting 24 h after culture initiation. The 48-h treatment was the most effective, inducing a significant increase in both chromatid-type CAs (SWCNTs and anatase) and chromosome-type CAs (SWCNTs and MWCNTs) at the highest doses, with clear linear dose-responses. Also the 72-h treatment yielded similar results with SWCNTs, but MWCNTs and anatase were negative. In the 24-h treatment, SWCNTs had no influence on CAs, and anatase and MWCNTs showed only isolated positive effects without a dose-response. Our results suggest that SWCNTs, MWCNTs, and TiO2 anatase are clastogenic in human lymphocytes in vitro after a continuous treatment for 48 h. (Supported by NMP4-CT-2006-032777)

MH038

COMPARISON OF MN FREQUENCIES IN MOTHERS VERSUS NEWBORNS: RESULTS FROM THE RHEA COHORT IN CRETE

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Maternal exposure to food and/or environmental contaminants leading to in utero exposure might contribute a an increased risk of cancer and immune disorders in childhood. The Newborns and Genotoxic Exposure risks project (NewGeneris), an integrated project within the European 6th framework programme focussing on food quality and safety, aims at investigating the role of early-life exposure to dietary compounds in the occurrence of early health effects. Within NewGeneris, our objective is to compare the genotoxic responses in mothers versus children, using the micronucleus (MN) assay as a validated biomarker for early genetic effects. Therefore we first developed an automated image analysis system for scoring of MN and an adapted protocol for slide preparation. MN are analysed in peripheral blood samples from mothers and umbilical cord blood samples from newborns, collected in different European mother-child cohorts. In addition, since it is known that deficiencies in folate and vitamine B12 can contribute to an increased chromosomal damage, plasma vitamine B12 and folate stores in erythrocytes are measured in mothers and newborns, and correlated with MN frequencies. Here we present the results obtained from 150 mother-child pairs for the Rhea cohort in Crete. Frequencies of MN in binucleated (‰ MNCB) and mononucleated cells (‰ MNMONO), and cytokinesis-bock proliferation index (CBPI) were assessed and are compared between mothers and newborns. Furthermore MN data are correlated with exposure data, folate and vitamine B12 concentrations. Future perspectives include the analysis of MN frequencies in other European mother-child cohorts, allowing comparison of genotoxic responses between mothers and children and between children from different countries with similar or different diet and environment. These data will provide information to define the positive predictive value of MN frequencies in newborns and children for a given disease. Acknowledgements: This work was financed by the EU Integrated Project NewGeneris, 6th Framework Programme, Priority 5: Food Quality and Safety (Contract no. FOOD-CT-2005-016320). NewGeneris is the acronym of the project 'Newborns and Genotoxic exposure risks' http://www.newgeneris.org

MH039

TRANSGENIC MICE FOR HUMAN SULFOTRANSFERASES WITH POLYMORPHIC SULT1A GENES

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Sulfotransferases (SULTs) conjugate nucleophilic groups of small

endogenous compounds and xenobiotics with a negatively charged sulfo moiety, thereby facilitating the excretion of these compounds. However, the sulfate group is a good leaving group in certain chemical linkages. Cations formed by this cleavage can react with DNA and other cellular nucleophiles. Therefore, a large number of food-borne compounds and environmental contaminants can be activated by SULTs to mutagens. Several epidemiological studies showed associations between the SULT1A1 genotype and the incidence of various neoplasias and non-neoplastic chronic diseases. In order to develop an experimental in vivo model for this polymorphism, transgenic mouse lines for the human SULT1A1*1 and SULT1A1*2 alleles and the SULT1A1*1/SULT1A2*1 gene cluster were created using large genomic DNA constructs. In this model the expression of the transgenes is regulated by their genuine human promoter sequences. One transgenic mouse line for each single construct and four transgenic mouse lines for the double construct were obtained. Complete integration of human genes in mouse genome was confirmed by genotyping. Varying copy numbers of the SULT1A1*1/SULT1A2*1 transgene in different mouse lines could be detected. They may be used as models for copy number polymorphisms that have been observed for SULT1A1 in humans. Expression and tissue distribution of human SULT1A1 protein in transgenic mice was similar to that in humans. After oral application of 90 mg/kg body weight of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), a heterocyclic amine that is formed during heating of meat and fish, higher DNA adduct levels in the tissues of transgenic mice compared to wildtype mice were found. To realize truly humanized mouse models, the transgenic lines are being crossbred with a knock-out line for the murine Sult1a1 gene.

MH040

IN VIVO MUTAGENICITY OF STRUCTURAL ISOMERS 2,4-DAT AND 2,6-DAT IN THE TARGET ORGAN FOR CARCINOGENICITY IN F344 GPT DELTA TRANSGENIC RAT

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To evaluate not only sensitivity but also specificity of in vivo mutagenicity test, it is important to perform a study in a target organ for carcinogenicity using carcinogenic and noncarcinogenic substances. The aromatic amines 2,4-diaminotoluene (2,4-DAT) and 2,6-diaminotoluene (2,6-DAT) are structural isomers that are genotoxic in the Ames/Salmonella assay. It is reported that 2,4-DAT induced liver tumors in rat, whereas 2,6-DAT was found not to be carcinogenic. In this study, to determine whether the results of in vivo mutagenicity test correspond to rodent carcinogenicity test, we investigated the mutation frequencies in male F344 gpt delta rats fed 125, 250 and 500 ppm 2,4-DAT and 500 ppm 2,6-DAT for 13 weeks. A feature of gpt delta transgenic rat is the incorporation of two different positive selection methods: gpt assay (6-thioguanine selection) using the gpt gene of E. coli mainly detects point mutations such as base substitutions and frameshifts. Spi- assay using the red/gam genes of lambda phage detects deletions including frameshifts. The gpt mutations were significantly induced in the liver of 2,4-DAT treated groups in a dose-dependent manner, but not induced by 2,6-DAT. The gpt mutant frequencies were at the background level in the kidney in any group. Sequencing analysis of the gpt mutants recovered from the liver indicated that the predominant base substitutions induced by 2,4-DAT were G:C to A:T transitions, G:C to T:A and G:C to C:G transversions. The Spi- mutant frequency in the liver was significantly increased in 250 and 500 ppm 2,4-DAT treated groups, but not in 2,6-DAT treated group. PCR analysis indicated that 2,4-DAT preferentially induce small deletions such as frameshifts rather than larger deletions. The results showed that in vivo mutagenicity of 2,4-DAT was observed in the target organ of carcinogenicity in rats. The difference in the mutagenicity of 2,4-DAT and 2,6-DAT in vivo and in vitro might be due to metabolism and detoxification system. It suggests that in vivo genotoxicity studies with gpt delta rat models are useful to assess mutagenicity and carcinogenicity.

MH041

GENOTOXIC EFFECTS OF SINGLEWALL AND MULTIWALL CARBON NANOTUBES IN HUMAN BRONCHIAL EPITHELIAL CELLS AND MESOTHELIAL CELLS IN VITRO

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Carbon nanotubes have shown toxic potential in various assays in vitro, but data on their possible genotoxicity are sparse. Here, we examined whether short singlewall carbon nanotubes (SWCNTs; <2 nm, 1-5 um) and multiwall carbon nanotubes (MWCNTs; 10-30 nm, 1-2 um) induce DNA damage in human bronchial epithelial BEAS-2B cells and mesothelial MeT-5A cells and micronuclei (MN) in BEAS-2B cells. Single cell gel electrophoresis (comet) assay was applied to study DNA damage (24-h and 48-h treatments), and the induction of MN was examined by the cytokinesis-block method (48-h and 72-h treatments). In the first series of experiments (doses 5-80 ug/cm²), both SWCNTs and MWCNTs induced DNA damage in MeT-5A cells in a dose-dependent manner, but only SWCNTs elevated DNA damage in BEAS-2B cells. An increase in MN in BEAS-2B cells was seen with the highest dose of SWCNTs. Similar results were obtained in the second series (doses 80-200 ug/cm²), with a dose-dependent increase in DNA damage in MET-5A cells by both SWCNTs and MWCNTs after both treatment times. SWCNTs increased DNA damage also in BEAS-2B cells, after both the 24-h and 48-h treatments, with a dose-dependent effect in the longer treatment. No induction of MN was seen in BEAS-2B cells by either of the carbon nanotubes. Our results show that short SWCNTs and MWCNTs induce DNA damage in human mesothelial cells, while only SWCNTs damage DNA in human bronchial epithelial cells. Neither of the materials shows a clear MN induction in epithelial cells. [Supported by NMP4-CT-2006-032777]

MH042

MECHANISM OF MICRONUCLEUS INDUCTION BY NANOSIZED TITANIUM DIOXIDE ANATASE IN HUMAN LYMPHOCYTES IN VITRO

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We have previously observed that nanosized titanium dioxide (TiO2) anatase induces micronuclei (MN) in cultured human cells. MN could arise from chromosomal fragments either through direct interaction with DNA, which requires that nanoparticles reach the nucleus, or indirectly via, e.g., oxidative stress. In addition, MN may be formed from whole chromosomes through an effect on the mitotic apparatus. We used the cytokinesis-block micronucleus assay and pan-centromeric and pan-telomeric fluorescence in situ hybridization, to examine what types of MN TiO2 induces and if this induction depends on possibility to reach the nucleus during mitosis when the nuclear envelope disappears. 72-h cultures of isolated lymphocytes from two female donors were treated for 48 h with 12.5, 25, or 50 µg/ml of nano-anatase (<25 nm). Binucleate lymphocytes with fragment-containing (centromere-negative) MN were increased at all TiO2 concentrations, when cytochalasin-B was added simultaneously with TiO2 at 28 h, but not when cytochalasin-B was added at 52 h. The latter schedule allowed lymphocytes to divide before cytokinesis block. Thus, the formation of fragment-containing MN did not require a preceding mitosis but seemed to occur during the first 24 h of the treatment. Centromere-positive MN with whole chromosomes increased regardless of the cytochalasin-B schedule at one concentration of TiO2 in both series. In conclusion, TiO2 appeared to induce MN by both clastogenic and aneugenic mechanisms, the fragment-containing MN apparently being formed during the first in vitro cell cycle. [Supported by NANOSH, NMP4-CT-2006-032777]

MH043

GENOTOXIC DAMAGE AND SUSCEPTIBILITY EVALUATION IN PATHOLOGY ANATOMY LABORATORY WORKERS

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Formaldehyde (FA) is an economically important chemical to which approximately 900,000 European workers are occupationally exposed. Classified by IARC, International Agency for Research on Cancer, as a human carcinogen, FA is a chemical traditionally used in pathology/anatomy laboratories as a tissue preservative. Biological evidence of FA-toxicity on distant-site such as peripheral lymphocytes and bone marrow is insufficient and conflicting, thus remains to be more documented. To evaluate the genotoxic effect of long-term occupational exposure to FA a group of 30 pathology/anatomy workers was tested for a variety of biological endpoints, including cytogenetic tests by means of micronuclei (MN), sister chromatid exchange (SCE), and comet assay (comet tail length, TL). In addition, the frequency of polymorphic genes of xenobiotic metabolising enzymes (GSTM1, GSTT1) and DNA repair enzymes (ERCC1, ERCC4, ERCC5-2, ERCC5-5) were also studied. A non-exposed group (n=30) from the same area and with same demographic characteristics was also studied and data obtained from both groups were compared. Controls did not differ from exposed workers in gender, age and smoking habits. Air sampling was performed in worker's breathing zone for representative working periods, and time weighted average (TWA) level of exposure for each exposed subject assessed. The TWA mean value for FA exposed workers was 0.44 ± 0.08 ppm. Both MN and SCE frequencies were significantly higher in the exposed subjects when compared with controls. Comet assay data showed significantly increase of TL in FA-exposed workers with respect to the control group. Gender, age and smoking habits did not have a significant effect on the frequencies of studied biomarkers. A positive correlation was found between TWA values and MN frequency (r=0.384, p=0.001) and TL (r=0. 333, p=0.005). Such results along with the recent implications of human carcinogenicity, point out for the need of close monitoring of FA exposures. Implementation of security and hygiene measures in this sector as well as good practices campaigns may be crucial to decrease risk. FCT (SFRH/BD/46929/2008) and Fundação Calouste Gulbenkian (Grant 76436) supports our current research.

MH044

EVALUATION OF GENOTOXIC AND ANTIGENOTOXIC ACTIVITIES OF Arrabidaea chica

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The aim of this study was to evaluate the genotoxic/antigenotoxic and mutagenic activities of crude extract and fractions of the plant *Arrabidaea chica* (Bignoniaceae) in mice. This plant is used in folk medicine due to its anti-inflammatory and astringent properties. Crude extract (CE) and aqueous (AF), chloroformic (CRF) and butanolic (BF) fractions from the aerial parts of this plant were prepared. CF-1 mice were divided in 7 groups with 5 males and 5 females. The negative control group received NaCl 0.9% solution; three groups received 500, 1000 or 2000mg/kg of the CE; e others three groups received 1000mg/kg of AF, CRF or BF. The administrations were made by gavage for three days and the animals were sacrificed on the 4th day. Samples of peripheral blood were collected 3, 24 and 72h after the first administration to assess genotoxic activities, using the alkaline comet assay (pH ³ 13); for the evaluation of the antigenotoxic activity the cells were treated *ex vivo* with H_2O_2 (0.25 mM). The results were expressed as damage index (DI) and damage frequency (DF). At 72h, bone marrow was collected for micronuclei analysis (in 2000 PCE per animal) and of the PCE/NCE ratio. The data were analyzed by ANOVA/Dunnett's test. CE and fractions did not induce DNA damages 3, 24 or 72h after the administrations in any of the experimental groups. However, there was a significant reduction in DI and DF in blood of male mice treated with CE, suggesting protection against induced oxidative damages by H_2O_2, reaching a percentage of inhibition of DI (I%) 83.7% at the highest dose. Similar results were observed in samples of females treated with CE (66.6%). Moreover, there was a reduction in DI and DF in blood samples of males treated with CRF (79.2%); however, this fraction did not have any effect in the females. The fractions AF and BF did not decrease DI nor DF. A similar PCE/NCE ratio was detected in all groups, indicating no toxicity in bone marrow of the animals. The micronucleus frequency was not altered by the treatment with CE or fractions, with values similar to those of the negative control group. In conclusion, no extracts presented mutagenic or genotoxic effects. CE showed antigenotoxic effect in a dose-dependent way. Support: FAPERGS and CNPq.

MH045

INFLUENCE OF GENETIC POLYMORPHISMS ON BIOMARKERS OF EXPOSURE AND GENOTOXIC EFFECTS IN STYRENE-EXPOSED WORKERS

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Styrene is a commercially important chemical widely used in the manufacture of resins, polyesters, and plastics. The highest levels of human exposure to styrene occur in occupational settings, especially during the production of reinforced plastic products, which involve manual lay-up or spray-up operations. The objective of this work was to study occupational exposure to styrene in a multistage approach, in order to integrate the following end-points studied: styrene in workplace air, mandelic and phenylglyoxylic acids (MA+PGA) in urine, haemoglobin (Hb) adducts, sister-chromatid exchanges (SCE), micronuclei (MN), DNA damage (comet assay) and genotypes of polymorphic genes of some metabolising enzymes. Seventy-five workers from a fibreglass-reinforced plastics factory and seventy-seven unexposed controls took part in the study. The mean air concentration of styrene in the breathing zone of workers (30.4ppm) was higher than the threshold limit value of 20ppm recommended by the ACGIH, and the biological exposure index adopted by the ACGIH for exposure to styrene prior to the next shift (MA+PGA=400mg/g cr) was exceeded, indicating that styrene exposure for this group of workers was higher than recommended. The level of Hb adducts and SCE in exposed workers were significantly increased as compared with controls. The DNA damage was higher among styrene-exposed workers than in controls. No significant differences were observed in the MN. Concerning the effect of the genetic polymorphisms on the different exposure and effect biomarkers studied, we observed the effect of microsomal epoxide hydrolase activity on Hb adducts of highly exposed individuals and on the levels of SCE of exposed workers. The present results suggest the importance of individual susceptibility factors in modulating genotoxicity, although cautious interpretations are required since the size of the study population limits the power of many of the analyses. Because the effects of these polymorphisms are relatively subtle, and some important alleles are relatively rare, a much larger study population will be necessary to evaluate their effects on biomarkers, especially when gene-gene interactions are considered. Fundação Calouste Gulbenkian (Grant 76436) supports our current research.

MH046

POSSIBLE ROLE OF ENVIRONMENTAL MUTAGENS ON UNEXPLAINED MALE INFERTILITY IN IRAN: A CASE CONTROL STUDY ON AHR, ANGIOGENIC MARKERS, SEX STEROID RECEPTORS AND GLYCODELINE

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Background/Aims: Infertility is one of the major health problems which affect more than 15% of couples by unknown mechanisms. Approximately one-third of unexplained human infertility has been attributed to adverse environmental factors, including exposure to hazardous organohalogen pollutants such as dioxins and PAHs. The biological effects of dioxins result from their binding to aryl hydrocarbon receptor (AhR) and subsequent activation of the AhR-mediated signal transduction pathway. Roles of mentioned toxicants on male unexplained infertility phenomenon as well as their role on angiogenic pathways, Glycodelin and sex steroid receptors remained unclear which were considered as the major goals of present study. Methods: Direct role of PAH exposure on testicular levels of Aryl hydrocarbon receptor (AhR), its interactions with sex steroid Receptors (ER, PgR, AR), MMP9 and VEGF as two major biomarkers of angiogenesis and Glycodeline were studied in mice after subchronic oral regimen with AhR ligands. Tissue levels of mentioned proteins in a clinical case-control study were studied in 30 idiopathic and 30 fertile testicular tissue specimens using immunohistochemal analysis. Results: Higher tissue levels of AhR were found in leydig, sertoli, and spermatid cells of PAH exposed mice but other cells showed no significant changes after PAH exposure. A similar pattern was found in infertile males. Significant association between AhR overexpression and MMP9 expression in leyidig and sertoli cells was one of the major findings of present study which could be interpreted as possible mechanisms of unexplained male infertility .ER overexpression, lack of AR expression in leydig cells and significant association between ER and PgR in leydig cells are other important findings of present research. Conclusion: Similar expression patterns of AhR in PAH exposed mice and unexplained infertile clinical cases suggest the possible role of environmental mutagens on male infertility .AhR overexpression as a biomarker of exposure may cause some critical changes on angiogenic pathways, Glycodeline and testicular levels of sex steroid receptors.

MH047

NICOTINE HAS NO EFFECT ON CELL CYCLE OR PROLIFERATION IN H1975 LUNG CANCER CELLS DESPITE OF INCREASED ERK1/2 PHOSPHORYLATION

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Nicotine may be involved in carcinogenesis by activating nicotinic acetylcholine receptors (nAChR) in non-neuronal cells, e.g. epithelial and endothelial cells. Nicotine-induced carcinogenesis-related processes include promotion of cell proliferation, angiogenesis and metastasis, and inhibition of DNA damage –induced apoptosis. Therefore, nicotine may act as a cancer promoter and also reduce the efficacy of chemotherapy. Recently, it has been also shown that polymorphisms in nAChR are risk factors for lung cancer. The aim of this study was to find out whether mitogen-activated protein kinase (MAPK) pathway is involved in putative nicotine-induced cell proliferation in human H1975 epithelial cell line originating from non-small cell lung cancer. As a marker for the activation of MAPK pathway, effects of nicotine (1 and 10 μM) on phosphorylation of extracellular signal-regulated kinase (ERK1/2) were studied at several time points (30 min, 6 h and 24 h) by immunoblotting. Cells exposed to epidermal growth factor (EGF; 100 ng/ml) were used as a positive control. According to immunoblots, nicotine increased phosphorylation of ERK1/2 at Thr-202/Tyr-204 most clearly at 24 h. In the three experiments carried out so far, the increase of ERK1/2 phosphorylation (normalized against the amount of total ERK1/2 protein) was consistent although not statistically significant. In addition, nicotine treatment for 30 min activated epidermal growth factor receptor (EGFR) as shown by nicotine-induced phosphorylation of EGFR at Tyr-1068. Nicotine exposure for 24 or 48 h, however, had no clear effect on cell cycle or proliferation in H1975 cells as analysed by propidium iodide staining and bromodeoxyuridine (BrdU) incorporation assay using flow cytometry. Likewise, nicotine had no clear effect on cell cycle or proliferation in other studied lung cancer cell lines, i.e. A549, H358 and H1650 cells. As a conclusion, our results suggest that although nicotine has no clear effects on cell cycle or cell proliferation at the studied time points, nicotine has the potential to activate EGFR and MAPK pathway.

MH048

ANTI-GENOTOXICITY OF NATURAL OPCs NEWLY EXTRACTED FROM THAI RED GRAPE SEEDS BY COMET ASSAY AND MICRONUCLEUS TEST IN TK6 CELLS

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Background**:** In 2008, Thailand Institute of Scientific and Technological Research (TISTR) was firstly isolated the natural oligomeric proanthocyanidins (OPCs) from Thai red grape seeds. A large number of publications reported that OPCs have antioxidant potential to protect cellular damage from reactive oxygen species (ROS). Aim: The aim of our study was to investigate OPCs anti-genotoxic activity in TK6 human lymphoblastoid cell line by comet assay and micronucleus test. Methods**:** Comet assay was conducted using the high alkaline version (pH≥13) and the micronucleus using cytokinesis-block technique. Prior to performing these two tests, cytotoxicity of OPCs was determined in TK6 cells by trypan blue exclusion method. The suitable doses of OPCS were chosen for each test. Results: Cytotoxicity test revealed the IC_{50} value of Thai OPCs was greater than1,000 μg/ml**.** Comet images of at least 50 cells per slide of each treatment were randomly analysed by Comet Assay III (Perceptive Instruments, UK). Two major parameters i.e. distance of DNA migration (tail length; TL) and DNA intensity in comet tail (tail moment; TM) were used as criteria of damage. Under our test condition, non-treated TK6 cells exhibited TL value = 59.67±18.83 μm and TM value = 2.48±1.23 %. A marked increase in DNA damage clearly found in TK6 cells solely treated with H_2O_2 (TL=139.07±13.25 μm, TM=20.97±1.67%). Interestingly the dose-dependent reduction in DNA damage was shown in OPCs-treated cells. We found that pre-treatment of cells with OPCS at 100, 250, 500 and 1,000 μg/ml could prevent TK6 cells from H_2O_2-induced DNA damage by 8.21, 30.62, 22.42 and 54.88 %, respectively. The results of mutagenic study revealed no significant increase in micronucleus formation of all OPCs doses tested and cell viability were above 70%. Moreover, OPCs exhibited anti-mutagenic activity indicated by a significant reduction in micronuclei frequencies by 13.13, 27.05, 36.07, 61.47, 51.64 % when tested at 10, 25, 50, 100 and 200 μg/ml, respectively. Conclusion: The present results suggest that natural Thai OPCs are non-genotoxin and exert anti-mutagenic potential over known mutagen MMC. Further genotoxicity studies will be performed to confirm their beneficial usage.

MH049

ANTI-MUTAGENICITY OF NATURAL OPCs EXTRACTED FROM THAI RED GRAPE SEEDS BY MICRONUCLEUS TEST IN TK6 CELLS

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Background: Oligomeric proanthocyanidins (OPCs) are phenolic compounds mostly found in grape seed extract (GSE) especially from red grapes. A large number of publications have reported that OPCs have antioxidant potential to protect cellular damage from reactive oxygen species (ROS) and hence lead to reduce risk of cancer, cardiovascular

disease and a number of the chronic diseases associated with aging. We had carried out this study on natural OPCs that just firstly isolated in 2008 from red grape seeds by Thailand Institute of Scientific and Technological Research (TISTR). Aim: The aim of our study was to investigate their anti-genotoxic activity in TK6 human lymphoblastoid cell line (ATCC CRL-8015) by cytochalasin B blockage micronucleus (CBMN) assay. Methods: Prior to anti-genotoxic experiment, mutagenicity test of OPCs in TK6 cells was performed at doses 10, 50, 100, 200 µg/ml RPMI for 4 and 24 h. For the anti-genotoxicity assay, five doses of OPCs (10, 25, 50, 100, 200 □g/ml RPMI) were chosen for treatment in combination with mitomycin C (MMC ; 1.5 µg/ml) for 4 h at 37°C. Following treatment, cytochalasin B (cyt B ; 3 µg/ml) was added and cells were further incubated for 18 h. All chemicals were removed by centrifugation. The cell suspensions were prepared onto glass slides using Cytospin®. Cells were fixed in methanol and stained by Geimsa. Micronuclei (MN) formation was scored in 1,000 binucleated cells (BNC) under light microscope (40x). Results: The results of mutagenic study revealed no significant increase in micronuclei frequencies of all OPCs doses in comparison to negative control cells. Cell viability of all doses was above 70%. Moreover, we found that OPCs exhibited anti-genotoxic activity indicated by a significant reduction in micronuclei frequencies in a dose-respond manner by 13.13, 27.05, 36.07, 61.47, 51.64 % for doses of 10, 25, 50, 100, 200 µg/ml, respectively. Conclusion: The present results suggest that natural Thai OPCs are non-genotoxin and exert anti-mutagenic potential over known mutagen MMC. Further genotoxicity studies will be performed to confirm their health beneficial usage.

MH050

DETECTION OF MUTAGENICITY AND ANTIMUTAGENICITY OF FLAVONOIDS USING AMES TEST AND PLASMID pBSII(-)SK

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Flavonoids are a large group of polyphenolic compounds, occurring in fruits, vegetables and plant-derived beverages. These compounds have been shown to exert a wide range of biological actions such as antioxidative, antimutagenic, anticancerogenic and antibacterial activities. Flavonoids and their derivates can act as effective scavengers of oxidizing molecules, including various free radicals and singlet oxygen. On the other hand some flavonoids have been reported to exhibit mutagenic effects and so it is important to know the character of possible genetic damage for the assessment of biological activities of these phenolic compounds. In this study, we have investigated a potential genotoxic effect of six flavonoid compounds (quercetin, isoquercitrin, rutin, silybin AB, DH-silybin and taxifolin). Detection of mutagenicity was carried out with the standard quantitative Ames test using variants with and without in vitro metabolic activation by applying the S9 liver microsomal fraction including a cofactor mixture. In the Ames test, the auxotrophic strains of Salmonella typhimurium His- TA100, TA98 and TA102 were used for the detection of mutagenicity of the samples. The potential antimutagenic activity of flavonoids against DNA damage induced by reactive oxygen species (ROS) was investigated using the Salmonella typhimurium His- strain TA102 and plasmid pBSII(-)SK, hydrogen peroxide as the oxidant mutagen was used. The method using plasmid DNA was based on the different electrophoretical mobility of DNA topological forms and the DNA strand breaks were measured by a conversion of supercoiled form to open circular and linear forms. In the Ames test, the mutagenic effect was detected in the case of quercetin (10–300 µg/plate) and taxifolin (50-500 µg/plate). The analysis of DNA strand breaks in plasmid DNA proved that the tested samples showed protective effects on DNA damage induced in the plasmid DNA by the presence of hydrogen peroxide and metal ions. The results indicate that when new compounds with possible antioxidative properties are developed, it is important to know their potential genotoxic risks. The work also documents the use of plasmid DNA as a suitable model system for the detection of compounds with protective effects on DNA damage.

MH051

LIMITATIONS IN THE GENOTOXICITY TESTING OF NANOPARTICLES ON PROKARYOTIC CELLS

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The rapid development of nanotechnology makes available a variety of nanomaterials with unique chemico-physical properties. However, little toxicological information and controversial data do not allow easy estimates of the potential risks for the human health. The European Centre for the Sustainable Impact of Nanotechnology (ECSIN) has been created to ascertain the biological risks related to the use of nanoparticles (NPs) by in vitro and in vivo studies. Aiming to evaluate selected NPs on rfa mutant Salmonella strains, colloidal water-soluble silica NPs (Ludox® of 13 and 21 nm ø) were tested on S. typhimurium TA 98, TA 100 (plate and fluctuation test) and TA 1535 pSK1002 (SOS umu test). Mild cytotoxicity but no mutagenic or genotoxic effects were detected. Therefore, quantum dots (Qtracker® of 10 nm ø) and two types of fluorescein-loaded silica NPs (ø~23-25 nm) were incubated with log-phase TA 100 cells at 37°C in PBS to estimate the intracellular NP uptake by flow cytometry and fluorimetry analysis. The internalization of electron dense NPs (Qtracker® and maghemite as Fe2O3 of ø~7-10 nm) was also evaluated by TEM imaging. After 3-6 h of contact both the silica NPs slightly increased the fluorescence of the bacterial cells compared to the controls, similar evidence was observed for Qtracker® NPs at 3-11 h. The overall results suggest that even the smallest tested particles are not able to enter the bacterial cells since, in the TEM pictures, quantum dots and maghemite aggregates remain sticked on the outer bacterial surface. These data do not exclude NP-related oxidative stress and possible indirect alteration of cellular functions. Despite protocols based on prokaryotes do not seem generally suitable for routine NP testing, the above assays could still be used to characterize the combined effect of NPs and genotoxic components in complex environmental mixtures.

MH052

DISTRIBUTION OF PARAOXONASE-1 ENZYME ACTIVITY, GENETIC POLYMORPHISM AND ITS CORRELATION WITH DNA DAMAGE IN PESTICIDE EXPOSED WORKERS

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Human paraoxonase-1 (PON1) is a high density lipoprotein associated enzyme that has a role in the detoxification of organophosphorous compounds by hydrolyzing the bioactive oxons. Two major polymorphisms have been described in the coding region of the PON1 gene as PON1 *Gln192Arg* and PON1 *Leu55Met*. Polymorphism at these positions has been found to be responsible for variations in catalytic activity and gene expression, and have been associated with susceptibility to organophosphate poisoning. Therefore, the present study was designed, (i) to evaluate PON1 phenotype and genotype frequency and (ii) to examine the role of PON1 polymorphisms in paraoxon's genotoxic potential in lymphocytes of pesticide exposed occupational workers (n=50) and control subjects (n=50). The results demonstrated that AChE activity was found significantly different in the exposed subjects (410µkat/gHb) as compared to control subjects (635µkat/gHb, p<0.05). BuChE activity was also found significantly lower in exposed subjects as compared to control subjects, respectively (64.96µkat/L and 98.05µkat/L, p<0.05). The enzymatic activity towards phenylacetate and paraoxon was also found significantly varied. The genotype frequency of *Arg-Arg* mutant was found to be 20.0% and 17.14% in exposed and control subjects (p=0.828), while that of *Met-Met* mutant was

7.5% and 5.71%, respectively (p=0.74). PON1 *Arg-Arg* and *Met-Met* showed decrease in paraoxonase activity in occupational workers. Significantly elevated DNA damage (as measured by comet assay parameters: olive tail moment, tail length, % tail DNA and tail extent) observed in PON1 *Gln-Gln*, *Gln-Arg*, *Leu-Leu* and with low activity of paraoxonase in pesticide exposed subjects. However, no such alteration was observed in the control subjects. The result clearly shows that PON1 *Arg/Gln*, *Gln/Gln* and *Leu/Leu* individuals have higher genotoxic effects caused by these pesticides. In conclusion, PON1 unfavorable metabolizing alleles are more susceptible to genotoxic effects than those with favorable alleles which could be helpful to identify individual markers of susceptibility and toxicity toward pesticides. This would help safeguard the proper care of occupational workers who might be affected by organophosphate poisoning.

MH053

INTERACTION BETWEEN CYP1A2 POLYMORPHISMS AND OCCUPATIONAL/ENVIRONMENTAL EXPOSURES IN BLADDER CANCER.

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Cytochrome P4501A2 (CYP1A2) is a key enzyme for activation of the major recognized lung and bladder carcinogens i.e., polycyclic aromatic hydrocarbons(PAHs), aromatic amines(AAs) and nitrosamine(NA). High CYP1A2 activity was therefore suggested as a susceptibility factor for lung and bladder cancer(BC). We have previously showed that polymorphisms, all located in the 5'-noncoding promoter region (mainly *-2467 T/delT* and *-163 C/A*), are crucial in increased CYP1A2 activity of smokers and in smoking-raised urinary mutagenicity. Lastly we found that *-2467 T/delT* is a significant risk modifier polymorphism of smoking-induced lung cancer. In this study we investigated whether *–2467 T/delT* and *-163 C/A* variants modulate the relationship between occupational/environmental exposures and BC risk. The case-control study included 185 BC cases, 180 non cancer controls, all Caucasian males. Data were collected on smoking pack years (PY), coffee, diet, and occupation: exposure to AAs and PAHs with cumulative exposure (CE), time since first (TSFE) and since last exposure (TSLE). A case-only design was applied for interaction between *CYP1A2 -2467 T/delT* (or *-163 C/A*) and occupational/environmental factors. At multiple logistic regression, a significantly increased risk was shown for smokers (especially >50 PY; OR 5.6, 95%CI:2.5-12.5) and for heavy coffee drinkers (>5 cups/day; OR 3.05, 95%CI:1.2-7.9). Exposure to AAs showed a significant trend of bladder cancer risk with increasing CE (p=0.03), but in this association PY is a confounder. A decreased risk was noted for large leaf vegetable (Veg) consumption, with significant trend from < 1/month to >3 times/week (p=0.01). In case-only analysis, interaction was evident between *CYP1A2 -2467 T/delT* and tobacco consumption (>25PY) among cases (p=0.04) but not controls. No interaction was detected between polymorphisms and coffee, Veg and AAs. No effects were shown with *-163 C/A* genotype. This is the first study demonstrating that *CYP1A2 –2467 T/delT* is a significant risk modifier polymorphism also of smoking-induced BC. The research confirmed the role of known personal habits and occupational factors in increasing BC risk and seem to show a protective effect for vegetable consumption.

MH054

POLYMORPHISMS IN NEP1 IN RELATION TO COLORECTAL CANCER WITH EVOLUTIONARY IMPLICATIONS

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By the use of RNAi screening, NEP1 (nuclear enriched protein) was identified to be a putative regulator of the Wnt signaling pathway. Dysregulation of the Wnt signaling plays a key role in colorectal carcinogenesis. We wanted to investigate whether genetic variation in NEP1 affects susceptibility to colorectal cancer (CRC). We used a tagging single nucleotide polymorphism (tagSNP) approach and selected eight tagSNPs, that represent a total of 123 SNPs and cover all the known common (allele frequency >10%) variation in NEP1 with a pairwise r^2 value of linkage disequilibrium >0.8. A case-control study was carried out using two well-characterized study populations from the Czech Republic (752 cases, 755 controls) and Germany (692 cases, 644 controls). The TaqMan® allelic discrimination method was used for genotyping. Odds ratios (OR) and 95% confidence intervals were calculated for association between genotypes and CRC. The ORs were calculated for the effect of the ancestral allele. Two of the chosen tagSNPs were significantly associated with the risk of CRC in the Czech and German population. For both SNPs, the ancestral allele showed a significant correlation with an increased risk of CRC. A comparison of the allele frequencies of the significantly associated SNPs among the Caucasian population (CEU), the Sub-Saharan African population (YRI) and the East Asian population (CHB and JPT) analyzed in the International HapMap Project, showed that the ancestral alleles decline from the YRI population to the CEU and the CHB/JPT populations. Considering the worldwide differences in allele distribution and the significant association of the ancestral allele of SNPs in NEP1 with a higher risk of CRC, the evolutionary history of the gene might have been affected by nutrition, environmental and life style factors.

This work was done in collaboration with the German HNPCC Consortium.

MH055

IN VITRO GENOTOXICITY OF TITANIUM DIOXIDES

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Titanium dioxide (TiO2) nanoparticles are known to induce oxidative stress, inflammation, and cytotoxicity in mammalian systems. However, the toxic mechanisms of nanosized TiO2 are not fully understood, and information on the genotoxicity of TiO2 is still limited. In our study, we assayed three types of commercial nano-TiO2 (nano-anatase, <25 nm; silica-coated nano-rutile, 10x40 nm; nano-rutile, 30-40 nm, with 10% anatase) for their genotoxicity. Two fine-sized forms of TiO2 (rutile, <5 um; anatase, <170 nm) were assayed for comparison. The alkaline single cell electrophoresis (comet) assay was used to assess DNA strand breaks (DNA damage) and the cytokinesis block micronucleus (CBMN) assay to detect chromosomal damage. DNA

damage was examined in cultured human mesothelial (MET5A) and bronchial epithelial (BEAS 2B) cells treated with the five TiO2 types for 24, 48 and 72 h. The induction of micronuclei was scored in BEAS 2B cells after 48-h and 72-h treatments. Five doses (10-160 µg/cm2) of each TiO2 were studied in both assays. Positive and negative controls were included in all experiments. Our results indicate that nano-anatase, nano-rutile and silica-coated nano-rutile significantly induced DNA damage in MET 5A cells at all treatment times. Similarly, fine-rutile and fine-anatase induced DNA damage in MET5A cells at all treatment times, except for fine-anatase after the 48-h treatment. In BEAS 2B cells, DNA damage was increased by nano-anatase after the 48-h and 72-h treatments and by silica-coated nano-rutile and nano-rutile after the 24-h and 72-h treatments. In the CBMN assay with BEAS 2B cells, none of the TiO2 nanomaterials (nano-anatase, silica-coated nano-rutile, nano-rutile) induced micronuclei. Studies are still going on for micronucleus analysis in BEAS 2B cells treated with fine-rutile and fine-anatase. In conclusion, our results indicate that all five TiO2 materials assayed induced DNA damage in human mesothelial MET5A cells, while their genotoxic effect was not equally strong in human bronchial epithelial BEAS 2B cells. None of the nanomaterials induced MN in BEAS 2B cells. [Funded by the European Commission (NANOSH, NMP4-CT-2006-032777)]

MH056
AUTOMATED CHROMOSOME ABERRATION SCORING FOR COSMETIC INDUSTRY

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Chromosome aberration scoring is a regulatory genotoxicity test widely used for compound screening and safety assessment in the cosmetic industry. Both the ban of animal testing and the empowerment of the REACH directive dramatically increase the number of tests to be performed. To face this situation, we validated an automated system to assist expert chromosome aberration scoring in a strongly regulatory environment. We present here the results of this validation study regarding time-savings, increase of operator-independence and data reliability through the implementation of an adapted system for optimal traceability, audit trail and access rights.

MH057
METHOD COMPARISON FOR AUTOMATED HIGH-THROUGHPUT HIGH-CONTENT-SCREENING OF COMET ASSAY

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COMICS is a European project (supported as EC STREP, contract LSHB-CT-2006-037575) aiming at developing new applications of the comet assay improving both its capacity and versatility to make it a genuine alternative to animal testing. We are thus designing and testing new high-throughput high-content imaging tools combined with new substrate formats. We are validating the new procedure against comets scored in the conventional way, and will demonstrate the added-value of high-content screening methods, specifically the Pathfinder™ automated scoring system, in terms of saving time and, more important, providing new information for empowered sensitivity and versatility of the test.

MH058
INDUCED DNA DAMAGE BY DENTAL RESIN MONOMERS IN SOMATIC CELLS

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This *in vivo* study investigated the genotoxicity of four dental resin monomers: triethyleneglycoldimethacrylate (TEGDMA), hydroxyethylmethacrylate (HEMA), urethanedimethacrylate (UDMA) and bisphenol A-glycidylmethacrylate (BisGMA). The Somatic Mutation and Recombination Test (SMART) in *Drosophila melanogaster* was applied to analyze their genotoxicity expressed as homologous mitotic recombination, point and chromosomal mutation. SMART detects the loss of heterozygosity of marker genes expressed phenotypically on the fly's wings. This fruit fly has an extensive genetic homology to mammalians, which makes it a suitable model organism for genotoxic investigations. The present findings provide evidence that the mechanistic basis underlying the genotoxicity of UDMA and TEGDMA is related to homologous recombination and gene/chromosomal mutation. A genotoxic pattern can correspondingly be discerned for both UDMA and TEGDMA: their genotoxicity is attributed to ~49% of mitotic recombination and ~51% of mutational events, including point and chromosomal alterations. The monomer UDMA is 1.6 times more active than TEGDMA to induce mutant clones per treatment unit. BisGMA and HEMA had no statistically significant effect on total spot frequencies suggesting no genotoxic action in the SMART assay. The clinical significance of these observations has to be interpreted for data obtained in other bioassays.

MH059
BIOMONITORING OF HUMAN POPULATIONS EXPOSED TO ARSENIC. ROLE OF GSTs POLYMORPHISMS IN ARSENIC METABOLISM AND GENOTOXIC DAMAGE.

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Background/Aims. Chronic arsenic exposure leads to hyperkeratosis, loss of skin pigmentation, neurotoxicity and hepatic injuries, as well as to significant increases in cancer incidence, particularly in bladder, liver, skin and lung. Although people are environmentally exposed to arsenic mainly via drinking water, copper industry workers, especially those working at smelting places are considered as a highly arsenic-exposed group. Arsenic health effects can be genetically modulated and, as consequence, polymorphisms in such genes are important in terms of individual risk. This works aims to determine whether polymorphisms in GST genes can modulate both the urinary arsenic profile and the level of genotoxic damage measured as the micronuclei frequency. Methods. 207 men working in a copper industry in the North of Chile were enrolled in the study. Determination of the levels of total arsenic, trivalent inorganic arsenic (AsIII), pentavalent inorganic arsenic (AsV), MMA and DMA were obtained by high-performance liquid chromatography coupled with mass spectrometry (HPLC-ICP/MS). Polymorphisms in GSTM1, GSTP1, GSTT1, GSTO1 and GSTO2 were determined. Genetic damage was detected by using the micronucleus (MN) assay in both peripheral blood lymphocytes and buccal cells. Results. In general, the urinary arsenic profiles of the selected workers were in agreement with the standard profile. Despite that inter-individual variations in the arsenic excretion profile were detected, no clear associations between haplotype forms and excreted percentages of iAs, MMA or DMA were detected. A detailed regression analysis, adjusted by age and arsenic excretion profiles, was also carried out taking into account separately the possible effect that each form of excreted arsenic (inorganic, MMA and DMA) could exert on the MN frequency It should be noted that we did not detect that any haplotype was significantly associated with MN frequencies. Conclusions. Our findings suggest that GSTs polymorphisms do not strong modulate nor urinary excretion profile nor the genetic basal damage detected by the MN assay in the occupational exposed popula-

tion. The only exception was a slight correlation between the GSTP1 variant and the increase of MN in lymphocytes.

MH060

TWO NOVEL MUTATIONS IN XPD ASSOCIATED WITH TRICHOTHIODYSTROPHY RESULT IN TEMPERATURE-DEPENDENT DYSFUNCTION OF THE TRANSCRIPTION/REPAIR COMPLEX TFIIH

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Trichothiodystrophy (TTD) is a rare autosomal recessive disorder characterised by hair abnormalities, ichthyosis, growth and mental retardation and, in many cases, photosensitivity. The majority of sun-sensitive TTD cases are mutated in XPD, the gene encoding a subunit of TFIIH, a multi-protein complex involved in RNA polymerase II-mediated transcription and in repair of DNA lesions induced by ultraviolet light. We have studied two genetically unrelated families with TTD affected members (one son and two siblings) showing mild clinical features that transiently worsen during episodes of fever. As typically observed in photosensitive TTD cases, patients' primary skin fibroblasts showed a partial inability to repair UV-induced DNA damage and a reduced steady-state level of TFIIH. By genetic analysis the patients were classified into the XP-D group. Sequence analysis revealed that the TTD siblings are compound heterozygous for a typical TTD allele (c.335G>A, p.Arg112His) and for a new XPD allele with a mutation that partially affects intron 22 splicing. The other patient is functional hemizygous for a novel mutation in XPD resulting in the Lys692Glu change. Incubation of patients' fibroblasts at 41°C resulted in increased TFIIH instability, aggravation of the DNA repair defect and reduction of the overall transcription. A similar situation has been previously described in four TTD patients with fever-dependent deterioration of clinical features due to the Arg658Cys change in the XPD protein (Vermeulen et al., 2001, Nat. Genet. 27:299-303). In human, documented examples of temperature-sensitive mutations with an associated feverish crisis have been rare. Nevertheless, our study led to the identification of two additional thermolabile mutations in the XPD gene associated with TTD. This may reflect the relative tolerance of TFIIH transcriptional activity toward amino acid changes in the XPD protein.

MH061

MUTAGENICITY AND DIOXIN-LIKE ACTIVITY OF BIODIESEL EMISSIONS

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Background/Aims: The aim of this study was to assess the toxicological properties of the soluble organic fraction of (bio)diesel emissions generated using various after-treatment technologies (i.e., diesel oxidation catalyst and exhaust gas recirculation) and diesel and biodiesel-blended fuel formulations (i.e., derived from soy, canola, and animal fats). Methods: Diesel emissions generated using diesel and biodiesel fuels were collected on Teflon-coated fiberglass filters and polyurethane foam plugs via a constant volume dilution tunnel. The soluble organic fractions were extracted using pressurized fluid extraction and the adsorbed organics were separated on open silica into polar aromatics and non-polar neutral compounds. Mutagenic and dioxin-like activities (i.e., induction of the aryl-hydrocarbon receptor pathway) were assessed using the Salmonella mutagenicity assay and the DR-CALUX assay respectively. Results: Results indicated that organic extracts of (bio)diesel particulate emissions contain direct- and indirect-acting polar aromatic mutagens as well as polar and non-polar Ah-receptor agonists. A reduction in the mutagenic activity of direct-acting compounds was observed for the polar aromatic fraction with increasing biodiesel content in the fuel (e.g., 48% reduction for biodiesel blend B20 compared to ULSD on Salmonella TA98 without metabolic activation). Conversely, an increase in dioxin-like activity with increasing biodiesel fuel content was observed for both the polar and non-polar fractions (e.g., 144% increase for the non-polar fraction and 111% increase for the polar aromatic fraction of biodiesel blend B20 compared to ULSD). Conclusions: The results obtained in this study support the hypothesis that the use of alternative fuels such as biodiesel blends and after-treatment devices reduce the risk of adverse health effects associated with diesel exhaust emissions. These results will provide a framework for evaluating the toxicological hazards of biodiesel emissions, and eventually identify fuel choice and engine design scenarios that minimize the risks of adverse health effects.

MH062

CYTOGENETIC ANALYSIS OF THE MESENCHYMAL STEM CELLS DERIVED FROM THE SUBENDOTHELIUM OF THE UMBILIACAL VEIN HUMAN CRYOPRESERVATED

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Mesenchymal stem cells (MSCs) are known as a population of multipotent progenitors cells able to proliferate and differentiate into multiple mesodermal tissues. The process of cryopreservation is essential for the maintenance of cell cultures, since the cell line is frozen, it can be maintained in liquid nitrogen for an indefinite period and then thawed for therapeutic or experimental purposes. Furthermore, concerns that adult human MSCs may be prone to malignant transformation have been recently raised. The aim of this study was to isolate a population of MSCs derived from the subendothelium of the umbilical vein human (MSCs-SUVH) to assess by cytogenetic analysis the possibility of appearance of chromosomal changes in MSCs-SUVH after the process of cryopreservation. Human umbilical cords from full-term deliveries (n=3) were collected after informed consent at the Januário Cicco´s Hospital (Brazil, Natal). MSCs-SUVH were isolated by using enzymatic digestion and cultivated in alfa-MEM supplemented with 10% FCS. Immunophenotypically, MSCs-SUVH were defined as cells expressing CD29, CD73 and CD90 and lacking hematopoietic lineage markers, such as CD14, CD34 and CD45. They also demonstrated capacity for osteogenic differentiation. The chromosomes obtained from the primary culture of MSCs-SUVH before and after the cryopreservation were analyzed by GTW banding, and results are described following the guidelines to International System for Human Cytogenetic Nomenclature (2005). A total of 245 metaphases were analyzed. There was no emergence of clonal chromosomal aberrations in the MSCs-SUVH in different situations analyzed. However, only after the cryopreservation, 14 metaphases showed nonclonal chromosomal aberrations, including monosomies and structural changes. These findings should be further investigated, since the use of MSCs for clinical approaches requires that the biosafety of these cells be carefully investigated through appropriate and sensitive tests.

MH063

MUTAGENIC EVALUATION OF 3'3-DITRIFLUOROMETHYLDIPHENYL DISELENIDE BY BONE-MARROW MOUSE MICRONUCLEUS ASSAY

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3´3-ditrifluormethyldiphenyl diselenide (DFDD) is a novel organose-

lenium compound which presents antigenotoxic, antimutagenic and antioxidant properties in bacteria, yeast and V79 mammalian cells. In addition, DFDD shows antipsychotic activities in mice. The aim of this work was to evaluate the mutagenic profile of DFDD by bone-marrow mouse micronucleus assay. Briefly, male CF-1 mice were treated intraperitoneally (i.p.) in a single dose with DFDD 1, 5, 25, 50 and 150 μmol/kg body weight (BW). Animals were sacrificed by cervical dislocation 24 h after treatment, and subsequently the femur was dissected to prepare bone marrow smears for the micronucleus test (MN). The mutagenicity of de DFDD was evaluated by scoring 2,000 polychromatic erythrocytes (PCEs) per animal per treatment group**.** To avoid false negative results and as a measure of toxicity of the compounds to bone marrow, the polychromatic erythrocyte (PCE)/normochromatic erythrocyte (NCE) ratio was scored in 1,000 erythrocytes. DFDD treatment did not induce neither decrease nor increase of the PCE number in relation to the NCE number at all doses tested when compared to negative control. Furthermore, none of the animals demonstrated toxic signals (such as diarrhea, seizures and ataxy) or have died during the experiment. In addition, DFDD was not able to increase the micronucleus frequency when compared to the negative control. These data are in agreement with the negative mutagenic results found in bacteria and yeast strains. In conclusion, our results suggest that DFDD is not mutagenic for mice.

MH064

EFFECTS OF AGING AND LONG-TERM OVARIECTOMY ON AVERSIVE AND RECOGNITION MEMORY AND DNA DAMAGE IN RAT HIPPOCAMPUS

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Background and Aim: Aging is a biological process that results in several disorders like a decreased of cognitive functions related to memory and learning, DNA damage and, in females, decreased of estrogen levels. In spite of aging be a multifactorial process, decreased of estrogen level have been indicated as a responsible for an increase of free radicals production and to cause injuries in macromolecules like the DNA. Moreover, some studies suggest that the decreased of estrogen by short-term ovariectomy impairs memory and reduces spine density in rats. By contrast, studies with long-term ovariectomy have showed unexpected results: long-term ovariectomy improves, instead of impairing, learning and memory process in rats. These controversial results justify this study for clarify the effects of long-term ovariectomy in the cognitive sensibility and in DNA damage induction. Methods and Results: In the present study we investigated the alterations in memory storage processes that occur in senescence by subjecting aged and young female Wistar rats in two distinct animal models of memory: object recognition task and inhibitory avoidance test. In addition, investigation of DNA damage in hippocampus by the Comet assay also was done. Twelve young (6 months) and twenty-four aged (18 months) female Wistar rats were employed in the present study. The animals were divided in 3 groups: adult sham, aged sham and long-term ovariectomized aged rats (ovariectomized 15 months before the test). The results showed that in the step-down inhibitory avoidance task aging affects test performance of rats. In the object recognition task assay our data demonstrated that just aged sham rats were unable to discriminate between familiar and novel objects. For the comet assay, sham aged rats showed a significant increase in DNA damage in relation to the adult group. Conclusion: Our findings demonstrate that aged sham female rats displayed memory impairments in both behavioral tests compared to young animals and that long-term ovariectomy can significantly improve recognition memory, but not aversive memory, in aged rats. Moreover the aged rats, but not the ovariectomized aged animals presents more hippocampal DNA damage in agreement with the recognition index findings.

MH065

INFLUENCE OF CHAMOMILLA RECUTITA AND MIKANIA LAEVIGATA EXTRACTS OVER THE GENOTOXICITY INDUCED BY ALKYLATING AGENTS.

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Background and Aim: The diversity of plants in Brazil is a potential source of biological active compounds. The utilization of certain plants in combating diseases is common practice among the people. Among various medicinal plants used in folk-medicine in Brazil, *Mikania laevigata* Sprengel (Asteracea), popularly known as “guaco” is an important medicinal species because of its multiple pharmacological properties, especially anti-inflammatory, anti-allergic, analgesic and antimicrobial activities. In addition, *Chamomilla recutita* (L.) Rauschert, which also belongs to the Asteracea family, is commonly used as a medicinal tea to treat inflammatory disorders, fever, menstrual pain and intestinal and hepatic tumors. However, in the majority of cases, there is no proof efficacy of treatment in popular use, or there has not been an adequate evaluation of medicinal plants for possible adverse effects or protective use. Thus, this work aims to evaluate*, in vivo*, the effect of *M. laevigata* and *C. recutita* extracts on the genotoxicity of some mutagens in CF-1 mice. Methods and Results: The animals were divided into 8 groups with 8 animals each. The treatment groups were: Group 1 and 2 (negative controls received water and chamomile or guaco extract, respectively), group 3 and 4 (pre-treatment) receive initially extract and 24 hours after receiving MMS (40mg/kg) and cyclophosphamide (CP) (25mg/Kg) intraperitonealy, respectively, and the groups 5 and 6 (post-treatment) initially received the MMS and CP respectively and 24 hours after received the chamomile or “guaco” extract; group 7 and 8 received only the alkylating agents MMS and CP. The results showed that the pre- and post-treatment with “guaco” extract could improve the DNA damage in erythrocytes of mice treated with MMS and CP reducing the damage in 52% e 34% (MMS, pre- and pos-treatment) and 60% and 65% (CP, pre- and pos-treatment). The results for chamomile showed a significant difference in the treatment with this plant only in relation to MMS in the post-treatment, with a significant reduction of 69,9% on the damage caused by this alkylating agent. Conclusion: These results showed that the extracts of both plants can protect or repair the DNA for damage caused by alkylating agents.

MH066

OXIDATIVE DAMAGE AND TRANSCRIPT DOWN-REGULATION OF DNA REPAIR GENES IN LYMPHOCYTES FROM PATIENTS WITH ALZHEIMER DISEASE, FRAILTY SYNDROME AND DIABETES MELLITUS TYPE-2.

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Damage to neurons can reflect both an increase in oxidative processes and a decrease in antioxidant defenses. Over the past decades, the scientific progress has been occurred to search the involvement of oxidative stress in several diseases, most notably those associated with age. In the present work, we analyzed the DNA damage by the comet assay (with or without hOGG1) and transcript profiles for DNA repair genes for lymphocytes taken from patients with Alzheimer disease (AD), frailty syndrome (FS) and diabetes mellitus type-2 (DM2), compared with paired healthy individuals. Inform consents were obtained from

all individuals (n=12-15 per group), and the research work was approved by the local Ethics Committee (HC-FMRP-USP). Lymphocytes from AD and FS were more sensitive to DNA damage induction compared with controls when treated with hydrogen peroxide. Hyperglycemic DM2 patients also showed significant higher levels of DNA damage than matched non-hyperglycemic patients and controls, probably due to the oxidative damage produced by glucosis. In addition, lymphocytes from DM2 patients also presented a transcriptional down-regulation of DNA repair genes (*ATM*, *ATR*, *OGG1*, *XRCC1*, *FEN1* and *APEX1*), as evaluated by the real time PCR (qPCR) method. Interestingly, for AD patients, 19 genes (*ATM, ATR, TREX1, OGG1, FEN1, FANCG, RAD17, DUSP, ERCC1, ERCC3, ERCC6, HUS1, RAD9, RAD1, PRKDC, ADAM17, APEX1, APP, and BACE1*) were found repressed by qPCR; most of them play roles in damage sensing and DNA repair pathways. These results showed that several signaling pathways associated to oxidative stress responses, including DNA repair pathways, may be compromised in lymphocytes from patients with DM2 and AD. Additionally, for patients with AD, transcriptional profiles analyzed by cDNA microarrays showed significant down-regulation of two genes (*TRAP1* and *KA35*) and an up-regulation of 39 genes (among them, *NOTCH1*, *MARK3*, *PAK*, *SMC1L1, PSMB2*, *GLE1L*, *MPP5*, and *PBX1*) playing roles in several biological processes. Thus, altogether, the present data provide a relevant contribution towards the elucidation of the molecular pathways involved in the processing of oxidative damage in human diseases related with ageing.
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MH067
TOXICOLOGICAL EVALUATION ON INDOOR-ENVIRONMENTAL EXPOSURE MODEL OF BENZENE ON MICE

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Background: Benzene is known to cause hematotoxicity and leukemia in occupational exposure workers. The lowest concentration at which an increased incidence of leukemia among exposed workers has been estimated to be in the range of 32–80 mg/m3. In recent years, benzene pollution emitted indoors from household materials is more serious with the high rate of new construction in China today. Although the indoor concentration is lower than potential carcinogen level on occupational exposure, but the indoor-environmental exposure may be more than 12h one day especially for the seniors and young kids. Methods: The BALB/C mice were continuously exposed to benzene by inhalation (20 mg/m3, 80 mg/m3) 12 hours a day within 90 days and raised for another 90 days. The following items were detected: general growth, micronucleus and comet rates, parameters of peripheral blood, bone marrow examination and the expression of NF-κB P65 and Bcl-2. Results: The toxic symptoms as wizened fur and indolence in action were observed in each treatment group. The micronuclei and comet rates in each treatment group were increased significantly compared with controls after 90 days with dose-dependent response. These two genetoxicity could be still observed lasting 90 days after exposure treatment finished. The anaemia happened in each exposure group without dose-dependent and myelodysplastic syndrome (MDS) happened in benzene (20 mg/m3). NF-κB and Bcl-2 expression increased significantly in each treatment group compared with controls after 90 days exposure treatment, but without dose-dependent response. Conclusion: It is suggested that the chromosome and DNA damage could be induced and hemopoietic function could also be influenced and malignant cloning disease in hemopoietic system could be induced by indoor-environmental exposure model at this concentration. The NF-κB and Bcl-2 anti-apoptosis pathway activation take part in the toxicity effect. (This work was supported by the National Natural Science Foundation of China 30671731, the Doctoral Program of Higher Education of China 20070286069, the Medical Research Foundation of Jiangsu Province H200538 and Innovation Foundation for Graduates of Jiangsu Province 2005-89)

MH068
MORTALITY ASSOCIATED WITH LIVER DISEASES IN EGYPT (1986-2005)

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Background: Hepatitis C Liver diseases are important health problem in Egypt with a national prevalence of anti-HCV of 12-14%. There is an evidence of transmission of HCV and HBV resulting in such high prevalence of HCV and a HBsAg carrier rate of over 4%. This was suggested to be a cohort infection through antischistomiasis campaigns between 1960 -1985. There are few studies about liver disease burden and mortality rates in Egypt. This study addresses the magnitude of liver diseases in Egypt 20 years after the initiation of the epidemic, over the period (1986-2005). Methods: All death records of Egypt during the period (1986-2005) were obtained from Central Agency of Public Mobilization and Statistics and National Information Center for Health and Population of Ministry of Health and Population. Data were analyzed to assess the Years of life lost for causes of death related to liver diseases (HBV infection, HCV infection, liver cancer and liver cirrhosis). Results: Mortality rates and number of YLL due to liver cancer showed a gradual increase from 1986 to 2005 (from 15 to 65 /100000). Proportionate Mortality Ratio rose from 1.5 to 10.5 of the total deaths (8.7 to 12.0 % among females and males respectively). There were no deaths recorded due to liver cirrhosis in year 1986. Rates due to liver cirrhosis showed a gradual increase from 1987 to 2005. Males showed higher mortality from liver diseases than females allover this period. The mortality was higher in old age groups than young ones. Conclusion: The study showed that the total mortality rates and YLL for liver diseases are steadily increasing with time with higher mortality from liver diseases among males than females all through the study period. Further modeling is needed to estimate the peak of this epidemic and estimate the therapeutic needs for antiviral therapy and liver transplant to avoid such an expected high impact during the next years.

MH069
AN ATTEMPT TO IDENTIFY GENES INVOLVED IN PROGRESSION OF TOBACCO SMOKE ASSOCIATED CANCER

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Tobacco smoking and alcohol abusing are the dominant environmental causable factors in laryngeal cancer. However, as only a fraction of exposed persons develops laryngeal cancer, a significance of genetic factor is to be studied. Further, a progression of cancer also seems to be associated with expression of specific oncogenes and tumor suppressor genes. Recently, molecular cytogenetics and biology provide promising techniques to identify an involvement of particular genes not considered yet. The study was done on 13 cell lines derived from laryngeal cancer with variable characteristics concerning TNM, survival time etc. To get orientation in genome changes related to cancer the cells were analysed by classical cytogenetics, array-comparative genomic hybridization, and gene expression. The selected regions were analysed by FISH using the appropriated probes. A combination of classical cytogenetics with FISH have shown the intersticial deletion at 8q22.1 present in three cell lines. The detailed analysis of breaking points targeted to PGCP and SDC2, already known as active in other types of cancer. Next, a frequent amplification of 11q13 region was extended on an estimation of amplicon size. It contains at least the following oncogens: CCND1, FG3 and FG4. An increase of copy number of the mentioned genes is currently confronted with mRNA expression studied by Affymetrix V 133 Plus 2. Another series of experiments concerns glob-

al genome analysis by CGH-array in laryngeal cancer cell lines. Several regions with a considerable gain (amplification) or loss (deletion) are at present under analysis. The gained regions potentially contain oncogenes and lost seem to be associated with tumor suppressor genes. A content of genetic material in the selected regions is being compared with mRNA expression. Oncogenes identified until now include: CRKL and MAPK1 present in the highly amplified region 22q11-12. The following tumor suppressor genes have been identified: GNG7 (19p13.3), CDKN2A (p16, 9p21.3) and STK17A (7p13). Genes involved in cancer progression could be further studied in respect to targeted therapy.

MH070

MICRONUCLEI FREQUENCY IN PERIPHERAL BLOOD LYMPHOCYTES AS BIOMARKER FOR COLORECTAL CANCER RISK.

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Colorectal cancer (CRC) is one of the most frequent neoplastic diseases in human population. Changes in chromosome structure and number, as well as genomic instability, are major events in the etiological pathway leading to cancer. Chromosomal instability is the most common type of genomic instability in CRC and occurs in 80-85% of colorectal tumours. The genome of colon cancer is often marked by chromosome rearrangement and alterations in chromosome number. It has been demonstrated that the level of genetic damage in peripheral blood lymphocytes reflects the amount of damage in the precursor cells that lead to the carcinogenic process in target tissues. In order to study biomarkers of genetic damage predictive for CRC risk, we have evaluated Micronuclei (MN) frequency in peripheral blood lymphocytes from subjects resulted positive to fecal occult blood test (FBT) and examined by colonoscopy, among the participants of screening program for CRC. At now, the study population comprise 44 subjects aged between 50-70 years, resulted positive to FBT and classified by histological lesion at the baseline colonscopy in two groups: normal colon (26 subjects, 10 female and 16 males, mean age: 59,3±6,1) and adenocarcinoma–CRC-subjects (18 subjects, 7 females and 11 males, mean age: 62,3±7,9). All the subjects have provided informed consent. Data collection regarding demographic characteristic, gastrointestinal history, occupational history, diet, and lifestyle habits. Interestingly, the frequencies of MN are significantly increased in CRC-subjects as compared with normal colon subjects (MN/1000 binucleated (BN) cells: 17,67±6,14 vs 7,66±1,48 $P<0.05$). Among the study population MN frequency was not affected by age or gender. Our preliminary results are interesting, and support the concept that biomarker study can provide new perspectives in prevention program for CRC. This project was supported by a grant from Fondazione del Monte di Bologna e Ravenna.

MH071

COMBINATION OF ACUTE IN VIVO MICRONUCLEUS AND COMET ASSAYS: CONSIDERATIONS AND NOVARTIS EXPERIENCE

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Regulatory genotoxicity testing of novel therapeutic molecules consists of a battery of in vitro tests as the first tier. In the case of positive results, scientific practice as well as regulatory guidance demands a clarification of the result, frequently by the conductance of in vivo tests While the bone marrow micronucleus test has been a standard first test since long, recently the comet assay has become popular as the second additional test, mostly due to its ease of conductance and versatility to analyze different organs. At Novartis, an approach has been used to combine micronucleus and comet assays into the same animals, using an acute treatment schedule, which allows an optimal correlation of both endpoints. Further, a significant saving in laboratory animals can be achieved by this approach, whereas treatment schedules and positive controls needed to be adapted in order to find a compromise suitable for both assay types. Currently, a three-dose design is used, with dosing at 0, 24, 45 hours and sacrifice at 48 hours, with minor modifications depending on the TK properties of the compound. The study is done with 7 males or 5 animals per gender per dose group, using three dose levels plus negative controls. Otherwise, 5 males and 5 females are used. In addition, a histopathological examination of the target organs complements the genotoxicity evaluation. Over the last 4 years, we have conducted 12 combined assays, generally triggered by positive results in in vitro mammalian tests. This approach is able to save 37% of animals if both genders, and 57% of the animals if only males are used, versus independent tests. The correlation between bone marrow MNT and comet assay, mostly in liver and blood cells, was good, with negative results in both enpoints in most studies. In summary, the proposed combination of micronucleus test and comet assay in liver or blood cells has provided a reliable design to clarify positive in vitro genotoxicity findings, allowing a significant reduction in animal numbers.

MH072

IN VITRO EVALUATION OF MUTAGENIC AND PROTECTIVE EFFECTS OF THE β-GLUCAN BOTRYOSPHAERAN IN MAMMAL'S CELLS

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The increasing of life expectancy has been leaded to a gradual increase in the prevalence of degenerative diseases which is strongly associated with mutational process. However, such events can be avoided by favouring the intake of protective factors and by modulating the body's defense mechanisms. Fungi are producers of exopolysaccharides (EPS), mainly □-D-glucan type that present health beneficial activities. In this study it were evaluated in human peripheral lymphocytes and V79 cells the possible citotoxic, mutagenic, protective effects and cell viability modulation of the EPS botryosphaeran, which consists of a □(1à3; 1à6)-D-glucan secreted by the ascomycetous fungus Botryosphaeria rhodina MAMB-5. The protective effect of EPS was evaluated against DNA damage induced by bleomicin, doxorubicin and hydrogen peroxide. It were used the cytokinesis-block micronucleus technique and the acridine orange/ethidium bromide staining using four treatments protocols. The obtained data showed that botryosphaeran itself did not present citotoxic and mutagenic activity in both cellular types. The three EPS concentrations tested on human lymphocytes reduced the frequency of micronuclei in the antimutagenicity protocols during the G1 and G2 cell cycle phase and throughout continuous treatment. In the G2 cell cycle phase there was a tendency of a dose-response curve indicating a desmutagenic mechanism. However, there were evidences that the protective effects exerted by this EPS may also have been related to a bioantimutagenic mechanism. In the V79 cells, the ten concentrations evaluated in MTT test showed no cell citotoxicity and the three concentrations evaluated on micronucleus test also reduced the frequency of micronuclei against damages induced by doxorubicin and hydrogen peroxide, suggesting some antioxidant action. Moreover, the botryosphaeran was able to increase cell viability with a decreasing in the apoptosis index. Among the mechanisms proposed to explain the observed protective effects, it can be cited the antioxidant activity, modulation of antioxidant enzymes expression and signals transduction by receptors coupled kinases with subsequent modulation of genotoxic stress response. Financial Support: CNPq/UEL

MH073

CADMIUM EFFECTS ON DNA REPAIR ACTIVITIES: ANALYSIS ON DEDICATED BIOCHIPS

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Background/aims: Cadmium is known to affect several cellular processes, including cell cycle progression, proliferation, DNA replication and some aspects of DNA repair but the molecular mechanisms underlying its carcinogenicity are not fully identified. We investigated the direct effects of cadmium exposure on the functionality of proteins involved in nucleotide and base excision repair using specialized DNA lesion microarrays. Methods: We used biochips bearing plasmid DNA or double stranded oligonucleotides carrying series of defined genotoxic lesions repaired by BER and/or NER. Upon incubation with a nuclear extract, these lesions are individually recognized and repaired. The activity of the enzymes involved in this process is quantified by the incorporation or the disappearance of fluorescence, according to the system and protocol used. These microarrays provide information on activities directed toward several types of DNA damage at the same time and define a "repair profile" for each sample; they were used to determine how the presence of cadmium modulates repair of the different types of lesions. Results: In a first set of experiments, we analyzed the repair of thymine glycols (TG), cyclobutane pyrimidine dimers (CPD-64), 8 oxoguanine (8 oxoG) and alkylated bases (AlkB) on plasmid DNA biochips. All activities were affected by cadmium in a dose dependant manner, but at different levels for each lesion. The recognition and excision of 8 oxoG and TG were relatively more inhibited by cadmium than that of CPD-64 and AlkB. Next, we used an oligonucleotide microarray designed specifically to monitor simultaneously the excision of several lesions. We found that excision of TG and of U opposite A were inhibited at lower cadmium concentrations than cleavage of abasic sites or of U opposite G. Conclusions: Cadmium has a direct broad inhibitory effect on the repair of damaged bases and nucleotides when added to acellular extracts. It affects both glycosylases and APE1, and excision by bi-functional glycosylases, which possess an AP lyase activity, appears to be more sensitive than excision by glycosylases which require APE1. This general DNA repair suppression most likely contributes to cadmium carcinogenicity.

MH074

BIOCHEMICAL AND GENOTOXIC EFFECTS IN PESTICIDE SPRAYERS: PRELIMINARY RESULTS

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In large cultivated areas in Ceres (Argentina), pesticide sprayers are generally exposed to pesticide mixtures. Oxidative stress and DNA damage have been proposed as mechanisms linking pesticide exposure to health effects. We determined biochemical and genotoxic effects in pesticide sprayers, compared with controls from the same area. Assay of malondialdehyde levels (MDA), reduced glutathione (GSH) and oxidized glutathione (GSSG) were estimated in blood samples to determine pesticides impact on redox potential. Lymphocytes were analyzed for DNA damage using Damage Index Comet Assay (DICA) and oxidative DNA damage introducing the modification by adding FPG protein (DICA FPG). The MN test was applied on buccal cells (BCMN). An increase in MDA levels were found among sprayers compared to controls ($P<0.003$). The oxidative stress produced was indicated by a significant decrease in the GSH/GSSG ratio observed in applicators ($P<0.003$), as a result of the decrease in the concentration of reduced glutathione and the increase of its oxidized form. Both DICA and FPG DICA as well as BCMN were higher from applicators when compared to controls ($P<0.003$). These results demonstrate that lipid peroxidation enhancement and antioxidant defense system alterations observed in sprayers correlate with DNA damage in both lymphocytes and buccal cells. The small sample size analized limits the implications of this study. However, the fact that these kind of alterations can be easily linked to the adverse health effects observed in chronic pesticide toxicity (in which oxidative damage plays a pathophysiological role) is an attractive hypothesis that justifies further investigation.

MH075

EFFECT OF ILEX PARAGUARIENSIS ON ESOPHAGEAL CYP EXPRESSION AND IN BACTERIAL CULTURES

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Esophageal cancer is the eighth most common cancer worldwide and sixth in cause of death from cancer, occurring mainly in developing countries. Approximately 93% of them are epidermoid carcinoma type and seems to be primarily caused by environmental factors. In southern Brazil, the incidence is related to the consumption of chimarrão, beverage ingested at high temperatures and made from the leaves of *Ilex paraguariensis*. There are, at least, three possible mechanisms which the infusion of *Ilex paraguariensis* (IIp) could increase the risk of esophageal cancer: (i) the presence of carcinogenic substances in yerba mate, (ii) the increase of carcinogens esophagus metabolism through the cytochrome P450 induction in this tissue, and (iii) chronic thermal injury. In this study, we analyzed first two points. For this, we evaluated the gene expression (RT-PCR), protein (Western blotting) and enzyme activities (COH and NDEAd) associated with CYP2A3 in rats oesophagus treated during 2, 4, 7, 14 and 21 days, with IIp 10% (w/v). Using the Ames test and SOS cromotest was verified the mutagenicity of IIp 0.1% (w/v), 1% (w/v) and 10% (w/v) in bacterial systems. No differences were observed in the expression of mRNA and apoprotein of CYP2A3 related to the control group, as well as on enzyme activities, suggesting that IIp 10% (w/v) does not interfere on the metabolism of carcinogens in the esophagus. Besides, no significant mutagenic effect (MI > 2 or $p < 0.05$) was observed on the standards strains (TA97, TA98, TA100, TA102) as well as on the more sensitive strains (TA1535, YG7104, YG7108, YG1021, YG1024, YG1041, TA98NR, TA98DNP-6) to detect nitro-compounds such as nitrosamines, regardless of metabolic activation. Thus, we observed that the risk of IIp in the patogenesis of esophagus cancer in southern Brazil does not involve the modulation of esophageal CYP enzyme or the presence of mutagenic compounds present in yerba mate. Support: CNPq, CAPES and FAPERJ.

MH076

MUTAGENIC AND REPROTOXIC ACTIVITY OF LOW CONCENTRATIONS OF TAURINE

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Introduction. The sulphured amino acid Taurine (TA) has been implicated in numerous biological functions based on its unique chemical structure (Lourenco and Camilo, 2002). TA has a role in bile acid conjugation, central nervous system neuromodulation and retinal development, among others. The deficiency of this a.a. increases the risk of patients requiring long-term parenteral nutrition, but also its effect on metabolism and antioxidant properties have contributed to use TA as ingredient in commercial beverages although, the effect of excessive intake on healthy individuals is unknown. The goal of this study is determine the effect of chronic exposure to TA on toxicity and genotoxicity parameters from Drosophila melanogaster. Methods. Wild type

(WT) and SMART standard larvae (SS) were chronically fed with TA. Fourteen successive dilutions from 50 mM TA [CAS 107-35-7] were assayed. As solvent and negative control, distilled water was used. For each concentration, adults recovered were sexed and counted to obtain the Survival Index (SI) as the fraction of experimental flies recovered compared with the control flies recovered; in addition, the probability to recover a male or female (Sx) was obtained for each concentration. WT males were individually mated with two untreated WT females to obtain the effect of TA on the fertility. The average of the progeny per male per concentration was compared to the control average. The genotoxic activity of TA was determined by mounting the wings of the SS adults and scoring the number and type of mutant spots to obtain: the frequency of mutant spots, the distribution of the size of the spots and the distribution of the number of spots per fly in experimental and control series. Results. The SI from WT flies exposed to 1.25 mM and those from flies treated with concentrations higher to 10 mM of TA was lower compared to the SI from control flies ($p<0.05$), and the effect was similar to females and males. The fertility of experimental males was lower compared to that from control males. The average of progeny per male increases with the concentration of TA, except al 50 mM in which was lower to that from control males. TA increases the frequency of spots from experimental flies treated with 10, 0.5 mM or lower concentrations.

MH077

IN VITRO EVALUATION OF DNA DAMAGE INDUCED BY LOW DOSES OF X RAYS

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X-rays are part of ionizing radiation that can act directly or indirectly on the genetic material and may cause damage depending on the dose that it is delivered. Low dose of X-rays have been studied for several years. It has been used in different treatments, and some of these doses were considered as non genotoxic in previous studies. The objective of this work was to evaluate the genotoxic effects by the cytokinesis block micronucleus test (CBMN) and the comet assay (CA), in human lymphocytes, exposed *in vitro* to low doses of X-rays. Briefly, the blood of a healthy individual was exposed to three different doses of X-rays: 0.1, 0.2 and 0.3 Gy. The frequency of micronuclei and micronucleated cells was determined. For the comet assay, DNA damage was measured by tail length (um), tail moment, and tail intensity (%). Statistical analyses were performed using the tests of Kruskal-Wallis and Mann-Whitney. We found that when the dose of X-rays increases, the frequency of micronuclei and micronucleated cells also increases, and there were significant differences between each of the evaluated doses compares with the control. For the CA, we found that the damage increases with the increasing dose. The CA has a lower threshold than the CBMN (0.1 and 0.2 respectively). This study shows that genotoxic effects in vitro are highly related to the X-ray dose used. In this work the best indicator of DNA damage in the CA is the tail intensity. The frequency of micronuclei and micronucleated cells is very similar at low doses. In conclusion, X ray causes DNA damage at chromosome levels with doses higher than 0.2 Gy, and at single strands breaks with doses higher than 0.1 Gy.

MH078

DNA INTERSTRAND CROSSLINK LESIONS PROMOTE AGE-RELATED DEGENERATIVE CHANGES

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Background: The stochastic theory of aging posits that the degenerative changes associated with aging arise as a consequence of random damage to cells. Previous work from our lab demonstrated that this includes damage to nuclear DNA. However, what remains unknown is what type of DNA damage promotes aging? The purpose of this study is to test the hypothesis that DNA interstrand crosslinks (ICLs), cytotoxic lesions that block DNA replication, promote aging but that monoadducts, affecting only one strand of DNA, do not. This is based on the observation that deficiency of ERCC1-XPF, a DNA repair endonuclease required for the repair of both ICLs and monoadducts, causes accelerated aging in humans and mice, but defects in repairing monoadducts alone does not. Methods: To test this hypothesis, ERCC1-deficient mice, which age rapidly and die by 7 mths, are exposed to two structurally related nitrogen mustards: 2-chloroethylamine (CEA) which is monofunctional and induces only monoadducts and mechlorethamine (MEC), which is bifunctional and induces monoadducts and ICLs. The age at onset of progeroid features and lifespan is recorded. In addition, post-mortem tissues are examined for degenerative changes associated with aging and markers of apoptosis and cellular senescence. XPA-deficient mice, defective only in the repair of monoadducts, are used as a control. If our hypothesis is correct, then chronic exposure of ERCC1-deficient mice to MEC, but not CEA, will accelerate/exacerbate their progeria, while not affecting XPA-deficient mice. Results: The age at onset of accelerated aging symptoms, including dystonia, trembling, kyphosis, ataxia, lethargy, urinary incontinence and muscle wasting were measured. The ERCC1-deficient mice treated with MEC had a significantly earlier onset of symptoms compared to ERCC1-deficient mice treated with CEA, and $Xpa^{-/-}$ mice treated with either drug. Conclusions: DNA ICLs, but not monoadducts, promote age-related degenerative changes. Future studies will focus on identifying environmental sources of ICLs by exposing the mice to cadmium chloride and chromium, two industrial contaminants that cause DNA ICLs. Identifying environmental sources if ICL damage will offers unique opportunities to prevent age-related degenerative diseases.

MH079

UNRAVELING A DISTINCT GENETIC NATURE IN BRAZILIAN XP-G PATIENTS

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NER (Nucleotide Excision Repair) is the most flexible of all known DNA repair mechanisms. XPG is a 3'-endonuclease that participates in the final steps of NER. Mutations in this gene may result in the group G form of Xeroderma Pigmentosum (XP) and, in some cases, in the severe early onset Cockayne Syndrome (CS). We are identifying the mutation present in two Brazilians patients, siblings and mildly affected, diagnosed as XP-G by the heterodikaryon complementation test. The cells from these patients demonstrate the high UV sensibility typical of this syndrome, but they are not sensitive to Methylene Blue plus light, an agent that causes oxidative stress. The sequence of both mRNA and gDNA revealed a mutation in heterozygosis, C(280)A (A28D). We also found some SNP's and one of particular interest, G(3507)C (D1104H), which has been reported as a polymorphism related to increased predisposition to some types of cancer. Analyzing the impact of the aminoacid substitution (through the internet tools SIFT, PMut and PolyPhen) we verified that A28D and D1104H have a negative impact in the protein function. Thus, apparently only one of the alleles is functionally affected by a mutation that has never been reported, while the other one encodes to a polymorphic protein also found in the DNA repair proficient individuals. The possibility of exon skipping was discarded by specific mRNA exon analyses. Now, we are testing if there is differential expression of these two alleles by Real Time RT-PCR, combining Relative Quantification with Allelic Discrimination. Although the mild clinical XP phenotype of these patients can still be due to a low expression of the functional allele, this may be the first clear example of a heterozygous genotype that leads to XP syndrome. Supported by FAPESP and CNPQ.

MH080

GENETIC POLYMORPHISM OF CLOCK GENES IN NURSES UNDER DIFFERENT WORK SHIFTS – PILOT STUDY

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Background: Night shift work, which affects circadian rhythm has been indicated as a risk factor of breast cancer. A hypothesis of circadian disruption is based on observed alterations in melatonin production and action by exposure to light at night. Genetic polymorphism in circadian genes may have a significant influence of light-modulated carcinogenesis. The circadian clock is controlled by several genes, including *Bmal1, Clock, Period (Per)* and *Cryptochrome (Cry)*. In humans, polymorphisms in clock genes have been implicated in sleep disorders, morning/evening preferences, mental health and probably shift work tolerance. The aim of this analysis, which is part of an ongoing cross-sectional study in nurses, is to determine frequency of genetic polymorphism of selected clock genes by shift-work pattern. Methods: The study of 350 nurses currently working rotating shifts night-shifts and 350 working the regular day shift, is being carried out in Lodz, Poland. The pilot study included 171 nurses (105 day, and 66 nigh-shift). We selected single nucleotide polymorphisms (SNPs) in *hBmal1* (rs2279287), *hClock* (rs1801260), *hPer1* (rs2735611), *hPer2* (rs2304672), *hPer3* (rs10462020), *hCry1* (rs8192440), *hCry2* (rs10838527). In the first course of the project we designed RFLP - PCR methods for *hBmal1, Clock, hPer3* and *hCry1* genotyping. Results: In the group of day-shift nurses, 52% of women with *Bmal* GG and GA genotype was found, while in the group of night-shift nurses, we found this genotype in 62% of the women. The frequencies of *Clock* TT, TC, CC as well as *Cry1* CC, CT, TT genotypes were similar in day and night-shift nurses. Variant *Per3* GG genotype was more frequent in day-shift nurses (6.7%) than in night-shift nurses (1.5%). Conclusions: Investigating clock polymorphisms, together with melatonin status in night- and day-shift women offers a unique opportunity to study mechanisms involved in circadian rhythm and to identify susceptible populations/individuals. Based on the observed differences in the distribution of the genotypes selected in this pilot study, clock SNPs analysis merit further investigation in a larger population. Acknowledgments: This project is supported from Polish-Norwegian Research Fund grant No. 243-AI-1/07

MH081

DNA DAMAGE IN LYMPHOCYTES OF SHORT BOWEL SYNDROME: SPONTANEOUS AND DXR-INDUCED CHROMOSOME DAMAGE (IN VITRO) BEFORE AND AFTER PARENTERAL NUTRITION

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Background: Short Bowel Syndrome (SBS) generally results in severe nutrient, water and electrolyte malabsorption. Parenteral nutrition (PN) is commonly prescribed to patients with SBS and includes amounts of essential vitamins assumed to meet metabolic needs but, even under PN treatment, SBS patients present nutritional deficiencies and exhibit poor clinical outcomes. It is well known nutritional deficiencies in vitamins, minerals and antioxidants, which are common in the population, could also contribute significantly to DNA damage. The present study aimed to investigate the levels of DNA damage and the sensibility to *in vitro* induced-DNA damage (challenge assay) in lymphocytes of patients with SBS before and after PN and healthy age-matched controls. *Methods:* Peripheral blood lymphocytes from eight healthy controls and four SBS patients were collected before and five days after PN infusion. Lymphocytes were cultured for 44 h when they were treated with doxorubicin (DXR) (0.01, 0.02 and 0.03 μg/mL) for DNA damage induction and with cytochalasin B for cytokinesis inhibition. Spontaneous and DXR-induced micronuclei (MN) were analyzed in 1000 binucleated cells and nuclear division index (NDI) was counted in 2000 cells. *Results:* SBS patients before PN exhibited higher frequencies of MN (2.5/100 cells) than controls (1.1/100 cells) ($p<0.01$). Frequencies of MN in SBS patients were similar before (2.5/100 cells) and after (2.6/100 cells) PN. Frequencies of MN were significantly increased after DXR treatments in patients as well in controls and patients response to DXR-induced damage was similar before and after PN. It was observed that the NDI in patients before PN (1.8±0.01) was significantly different from controls (2.1±0.02, $p<0.05$), however, after PN, the NDI was completely restored in patients (2.2±0.01). *Conclusions:* These preliminary results indicate that patients with SBS exhibit higher levels of chromosome damage than healthy controls and that DXR-induced damage is not differently modulated by PN treatment in SBS patients. However, the results also point that SBS patients are under cellular cytotoxic conditions, as demonstrated with NDI, which can be reduced after treatment with PN. Financial Support: FAPESP proc. Nº 2007/58409.

MH082

THE ROLES of DNA REPAIR GENE POLYMORPHISMS and ANTIOXIDANT ACTIVITY in ISCHEMIA – REPERFUSION STATUS in CLINIC

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Although cardiac coronary artery by-pass grafting (CABG) surgery has become a routine procedure worldwide, patient morbidity and mortality due to adverse complications are still unacceptably high. Cardiac surgery may lead to oxidative stress due to formation of oxidation products generated during ischemia and reperfusion. From these oxidative products, 8-hydroxy-7, 8-dihydro-2-deoxyguanosine (8-OHdG) is a mutagenic lesion and can be formed by 8-OHG repair pathway and also, occurs in the nucleotide pool. This lesion is repaired by both BER (such as OGG1 and MTH1) and NER enzyme systems. On the other hand, antioxidants act as "free radical scavengers" and they prevent and repair oxidative damage. The superoxide dismutase (SOD) is a major enzyme of the antioxidant defense system and catalyzes the dismutation of the superoxide anion into hydrogen peroxide and molecular oxygen. The aim of the present study was to investigate the roles of OGG1 and MTH1 gene polymorphisms on ischemia-reperfusion status and demonstrate their associations with 8-OHdG and SOD activity. Thus, the serum 8-OHdG levels and SOD activity of patients (n=60) were measured at five time intervals during CABG operation (before and after operation; T1 and T5; respectively, after anesthesia, ischemia and reperfusion; T2, T3 and T4; respectively) and OGG1 Ser326Cys and MTH1 Val83Met polymorphisms were genotyped by RFLP-PCR. We found that the frequencies of OGG1 Ser/Ser, Ser/Cys and Cys/Cys genotypes were 55%, 42% and 3%, respectively; while the frequencies of MTH1 Val83Val and Val83Met genotypes were 93.3% and 6.7%. We observed a decrease in the levels of 8-OHdG among time intervals (T2, T3 and T4) when compared to T1, while the levels of 8-OHdG in T5 was higher than in T4 ($p<0.05$). There was no significant association between the variant forms of each gene and serum 8-OHdG levels. However, SOD activity in T4 significantly increased compare to T1, T2, T3, but SOD activity in T5 decreased compare to T4. The increased in T4 suggested that some free radicals occurred during operation might be scavenged by SOD antioxidant system. This study was supported by The Scientific & Technological Research Council of Turkey (Project No:106S176).

MH083

LOOKING FOR NEW MEGAZOL DERIVATIVES AGAINST TRYPANOSOMA CRUZI (CHAGAS DISEASE)

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Chagas disease is an endemic infection disease in Latin America caused by *Trypanosoma cruzi* protozoan. There is no efficient drug for the Chagas disease treatment due to the acquired resistance of the parasite and because the drugs in use, as nifurtimox and benznidazol, are cytotoxic. Derivatives from megazol, a nitroimidazole, could be good alternative for this purpose. Although megazol presents a high efficiency against *T. cruzi* it is not clinically available once its mutagenicity and carcinogenicity is controversial. Positive mutagenic data was already described without metabolic activation for *Salmonella typhimurium* strains TA98 and TA102. In our laboratory, a first investigation with megazol indicated positive mutagenic response, with no cytotoxic effects, in TA97, TA98 and TA100 at the tested concentrations (0.05, 0.1, 0.2, 0.5, and 1.0 microg/mL in dimethylsulfoxide), with or without metabolic activation (S9). No induction was observed in TA102 (+/-S9). Our results showed that this potential trypanocidal agent acts as a strong inducer of base-pair substitution and frameshift mutation. As *S. typhimurium* expresses high levels of nitroreductase activity, positive results observed in the Ames test should be interpreted with caution. The presence of a nitro-group could play a key role in the overall mutagenic activity of megazol. This result has encouraged us to further explore structurally-related megazol derivatives, in order to obtain a better understanding of their structural and antiprotozoal activity relationships. Support: CNPq, FAPERJ and SR2/UERJ.

MH084
OXIDATIVE STRESS, PAH-DNA ADDUCTS AND BREAST CANCER

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Background: Reactive oxygen species (ROS) damage DNA, and may play a role in the etiology of breast cancer as well as progression and survival. Tobacco smoking, dietary exposure and environment are sources of polycyclic aromatic hydrocarbons (PAHs) exposure which produce ROS and may be associated with breast cancer risk. Objective: We estimated the breast cancer risk associated with multiple polymorphisms in MPO, CAT, MnSOD and the interaction with PAH–DNA adducts and cigarette smoking. Methods: We conducted unconditional logistic regression using data from a population-based sample of women (873 cases/ 941 controls), PAH–DNA adduct blood levels were measured by competitive enzyme-linked immunosorbent assay (n = 873 of 941), and smoking status was assessed by in-person questionnaires (n = 943 of 973). Results: We did not find evidence for the main effects of MPO, CAT, or MnSOD on detectable PAH-DNA adducts among controls. We do not report evidence of interaction between PAH-DNA adducts and MPO (p=0.62), CAT (p=0.54), and MnSOD (p=0.59) and risk of breast cancer. Conclusion: We found little statistical evidence that PAHs interacted with *MPO*, *CAT*, *MnSOD* polymorphisms to further increase breast cancer risk.

MH085
THE EXPERIMENTAL STUDY OF IMMUNOLOREGULATION FUNCTION OF RAT MESENCHYMAL STEM CELLS ON SPLEEN MONONUCLEARCELL AND ITS MECHANISM IN VITRO

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Aims: The immunological regulation mechanism of mesenchymal stem cells (MSCs) was analyzed, and hopeful to provide scientific evidence for clinical application. Methods: MSCs cell cycle distribution and cell surface markers were detected by flow cytometry (FCM). The multiple differentiation and proliferation potentials were confirmed by evaluating cell growth curve. The inhibitory effect on MNC proliferation stimulated by Con A was evaluated by MTT. CD25, TGF-β1, IL-2, IL-10 and IFN-γ expression were confirmed. Results: MNC proliferation was inhibited significantly (*P*<0.01). After treatment with MSCs or MSCs supernatant by 20μg/ml Con A stimulating for 24h, CD25 expression was lower than that of spleen MNC cultured alone (*p*<0.01). MSCs supernatant could inhibit the activation of spleen MNC stimulated by Con A (*P*<0.01). IL-2 and IFN-γ mRNA expression were lower than that that without MSCs (*p*<0.05), while IL-10 mRNA expression was higher (*p*<0.05). Spleen MNC stimulated by Con A in the presence of MSCs was arrested to G_0/G_1 phase. MSCs could upregulate p27 expression and downregulate cyclinE expression significantly. The cytotoxcity to inhibit tumor cell decreased significantly after MSCs co-cultured with spleen MNC for 48h (*p*<0.05). The proportion of $CD4^+CD25^+$T cells subset and Foxp3 mRNA expression in MNC cultured with MSCs were higher than that without MSCs P<0.01. TGF-β1 and IL-10 level secreted by co-culture group was significantly higher than that of control (*p*<0.01). Conclusions: MSCs may promote Th0 cells differentiating into Th2 cells, but supressing into Th1 cells differentiation. Upregulation of p27 expression and downregulation cyclin E expression can result in MNC arresting to G_0/G_1 phase. MSCs could not induce MNC apoptosis, which might related to the MNC subsets changes and improving secretion of inhibitory cytokines by MSCs.

MH086
DO ANTIOXIDANTS PREVENT THE LYMPHOCYTE DNA DAMAGE INDUCED BY EXHAUSTIVE EXERCISE?

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The beneficial effects of regular and nonexhaustive exercise have been well-known for along time. Effectiveness of regular physical exercise includes reduced risk of various diseases such as cardiovascular disease, diabetes, osteoporosis and cancer. Besides the known benefits of physical activity, exercise appears to increase ROS, which can result in damage in all cellular macromolecules such as DNA, lipids, and proteins. Oxidative DNA damage may play an important role in biological processes such as mutagenesis, carcinogenesis, chronic inflammation and aging in humans. Antioxidants protect the integrity of DNA from genotoxicants and are capable of eliminate ROS generated in situations of oxidative stress. Therefore, the purpose of this study was to investigate the possible protective effect provided by antioxidant supplementation of vitamin E, by comet assay, before and after an exhaustive physical activity. The study participants were competitive rowers (CR) (n=12), sport academy students (SAS) (n=11) and control group (CG) (n=12). An exhaustive exercise tests were performed by competitive rowers and sport academy students. Vitamin E supplementation was given to competitive rowers and sport academy students for 2 months. At the end of vitamin E consumption, the comet assay was again carried out in all subjects. Mean tail %DNA for each cell was calculated as 100-Head %DNA. The same application was carried out for CR and SAS subjects after 2 months of vitamin E supplementation. The preli-

minary of this study shows that the DNA damage increases after exhaustive exercise and vitamin E seems to have slight decrease in the mean tail %DNA.

MH087

ASSESSMENT OF INTRA-INDIVIDUAL VARIATION IN MICRONUCLEATED RETICULOCYTES IN HUMANS USING FLOW CYTOMETRY AND COMPARISON WITH CHROMOSOME ABERRATION DATA. A POTENTIAL BIOMARKER FOR HUMAN GENOTOXICITY?

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Cytogenetic endpoints (chromosome aberrations and micronuclei) have traditionally been used to assess the burden of genetic damage in animal models and humans. It is well recognized that the sensitivity of standard microscopy methods is limited by the small numbers of cells that can be scored routinely. This is particularly true for human populations, where cytogenetic endpoints have not provided reliable translational biomarkers of individual genetic damage. The development of flow cytometry micronucleus assays, therefore, is one of the most promising new developments for a translational biomarker of individual human genetic damage. Preliminary data from human clinical studies have been reported and there appears to be consistency in the mean spontaneous frequency of micronucleated reticulocytes in normal human adult, juvenile and paediatric populations. Interestingly, baseline frequencies are similar to those previously reported for rodents and dogs. To explore human intra-individual variation in the production of micronucleated reticulocytes, blood samples from 8 healthy adult volunteers (4 males and 4 females) were collected on 4 occasions over a period of 3 months (day 1 am and pm, day 3, week 3 and month 3). Chromosome aberration frequencies were determined at the start and end of the study. In addition, red blood cell folate and B_{12} levels were measured. The results of the study will be presented. These data represents a step forward for the development of a translational biomarker of genetic damage from animal models to humans. Although the ‘memory’ of the biomarker is limited because of the short half-life of reticulocytes expressing the CD71 transferrin receptor, such a translational biomarker could provide a powerful tool for determining genotoxic risk in humans.

MH088

SERUM LEPTIN, ESTRADIOL AND TGF-α LEVEL IN MALAYSIAN PRE-MENOPAUSAL BREAST CANCER PATIENTS

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Introduction: Malaysian women of less than 50 years of age have a higher prevalence of breast cancer (65%). Evidence indicates that apart from the known risk factors of breast cancer, the emerging role of associated risk factors such as leptin and other growth factors remains uncertain. Therefore the present study is undertaken to measure the levels of serum leptin, TGF-α and estradiol in control and pre-menopausal breast cancer patients. Methodology: The present case-control study was conducted in Hospital Universiti Sains Malaysia (HUSM). A total of 20 controls and 20 newly diagnosed pre-menopausal breast cancer patients were recruited. After recruitment, age, anthropometrical measurements and fasting venous blood samples were obtained for serum leptin, estradiol and TGF-α assays. Detailed clinical examinations of TNM staging, histopathological examination and immuno-histochemistry of estrogen receptors (ER), progesterone receptors (PR) and epidermal growth factor (C-erb-2) were performed. Data were analyzed using Mann-Whitney U test. Results were expressed as mean ± S.E.M and a ‘p’ of < 0.05 was considered statistically significant. Results: No significant difference observed between patients and controls of age (41±1.9 vs 39±2.0; p=0.383), height (1.55±0.01 vs 1.53±0.01 m; p=0.379), weight (55±3.0 vs 60±2.4 kg; p=0.173) and body mass index (23±1.3 vs 26±1.2 kg/m2; p=0.101). Clinical results showed that 80 % of the patients had locally advanced stages of T4, N1, M1 with ER and PR positive while C-erb-2 negative receptor status and were of ductal carcinoma. Biochemical assays showed a significantly decreased levels of serum leptin (16±4 vs 47±6 ng/mL; p=0.000) and estradiol (259±66 vs 491±80 pmol/L ; p=0.042) in breast cancer patients compared to controls. Conversely, serum TGF-α level was significantly higher in patients compared to controls (128±22 vs 33±7 pg/mL; p=0.000). Conclusion: The increased TGF-α concentration in breast cancer patients suggest a possible role as a prognostic factor in pre-menopausal breast cancer. Further studies are needed to elucidate the possible role of reduced levels of leptin and estradiol in pre-menopausal breast cancer.

MH089

ANTIOXIDANT ENZYME ACTIVITIES AND mRNA LEVELS IN THE KIDNEY OF PRE-HYPERTENSIVE SPONTANEOUSLY HYPERTENSIVE RATS (SHR) AND WISTAR-KYOTO RATS (WKY)

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Oxidative stress has been implicated in essential hypertension. However, the precise status of antioxidant enzyme (AOE) activities and their expressions during the pre-hypertensive stage remains inconclusive. This study was therefore undertaken to measure and compare the AOE activities in the kidney of pre-hypertensive SHR and age-matched WKY rats. After the measurement of body weight in 2 and 3-week old, and body weight and blood pressure in 4-week old pre-hypertensive SHR and age-matched WKY rats, (n = 8 per group), the rats were sacrificed and their kidneys were harvested for the measurement of mRNA levels and activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), and activities of glutathione reductase (GR) and glutathione-S-transferase (GST). In addition, the total antioxidant status (TAS) was also estimated. Data were analysed using ANOVA, and a ‘p’ of < 0.05 was considered statistically significant. When compared to age-matched WKY rats, body weight was significantly lower in 2, 3 and 4-week old pre-hypertensive SHR (p<0.001). No significant difference was present in the mean systolic blood pressure at 4 weeks between the two groups. CAT activity was significantly higher at 2, 3, and 4 weeks (p<0.001) but GPx activity was significantly lower at 3 and 4 weeks (p<0.05) in pre-hypertensive SHR. GR activity was significantly lower at 2 weeks (p<0.001) whereas GST activity was lower at 4 weeks (p<0.05) in pre-hypertensive SHR. No significant differences were evident in the SOD activity at 2, 3 and 4 weeks between the two groups. CAT mRNA levels were significantly higher in SHR at 4 weeks (p<0.05) but no significant difference was evident in mRNA levels of Mn-SOD, CuZn-SOD and GPx between the two groups. Interestingly, TAS levels were found to be significantly higher in the pre-hypertensive SHR at 3 and 4 weeks (p<0.05). The results indicate that there was an altered pattern of mRNA expression and activity of some AOE in the kidneys of young pre-hypertensive SHR and this might have some influence in the pathogenesis of hypertension in this species.

MH090
FUNCTIONAL CHARACTERIZATION OF LYMPHOBLASTOID CELL LINES DERIVED FROM PATIENTS AFFECTED BY MUTYH-ASSOCIATED POLYPOSIS

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Background The recessively inherited adenomatous polyposis (MAP) disease is associated with mutations in the DNA base excision repair (BER) MUTYH gene. MAP patients present multiple colorectal adenomas that can evolve with high probability into colorectal cancer. The main somatic mutations found in MAP patients are G:C>T:A transversions typically associated with a defective BER pathway. These most likely derive from one of the major oxidation products, 8-hydroxyguanine (8-oxodG), which can easily mispair with adenine and, if unrepaired, results in G>T transversions at the next round of replication. Aims To investigate the functional significance of different MUTYH mutations (Y165C/Y165C, G382D/R231C, G382D/1186-7insGG, IVS10+3A>C/1105delC), we characterized their biological effects in lymphoblastoid cell lines derived from MAP patients. Methods Expression levels of the MUTYH protein were quantitated by Western blots and transcripts were investigated by RT-PCR. Basal and oxidant-induced levels of DNA 8-oxodG were measured by HPLC/EC. Co-immunoprecipitation and subsequent Western Blot analyzed MUTYH interaction with other proteins involved in the repair of oxidized bases and/or cell cycle checkpoints. Results A comparison with two independent wild-type cell lines indicated that cells expressing an homozygous Y165C mutation show wild-type levels of the MUTYH protein. In contrast less than 50% of wild type were observed in G382D/R231C and G382D/1186-7insGG and no expression was detectable in IVS10+3A>C/1105delC. Significant amounts of aberrantly spliced out-of-frame transcripts were evidenced by RT-PCR only in the IVS10+3A>C/1105delC cell line. Basal levels of 8-oxodG were increased in all MUTYH mutants, when compared to wild-type cells (1.2-2.3 fold increase). To confirm a defective repair of 8-oxodG in these mutants, repair kinetics of the oxidized base following exposure to the $KBrO_3$ oxidant are currently under investigation. An interaction of MUTYH with MSH6, RAD9 and PCNA was confirmed by co-immunoprecipitation experiments. We are currently studying whether these MUTYH mutations affect these interactions.

MH091
MOLECULAR AND GENETIC FACTORS INFLUENCE ON DNA REPAIR EFFICIENCY AND HEALTH HAZARD

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The aim of our human monitoring studies performed on subjects from various cities and countries was to find out if occupational exposures to genotoxic agents can alter cellular radiosensitivity or alter health risk. Exposures to pesticides, mercury ions, polycyclic aromatic hydrocarbons (PAHs) elevated levels of cytogenetic damage and increased risk of cancer. Our research also displayed that results of repair competence assay, applying the challenging dose of X-rays, combined with the detection of DNA damage by SCGE assay, correlated to the induced level of cytogenetic damage, so could be used as phenotype related biomarker of health risk. Observed reduction in cellular capacities were associated to genetic and life styles related factors; smoking or levels of vitamins. Results from studies on the PAHs influence on efficiency of repair of the DNA damage induced by radiation, showed a strong variability between donors. A significant decrease of repair efficiency in subjects occupationally exposed to PAHs was also observed when those groups were stratified first according to various genotypes for genes, encoding enzymes involved in the process of biotransformation (CYP1A1(Ile/Val), GSTM1, NAT2) or DNA repair (EPHX4 or XRCC1)[1] and then to the occupational exposure. Results of our studies have also shown that significant reduction of cellular repair efficacy was observed in a various groups of cancer patients. Presented results point toward that environmental exposure to genotoxic agents; *via* alteration of the DNA repair processes can alter the level of cytogenetic damage and result in increased health risk from cancer. Our results are also indicating that the DNA repair competence assay might be a sign of the phenotype related cellular susceptibility to various (environmental, occupational or therapeutic) radiation or chemical treatments, in addition to significantly reduced or increased the efficiency of DNA repair.

[1] Cebulska-Wasilewska, A., Binkova B., Sram R.J., Kalina I., Popov T., Farmer P. B., (2007).Repair competence assay in studies of the influence of environmental exposure to c-PAHs on individual susceptibility to induction of DNA damage. Mutat. Res./Fundamental and Molecular Mechanisms of Mutagenesis .620, 1-2, 1, 155-164.

MH092
BACTERIAL MUTAGENICITY SCREENING: AUTOMATION OF THE AMES TEST

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The bacterial reverse mutagenicity test on Salmonella typhimurium, known as the Ames test, is generaly used to assess the mutagenic potential of chemicals and is part of standard regulatory testing for non clinical safety assessment of new pharmaceuticals. As mutagenic activity is very critical for the development of potentially new drug candidates reliable information on Ames test outcome is needed early on. Bacterial indicator assays to predict the Ames test have advantages like very fast performance, higher throughput and the need for only very small amounts of compound. On the other side such assays are generally limited by lower predictivity for the outcome of the OECD 471 guideline assay compared to miniaturised versions of the Ames test. In order to fit the industrial constraint of screening more products at the low quantities available while keeping excellent predictivity we fully automated a mini version of the regulatory Ames test protocol that allows a significant reduction of the quantity of test substance needed (30 mg) but remains applicable to all Salmonella strains used in the regulatory protocol. This robot system consists of two integrated modules processing all five tester strains in parallel and performing for the first time all steps of a complete Ames test automatically. The plate preparation module encompasses the preparation of the compound and bacteria mixtures into 4 microtiter plates including all dispensing and incubation steps. The overlay module comprises the handling and manipulation of the Petri dishes and the growth media as well as the pipetting of the bacterial mixtures onto the pre-cast agar dishes and the storing of the dishes in easy-to-handle containers for incubation. We confirmed for accuracy and stability of continuous operation of the system and first test results show identical dose relationship curves as the manual procedure. Despite of increased throughput and replacing long repetitive working procedures this Ames test robot system might also enhance assay performance by reducing variabilities caused by manual operation. Adapting the plate preparation module to the handling of larger volumes would also allow to apply the robot to the OECD 471 guideline protocol used for regulatory testing.

MH093
RESPONSE KINETICS OF PIGA and LACZ MUTATIONS, AND MICRONUCLEOUS INDUCTION BY BENZO(A)PYRENE IN MUTA™MOUSE HAEMATOPOIETIC CELLS

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While there is great interest in pigA as a new mutation marker, there is little data showing how it relates to established mutagenicity endpoints. In order to provide insight into this issue, we have conducted a comparative investigation of pigA mutation response with lacZ mutation and micronucleus induction in MutatmMouse. 25-week old male transgenic mice, (i.e, Muta™Mouse, strain 40.6) were dosed daily for 28 days with benzo[a]pyrene (0, 25, 50 and 75 mg/kg/day by gavage). Following a subsequent 72h sampling period, mice were sacrificed, and tissues and blood were collected. The lacZ transgene mutant frequency (MF) was determined by PGal positive selection in DNA isolated from bone marrow. pigA mutants were measured by antiCD59 flow cytometry of reticulocytes (RET). Micronucleus frequencies were also measured in RETs and normochromatic erythrocytes (NCE) by flow cytometry. A significant dose-dependent increase in pigA phenotypes were observed in RET. Matched samples from the same animals showed a significant dose-related increase in lacZ MF in the bone marrow, which was approximately 25x higher than that observed for pigA. This difference could be due to factors such as the greater target size of lacZ and/or differences in the optimal sampling time for these endpoints. Significant dose-related increases in the % micronuclei were also observed for NCE and RET in these same animals. The dose responses of all three endpoints followed virtually the same linear kinetics, suggesting that similar mechanisms were at play in their induction. Furthermore, the similarity in the response of pigA and lacZ provides evidence that pigA mutant phenotypes likely result from mutant genotypes, since there is ample evidence for the gene mutation origin of lacZ mutant phenotypes.

MH094

GENOTOXIC EVALUATION OF PIPER ELONGATUM EXTRACT AND AMPHOTERICIN B IN THE SOMATIC MUTATION AND RECOMBINATION TEST

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In Bolivia parasitic diseases are very frequent, especially the leishmaniasis is considered as neglected disease. Nowadays new anti-parasitic agents from medicinal plants are showing effective response against leishmaniasis. The present work studies the genotoxicity level of two anti-parasitic therapeutic alternatives. As drug we evaluated Amphotericin B and as natural therapeutic alternative we selected *Piper elongatum* Vahl commonly called "matico". Biological activity of hydroalcoholic extract has demonstrated an interesting anti-parasitic activity against *Leishmania*. The genotoxic evaluation was done using the Somatic Mutation and Recombination Test (SMART), which uses *Drosophila melanogaster* as experimental organism to determine recombinogenic and mutagenic activity for plant extracts and drugs. Mitomycin C was used as positive control. Two types of crossing were used: standard (ST) and high bioactivation crosses (HB). Larvaes of third instar coming from both crossings were treated with sub-toxic concentrations, for each one of tested compounds. The results in the experimental conditions of the present work suggest that the hydroalcoholic extract of *Piper elongatum* Vahl at concentrations well below 100mg/mL and Amphotericin B at 200 uM, do not show genotoxic activity. * Acknowledge: MAEC-AECID

MH095

MITOCHONDRIAL DNA HAPLOGROUPS AND ETHNICAL ORIGINS IN PATIENTS WITH MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system in which complex genetic factors exert influence on susceptibility. It is a multifocal demyelinating central nervous system disorder in which interplay between genes and the environment are supposed to be involved. Mitochondrial DNA (mtDNA) has the none-coding regions at the displacement loop (D-loop) region that contains two hypervariable segments (HVS-I and HVS-II) with high polymorphism. Several authors have mentioned increased prevalence of multiple sclerosis (MS) in some ethnical groups. To asses the relantionship between mtDNA haplogroups and MS, we sequenced the hypervariable segments in 52 patients and 50 control subjects to determine their ethnic origin in an heterogeneous population in Bogotá, Colombia. The haplogroups found in the subjects (102) were: A (35.3%); B (26.5%), C (15.6%); D (8.8%); L (5.8%); T (2%); and J (4%). No differences were found in the prevalence of EM between patients and controls.

Prevention of mutation-related diseases

PD001

PHENOTYPIC REVERSION OF HUMAN KERATINOCYTES FROM A PATIENT SUFFERING FROM COCKAYNE SYNDROME

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As cellular DNA is under constant challenge by genotoxic stress, all cells are equipped with DNA damage response pathways that trigger DNA repair, cell cycle arrest, and apoptosis, to eliminate damaged cells. The consequences for stem cells (SC) is diminution in SC pools or increase of SC differentiation /malignant transformation. A number of DNA repair abnormalities are linked to premature aging syndromes, and these are associated with defects in the SC population. Our studies are focused on recessive autosomal syndromes, such as Xeroderma Pigmentosum (XP), Cockayne syndrome (CS) and Tricho-thiodystrophy (TTD), caused by defects in nucleotide excision repair mechanisms and characterized by skin premature aging. Self-renewal of epidermis relies on the presence of SCs and transient amplifying (TA) cells. Transition from SCs to TA cells, named clonal evolution, is a process that occurs during natural aging and keratinocyte subcultivation. Replicative senescence, considered the cellular counterpart of in vivo aging, occurs when all SCs have completed their clonal evolution. We have stably transduced CS-A deficient keratinocytes with a transduction efficiency near to 100%, using a retroviral infection method. In order to study defects in replicative senescence, CS-A-transduced keratinocyte cultures were serially passaged and showed a proliferative potential similar to normal keratinocytes, different from patient and empty vector-transduced cultures. Exogenous protein was translocated into the nucleus and its expression was maintained during serial passages. Moreover, CS-A-transduced keratinocyte cultures were able to survive and to recover RNA synthesis after UVC irradiation such as normal keratinocytes, different from defective keratinocytes. CS-A protein was able to restore 8-oxo-guanine levels, physiologic clonal evolution and a normal ratio of proliferation/differentiation markers in deficient keratinocytes. Further functional studies will let us to obtain new knowledge about a link between DNA repair systems and SC maintenance.

PD002

PHARMACOLOGICAL ANTIOXIDANTS PROTECT FROM RADIATION INDUCED DNA DAMAGE AS WELL AS GENETIC INSTABILITY AND LYMPHOMA IN ATM DEFICIENT MICE

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We examined the role of N-acetyl-L-cysteine (NAC), a clinically proven safe agent, for it's ability to protect against γ-ray-induced DNA strand breaks in human cells and in mice as well as against DNA damage and lymphoma in Atm deficient mice. Human lymphoblastoid cells were examined for the frequency of γ-H2AX foci formation, indicative of DNA double strand break formation. DNA strand breaks were also measured in mouse peripheral blood by the alkaline comet assay. NAC reduced γ-H2AX foci formation in human lymphoblastoid cells but had no protective effect in the colony survival assay. NAC administration via drinking water fully protected against DNA strand breaks in mice whole-body irradiated with 1 Gy. Ataxia Telangiectasia (AT) is an autosomal recessive disorder characterized by motor dysfunction, chromosomal instability, radiosensitivity, oxidatively stressed phenotype and high incidence of cancer. We measured deletion events occurring in somatic cells in pun/pun mouse embryos caused by reversion of the pun mutation to wildtype p gene and resulting in the assembly of a black pigment melanin complex in melanocytes. These deletion events result in black spots on the gray fur and patches of black cells (eye spots) on the transparent retinal pigment epithelium (RPE) and are significantly elevated in Atm deficient mice. We hypothesized that dietary intake of antioxidants may eliminate oxidative stress in AT and thereby reduce the frequency of DNA deletions and oxidative DNA damage. To test our hypothesis we crossed Atm+/- pun/pun mice with each other and the dams were treated with NAC in drinking water during pregnancy. The frequency of DNA deletions and oxidative DNA damage was determined in the offspring. NAC treatment reduced the levels of DNA deletions as well as oxidative DNA damage in Atm-/- mice to the wild-type level. Furthermore, continuous NAC treatment significantly prolonged the life of and reduced the frequency of lymphomas in Atm-/- mice. This finding suggests that NAC is a prospective nutritional antioxidant to counteract DNA damage, DNA instability and cancer in AT and it may be useful in radiation therapy to prevent radiation-mediated genotoxicity, but does not interfere with efficient cancer cell killing.

PD003

EVALUATION OF AGARICUS BLAZEI IN VIVO FOR ANTIGENOTOXIC, ANTICARCINOGENIC, PHAGOCYTIC AND IMMUNOMODULATORY ACTIVITIES.

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In Brazil, cancer represents the second leading cause of death in the adult population. The development of various more common forms result from the interation among endogenous, environmental and hormonal factors, where the most notable of these factors is diet. The aim of the present study was to determine the antigenotoxic, anticarcinogenic, phagocytic and immunomodulatory activities of Agaricus blazei. Supplementation with the mushroom was carried out under pre-treatment, simultaneous treatment, post-treatment and pre-treatment+continuous conditions. Statistical analysis demonstrated that the mushroom did not have genotoxic activity but showed antigenotoxic activity. Supplementation caused an increase in the number of monocytes and in phagocytic activity, suggesting that supplementation increases a proliferation of monocytes, consequently increasing phagocytic capacity especially in the groups pre-treatment, simultaneous and pre-treatment+continuous. The data suggest that Agaricus blazei could act as a functional food capable of promoting immunomodulation which can account for the destruction of cells with DNA alterations that correlate with the development of cancer, since this mushroom was demonstrated to have a preventive effect against pre-neoplastic colorectal lesions evaluated by the aberrant crypt foci assay. According to these results and the literature, it is believed that supplementation with Agaricus blazei can be an efficient method for the prevention of cancer as well as possibly being an important coadjuvant treatment in chemotherapy, which could improve quality of life in both the healthy population and patients on chemotherapy. Supported by CNPq and CAPES.

PD004

ANTIMUTAGENIC AND ANTICARCINOGENIC EFFECT OF OLIGOSACCHARIDE INULIN IN VIVO.

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This study evaluated the antimutagenic and anticarcinogenic action of inulin by means of testing the comet, micronucleus and aberrant crypt foci in vivo. The experiment was conducted with Swiss adult males mice, which received administration 1,2-dimethylhydrazine (DMH), for two weeks in four doses of 20mg/kg of body weight (b.w), the inulin was administered orally daily at a concentration of 50 mg/kg b.w, beyond the control groups. The collection of peripheral blood for the testing of micronucleus occurred 24 (T1) and 48 (T2) hours after the last administration of DMH, the collection for testing the comet occurred in T1. On the other hand there was a high potential antimutagenic in all protocols in the micronucleus test. The rate of reduction of damages in T1 were: 47.25% for post-treatment, 111.45% for pre-treatment, and simultaneously to 118.67% 103.80% to pre+continuous. In T2 the reduction of damage is 141.75%, 108.21%, 121.93% and 77.46%. The anticarcinogenic activity was observed in all protocols and the percentages of reduction of neoplastic lesions were 69.12%, 87.56%, 55.78%, 72.89% for the same protocols above. Thus, further studies may investigate the potential of this compound in the prevention of damage related to the initiation and/or promotion of cancer.
Supported by CNPq

PD005
CHEMOPREVENTION OF DNA DAMAGE IN BRONCHOALVEOLAR LAVAGE CELLS OF MICE EXPOSED TO CIGARETTE SMOKE

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Cigarette smoke is the major risk factor for lung cancer and a variety of chronic degenerative diseases. We tested the ability of the glucocorticoid budesonide and of the natural agent phenethyl isothiocyanate (PEITC) to inhibit DNA damage in bronchoalveolar lavage cells (BAL) of CD–1 mice exposed to environmental cigarette smoke (ECS). A total of 197 mice were divided into following experimental groups: (A) untreated (sham); (B) ECS; (C) ECS +PEITC; (D) ECS + budesonide. Exposure to ECS started within 12 h after birth and continued throughout the weaning period and for 2 additional weeks after weaning, for a total of 7 weeks. After treatment, the mice were sacrificed and subjected to BAL in order to evaluate DNA damage in BAL cells by using the alkaline–halo test. DNA damage was significantly increased in ECS exposed mice vs. Sham (3.3-fold). Both PEITC (2.4-fold) and budesonide (2.0-fold) significantly attenuated ECS-induced DNA damage. In conclusion, the analysis of sentinel cells collected by BAL showed that the investigated agents, which are among the most promising chemopreventive agents, are able to revert the DNA damage produced by passive exposure to cigarette smoke.

PD006
MOLECULAR AND CYTOGENETIC ALTERATIONS INDUCED BY CIGARETTE SMOKE IN MICE AND THEIR MODULATION BY DIETARY AND PHARMACOLOGICAL AGENTS

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Tobacco smoke is the major risk factor for human cancer, being responsible for the 85–90% of lung cancer, and for cancers in several other anatomic sites. Environmental cigarette smoke (ECS), which is inhaled by involuntary smokers (or passive smokers), is composed of side-stream smoke (SCS), released from the smoldering distal part of the cigarette, and, in minor proportion, of that portion of mainstream cigarette smoke (MCS) exhaled by active smokers. ECS is classified as a lung carcinogen to humans. In the present study we evaluated the protective effects of the glucocorticoid budesonide and of the dietary agent phenethyl isothiocyanate (PEITC) in CD-1 neonatal mice exposed to ECS for 4 weeks. The mice exposed to ECS since birth underwent a variety of alterations of molecular and cytogenetic end-points. In particular, a diagonal radioactive zone appeared in 32P postlabeling autoradiographs of lung DNA from ECS–exposed mice, with a 3.7–fold increase of the signal as compared with sham–exposed mice. Similarly, there was a 2.2–fold increase of 8–oxodGuo levels, showing induction by ECS of oxidative DNA damage in the lung. In addition, ECS produced a systemic cytogenetic damage. In fact, there was a 2.1–fold increase of MN frequency in bone marrow PCE, accompanied by a significant decrease of the PCE/NCE ratio. Cytogenetic damage was also detected in peripheral blood, with a 2.2–fold increase of MN frequency in the NCE from ECS–exposed mice. Both PEITC and budesonide, administered daily with the diet after weanling, decreased these alterations. In particular, budesonide had a quite powerful effect in protecting the lung from the oxidative DNA damage generated by ECS. This finding is likely to be related to the anti–inflammatory properties of this glucocorticoid, which by attenuating inflammation blocks a major source of oxidative damage. In addition, budesonide significantly attenuated ECS–related cytogenetic damage in bone marrow and peripheral blood. The major protective effects of PEITC were a reduction of bulky DNA adducts and 8–oxodGuo. Inhibition of ECS–induced bulky DNA adducts by PEITC is likely to be related to the ability of this chemopreventive agent to stimulate the metabolic activation of smoke components.

PD007
CHEMOPREVENTION OF CIGARETTE SMOKE-INDUCED ALTERATIONS OF microRNA EXPRESSION IN RAT LUNG

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We previously showed that exposure of rats to environmental cigarette smoke (ECS), for 28 days, causes extensive downregulation of microRNA expression in the lung, resulting in overexpression of multiple genes and proteins. In the present study, we evaluated by microarray the expression of 484 microRNAs in the lung of rats, either ECS-free or ECS-exposed, which were treated orally with chemopreventive agents, including N-acetylcysteine, oltipraz, indole-3-carbinol, 5,6-benzoflavone and phenethyl isothiocyanate, or combinations thereof. Scatterplot, hierarchical cluster, and principal component analyses of microarray and qPCR data showed that none of the above chemopreventive regimens appreciably affected the baseline microRNA expression. On the other hand, all of them were successful in attenuating ECS-induced alterations but to a variable extent and with different patterns. The main ECS-altered functions that were modulated by chemopreventive agents included cell proliferation, apoptosis, differentiation, Ras activation, P53 functions, NF-κB pathway, TGF-related stress response, and angiogenesis. Some microRNAs known to be polymorphic in humans were downregulated by ECS and protected by chemopreventive agents. Thus, microRNA analysis provides a new tool for predicting both safety and efficacy of cancer chemopreventive agents at early carcinogenesis stages.

PD008

MODULATION OF PROLIFERATION, APOPTOSIS AND HISTOPATHOLOGICAL ALTERATIONS INDUCED BY CIGARETTE SMOKE IN MOUSE LUNG

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We previously demonstrated that mainstream cigarette smoke (MCS) is a quite potent carcinogen in neonatal mice. Further on, we showed that exposure of mice to environmental cigarette smoke (ECS), starting at birth, results in alterations of a variety of intermediate biomarkers. In the present study we evaluated the protective effects of the glucocorticoid budesonide and of the dietary agent phenethyl isothiocyanate (PEITC) in CD-1 mice exposed to ECS for 9 months, followed by 2 months of recovery. ECS stimulated the proliferation of bronchial epithelial cells, as shown by an 8–fold increase of PCNA. In parallel, there was a 5.5–fold increase of the apoptotic index, as evaluated by TUNEL method in the same cells. Eleven months after birth, these animals exhibited significant histopathological changes, such as pulmonary anthracosis, emphysema, hemorrhagic areas, alveolar bronchiolarization, bronchial hyperplasia, and tumors, both benign and malignant. The carcinogenic response was less evident in dams exposed to ECS under identical conditions. The two chemopreventive agents under investigation, administered daily with the diet after weanling, modulated ECS–related early biomarkers and medium-term damages to a variable extent. In particular, the ECS–induced hyperproliferation of bronchial epithelial cells was slightly but significantly attenuated by budesonide. Both PEITC and budesonide were able to inhibit the ECS–induced increase of the apoptotic index in the same cells. Furthermore, they moderately protected the lungs from histopathological alterations, including tumors. Thus, although not as efficiently as the bioassay in MCS–exposed mice, the model in neonatal mice is suitable to evaluate both ECS carcinogenicity and its modulation by chemopreventive agents.

PD009

ANTIMUTAGENIC EFFECTS OF WATER-SOLUBLE CHITOSAN

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Chitosan is derived from chitin by deacetylation in the presence of alkali. Chitin is usually prepared from the shells of crabs and shrimps. Chitosan has been reported to have antitumor and anticancer activities although those details have not been clarified yet. In this experiment using the Ames test, there was antimutagenic effect by the water-soluble chitosan (WSC). For this study, WSC was supplied by Chitosan Food Industry, Ltd. The Ames test was performed using *Salmonella typhimurium* TA98 and TA100. For the antimutagenic test, and the desmutagenic and bioantimutagenic tests, Trp-P-2, 2AA and AF-2 were used as mutagens. Tests for spontaneous mutation rate without mutagens were performed using *Salmonella typhimurium* TA98 and TA100. When several concentrations of WSC were directly added to Minimal Glucose Plates, there were no changes in the number of His+ revertants of TA98 and TA100. In desmutagenic and bioantimutagenic tests, the number of His+ revertants of TA98 and TA100 decreased. In the test for spontaneous mutation rate, the number of His+ revertants of TA98 and TA100 decreased slightly. When a concentration of WSC was added to examine for antimutagenic effects, there was no change in the viable cell count of TA98 and TA100. From these results, AF-2 that is a direct mutagen may combine with WSC, and that may also be carrying out the inactivation. WSC possibly acts directly on the DNA by decreasing the rate at which mutation is caused to the strain's promutagens Trp-P-2 and 2AA. This is evident from the decreased spontaneous mutation rate without a decreased viable cell count. For the above-mentioned reasons, WSC is suggested as a possible preventive medicine for carcinogenesis.

PD010

POTENTIAL PREVENTIVE ROLE OF MONTMORILLONIATE CLAY AGAINST CLASTOGENICITY AND GENOTOXICITY PRODUCED BY ZEARALENONE MYCOTOXIN

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Zearalenone (ZEN) is a potent estrogenic metabolite produced by some Fusarium species. No treatment has been successfully employed to get rid of the ZEN contained in foods. This study was conducted to evaluate the ability of Tunisian montmorillonite (TM) to protect Balb/c mice against cytotoxicity and genotoxicity induced by ZEN. TM was added alone or simultaneously with a toxic intragastric ZEN dose by orally route. The experimental approach consisted of 7 mice treatments. The first three groups received 400, 600, 800 mg/kg bw of TM. Two experimental groups received respectively ZEN alone (40 mg/kg representing 8% of LD50) and ZEN with the lowest dose of TM (400 mg/kg bw). The two control groups received respectively distilled water and olive oil. The positive control groups received Colchicin (4 mg/kg bw) for the micronucleus assay and mitomycin C (1 mg/kg bw) for the chromosome aberrations assay. 48 h after treatment, the femur and tibia are dissected out. The results show that ZEN was cytotoxic and genotoxic to Balb/c mice as indicated by the increase in frequencies of polychromatic erythrocytes micronucleated (PCEMN) and chromosomal aberrations in bone marrow cells. The simultaneous intragastric administration of TM with ZEN resulted in a decrease of PCEMN number and chromosomal aberrations frequency and in an increase of polychromatic erythrocytes (PCE) in bone marrow cells compared with the group treated with ZEN alone. It could be concluded that TM itself was safe and efficient in the prevention of ZEN toxic effects in gastro-intestinal tract.

PD011

IN VITRO AND IN VIVO GENOTOXICITY INDUCED BY ZEARALENONE: POSSIBLE PROTECTIVE EFFECTS BY ISOTHIOCYANATES EXTRACTED FROM TUNISIAN RAPHANUS SATIVUS

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Zearalenone (ZEN), is a naturally occurring contaminant of animal feed, that has been implicated in several mycotoxicoses in farm livestock. Recently, there is little information indicating that ZEN caused cancer or at least increases its prevalence, although the mechanism of action is unknown. Many papers mentioned that exposure to ZEN results in oxidative stress, genotoxicity and DNA damage. Therefore, we investigated the chemopreventive role of 4-(Methylthio)-3-butenyl isothiocyanate (MTBITC) extracted from Tunisian Raphanus sativus (radish) on the cytogenetic effect of ZEN in Balb/c mice and in vitro cultures of mouse lymphocytes. We determined chromosome aberrations and micronuclei as well as the mitotic index and DNA fragmentation following ZEN treatment alone or in combination with MTBITC. This report is the first to provide evidence of a statistically-significant decrease of structural chromosome aberrations and micronuclei associated with an augmentation of the mitotic index and inhibition of DNA fragmentation in all ZEN-MTBITC treated mice or mouse lymphocyte cultures. The MTBITC alone was safe and succeeded to inhibit the availability of ZEN by counteracting the oxidative stress and protect against the genotoxicity resulting from ZEN.

PD012

SPECTRUM OF SOMATIC KRAS MUTATIONS ASSOCIATED WITH CONSTITUTIONAL BIALLELIC MUTATIONS OF THE HUMAN BASE EXCISION REPAIR GENE MUTYH.

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Background: Biallelic mutations in the BER gene MUTYH cause MUTYH-associated polyposis (MAP), a condition predisposing to colorectal cancer. A few studies have provided evidence for an excess of somatic G>T transversions in the APC and KRAS genes, as expected with defective BER function, in colorectal tumors from biallelic MUTYH mutation carriers. Moreover, a specific KRAS G>T substitution (c.34G>T) is characteristically associated with MAP. To date, the clinical phenotype of MAP is not yet fully established. Few extra-colonic manifestations have been observed in MAP patients, but it is not yet clear if they are related to MUTYH deficiency. Aim: To investigate the frequency and type of KRAS somatic mutations in tumors from patients with biallelic MUTYH mutations. We report 5 cases with a typical MAP intestinal phenotype. 3 cases presented with different extracolonic manifestations (breast, endometrial cancer and pilomatricomas). Methods: Tumor samples were obtained from 5 patients diagnosed with MAP based on the presence of biallelic MUTYH mutations in constitutional DNA. KRAS codon 12 mutation analysis was performed by direct sequencing and RFLP-PCR on genomic DNA samples from colorectal tumors (n=6), endometrial carcinomas (n=2), breast carcinoma (n=1) and pilomatricoma (n=1). In 2 patients with endometrial cancer, the possibility of Lynch syndrome was originally considered, and subsequently ruled out by the results of immunohistochemical analysis of mismatch repair proteins and/or microsatellite instability testing. Results: Somatic mutation analysis revealed the presence of KRAS mutations in 2 of 5 MAP patients. The specific molecular signature of MUTYH inactivation (the KRAS c.34G>T transversion) was identified in both colorectal and endometrial tumor tissue from the same patient. Another patient with colorectal tumor associated with pilomatricomas showed the same c.35G>A transition (p.Gly12Asp), in both tumour types. No mutations were found in the other tumors investigated. Conclusion: The occurrence of the same KRAS alteration in different tumors from the same patient suggests that other factors, in addition to BER deficiency, may be involved in determining the spectrum of and type of somatic mutations and the phenotypic manifestations in MAP patients.

PD013

KAHWEOL INHIBITS STAT3 SIGNALING ASSOCIATED GROWTH ARREST AND APOPTOSIS IN HUMAN LUNG ADENOCARCINOMA A549 CELLS

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Kahweol, the coffee-specific diterpene, has been reported to have anti-carcinogenic properties. Animal data support such a chemopreventive effect of coffee. However, the precise underlying protective mechanisms are poorly understood. In this study, the apoptotic effect of kahweol in human lung adenocarcinoma A549 cells was investigated. In cell viability assays and cell proliferation assays, kahweol exhibited anti-proliferative and pro-apoptotic effects on A549 cells in a time- and dose-dependent manner. Kahweol considerably inhibited the expression of Bcl-2 but increased that of Bax; it also stimulated the cleavage of caspase-3 and PARP (poly ADP-ribose polymerase). In addition, kahweol-induced apoptosis was confirmed by TUNEL assays. Furthermore, kahweol inhibited dose-dependent phosphorylation of signal transducer and activator of transcription 3 (STAT3). An overexpression in STAT3 led to resistance to kahweol-induced apoptosis, suggesting that STAT3 was a critical target of kahweol. These findings suggest that kahweol inhibited A549 cell growth and induced apoptosis via down-regulation of STAT3 signaling pathway.

PD014

PROTECTIVE EFFECT STUDIES OF POLYSACCHARIDES FROM TREMELLA FUCIFORMIS ON HEMATOPOIETIC FUNCTION IN CHEMICAL INJURED MICE AND ITS TOXICITY

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Objective: Tremella Fuciformis Berk belongs to tremellaceae of Heterobasidiomycetae, it is known as nutritious mushroom and important medicine by people all over the world. Polysaccharide is one of the main bioactive components of Tremella fuciformis Berk. Our aim in this paper is to study the protective effects of polysaccharides from Tremella fuciformis on hematopoietic function in chemical injured mice and its toxicity. Method: Number of white blood cell, quantity of DNA in bone marrow and spleen index were used to investigate the effect on hematopoietic function of mice treated with cyclophosphamide and cytarabine hydrochloride. The maximum single dosage was adopted to exam acute toxicity of polysaccharides on mice. Results: Compared with administered cyclophosphamid and cytarabine hydrochloride groups , the number of white blood cell of mice treated with polysaccharides administered 24 mg/kg ip increased markedly. Toxicity on mice weren't found at 2000mg/Kg ip. Conclusion: polysaccharides from Tremella fuciformis have protective effect on hematopoietic function of injured mice by chemicals. It is safe and effective. [Key words] polysaccharide;Tremella fuciformis; cyclophosphamide; cytarabine hydrochloride; hematopoietic, toxicity

PD015

ANTIMUTAGENIC COMPONENTS IN THE EDIBLE MUSHROOM AGROCYBE CYLINDRACEA

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There are many investigations showing that antigenotoxic ingredients are contained in our daily foods, including a variety of mushrooms. Identification of antigenotoxic factors is expected to lead to the development of cancer-preventing agents. Recently, we demonstrated heat-unstable antimutagenic activity in a water-soluble extract of Agrocybe cylindracea, and active components were precipitated in 30-40, 40-50 and 50-60% ammonium sulfate fractions. Precipitates at 30-40 and 40-50% saturation fractions were thought to contain the same substances and showed strong antimutagenic activity against MeIQx and NDMA in the Ames test, but not in the Drosophila in vivo DNA repair test. In contrast, the 50-60% precipitated fraction showed antigenotoxic activity against MeIQx in Drosophila but not in bacteria. In this study, we attempted to purify the antimutagenic components contained in each ammonium sulfate fraction. Each 30-50 and 50-60% fraction was loaded onto a DEAE-Sepharose column, equilibrated with 20 mM Tris–HCl buffer (pH 8.8), and eluted by the same buffer containing 0 to 1 M NaCl in liner gradient. Antimutagenic activity in the 30-50% ammonium sulfate fraction was detected in a non-adsorbed fraction eluted just after the void volume. When this fraction was analyzed by electrophoresis with 15% SDS-polyacrylamide gels, only a single band was detected at around 25 kDa using CBB-staining. In addition, this peptide was also detected as one spot of neutral peptide using 2D-gel electrophoresis. Separation using gel filtration chromatography (Sephadex G-100) revealed that the molecular weight of this component was 12–30 kDa. On the other hand, antimutagenic component(s) in the 50-60% ammonium sulfate fraction were eluted in fractions containing 0.5 M NaCl. As several bands were observed following SDS-PAGE analysis, we could not identify the number of active components in this fraction. Taken together, A. cylindracea contains at least two unique antimutagenic components that could be separated by ammonium sulfate precipitation and DEAE-Sepharose chromatography, and one component responsible for antimutagenicity as shown in the Ames test may be a peptide with a molecular weight of around 20 kDa.

PD016

ANTIGENOTOXIC EFFECTS OF LIPOIC ACID AGAINST MITOMYCIN-C ON HUMAN LYMPHOCYTE CULTURES

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Lipoic acid (LA) is known as one of the most powerful antioxidants. In this study, antigenotoxic effects of lipoic acid on mitomycin-C (MMC) induced chromosomal aberrations (CA), sister chromatid exchanges (SCE) and micronucleus (MN) in human lymphocytes were investigated. All cultures were treated with 0.2 µg/ml MMC for 48 hours. Three different concentrations (0.5, 1, 2 µg/ml) of LA were added to the MMC induced cultures for three treatment times; 1 hour pre-treatment, simultaneous treatment and 1 hour post-treatment. A negative control, a positive control and a solvent control were also included. In all the cultures treated with MMC and LA in combination, the frequency of abnormal cells and CA/cell significantly reduced compared with the culture treated with MMC alone. Statistically significant reduction was also observed in SCE/cell and MN frequencies in all treatment times. These results show anticlastogenic and antimutagenic effects of LA against MMC induced genetic damage. On the other hand, LA treatments induced significant reduction in the mitotic index in all the treatments. These results indicate that this antioxidant have antimitotic effects in human lymphocyte culture.

PD017

GENOTOXIC EFFECT OF QUERCETIN/LANTHNUM COMPLEX ON HUMAN CERVICAL CARCINOMA CELLS

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Abstract: Quercetin is the main flavonoid present in our diet. Because of its specific chemical and biological properties, quercetin has been one of the most studied flavonoids for potential cardiovascular, neurodegenerative diseases and cancer treatments. Quercetin readily forms chelates with metal ions as a result of specific chemical structure. The aim of this study was to evaluate potential genotoxic effect of quercetin/lanthanum complex on human cervical carcinoma cells. First the influence of physiological solution on cells viability was investigated due to it's instability in physiological conditions and stability in isotonic solution of sodium chloride. Investigated complex showed considerable cytotoxicity within 100-1000 µM concentration during 3 hours of the cells exposure. Also, complex showed dose/response depended prooxidant activity and induction of single strand and double strand breaks of DNA. If future experiments show selectively effects of quercetin/lanthanum complex to cancer cells and it's stability in physiological conditions, it can be used in treating patients.

PD018

EXTRACTS OF EGYPTIAN FLORA ACTIVE AS ANTIOXIDANTS AND ANTICANCERS

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Natural products from plants are rich sources of chemical diversity. Mixtures of natural products were used historically for treating several diseases instead of single chemical therapy. Many of the pharmacological principles currently used as anticancer agents were first isolated from plants. Especially abundant resources come from plants that grow in semi arid regions, like most of Egypt. Here the aim was to examine extracts from some Egyptian flora for antioxidant and anticancer agents. Used were leaves of Luffa aegyptiaca (sponge gourd), Solenostemma arghe(argel), Cassia italica (senegal senna), Ocimum basillcum(basil), Colocasia antiguorum(taro), Beta vulgaris(beet) and fruit of Capsicum frutescens(chili pepper). Antioxidant activity was assayed using the 2,2'-diphenylpicrylhydrazyl (DPPH) radical method. Anticancer activity was assayed against both acute myeloid leukemia (AML) and acute lymphocyte leukemia (ALL) in-vitro); and Ehrlich ascites carcinoma cells (EACC, In-vivo study). The results showed that in the DPPH assay most of extracts showed significant antioxidant activity. Further, EACC derived tumor growth was most significantly reduced by the Solenostemma arghel hot water extract. Death of tumor transplanted animals was delayed by about 29 days. In in-vitro experiments all the plant extracts could kill the majority (66-90%) of abnormal cells among primary cells harvested from patients with ALL and AML, but most significantly by the hot water extract from Solenostemma arghel. DNA fragmentation patterns within treated cells inferred targeted cell death by apoptosis was altered. Therefore, the effects of plant extracts on tumor cells may due to the promotion of apoptosis, reduced oxidative DNA damage, and/or a reduction in protein denaturation by oxidation.. In conclusion, natural products from Egyptian flora may have potential for use as sources of therapies for diseases such cancer. Keywords: Natural products, Anticancers, Antioxidants, Acute myeloid leukemia, Ehrlich Ascites Carcinoma Cells.

PD019

ANTICANCER EFFECT OF CURCUPHENOL: AN SESQUITERPEN PHENOL

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Background: Chemical and pharmacological investigations have indicated that sesquiterpene phenols are important bioactive components in several plants. Curcuphenol, isolated from the sponges Myrmekioderma and plants such as Baccharis genistelloides, has been reported to have antifungal and antimalarial activity and preliminary data indicate an antitumor activity in murine leukaemia, lung, mammary and colon cells. In this work we investigated if curcuphenol had any effect on cell proliferation and also the possible mechanisms of its antiproliferative activity by measurement of DNA synthesis, apoptosis and caspase-3 activity. Methods and Results: Cell proliferation was evaluated in cultured CaCo2 human colon cancer cells by use of the reagent WST-1. It was demonstrated that curcuphenol in the concentration range 29-116 µg/ml inhibited cell proliferation and induced cell death. DNA synthesis as measured by the amount of [3H]thymidine incorporation, was inhibited by curcuphenol treatment (29-116 µg/ml) in a dose-dependent manner with an IC50 of 43 µg/ml. Apoptosis was assayed by a Cell Death Detection ELISAPLUS kit. At curcuphenol concentrations between 14.5-116 µg/ml, the rate of apoptosis increased significantly from 14.5 to 58 µg/ml (2.6-, 3.3- and 3.0- fold increase as compared to untreated cells at 29, 58 and 116 µg/ml respectively). The activity of caspase-3 was measured by the proteolytic cleavage of Ac-DEVD-pNA. After treatment for 6 h with curcuphenol (14.5-58 µg/ml) we found a dose-dependent increase in the caspase-3 activity at 30 min of incubation, 6.6- and 8-fold increase as compared to untreated cells at 29 and 58µg/ml, respectively. Conclusion: The findings presented here suggest that curcuphenol induces cell death in CaCo2 colon cancer cells which can be at least partly explained by a stimulation of apoptosis.

PD020

PROMOTER METHYLATION OF METABOLISM AND DNA REPAIR GENES IN PLASMA AS ONE OF MARKS THE EARLY DETECTION OF ESOPHAGEAL CANCER IN HUAIAN POPULATION, CHINA

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Background: Huaian county is a high-risk area for esophageal cancer in southern China. The study was performed to evaluate whether promoter methylation profile of metabolism gene GSTP1, NQO1 and DNA repair gene MGMT associated with the risk for esophageal cancer in plasma could be used for the early detection of esophageal cancer in Huaian population. Methods: 95 patients with newly diagnosed, untreated esophageal cancer from Huaian county were recruited. Esophageal cancer tissues, tissues adjacent to the tumors and plasma from peripheral blood were collected to determine CpG island hypermethylation of promoter of GSTP1, NQO1 and MGMT genes with nest-methylation-specific PCR. Results: The results showed that the methylation rates of GSTP1, NQO1, MGMT genes in tumor tissue were significantly different from that in tissues adjacent to the tumors ($\chi 2$=13.37, 18.24, 25.0 respectively; P<0.01). The conditional logistic regression analysis exhibited the significant association between the risk for esophageal cancer and aberrant hypermethylation of GSTP1 (OR=5.75, 95%CI: 1.99~16.63), NQO1 (OR=8.67, 95%CI: 2.62~28.63) or MGMT (OR=11.0, 95%CI: 3.37~35.86). The methylation of one and above genes increased the risk for esophageal cancer rapidly (OR=14.99, 95%CI: 3.58~62.72).The sensitivity and specificity with combined three markers were 90.5% and 38.9% in tissue samples. Promoter hypermethylation of GSTP1, NQO1, or MGMT gene in circulating DNA from plasma was demonstrated to be significant correlation with that in tumor tissue ($\chi 2$=16.83, 25.03, 46.75, P<0.001). The promoter hypermethylation of GSTP1, NQO1, or MGMT genes in plasma was accordant with that in tumor tissue (κ=0.33, 0.51, 0.66, P<0.01). Conclusion: The results suggest that aberrant methylation of GSTP1, NQO1, or MGMT genes increase the risk for esophageal cancer significantly, which could be taken as a potential biomarker for the screening of the early carcinoma. As a surrogate for target tissues, the plasma of peripheral blood provides a noninvasive method for epigenetic markers detection related with esophageal cancer. (This work was supported by the National Natural Science Foundation of China (No.30571540, 30671732, 30800891).

PD021

EFFECT OF AFOBAZOLE ON DNA DAMAGE IN SLE PATIENTS

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OBJECTIVE: The purpose of this study was to evaluate the effects of afobazole (anxiolityc drug with antimutagenic activity) on DNA breakage and oxidative DNA damage in nucleated peripheral blood cells of 60 patients with systemic lupus erythematosus. METHODS: Patients were randomized to receive either placebo or afobazole for 30 days in addition to standard therapy. The levels of DNA damage in fresh isolated blood were evaluated using the alkaline comet assay. A modified version of comet assay (8-oxoguanine Comet FLARE) was used to quantify oxidative DNA damage. Also, we exanimate susceptibility of blood cells to ex vivo H_2O_2-induced DNA breakage before and after therapy. RESULTS: Before therapy SLE patients had significantly increased background levels of 8-oxoguanine in peripheral blood cells as compared to healthy subjects (3.2±1.2 vs. 1.6±0.4 a.u., respectively). After treatment, 8-oxoguanine levels remained unchanged in both patient groups. Surprisingly, the mean basal DNA damage, estimated as %DNA in tail, in SLE patients was lower in comparison with controls (4.9±0.9 and 6.4±0.6 %DNA, respectively), but varied widely from 0.4 to 24.2 %DNA. After therapy mean DNA damage were significantly decreased in the afobazole-treated group, mainly in patients with an initial high level of DNA damage. No changes were found in the placebo group. Blood cells of SLE patients demonstrated increased ex vivo sensitivity to H_2O_2-induced DNA damage in comparison with control subjects (11.1±6.8 vs. 2.8±1.1 Δ%DNA tail, respectively). After treatment, decreasing in sensitivity to H_2O_2 was observed in cells from patients of afobazole-treated group, as estimated by decreasing in Δ%DNA in tail after H_2O_2 exposure (12.9±1.7 before and 6.5±1.4 after). Patients receiving placebo showed no a statistically significant differences. CONCLUSION: Afobazole treatment was associated with decreased basal DNA damage and decreased sensitivity of blood cells to ROS-mediated DNA-damage in patients with SLE. However, afobazole treatment, as well as standard therapy no affects oxidative DNA-damage. This is evidence that increased levels of 8-oxoguanine in SLE caused primarily by its disturbed repair.

PD022

GENOTYPE AND HAPLOTYPE ANALYSIS OF TP53 IN PATIENTS WITH PANCREATIC CANCER FROM THE CZECH REPUBLIC.

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Background: Pancreatic carcinoma is the fourth leading cause of cancer-related deaths in the Czech Republic, with only a minimum of patients surviving 5 years. The etiology and molecular pathogenesis is still weakly understood. Alterations in cell cycle regulation genes are commonly found in many cancers, including pancreatic. TP53 has a fundamental role in cell cycle and apoptosis and is frequently somatically mutated in solid tumors and particularly in pancreatic cancer. Based on the assumption that genetic variation may affect susceptibility to cancer, the role of polymorphisms in TP53 in modulating the risk of pancreatic cancer development is of major importance, although scarcely investigated so far. Aims/Methods: In a recent association study we have found a significant association between specific haplotypes constructed on TP53 polymorphisms and colorectal cancer risk in a population of the Czech Republic (Polakova et al, 2009). Thus, we have decided to further investigate the possible role of the same genotypes and haplotypes in modulating the risk of pancreatic cancer. The selected four polymorphisms in TP53 (PIN3, Arg72Pro, Int7 +72C>T, and Ex11 -363G>A), for which haplotype is constructed, are assayed for in association with pancreatic cancer risk in a case-control association study including 235 cancer cases and three different population of controls (a total of 1791 individuals) from the same country. Results and Conclusions: Preliminary results show that carriers of the variant allele of TP53 Arg72Pro polymorphism are at a moderate increased risk of pancreatic cancer (OR 1.47, 95% CI 1.09-1.98, P<0.05). Haplotype analysis is currently ongoing in concomitance with investigation on the possible role of main confounders. Genetic variants of TP53 gene may contribute alone or in concert with other risk factors on modifying a patient's risk for pancreatic cancer. The severity of this cancer and the difficulty to analyze, for the moment, large sample size cohorts represents a challenge for a combined epidemiological and biological approach in understanding the individual susceptibility to this cancer. Acknowledgements This work was supported by the Grant Agency of Czech Rep. Ministry of Health, grants No. 9422–3/2007 and 8563-5/2005.

PD023

XPC ALTERATION AND HEAD AND NECK CANCER

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Background: Xeroderma pigmentosum group C (XPC) participates in the DNA nucleotide excision repair in global genomic repair. We previously reported that loss of XPC expression as a risk for squamous cell carcinoma of the head and neck (SCCHN) in Korean population. Considering complexity and multiple steps of cancer, full genome wide studies are necessary to clarify etiology of SCCHN. Recently, chromosomal comparative genomic hybridization (CGH) is developed to evaluate the entire tumor genome. Aim: we performed CGH analyses to confirm our previous result, i.e. effects of XPC, and finally, to clarify the etiology of SCCHN. Methods: Following IRB, we newly recruited 16 subjects who were firstly diagnosed to SCCHN in Hanyang University Hospital at Seoul, Korea (Male,15;age, 42-73yrs). DNA was extracted from tissue samples, which were residues of biopsy, using the PureGene kit and applied to the MacArray™ Karyo1400 (Macrogen, Inc., Seoul). Macrogen's MacViewer™, a CGH analysis software, was used for quantification and image analysis of array CGH data. Results: we found multiple genetic aberrations in them throughout the genome including gains at 7q35, 8q24.12, and losses at 1p21.1, 3p22.1, 3p22.2, 3p22.3, 3p25.1, 3q25.2 and 3q26.3. The XPC is located at 3p25.1 with loss of gene expression. Conclusions: we confirmed that XPC is involved in SCCHN through genome wide analyses. Recently, XPC has been also emphasized for susceptibility to chemotherapy. In the near future, XPC should be further studied for its applications of SCCHN prevention and therapy.

PD024

ASSOCIATION OF POLYMORPHISMS WITH BACTERIAL AND ASEPTIC MENINGITIS.

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Background/Aims: Bacterial meningitis (BM) is still an important infectious disease causing death and disabilities. As part of the host inflammatory response to the pathogen invasion, factors such as reactive oxygen species are generated, which may damage DNA and trigger DNA repair mechanisms. Furthermore, Kynurenine (KYN) pathway is also associated to oxidative stress and has been shown to be altered in several diseases which compromise the CNS including infectious diseases such as BM. Methods: In this work, we investigated the association of SNPs APE1/Ref-1*Asn148Glu*, OGG1*Ser326Cys*, PARP-1*Val762Ala*, KYNU+715G/A and KATII+401C/T with meningitis in 81 patients with BM, chronic meningitis (CM), aseptic meningitis (AM) and not infected using PIRA-PCR and PCR-RFLP. The allelic and genotypic frequency was also associated with levels of the main chemo/cytokines and immunoglobulins during the meningitis. Results: A major incidence of polymorphic alleles and combined SNPs was found in BM and AM patients, giving evidences that these SNPs may be related to increased susceptibility to these diseases. Levels of IgG and IgA was altered in the presence of APE1/Ref-1*Asn148Glu* and OGG1*Ser326Cys*. In the presence of APE1/Ref-1*Asn148Glu* the levels of CCL2/MCP-1 and CXCL8/IL-8 decreased in patients with BM. To KYN pathway genes, the analysis also identified a prevalent frequency of the variant allele of SNP KATII+401C/T in patients with BM and AM. In BM group, a decrease of TNF-α and IL-6 levels was observed in patients that are homozygous (TT) to the SNP KATII+401C/T. A reduction of IL-1β, CCL3/MIP-1α and CCL4/MIP-1β was also observed in this group. This genotype was also associated to the increase of IgG level. The variant allele for KYNU+715G/A was only found in the control group of patients (without infection in CNS) and may have a protective role. Conclusions: Our current data demonstrate that SNPs in DNA repair and KYN pathway genes seem to regulate important chemo/cytokines to BM and AM inflammatory response and affect the imunoglobulins production, suggesting a immune regulation during meningitis in the presence of the analyzed SNPs.

PD025

HUMAN PAPILLOMA VIRUS IN BARRETT'S OESOPHAGUS

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Oesophageal adenocarcinoma and its precursor condition, Barrett's oesophagus (BE), are strongly associated with gastroesophageal reflux disease (GERD). Oesophageal adenocarcinoma shares a number of features of its appearance and behaviour with cervical cancer. We hypothesized that HPV, the primary causative agent for cervical cancer, might also play a role in the development of Barrett's oesophagus and oesophageal adenocarcinoma. This initial study aims to determine whether HPV infection is more common in Barrett's oesophagus than in gastroesophageal control tissue. Tissue samples were obtained during standard endoscopy from a consecutive series of consenting patients aged between 18 and 80 years who were undergoing routine upper intestinal endoscopy for investigation of heartburn and similar symptoms. The biopsy samples were taken from the squamous columnar (SC) junction and from the Barrett's lesions or for control samples from 2 cm above the SC junction. Patients being investigated for major stomach bleeding and those for whom biopsy would be dangerous were excluded from the study. Tissue biopsies were tested for the presence of HPV DNA by PCR using two established primer sets: MY09/MY11 and GP5/GP6+. HeLa cells were used as a positive control for HPV-18. A high HPV detection rate was observed in all patient groups with a statistically significant decreasing trend in HPV prevalence with increasing disease progression to BE (p = 0.045). A total of 7/11 (82%) of the control samples, 6/9 (67%) of the GERD controls and 14/29 (48%) of the Barrett's oesophagus samples tested positive for HPV with one or both of the primer sets. The number of samples in all groups positive for the internal primer set (GP5/GP6+) was smaller, but the trend was similar. The observed decrease in prevalence with progression may be due to a number of factors, none of which can be ruled out as yet. In summary, further research needs to be conducted with inclusion of oesophageal adenocarcinoma patient samples and larger sample sizes to gain a better understanding of the association between HPV and progression of oesophageal disease. This full study is ongoing and additional samples, including biopsies of gastroesophageal adeneocarcinoma, will be sought.

PD026

SIMULTANEOUS QUANTIFICATION OF N-ACETYL-S-(2-CYANOETHYL)CYSTEINE (CEMA) AND COTININE IN URINE OF ACRYLONITRILE-EXPOSED WORKERS USING ISOTOPE-DILUTION ON-LINE LC-MS/MS

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Acrylonitrile (AN) has been classified as a probable human carcinogen (Group 2B) by IARC in 1999. AN is an important industrial chemical for the production of artificial fibers, household articles, and resins. Occupational exposures have been of great concerns. It is also present in tobacco smoke, about 1-2 µg/cigarette. AN can be detoxified by glutathione transferase by direct conjugation with glutathione. The conjugates can be further metabolized to N-acetyl-S-(2-cyanoethyl)cysteine (CEMA). The objective of this study was to develop an isotope-dilution on-line solid-phase-extraction high performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) method to quantify urinary CEMA and cotinine for AN-exposed workers to validate CEMA as a biomarker for AN exposure. Personal sampling was conducted to assess AN personal exposure. Quantification of CEMA and cotinine was by monitoring $m/z = 218 \rightarrow 165$ and $177 \rightarrow 80$, respectively. The limits of quantification (LOQs) for CEMA and cotinine in urine were 4.6 µg/L and 1.0 µg/L, respectively. Thirty eight samples were analyzed. The results show that this method could simultaneously analyze CEMA and cotinine in urine and validate CEMA as a biomarker for AN exposure

successfully. It also demonstrates the high-throughput capacity of this method for future molecular epidemiology studies on the potential health effects resulting from the AN exposure.

PD027

THE REDOX MODULATION OF TUMOR SUPPRESSOR P53 IN METHYL METHANE-SULFONATE (MMS)-INDUCED BASE EXCISION REPAIR (BER) UNDER SELENOMETHIONINE (SEMET)

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Selenium has been known as an important component of selenoprotein modulating reduction/oxidation (redox) status in organisms. Recently, selenium has been also reported to prevent cancer in variety of animal studies. Furthermore, the Selenium and Vitamin E Cancer Prevention Trial (SELECT) project has been in force from 2002 to a national cancer institute (NCI). However, chemopreventive mechanism of selenium was understudied yet. We investigated that in here the effect of selenium on p53-dependent base excision repair (BER). Our data showed that the enhancement of BER activity and decrease of DNA damaging sites, apurinic/apyrimidinic sites (AP sites) were induced by pretreated selenomethionine against base damaging agent MMS in p53 wild-type human cells, implying that selenium might induce p53-dependent BER. We suggest the first time that the activation of p53 and Gadd45a in response to selenium might provide a novel chemopreventive mechanism of selenium protecting cells from the oxidative DNA damage under the presence of environmental mutagenic stresses.

PD028

SACCHAROMYCES CERVISIAE AND PROBIOTEC BACTERIA POTENTIALLY INHIBIT AFLATOXINS PRODUCTION IN VITRO AND IN VIVO STUDIES

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Saccharomyces cervisiae (SC) and Lactic acid bacteria (Lactobacillus rhamnosus GG and Lactobacillus rhamnosus LC705) potentially inhibited Aspegillus flavus growth and aflatoxins production in YES liquid media. Six groups of rats orally administrated SC (1011 CFU ml-1) and LGG & LC705 (109 CFU ml-1) daily for 15 days alone or with 2 mg ml-1 aflatoxin B1 (AFB1) in corn oil, significantly reduced serum ALT, AST, GGT, creatinin, and BUN compared with AFB1-tereated group. No deaths occurred in any combined treatment with AFB1, while 30% of mortality rate was recorded in AFB1-treated group. Blood glutathione (GSH) level significantly increased in groups treated with single-treatment of SC, LGG & LC705 or concomitant with AFB1; However, AFB1-treatment severely depleted GSH level than other treatments. Histopathological examination of liver and kidney in rats treated with AFB1 showed necrosis, vacuolar degeneration and fatty changes in hepatocytes; cellular swelling and pyknotic nuclei of proximal convoluted tubules in renal tissue. DNA content decreased significantly in liver and kidney tissues by AFB1-administration, these findings were ameliorated by probiotec bacteria and S. cervisiae treatment. Conclusion: These probiotec are safely to be food additives or preservative due to their antioxidant activities. Our study needs further continuation in this respect.

PD029

PHYTOCHEMICAL AND ANTIOXIDANT PROPERTIES OF CERTAIN PLANTS BELONGING TO ASTERACEAE FAMILY IN ALBINO RAT

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Phytochemical investigation was performed to aerial parts of three herbal plants belonging to Family Asteraceae. Phytochemicals isolated from Inula crithmoides were: isopimpinellin (I), Scoparone (II), Psoralene (III), Kaempferol-3-O-α-L-rhamnopyranoside (IV), Kaempferol - 3 ,7- di- O- α- L- rhamnopyranoside (V), Kaempferol- 3-O-β- D- glucopyranoside -7 – O -α- L- rhamnopyranoside (VI) and isorhamnetin-3-O-β-D-glucopyrano-side-7-O-α-L-rhamnopyranoside (VII). While the isolated phytochemical compounds from Iphiona scarab were umbelliferone (VIII), xanthotoxol (IX), marmesin (X), α-amyrin (XI), stigmasterol (XII) and β-sitosterol (XIII). Moreover, Jasonia montana were found to contain 7-hydroxy-3, 5, 6, 3`, 4`-penta methoxyflavone (XV) and 5,7-dimethoxy-3,3`,4`-trimethoxyflavone (XVI) In addition to ferulic acid, caffeic acid, chrorogenic acid, α-amyrin (XI), stigmasterol (XII) and β-sitosterol (XIII). Inula crithmoides aqueous methanolic extract showed significant inhibitory effect on mouse lymphoma (Cell line L1210) in vitro study in concentration dependant manner starting from 12.5 up to 250 μg/ml (20 – 100 % inhibition), respectively . In vivo study, the results indicated that Inula crithmoides methanolic extract had significant antioxidant effect against mycotoxin (Ochratoxin A)–induced oxidative stress in rat. Iphiona scarab and Jasonia montana ethanolic extracts showed potent acute antiinflammatory, anticoagulant and hypotenssive activities comparing to the standard drugs (indomethacine).

PD030

INFLUENCE OF DIETARY FACTORS ON RADIATION SENSITIVITY AND TELOMERE LENGTH.

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Extensive evidence highlighted the pivotal role of micronutrients in the maintenance of genome stability: dietary micronutrients provide protection from oxidative DNA damage, and contribute to genomic stability as cofactors or structural components of enzymes involved in DNA metabolism and repair. Much less is known about the influence of unbalanced micronutrient intake on the susceptibility to induced DNA damage. To this aim, a study involving 82 healthy volunteers was performed to investigate whether dietary intake of micronutrients, assessed by a food frequency questionnaire, could affect individual response to γ-ray-induced chromosomal damage, evaluated by the cytokinesis-block micronucleus assay. The results obtained did not show a significantly influence of diet on the frequency of radiation-induced micronuclei (Mn), confirming the prevailing role of hereditary factors on individual sensitivity to ionising radiation. Interestingly, a clear effect of aging on radiation-induced Mn was observed, older individuals (>55 y) having higher frequency of Mn than younger ones (250.8±54.1 *vs* 192.8±73.4 Mn‰, respectively; $p<0.001$). In agreement with previous findings, a low intake of vitamins B turned out to be associated with higher baseline incidences of Mn, possibly related to the important role of folic acid metabolism in the preservation of genome stability. The well known effects of aging and gender on spontaneous Mn frequency were confirmed. This study also highlighted an influence of dietary factors on telomeres, the terminal chromosome regions controlling chromosomal stability. Telomere length was evaluated in a subgroup of 56 individuals, applying the terminal restriction fragment analysis by Southern hybridisation. Data indicated that telomere shortening was significantly modulated by the deficiency of dietary antioxidants and by aging. In view of the high incidence of guanine

residues in telomeric DNA (TTAGGG) that should make telomeres particularly sensitive to oxidative damage, it is plausible that an age-related increase of oxidative damage as well as a dietary antioxidant deficiency may lead to telomere dysfunction. To our knowledge this is the first report showing an impact of micronutrients on telomere maintenance, which confirms the pivotal influence of dietary habits on genome stability.

PD031

VIRGIN COCONUT OIL IS NONMUTAGENIC AND ANTIMUTAGENIC: RESULTS OF AMES TEST AND COMET ASSAY

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Background/Objectives: Virgin coconut oil (VCO) is one of the most popular and widely used natural oils here in the Philippines because of its purported benefit as energy supplement, anti-microbial agent, HIV treatment, etc. However, assessment of its safety is still very limited. This study aims to investigate the safety profile of VCO in terms of its mutagenicity. Anti-mutagenic property was also studied, due to VCO's claims of anti-carcinogenicity. *Methods*: This is a randomized-controlled study using the (1) Ames Salmonella mutagenicity assay on S. typhimurium TA98 and S. typhimurium TA100 (in vitro) and (2) alkaline single-cell gel electrophoresis (comet) assay using Swiss Webster ICR mice (in vivo). The dosages of VCO used were 54 µL (No Adverse Effect Level, or NOAEL) and 168 µL (equivalent to the usual human dietary consumption dose), based on a previous study on the toxicity of VCO. Methyl methanesulfonate was used as the reference mutagen. *Results*: Ames test showed that VCO does not cause frameshift mutation in S. typhimurium TA98, or base-pair substitution in S. typhimurium TA100. Comet assay results showed that VCO does not cause damage to the DNA at both doses. In the anti-mutagenicity arm of the study, VCO appeared to be anti-mutagenic at the usual dose in both Ames test and comet assay. NOAEL dose of VCO exhibited anti-mutagenic effect in Ames test TA98 and comet assay. Based on statistical analysis, there was a trend of increasing anti-mutagenic potential at increasing dose of VCO on TA100. This dose dependence was also observed in vivo using the comet assay. *Conclusion*: Based on the Ames test and the comet assay, VCO is non-mutagenic and is even anti-mutagenic at the NOAEL and usual human dietary consumption dose.

PD032

MECHANISTIC ASPECTS OF GENOTOXICITY OF FURAN AND ITS KEY METABOLITE CIS-2-BUTENE-1,4-DIAL IN MAMMALIAN CELLS IN VITRO

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Furan, a possible human carcinogen present in a wide variety of human foods is formed during its processing results in widespread human exposure. It is a potent carcinogen in rats and mice showing a dose-dependent increase in hepatocellular adenomas and carcinomas. The mechanism for carcinogenicity of furan is not well understood. Furan is metabolized by cytochrome P450 2E1 to a bifunctional electrophilic metabolite, cis-2-butene-1,4-dial a DNA cross-linking agent which could probably be responsible for its carcinogenic properties. However, available data on the genotoxicity of furan are highly inconsistent and controversial both *in vivo* and *in vitro* due also its elevated volatility. In the present study we evaluated cytogenetic effects of furan and cis-2-butene-1,4-dial in human lymphocytes cultured *in vitro* and in two Fanconi's Anemia lymphoblastoid cell lines hypersensitive to DNA cross-linking agents. For furan, treatments in the presence of rat liver S9 metabolism (CYP 450, 2E1) were also performed. In the presence of S9 metabolism furan proved to be clastogenic only in Fanconi's Anemia cell lines while in its absence showed cytogenetic activity only in the prolonged treatment time at the highest dose-level (10mM) allowed *in vitro*. On the other hand, cis-2-butene-1,4-dial proved to be highly clastogenic in the short treatment time in Fanconi's Anemia cell lines and hardly positive in human lymphocytes but only in the prolonged treatment time.This outcome indicates that furan is a subtle clastogenic agent which cannot be detected with conventional short term genotoxicity assays. Acknowledgements:The work is financially supported by European Commission, call FP6-2005-SSP-5A (contract no. SSPE-CT-2006-44393, Specific Targeted Research Project) "Role of genetic and non-mechanisms in furan risk.

PD033

THAI OOLONG TEAS : PREVENTION OF MUTATION AND CANCER

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The role of dietary factors in prevention of major chronic diseases, cancer in particular, has been under intensive investigation. Evidences from epidemiological studies and tests in laboratory animals suggested a number of compounds found in foods and drinks may play roles in reduction the risk of cancers. Among the drinks, Japanese green tea and Chinese oolong tea are popularly studied. They have been found to have the appreciably antimutagenic and anticarcinogenic potentials. The present investigation was carried out on Thai oolong tea produced from tea grown in northern Thailand. The preliminary study on antimutagenicity under Ames test using Salmonella typhimurium TA 98 as the tester strain and 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (AF2) as the tested mutagen showed the boiled water extracts from oolong teas of 5 marketed brands had similar antimutagenic potentials in reducing the mutagenicity induced by AF2. One branded Thai oolong tea was then selected for further antimutagenicity evaluation by which was revealed to have the appreciable activity. This tea was able to inhibit the mutagenic induction of 2-aminoanthracene (2AA), aflatoxin B1 (AFB1), benzo(a)pyrene (BP), cyclophosphamide (CP), 4-nitro-o-phenylenediamine (NPD) and 4-nitroquinoline-1-oxide (4NQO) in S. typhimurium strains TA98 and TA100, and mitomycin C (MC) in Bacillus subtilis H17 and M45 tested by Rec assay. The antimutagenic activity of Thai oolong tea was found to be better if added before the application of mutagens. This tea was also found to be effective in reducing the micronucleus formation in mouse polychromatic erythrocytes induced by AFB1, BP, CP and MC. Again, the oral administration of tea before the intraperitoneal injection of mutagens to mice provided better protection than after administration. The tested Thai oolong tea was finally evaluated for cytotoxicity on P388 cells (mouse lymphocytic leukemia cells) and demonstrated to have the appreciable cytotoxic potential.

PD034

CHEMOPREVENTIVE EFFECT OF GLUTATHIONE S-TRANSFERASE ISOZYMES AGAINST AFB1-8, 9-EPOXIDE IN ALPHA MOUSE LIVER 12CELLS

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Background/Aims: The AFB_1-8, 9-epoxide is the ultimate hepatocarcinogenic intermediate of AFB_1 which is formed by Aspergillus flavus and detoxified by glutathione S-transferase (GST). The aim of this

study was to propose a possibility method, which could detect GST activities through conjugation of AFB_1-8, 9-epoxide with glutathione (GSH) and to examine which GST isoform could decompose the ultimate intermediate. The sulforaphane (SFN) and phloretin were used to induce GST isozymes in AML 12 cells and their chemopreventive effect have been evaluated. Methods: AFB_1-8, 9-epoxide was formed by human liver microsomal CYP activities and the GSH conjugated form of the epoxide was detected by HPLC with UV detector. The expression of major GST isozymes and glutamate-cysteine ligase catalytic subunit (GCLC) were demonstrated by RT-PCR respectively and the expression of each GST isozymes was blocked by a transfection with its siRNA respectively. Results: We detected GST activities using conjugation of AFB_1-8, 9-epoxide with GSH. The conjugation potential was proved to be correlated with the activities of GST because the formation was inhibited with curcumin, an inhibitor of GSTs, in a dose dependent manner. SNF and phloretin induced the expression of GST A3, A4, T1, P1, M1 and GCLC respectively. The cell lysates increased the conjugation potential of AFB_1-8, 9-epoxide up to 35 fold at 10 μM SFN and 40 fold at 10 μM phloretin compared with vehicle-treated cell lysate. The conjugation potential was blocked partially in the presences of each GST isozyme siRNA. GST A3, T1 and P1 were proved to be powerful to conjugate the epoxide with GSH in SFN treated cells, whereas GST P1, M1 and T1 were observed in phloretin-treated cells. However, SFN and phloretin treatment did not increase the total GST activities significantly which were detected with CDNB. Conclusions: The chemopreventive effects of SFN and phloretin on decomposition of AFB1-8, 9-epoxide are related with the up-regulation of several GST isozymes genes. The increase of GST activities by SFN and phloretin were extremely specific toward the conjugation step of AFB1-8, 9-epoxide and this detecting system of GST activity seems to be excellent to screen chemopreventive compounds toward AFB1 toxicity.

PD035

MOLECULAR EPIDEMIOLOGY OF GASTROINTESTINAL STROMAL TUMORS (GISTS) IN PATIENTS FROM COLOMBIA FOCUSED ON THE PRESENCE OF KIT AND PDGFRA MUTATIONS

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Background: Most GISTs contain activating mutations of KIT or less frequently the PDGFRA gene. These mutations result in constitutively phosphorylated and activated receptors and are found in 80-90% tumors in studies from developed countries; very few studies had reported the molecular composition of these tumors from countries composed by a heterogeneous genetic background (Native American, European, and African ancestries) like Colombia. Objective: To evaluated the status of KIT and PDGFRA genes of (GIST) in Colombian patients and to compare with other Latin American studies. Methods: 100 cases of GIST were selected. DNA was successfully extracted from paraffin blocks in 58 cases and evaluated for the presence of Exon 9, 11 and 13 mutation of KIT and exon 12, 18 of the PDGFRA were analyzed by direct sequencing. Results: GIST mutation were found in 70% (40/58) cases; 70% (28 /40) of the mutations involved exon 11; 12.5 %(5/40) exon 9 and 5% (2/40) in exon13 of the KIT gene. Mutations in the exon 11 were heterogeneous including deletions 57.2% (16/28), point mutations 17.8% (5/28), internal tandem duplication (ITD) 17.8% (5/28), deletion-insertion 3.6% (1/28) and deletion-inversion 3.6% (1/28). Mutation involving PDGFRA were found exclusively in exon 18 (D842V) in 12.5% (5/40). The wild-type (WT) GIST comprised 18 cases (31%). KIT mutations in Colombia (COL) were similar to Brazil, 72.7% and 70% respectively. Exon 11 in 69.1%(BZ) and 70% (COL) and exón 9 3,6%(BZ) and 5%(COL). Regarding the PDGRFA, we found 7% mutation compared with7.3 %(BZ) , in contrast with that series our mutations were exclusively found in exón 18 instead of the exón 12 predominantly reported in Brazil, we confirmed the gastric location in all of the cases studied. KIT and PDGRFA mutations in Argentinian cases (ARG) were similar, 72% and 70% respectively for the KIT and 10.5% (ARG) and 12.5% (COL). We found a increase number of WT cases 30% (COL) compared with 17.5% (ARG). Conclusion: The molecular epidemiology of GIST appears to be similar in the Latin American series regardless of the heterogeneous genetic background. In Colombia Wild Type GIST exceeds what was reported elsewhere, however before making definitive conclusions a large amount of cases should be evaluated.

PD036

EFFECT OF TOCOTRIENOL SUPPLEMENTATION ON THE RENAL OXIDANT-ANTIOXIDANT STATUS IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR)

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Studies assessing the effect of antioxidant supplementation on the activities of antioxidant enzyme (AOE) during the development of hypertension in SHR are limited. This study therefore investigates the effects of supplementation of Tocomin® 50%, a tocotrienol rich compound (TRF), on renal oxidant-antioxidant status during the development of hypertension in SHR. Four-week old SHR were given a daily oral dose of Tocomin® 50% (100 mg/kg body weight) dissolved in oleic acid for either 2 or 4 or 6 weeks. Controls (SHR) were given an equal volume of oleic acid. Systolic blood pressure and body weight were measured at the end of each treatment period. Groups of rats (n=6) were then sacrificed at 4, 6, 8 and 10 weeks and their kidneys were collected for the measurement of activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST), and for estimation of total antioxidant status (TAS) and lipid peroxidation marker (MDA. Data were analyzed using ANOVA and a 'p' of < 0.05 was considered statistically significant. Compared to age-matched controls, mean body weight was significantly higher in all Tocomin supplemented animals. Systolic blood pressure was significantly lower only following two weeks ($p<0.05$) of Tocomin supplementation to SHR but not after 4 and 6 weeks. GR activity was significantly higher following Tocomin supplementation in all groups ($p<0.01$), while GST activity was significantly higher after 6 weeks of Tocomin supplementation ($p<0.05$). No significant changes were observed in the activities of SOD, CAT, GPx and TAS levels between the treated and age-matched SHR controls. MDA levels were lower following 4 and 6 weeks of weeks of Tocomin treatment, although statistical significance was only evident after 4 weeks of treatment ($p<0.001$). In conclusion it appears that supplementation of Tocomin, a tocotrienol rich compound, delays slightly but does not completely ameliorate the rise in blood pressure or significantly affect the activities of primary AOE in the kidneys of young SHR. However, changes observed in the levels of MDA, GR and GST activities indicate the need for further studies with perhaps higher doses and a longer duration of TRF administration.

PD037

GENOTOXIC AND ANTIGENOTOXIC EFFECTS OF (-)-EPIGALLOCATECHINE GALLATE (EGCG) AGAINST MITOMYCIN-C INDUCED CHROMOSOMAL ABERRATIONS AND SISTER CHROMATID EXCHANGES IN HUMAN LYMPHOCYTES IN VITRO

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The chemical composition of green tea contains many polyphenolic compounds, generally known as catechins. (-)-epigallocatechine gallate (EGCG) is the most active polyphenol of green tea and primarily responsible for the green tea effect. Plant polyphenols are naturally occuring antioxidants but they also have prooxidant properties. In this study, the genotoxic effects of EGCG as well as its possible antigenotoxic effects against mitomycin-C (MMC) induced chromosomal aberrations (CA's) and sister chromatid exchanges (SCE's) in human lymphocytes were investigated. Human peripheral lymphocytes were treated with four different concentrations of EGCG (0.01, 0.05, 0.1, 0.5 µg/ml) alone and in combination with 0.2 µg/ml MMC for 24h. A negative control, a solvent control and a positive control were also included. EGCG significantly increased the frequency of CA's in all concentrations compared to the negative control. When compared to solvent control, the variations in CA's were not significant (except the highest concentration; 0.5 µg/ml) in EGCG treatment alone. On the other hand, MMC+EGCG treatments increased the frequency of CA's in human lymphocytes. This increase was not significant compared to the positive control (except the lowest concentration; 0.01 µg/ml) but it was highly significant compared to the negative and solvent controls. In SCE induction, EGCG caused significant increase at all concentrations except the lowest dose (0.01 µg/ml) compared to the negative control. When compared to solvent control, only the highest (0.5 µg/ml) concentration caused significant increase. On the other hand, MMC+EGCG treatments decreased SCE/cell compared to MMC treatment alone. These results show that EGCG prevents SCE induced by MMC alone, however, EGCG potentiates CA's when used together with MMC.

PD038

THE PROTECTIVE EFFECT OF THE FLAVONOIDS ON FOOD MUTAGEN INDUCED-DNA DAMAGE IN HUMAN LYMPHOCYTES FROM COLON CANCER PATIENTS

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The heterocyclic amines 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) formed during cooking procedures are associated with a Western diet and increased risk of colon cancer. Flavonoids – polyphenolic compounds widely present in foods of plant origin are known to have biological effects, among which anti-inflammatory and anti-oxidant activity seem to be the most important. The aim of this study was to investigate the response of lymphocytes isolated from healthy individuals and colon cancer patients to food mutagens in the presence of the flavonoids, rutin and quercetin. A constant dose was used of 75 µM of PhIP and 150 µM of IQ, to treat lymphocytes from both groups of participants in the presence of variable doses of flavonoids of 100 (50 for rutin), 250 and 500 µM. DNA damage has been examined with the Comet assay. Both quercetin and rutin in combination with the food mutagens, IQ and PhIP, were shown to reduce DNA damage caused by HCAs. There was a statistically significant reduction of damage which appeared to be dose-dependent: at higher doses of flavonoids the response was almost decreased to control levels. DNA damage observed in healthy individuals was lowered as evident from a decreased Olive Ttail moment and % Tail DNA when compared with colon cancer patientscontrols. Factors such as gender, general diet, smoking and drinking habits did not interfere with the responses observed. This study has shown that peripheral lymphocytes can be used satisfactorily as surrogates for colon cancer cells.

PD039

PLANT EXTRACTS – A TOOL FOR GENOTOXIC REPAIR

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There has been extensive use of chemical pesticides in order to enhance the agricultural yields. The present paper deals with the antimutagenic potential of the plant extracts viz. *Azardiractha indica* A Juss (A),*Chrysanthemum cinerarifolium* Vis (C) and *Blumea lacera* L (B) along with chemical pesticides in the meristematic root tips cells of *Vicia faba* L. Seeds of *Vicia faba* L were treated with different concentrations (0.12%,0.25%,0.50%,1.00% and 2.00%)of the three pesticides viz. Rogor (R), Endosulphan (E) and Dichlorovos (D) separately for four hrs at room temperature. The *Vicia faba* L root tips assay is a well recognized system for detection of chromosomal aberrations due to environmental chemicals (Ma, 1982). The pretreated root tips were squashed and stained in 2% acetocarmine solution. The results showed that1.00 %concentration of chemical pesticides reduced the mitotic index but induced the chromosomal abnormalities significantly. The type and degree of chromosomal abnormalities were noted as Stickiness>Fragment >Disturbed metaphase/anaphase > Bridges>Laggards >Micronuclei. However, the synergistic effect of chemical pesticides and plant extract significantly amplified the mitotic index and depressed the chromosomal abnormalities as compared with the chemical pesticides alone. The gradation of synergistic effect was noted as N+D > N+E > N+R > B+D > B+E > B+R > C+D > C+E > C+R. It is clear that the plant extracts repair the damages caused by chemical pesticides to a significant level. Obviously, the plant extracts with the commercial chemical pesticides may be better substitutes in ordered to reduce the risk of genotoxic hazards to agriculturally important plants.

Risk assessment

RA001

DIFFERENTIAL RESPONSE RELATED TO GENOTOXICITY IN MULTIPLE ORGANS OF CIRRHOTIC RATS

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The aim of this study was to investigate whether blood, liver, heart, kidney and brain are particularly sensitive organs for DNA damaging in cirrhotic rats by the single cell gel (comet) assay in order to predict genetic instability induced by this pathological condition. A total of 16 male Wistar rats were divided into two groups: negative control (n=8) and experimental (n=8), in which was submitted to bile duct ligation during 28 days. The results showed that cirrhosis was able to induce genetic damage in liver, and brain cells as depicted by the mean tail moment. No genetic damage was induced in blood, heart or kidney cells, i.e. no significant statistically differences ($p>0.05$) were noticed when compared to negative control. In conclusion, our results suggest that cirrhosis could contribute to the damage of DNA in liver and brain cells. Since DNA damage is an important step in events leading to some degenerative diseases, as for example, cancer, this study represents a relevant contribution to the correct evaluation of the potential health risks associated with cirrhosis.

RA002

COCAINE OR ECSTASY INDUCE DNA DAMAGE IN MICE PERIPHERAL BLOOD CELLS: AS ASSESSED BY SINGLE CELL GEL (COMET) ASSAY

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Cocaine and ecstasy abuse is a serious and socially damaging illegal drug problem. Different doses of administration are associated with a specific progression of effects and propensity for dependence. However, the effects of first exposure of drugs on DNA damage are still unknown. Thus, the study aimed to evaluate the overall genetic damage in peripheral blood cells induced by different doses of cocaine or ecstasy in male mice. After one hour after the administration (cocaine: 1.75, 3.5 and 7 mg/kg, and ecstasy: 1.25, 2.5 and 5 mg/kg, ip), all C57BL/6J mice were euthanized and peripheral blood was collected and the cellular suspensions were used for the single cell gel (comet) assay. The results pointed out that cocaine or ecstasy were able induce DNA damage in blood cells for all concentrations evaluated in a dose related-fashion being the strong effect encountered for cocaine as depicted by the tail moment data. Taken together, such findings demonstrate that cocaine or ecstasy are potent genotoxins. Since DNA damage is an important step in events leading to genomic instability, in so far as tumorigenesis process this study represents a relevant contribution to the understanding of the potential health risks associated with users of some illicit drugs.

RA003

IN VITRO EVALUATION OF GENETIC DAMAGE INDUCED BY THREE RADIOPACIFIERS USED IN CLINICAL PRACTICE

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The aim of the present study was to evaluate the capacity of radiopacifiers to induce genetic damage in vitro. Chinese hamster ovary cells or murine fibroblasts cells were exposed to calcium tungstate, lead oxide or calcium carbonate, at final concentrations ranging from 10 to 1000 ug/mL for 1 hour at 37 oC. The negative control group was treated with vehicle control (phosphate buffer solution - PBS) for 1 hour at 37°C, and the positive control group was treated with methylmetanesulfonate (at 1 uM) for 1 hour at 37°C . Genotoxicity was assessed by the single cell gel (comet) assay. The results pointed out that radiopacifiers did not induce DNA breakage in all tested cells as depicted by the mean tail moment in all concentrations. In summary, our results indicate that exposure of mammalian cells to these radiopacifiers may not be a factor that increases the level of DNA lesions.

RA004

CHARACTERISTICS AND PERFORMANCE OF A BHAS 42 CELL TRANSFORMATION ASSAY FOR PREDICTION OF CHEMICAL CARCINOGENICITY

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The carcinogenicity of chemicals has been predicted with genotoxicity assays. Cell transformation assays can also detect chemical carcinogens, but are classified into a category different from genotoxicity assays. The BALB/c 3T3 is one of cell lines which have been used for cell transformation assays. The Bhas 42 cells were established from the BALB/c 3T3 cells through transfection of an oncogenic murine ras gene and regarded as initiated cells in the two-stage carcinogenesis theory. Using the Bhas 42 cells, a short-term cell transformation assay was developed. The Bhas 42 cell transformation assay is superior to conventional cell transformation assays in cost and labor performance. The Bhas 42 cell transformation assay has previously been reported to be capable of detecting initiating and promoting activities of carcinogens and consists of an initiation assay and a promotion assay to detect initiating activity and promoting activity, respectively. In the promotion assay, the initiating treatment with a known carcinogen is not required to detect promoting activity of test chemicals. We applied this short-term assay to almost 100 chemicals to characterize the assay and evaluate its performance for the prediction of chemical carcinogenicity. The promotion assay could detect a considerable number of Ames-negative carcinogens, confirming that Bhas 42 cells act as initiated cells in the cell transformation assay. The concordance, sensitivity, specificity, positive predictivity, negative predictivity, false positives and false negatives were calculated from the assay results for known carcinogens and non-carcinogens. Among the performance characteristics, the specificity and positive predictivity were high and the specificity was superior to those of genotoxicity tests. As overall evaluation, the other performance characteristics were equivalent to those of genotoxicity tests and conventional transformation assays. From these results, we concluded that the accuracy of prediction for chemical carcinogenicity would be increased by including the Bhas 42 cell transformation assay in the battery of in vitro assays. [This work was supported by the New Energy and Industrial Technology Development Organization/the Ministry of Economy, Trade and Industry of Japan.]

RA005

ASSESSMENT OF GENOMIC INSTABILITY IN NORMAL AND DIABETIC RATS TREATED WITH METFORMIN

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To examine if a single or multiple oral administration of motorman, a

member of the iguanid class of anti-diabetic agents, has any geotaxis and catatonic potential in normal and diabetic rats, a mammalian model, cytogenesis assays through several endpoints such as induction of micronuclei, chromosome aberrations, mitotic activity of bone marrow cells, sperm-head anomaly and assays of some oxidative stress markers have been conducted by the use of standard techniques. Diabetes was induced by streptozotocin injection. Metformin was administrated to both diabetic and non-diabetic rats in single doses of 100, 500 or 2500 mg/kg along with vehicle control groups for diabetic and non-diabetic rats. The animals were killed by cervical dislocation at 24 h after treatment, and then bone marrow cells were sampled. Also, a multiple dose study has done in which diabetic and non-diabetic animals were treated with 100 or 500 mg/kg of metformin daily for 4 or 8 weeks after which the animals were killed by cervical dislocation, and then bone marrow and sperm cells were collected. Concurrent control groups were also included in each experiment. The obtained results revealed that metformin was neither genotoxic nor cytotoxic for the rats in all groups at all tested doses. Moreover, metformin significantly reduced the diabetes-induced genomic instability and cell proliferation changes in somatic and germinal cells in a dose-dependent manner (2500, 500, >100 mg/kg). In addition, diabetes induced marked biochemical alterations characteristic of oxidative stress including, enhanced lipid peroxidation and reduction in the reduced glutathione level. Treatment with metformin ameliorated these biochemical markers. In conclusion, metformin is a non genotoxic or cytotoxic compound and may protect from genomic instability induced by hyperglycemia. Apart from its well-known anti-diabetic effect, the anti-genotoxic effect of metformin could be possibly ascribed to its radical scavenger effect that modulated the genomic instability responses and cell proliferation changes induced by hyperglycemia. Other anti-genotoxic mechanisms such as DNA repair and increasing in apoptotic elimination of heavily-damaged cells are in progress. The results of this part will be finished in the upcoming weeks.

RA006

THE IMPACT OF SURFACE MODIFICATIONS ON CYTOTOXIC AND GENOTOXIC EFFECTS OF SUPERPARAMAGNETIC MAGNETITE NANOPARTICLES

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Magnetite nanoparticles (MNPs) are used for in vivo biomedical application including magnetic resonance, magnetically controlled transport of anticancer drugs as well as hyperthermia generation. In order to prevent aggregation and to increasing the circulation time in the blood stream, the surface of MNPs must be modified with various surfactants to make them more stable, biodegradable and non-toxic. Multiply functionalized MNPs, prepared by loading of specific ligands, antibodies, peptides and drugs may offer an exciting tool to make MNPs target-specific and increase their therapeutic benefit. The objective of this study was to investigate the biological activity of nanospheric superparamagnetic magnetite particles (Fe_3O_4, 10 nm in diameter) in dependence on surface modifications. MNPs were prepared by the coprecipitation method of ferric and ferrous salts in an alkali aqueous medium. X-ray diffraction measurement was performed to identify the crystallographic structure of prepared MNPs and the magnetic properties were characterized by SQUID magnetometer at room temperature. Two surfactans - sodium oleate ($C_{17}H_{33}COONa$) and polyethylene glycol (PEG Mw=1000) were used for coating of MNPs (10 nm). The biological activity of sodium oleate-coated MNPs (SO-MNPs) and sodium oleate- plus PEG-coated MNPs (SO-PEG-MNPs) was investigated using the human alveolar epithelial carcinoma cell line A549. Three approaches were applied to evaluate the cytotoxicity of particular MNPs and individual surfactants, MTT assay, trypan blue staining and assessment of proliferating activity. The ability of particular MNPs to produce DNA strand breaks was measured using the alkaline single cell gel electrophoresis. The effects of MNPs were analyzed after short-term (4h) and long-term (24h) exposure. Our preliminary results have shown differences in both cytotoxic and genotoxic effects of nanosphered magnetite particles in dependence on surface coating. This study was supported by grants: VEGA 2/0051/09, APVV 99-026505 and CEX SAS NANOFLUID.

RA007

CHARACTERIZATION OF INDUCED RAT S9 FOR AN APPROPRIATE EXTRAPOLATION FROM IN VITRO TO IN VIVO IN GENOTOXICITY STUDIES

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There are some discrepancies in genotoxic responses between in vitro and in vivo genotoxicity studies. However the mechanisms of these discrepancies are unknown and this makes the extrapolation from in vitro to in vivo study difficult. To examine one of the mechanisms, we focused on the induced rat S9 generally utilized in vitro studies, and evaluated its characteristic and impact on the interpretation of in vitro studies. Enzyme activities and expression levels of cytochrome P450 (CYP) isozymes in rat S9 induced with phenobarbital and 5,6-benzoflavone were compared to those of untreated rat S9, and both S9s were subjected to in vitro micronucleus tests for 8 compounds which were selected as indirect genotoxicans. The P450 content and MCD, ECD and PCD activities in induced rat S9 were higher than those in untreated rat S9. In terms of protein expression, induced S9 showed higher levels of CYP1A1, 1A2, 2B1/2, 2C6, 3A1 and 3A2, and lower levels of CYP2C11 and CYP2E1 compared with untreated rat S9. In vitro micronucleus tests with induced rat S9, no differences in the intensity of micronucleated cells induction or cytotoxicity were observed between induced rat S9 and untreated rat S9 in 3 diclophenac, piroxicam and lansoprazole. On the other hand, stronger inductions of micronucleated cells and cytotoxicity than untreated S9 were observed in benzo[a]pyrene, cyclophosphamide, PhIP, coumarin and chlorpheniramine. In the cases of coumarin and chlorpheniramine, the metabolites which are not typically seen in vivo were produced by induced S9. And also negative results have been reported in in vivo micronucleus studies. Therefore, in these 2 cases, it is considered that the judgment of genotoxicity by induced rat S9 could not be extrapolated to in vivo situations. This study demonstrates that the use of induced rat S9 caused discrepancies of the genotoxic responses between in vitro and in vivo studies in the case of the generation of untypical metabolites in vitro. Consequently, when induced S9 is used it is necessary to consider the characteristics of this S9, and when these characteristics are not consistent with those of in vivo studies, another genotoxicity studies should be conducted with more appropriate metabolic activation systems.

RA008

MOBILE PHONE AS A RISK FACTOR FOR EARLY BRAIN CHANGES IN CHILDREN

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During the last decade there has been a steep increase in the number of mobile phone (MP) users that invaded the world of children. Aim of the study: to detect the early effects of MP use on the electrical activity of the brain in children. Subjects and methods: cross-sectional study recruiting school-aged children attending a Teaching Hospital for mild illness. MP users and non-users were given appointments after recovery to participate (30 and 40 respectively). In their second visit, filling psychiatric questionnaire and recording electroencephalographic waves (EEG) after 5 minutes of MP use were done. Results: The frequency of the psychiatric symptoms and EEG changes were significantly higher in the MP users than in non-users ($p < 0.05$ & 0.01 respectively). Recommendations: the hazardous effect of MP use alarms the restriction of MP use by children.

RA009

CARDIOVASCULAR DISEASE PREDICTORS IN RELATION TO SMOKING IN EGYPTIAN MEN

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Cigarette smoking increases the risk of coronary heart disease (CHD). The aim of the present study was designed to compare the influence of smoking on novel CHD predictors with the traditional predictors. Methodology: Estimation of the levels of novel CHD predictors sush as serum albumin, bilirubin, lipid peroxides and free fatty acid (FFA) pattern, in addition to traditional predictors such as blood pressure, body mass index (BMI), serum total cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-C) and iron between smokers and non-smokers. The results of this work revealed that the prevalence of cardiac arrhythmias was higher in smokers than in non- smokera. Both of BMI and blood pressure were not significantly changed between the two groups. Biochemical parameters showed an extreme increase of serum lipid peroxides, triglycerides and cholesterol / HDL-C ratio in smokers relative to non- smokers. On the other hand, smokers had severly reduced HDL-C levels and slight decreased total cholesterol / total bilirubin as compared to those of non- smokers. Serum albumin and iron levels showed non-significant changes between smokers and non-smokers. FFA pattern demonstrated a marked reduction in oleic acid level and a significant increase in euric acid concentration in smokers regarding to those of non- smokers. Each of palmitic, stearic and arachidonic acids decrease in smokers than in non-smokers. Younger smokers had extremerly higher levels of serum lipid peroxides, total cholesterol, triglycerides and total cholesterol / HDL-C ratio than their aged matched controls. Serum HDL-C levels were severly reduced in younger smokers as compared with their corresponding controls. Older smokers showed a significant decrease in HDL-C level and a marked increase in its ratio with cholesterol regarding to non-smokers who had the same age.

RA010

PREDICTION OF BLADDER CANCER IN EGYPTIAN TANNERY WORKERS

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Carcinoma of urinary bladder is the fifth commonest form of cancer all over the world. In Egypt, bladder carcinoma is the most common solid tumor in men. The aim of the present study was evaluation of the carcinogenic effect of tannic on the urinary bladder of tannery workers, and comparison of the diagnostic value of urinary cytology, nuclear matrix protein (NMP) and Kontron Image Analysis System for the detection of occult bladder cancer. Methodology: The study included 38 tannery workers and 40 unexposed subjects. Urine samples were tested for abnormal cells through cytopathological examination and DNA image analysis for any abnormality in the cell life cycle, in addition to NMP, a tumor marker specific for bladder cancer, to detect suspected bladder lesions. Results: Statistical analyses found that there were significant differences between the examined groups according to the cytopathological examination of the urine samples and DNA images. In smoking tannery workers, 4C and S phase were significantly higher and 2C was significantly lower compared to those not smoking. There was no significant difference between the two groups according to NMP. But, the percent of positive NMP in smoking workers was higher compared to non-smokers in both groups. Conclusion: Tannery workers were at high risk for bladder pre-cancerous lesions. Smoking increases this risk. Urinary cytology as well as NMP can provide useful excluding information, and can be used in screening for bladder cancer in the population at risk to exclude the presence of the condition, but not as a diagnostic methods.

RA011

ASSESSMENT OF RESPIRATORY HEALTH PROBLEMS DUE TO EXPOSURE TO AIRBORNE FUNGI IN CERAMICS INDUSTRY

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Ceramics industry needs constant humidity and high temperature, which creates conditions for microbial growth. Aim: The study aimed to assess the role of humidifiers in ceramics industry as a microbial source, and to assess respiratory health problems secondary to the microbial exposure among ceramics workers. Methodology: Air samples were collected from the different departments for counting of airborne microorganisms. Medical and occupational questionnaire, clinical examination, ventilatory functions (VFT) and sputum culture were done for ceramics workers (30 from compressors, 26 from mills, and 14 from quarrying departments) and compared with 29 non-exposed workers. Skin prick tests (SPT) of Penicillium spp., Asp. fumigatus, and Asp. niger were done for ceramics workers. Results revealed that indoor airborne microorganisms were at higher levels in comparison to outdoor samples. Penicillium was the most dominant indoor fungi spores followed by Asp. fumigatus and Asp. niger. Prevalence of chronic respiratory symptoms and clinical signs were significantly higher in exposed workers than in their controls. FEV1 and FEV1/FVC ratios of the compressors workers were significantly lower compared to their controls. Prevalence of positive SPT in ceramics workers was 8.6% to Penicillium spp, 4.3% to Asp. fumigatus and 2.9% to Asp. niger. Sputum cultures of the ceramics workers revealed that 34.3% were positive for Penicillium spp, 5.7% for Asp. fumigatus and 5.7% for Asp. niger. Prevalence of SPT reactivity and positive sputum cultures were not influenced by the duration of exposure. There were no significant differences in VFT of the workers concerning the results of SPT and of sputum cultures. Conclusion: Ceramics workers found to be at risk to develop respiratory problems not only due to their exposure to dusty environment, but also due to their exposure to airborne fungi, and this risk was not influenced by the duration of exposure. It was recommended to consider fungal exposure in ceramics industry as a risk factor for increasing chest problem.

RA012

RESPIRATORY HAZARDS, GENOTOXICITY, AND CARCINOGENICITY OF OCCUPATIONAL EXPOSURE TO FORMALDEHYDE IN MEDICAL LABORATORIES

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A relatively large number of workers are being exposed to formaldehyde because of its wide spread use. Aim of work: The present study aimed to reveal whether prolonged or repeated formaldehyde exposure can induce human health hazards. Methodology: A study of 48 exposed employees (pathology laboratories) was undertaken using the yield of ventilatory pulmonary function tests, specific IgE to formaldehyde-human serum albumin (HSA), chromosomal aberrations, DNA-protein cross links in peripheral blood lymphocytes and serum p53. Results: Chronic occupational formaldehyde exposure appeared to be associated with an increase in the prevalence of the respiratory and eye symptoms. The mean values of FEV1, and FVC in percent of the predicted values were significantly lower in the exposed workers than in the controls. At the same time, the mean levels of DNA-protein crosslinks and the mean frequency of mitomycin C induced chromosomal breakage were significantly higher in the exposed group compared to the controls. There was no significant difference between the both groups in the mean values of PEFR%, specific IgE to formaldehyde-HSA, serum p53, and the mean frequency of spontaneous chromosomal breakage. Unexpectedly, no obvious correlation was seen between either the duration of employment, smoking index, or other parameters analysed. Conclusion: The study confirms the allergenic and genotoxic haz-

ardous effects of formaldehyde vapor on the health of exposed workers and calls for establishing protective measures for them.

RA013

EFFECT OF MDI EXPOSURE ON THE LIVER: EVALUATION OF ALPHA-FUCOSIDASE AS A USEFUL MARKER FOR HCC DETECTION

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Aim: The study was aimed to investigate the hepatotoxicity of methylene diphenyl diisocynate (MDI) and its metabolite methylene dianiline (MDA) in a group of workers occupationally exposed to polyurethane thermal degradation compounds in the flexible packing industry. Evaluation of serum AFU activity as a useful marker for early detection of HCC was also done. Methodology: The study was carried on 45 workers exposed to MDI, 34 controls who have never been exposed to chemicals (negative controls), and 25 hepatocellular carcinoma patients (HCC) as a positive controls. Results: The levels of serum ALT, alkaline phosphatase (ALP) and total bilirubin were statistically significantly elevated in the exposed workers compared to the normal controls. On the other hand, the levels of serum total protein, albumin, and A/G ratio of the workers were significantly lower compared to the normal controls. There was no significant difference in serum AST and liver tumor markers alpha-fetoprotein (AFP) and alpha fucosidase (AFU) levels between the workers and their normal controls. However, with 10.5 Umol/L/min (mean value of controls+3.0 SD) considered as the cut-off point, about 29% of exposed workers had higher AFU values than the cut-off points. Whereas, none of our workers had higher than normal AFP levels. The mean levels of liver function tests and the tumor markers used in this study were significantly different in the HCC patients (positive group) compared with those of workers except for the albumin level. In the HCC group, AFU did not correlate with with AFP, suggesting that the positive detection rate can be increased when these 2 markers are used in conjunction. Whereas, AFP positively correlated with serum ALT and tumor size. AFU did not, suggesting that AFU can be used as a useful marker for detecting small HCC particularly in cirrhotic patients. Conclusion: It is apparent from this work that MDI exposure has an adverse effect on human hepatic function. Regular screening program among this high risk population, using ultrasonography, liver function tests including prothrombin time and serum tumor markers (AFP and AFU) are highly recommended.

RA014

A PROPOSAL METHODOLOGY TO CANCER RISK ASSESSMENT TO FORMALDEHYDE OCCUPATIONAL EXPOSURE

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Background: Very recently, the International Programme on Chemical Safety has convened a working group to promote harmonisation of approaches to the assessment of cancer risk from exposure to chemicals. For chemicals that promote local genotoxicity, similar to formaldehyde, it's accepted a practical threshold supported by toxicokinetics and experimental studies that can be used to developed cancer risk assessment. Nowadays, formaldehyde is classified as a human carcinogen by the International Agency for Research on Cancer and has determined that there is "sufficient evidence" that occupational exposure to formaldehyde causes nasopharyngeal cancer in humans. It's also considered an important genotoxic occupational agent. The aim of this studied was too developed and applied a cancer risk assessment methodology that can be useful in cases of formaldehyde occupational exposure. Methods: It was developed a methodology to assess the cancer risk of workers exposed to formaldehyde. It was used the results from environmental monitoring and data about biologic adverse events caused by formaldehyde exposure. The methodology was applied in 88 work activities from 11 anatomy and pathology laboratories and 46 work activities from 5 industrial units from Portugal. Results: In the 46 work activities evaluated from industrial units only 2% have high cancer risk and all the others activities have low risk. With regard to laboratories activities, 14,7% have high cancer risk, 20,45% medium risk and 50% low cancer risk. These results are in line with other studies that point workers from anatomy and pathology laboratories with the highest cancer risk from occupational exposure to formaldehyde. Conclusions: While industrial production processes are currently automated, anatomy and pathology laboratories activities require a great proximity to anatomical species and frequent handling of solution based on formaldehyde. The obtained data draw attention to the importance for risk assessment be performed for each effect to health caused by exposure to a specific chemical agent and to the value of existing data about biologic adverse events from experimental and epidemiological studies to permit define and applied a cancer risk assessment methodology.

RA015

CYTOTOXICITY AND GENOTOXICITY INDUCED IN HUMAN CELLS INCUBATED WITH COMMERCIAL NANOMATERIALS

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The environmental and health impact associated with exposure to nanomaterials is an emerging area in toxicology and health risk assessment. In fact, the same properties which make nanoparticles so attractive in industry and medicine, such as small size, chemical composition, structure, large surface area and shape, may contribute to the toxicological profile in biological systems. In this work we investigated about the cytotoxicity and the mechanisms of action of two industrial nanostructures, purified and characterized at the Department of Chemical Sciences (University of Padova): Elicarb® single-wall carbon nanotubes (SWCNTs) and Ludox® nanoparticles (commercial colloidal silica nanoparticles in aqueous phase) with two different diameters (20 nm and 7 nm). Various cell lines were treated with these different nanostructures: fibrosarcomeric cells HT-1080, lung cancer cells A-549 and normal human lung fibroblasts, CCD-34Lu. At the end of treatments performed with increasing concentration of nanomaterials in culture medium with or without foetal calf serum (FCS) for different incubation times, we measured cell viability with MTS (CellTiter 96® AQueous One Solution Cell Proliferation Assay, Promega) and clonogenic assays. Our results show that: 1) the toxicity of Elicarb® SWCNTs solubilized in water increases for long incubation time (48 h and 72 h); 2) smaller Ludox® (SM30, 7 nm) are more toxic than Ludox® AS30 (20 nm); 3) A-549 e CCD-34Lu cell lines are the more sensitive cells. Moreover, the presence of FCS in the culture medium during the treatments with Ludox® reduces cell toxicity, by promoting nanoparticle aggregation. In A-549 and HT-1080 cells was measured apoptotic index by DAPI staining and caspase-3 activity to investigate the mechanism of cell death induced by Ludox® nanoparticles: apoptosis induction was higher in HT-1080 after treatment with Ludox® SM30 nanoparticles. Finally, we analysed DNA damage induction by the immunofluorescence of the phosphorylated form of the histone H2AX (□H2AX), the production of reactive oxygen species (ROS) in cells treated with Ludox® and gene expression alteration through microarray analysis after treatment with Ludox® AS30 and SM30.

RA016

MORPHOLOGY AND DNA DAMAGE OF SPERMATOZOA OF ADULT MEN EXPOSED TO THE PROCESSING OF ELECTRONICS WASTE

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Background Electronic wastes (e-wastes) are being the fastest growing type of solid waste. Most of them were exported to Asia and Africa. It has become a major global environmental problem to manage and recycle the e-wastes. E-wastes were widely processed by manually disassembling or burning in Jinghai, a county of China, which has become the largest disposal centre in northern China in recent years. This study analyzed the potential damaging effects of pollutants generated by the electronic waste disposal process on human reproductive function, especially to the spermatozoa of adult men. Methods Sixty-six couples were randomly selected as exposed group from permanent residents in the local area, where there was massive disposal of electronic waste. Fifty couples were recruited from the neighboring areas that were not exposed to electronic waste as control group. Routine semen analysis, spermatozoa motility and morphology were performed to detect the cytotoxic effect. DNA damage was detected using comet assay, the DNA percentage in the comet tail (TDNA%), tail moment (TM) and Olive tail moment (OTM) were recorded to describe DNA damage to spermatozoa. Results The volume of semen in exposure group was less than that of control (P=0.039), but the sperm concentration didn't show statistical significance between the two groups (P=0.693). Remarkably, the sperm motility and abnormity rate showed significant statistical difference, which demonstrated that the quality of spermatozoa in exposure group was worse than that of control group. The parameters detected by comet assay didn't show significant difference between the two groups. The gender ratio (boys : girls) of exposure group is less than that of population in the local area. Conclusions The toxic substances that released during the process of e-waste recycling by inappropriate ways can have harmful effects to the quality of spermatozoa and reduced consequence on sperm counts. Further study about the recycling of e-wastes should be performed and further follow-up investigation on the growth and development of the offspring and the gender ratio of newborn in the exposure area should be performed urgently according to the discovery of this study.

RA017

CYTOTOXICITY AND GENOTOXICITY OF PALYTOXIN-LIKE COMPOUNDS FROM OSTREOPSIS SPECIES.

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During the last decade, the occurrence of harmful algal blooms has increased both in frequency and in geographic distribution in many regions of the world. Benthic dinoflagellates of the genus Ostreopsis have expanded their distribution from tropical–subtropical to temperate waters, such as the Mediterranean Sea. Among the thousands of species of microscopic algae at the base of the marine food chain, there are a few dozen which produce potent toxins that impact human health through the consumption of contaminated fish or shellfish, or through water or aerosol exposure. Palytoxin (PLT) is one of the most potent marine biotoxins known to date, exhibiting extreme toxicity in mammals. Analyses of plankton samples collected along the Ligurian coasts (Italy) during a massive bloom of the tropical microalga Ostreopsis ovata indicated the presence of putative palytoxin (p-PLT) together with a palytoxin-like molecule which was named ovatoxin-a. The aim of our study was to characterize the toxic and potential genotoxic properties of algan toxins accumulated in shellfish tissues. A number of extracts from mussel samples, collected in the framework of a specific monitoring program for marine biotoxins in Liguria, were tested in vitro in human hepatoma HepG2 cells. Genotoxicity was evaluated using the comet assay and micronucleus test. A dose-response curve for cell-death was observed for 1 and 3 hours of exposure with 100% of mortality at the concentration 20 ng/ml p-PLT. Preliminary data revealed an increase of DNA fragmentation index and of micronuclei frequency starting from 0.01 ng/ml p-PLT.

RA018

MICRONUCLEUS FREQUENCY IN BUCCAL MUCOSA CELLS OF MOBILE PHONE USERS

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Mobile phone use is still increasing throughout the whole world. Currently there are more than three billion subscribers. In Western Europe the penetration rate (subscribers / person) exceeds 110 %. Health effects of the transmitting radiation (≈900 MHz and ≈1800 MHz) have been discussed and investigated for a long time. Nevertheless there has not been a final answer because researchers found diverse and even contradictory results. This study was carried out to examine the in-vivo effect of mobile phone use on genetic stability in the oral mucosa of humans. More than 100 samples of buccal mucosa cells of young adults (including non-users) were taken using a wooden stick with some cotton attached to it. The cells were washed, brought onto microscopy slides and fixed with methanol. For evaluation cells were stained using chromomycin A3 and α-tubulin antibody. For each sample 2000 cells were scored counting binucleated cells, micronuclei and other nuclear anomalies. In questionnaires information on the mode of use of mobile phones was requested from each study participant. Questions included average daily duration of use, overall time of using a mobile phone and whether head-sets are used. Furthermore drinking and smoking habits, diseases, medication and special nutrition were registered. Results are presented in relation to those parameters.

RA019

CHANGES IN ANTIOXIDANT DEFENSE SYSTEMS INDUCED BY DIETHYLDITHIOCARBAMATE (DETC) IN V79 CHINESE HAMSTER FIBROBLASTS

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Diethyldithiocarbamate (ditiocarb, DETC) is a first mammalian metabolite of disulfiram. DETC and its analogs are compounds with diverse applications, both as an important class of agricultural pesticides and as pharmacological agent - cancer chemo-preventive agent, potential adjuncts to traditional oncological chemotherapy, along with protection against tissue toxicity of cisplatin treatment. Although dithiocarbamates display low acute and chronic toxicities in human and experimental animals, the extreme reactivity mainly related to their metal-chelating ability, and high affinity for –SH group containing proteins, underlies the wide range of their adverse effects. We have currently explored the effect of DETC on antioxidant enzymes activity and intracellular glutathione in vitro to elucidate their role in cellular damage and molecular mechanism responsible for drug-induced cytotoxicity. Chinese hamster fibroblast (V79) cells were treated with 100-200 μM DETC for 1h. Activities of catalase, superoxide dismutases (SOD1 and SOD2) glutathione peroxidase, and glutathione reductase, ratio of GSH/GSSG, level of protein carbonyl groups (PC), and lipid peroxidation (TBARS) have been determined. DETC increases GSH level. However, the ratio of GSH/GSSG in treated cells was reduced comparing to the control, due to significant increase of GSSG level, which is harmful to cells. GSH is considered to be an important mediator of cancer cell resistance to anticancer agent-based chemotherapy. Oxidative

damage via GSH/GSSG depletion might affect many important biological molecules, e.g. proteins and lipids. To test whether oxidatively modified proteins and lipids appeared in the course of the tested compound exposure we measured the amount of PC and TBARS as biomarkers of the oxidative stress. DETC resulted in increase in protein oxidation and in lipid peroxidation. Analysis of GSH related enzymes showed a significant decrease in glutathione peroxidase and an increase in glutathione reductase as well as catalase activity. We have also shown decrease in both SOD1 and SOD2 activities as compared to those in the controls. Presented data show that DETC induced cytotoxicity occurred via an oxidative shift in the intracellular redox state.

RA020

DISULFIRAM-INDUCED CYTOTOXICITY IN V79 CHINESE HAMSTER FIBROBLASTS

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Disulfiram (DSF) (thiuram disulfide of diethyldithiocarbamate) has been used clinically in alcohol aversion therapy for nearly 60 years. As a member of dithiocarbamates it shows high reactivity with strong metal-binding characteristics, and is capable of interacting with protein and nonprotein thiol groups. Despite many in vivo and in vitro studies considering dithiocarbamate toxic, mutagenic and genotoxic activities, few investigations have been performed to evaluate the cellular damage and molecular mechanisms responsible for drug-induced cytotoxicity. Glutathione (GSH) has a central role in the maintenance of the thiol-disulfide redox state in mammalian cells. The purpose of this study was to determine the effect of Disulfiram on the redox state of GSH/glutathione disulfide (GSSG) pool and antioxidant enzymatic defense in Chinese hamster fibroblasts (V79 cells). Cells were cultured without or with N-acetyl-L-cysteine (NAC) - the GSH biosynthesis precursor, or L-buthionine sulfoximine (L-BSO) - the GSH synthesis inhibitor. A significant increase in oxidative stress was observed, evidenced by decreased GSH/GSSG ratio, elevated total protein carbonyl content, but no change in lipid peroxidation (measured as TBARS) in cells treated with 100-200 μM DSF. Disulfiram increased both intracellular GSH and GSSG levels. Disulfiram increased catalase activity but did not change SOD1 and SOD2 activities. Analysis of GSH related enzymes showed a significant increase in glutathione reductase, and no change in glutathione peroxidase (H_2O_2 as a substrate) with concominant significant decrease of GSH peroxidase (cumene peroxide as a substrate) activity. In addition, modulation of intracellular GSH level with NAC pretreatment altered DSF-mediated changes both in protein oxidation and in antioxidative enzyme activities. NAC pretreatment protected V79 cells against oxidative damage of proteins induced by Disulfiram. Data show that oxidative properties are at least partially attributable to cytotoxic effects of the drug. Disulfiram affects both cellular nonenzymatic and enzymatic antioxidant defense systems.

RA021

ENVIRONMENTAL RISK INDEX (ERI) : A NEW PROPOSAL FOR RISK ASSESSMENT INTEGRATING IN VITRO TOOLS.

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A balanced and reliable index for environmental and health risk assessment might be a valid tool to evaluate and compare potentially critical sites like areas surrounding incinerators, landfill or industries. We elaborated Environmental Risk Index (ERI), a multiparametric tool to quantify the global risk posed by diverse hazardous chemicals distributed in the investigated area focusing on both human and ecosystem targets. The risk assessment strategy behind ERI comprehends three main parts: human toxicology, ecotoxicology, environmental fate and distribution. A database containing 186 chemicals (e.g. PCDD/F, PCBs, PAHs, VOC, metals, etc.) was assembled and each class of compound was investigated starting from chemical structures, physico-chemical properties, toxicological and ecotoxicological data and distribution predictions. Even innovative instruments to calculate the indicators now offered by regulators and research activities (i.e. EU projects CAESAR and OSIRIS) were included, when necessary, on the basis of reliability and uncertainty of data. 16 priority compounds are the minimum required set to calculate the index on the basis of the relative knowledge of their toxicological profiling, environmental distribution and anthropic emissions. We are now further improving the main index structure with the information coming from in vitro experiments that allow evaluating the synergic or addictive effects due to hazardous chemicals on target cells. Particularly we focus on human health, selecting a test battery on A549 and HepG2 cell lines, used as in vitro models for respiratory tract and the epatic tissue respectively. Assay-based results will be integrated into ERI main structure using a scoring system set on the statistical differences between exposed and control cells. The proposed index, enriched with the in vitro information, allows considering the risk of the single compound in a broader way and also the site specific chemical charges and its global effect on possible target cells. ERI index should be considered an innovative diagnostic/screening method to get a comprehensible picture of the global situation of a critical area. This will be an useful tool for detailed risk analysis and also for comparison along time and space dimensions.

RA022

NEROLIDOL CAUSES GENOTOXICITY IN DIFFERENT CELLS OF MICE BY COMET ASSAY AND MICRONUCLEUS TEST

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Nerolidol is a sesquiterpenoid component of essential oil used to enhance flavor and aroma, has been studied as a topical skin penetration enhancer, has inhibitory activities against S. aureus and E. coli, among others activities. The objective of this study was to evaluate the ability of acute treatment of nerolidol to induce DNA damage in peripheral blood and liver cells of mice and micronuclei in polychromatic erythrocytes (PCEs) in bone marrow cells. In the dose range-finding assays, the maximum tolerated dose was superior than 2000 mg/kg. The doses used in the experiments were 250, 500 and 2000 mg/kg. At least 100 nucleoids per cell type/animal were analyzed for determine DNA damage scores and 2000 PCEs per animal for micronuclei in PCEs. The positive control was N-Nitroso-N-Ethylurea 50 mg/kg. Cytotoxicity was assessed by scoring a 200 consecutive total polychromatic (PCE) and normochromatic (NCE) erythrocytes (PCE/NCE ratio). The results showed that nerolidol induced significantly elevated dose-dependent cell DNA damage in both type of cells analyzed and enhanced the average number of micronucleated cells in the two high tested doses. PCE/NCE ratio showed cytotoxicity for the two lower doses of the compound. The data obtained provide support to the view that nerolidol induces genotoxicity and clatogenicity in the mice cells tested. Hence, great care should be taken while using its as a therapeutic agent or to enhance foods flavor.

RA023

eRF3A/GSPT1 (GGC)N ALLELES AS GENETIC SUSCEPTIBILITY MARKERS FOR CANCER DEVELOPMENT

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The human eukaryotic release factor 3a (eRF3a), encoded by

eRF3a/GSPT1 gene, is up-regulated in various human cancers. However, the mechanism responsible of transcription enhancement in cancer tissues is still not understood. eRF3a/GSPT1 contains a (GGC)n polymorphism in exon 1, encoding a polyglycine expansion in the N-terminal of the protein. The longer allele was associated with some cancer. Here we show that the longer allele (12GGC) is present in colorectal cancer patients (F=1.09%) but not in Crohn Disease patients and the control population. Using real time quantitative RT-PCR we show that the longer alleles present higher transcription levels both in primary lymphocyte cultures from the patients ($p<0.001$) and in Jurkat-transfected cell lines ($p<0.0001$). However, no eRF3a/GSPT1 amplifications were detected. The methylation levels of the CpG sites inside the GGC expansion were analysed by pyrosequencing and were totally unmethylated regardless of the length of the alleles. Using flow citometry, we compared the levels of apoptosis and the proliferation rates between cell lines with different genotypes, but no significant differences were detected between them. Finally, we used a cytokinesis-block micronucleos assay (CBMN) to evaluate the frequency of MN in the same cell lines. Our results show that cell lines with the longer alleles have higher frequencies of MN in binucleated cells, which is probably a result of defects in mitotic spindle formation. We discuss how eRF3a could affect the spindle formation, and its impact in cancer development/progression. Taken together, our results show that eRF3a should be considered as a potential proto-oncogene.

RA024

AN IN VITRO MICRONUCLEUS ASSAY WITH SIZE-CLASSIFIED MICRONUCLEUS COUNTING TO DISCRIMINATE ANEUGENS FROM CLASTOGENS

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In the in vitro micronucleus (MN) assay, genotoxic chemicals can be characterized as aneugens and clastogens by the presence and absence of kinetochore protein or centromere regions in the micronuclei, respectively. Aneugens preferentially induce kinetochore- or centromere-positive micronuclei which can be detected by the immunofluorescence staining method or the fluorescence in situ hybridization (FISH) method. Both methods are robust and reliable; however, these assays require a considerable time and cost to obtain a result that suggests that the genotoxic chemicals cause aneuploidy. This is why these methods are not adequate to evaluate dozens of chemicals which are mixtures of aneugens and clastogens. To evaluate a batch of chemicals, a quicker and more convenient assay is desirable. In the present study, we examined whether the size-classified counting of MN is as effective as FISH method to characterize aneuploidy in the in vitro MN assay using Chinese hamster lung (CHL) cells. As aneugens, 9 substances (colcemid, vincristine sulfate, paclitaxel, thiabendazole, diethylstilbestrol, griseofulvin, bisphenol A, fisetin and okadaic acid) were used; as clastogens 6 substances (methylmethane sulfonate, N-methyl-N'-nitro-N-nitroso-guanidine, etoposide, mitomycin C, hydroxyurea and actinomycin D) were used. The size-classified counting revealed that all the 9 aneugens increased both the frequency and proportion of large-size MN as compared with the vehicle control. Although N-methyl-N'-nitro-N-nitroso-guanidine, etoposide and mitomycin C increased the frequency, no increase was observed in the proportion. Meanwhile, with the FISH method, all the aneugens induced centromere-positive micronuclei but the clastogens did not. Based on these results, it is considered that the frequency of large-size MN in the in vitro MN assay is an alerting index for aneugenic effects and that its proportion is a simple and reliable index which is as effective as the FISH analysis for discrimination of aneugens from clastogens.

RA025

PRACTICAL DECISION TREE FOR GERM CELL MUTAGENS IN GHS CLASSIFICATION

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The Globally Harmonized System of Classification and Labelling of Chemicals (GHS) requires the classification of chemicals for germ cell mutagenicity. Simple decision logic for germ cell mutagens (GCM) has been devised within the GHS, but expert judgment on a weight of evidence approach is always needed for application of the GHS criteria. It is difficult for users of the GHS, who are not expert in this field, to classify chemicals without additional guidance if they apply it in combination with criteria of the hazard categories detailed in the GHS text. Therefore, a practical decision tree for classification of GCM has been developed. The judgment in the tree flows from upstream (i.e., Category 1A as "Human heritable germ cell mutagen") to downstream (i.e., Category 1B as "Mammalian germ cell mutagen", Category 2 as "Mammalian somatic cell mutagen", or Not yet classified as genotoxic [as Not classified in the GHS] as "Not likely to be mammalian mutagen") as well as the original tree given in the GHS. The basic concept of the tree is that positive results outweigh negative results in each test because, when conflicting results were obtained, negative results sometimes arise from inadequate experiments. Judgment of accuracy of the negative results will be difficult for non-expert classifiers. While a single positive result can sometimes be pivotal to a decision about a classification, such findings should be considered on a case-by-case basis. Therefore, expert judgment is required in some cases in applying the tree. The usefulness of the tree for GCM classification has been investigated with some chemicals including "problematic" examples (acrylonitrile, phenol, nitrilotriacetic acid, ethanol, etc) for non-expert classifiers. As positive findings are given more weight than negative ones for decision making, there is a danger of overestimating risk categories without expert judgment. For example, the positive findings of a dominant lethal test had an impact in the classification of ethanol despite a lack of clear supportive evidence in other in vivo and in vitro tests. Therefore, the decision tree including expert judgment is useful for GCM classification in GHS.

RA026

TOXICITY ASSESSMENT OF THE AQUEOUS EXTRACT OF CALOTROPIS PROCERA IN RABBITS

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Background: Calotropis procera is an evergreen tree belonging to the category of spreading shrubs and exudes copious milky sap when cut. C. procera has been reported to possess medicinal properties but equally pose deleterious effect on animals. To investigate the extent of damage, a toxicological evaluation of the aqueous extract of fresh leaves of the plant was conducted. Methods: Crude extract was prepared from 3.5kg of the fresh leaves of the plant by crushing, diluting with water, filtering using a wire filter paper and dried at 500C in a Gallenkamp 300. Phytochemical Screening of Alkaloids, Saponins, Tannins, Cardiac glycosides, and Flavonoids was conducted on the crude extract. Acute toxicity study was carried out with oral administration of 200, 400, 800, and 1600mg/kg of the extract once to groups I, II, III and IV respectively within a 24 hours observation period. Four rabbits died within 24 hours and LD50 was estimated (940mg/kg). 80, 40 and 20mg/kg of the extract were administered daily to groups I, II, and III respectively during sub-acute toxicity study for 14 days. Results: Phytochemical Screening revealed the presence of alkaloids, saponins, tannins, cardiac glycosides, and flavonoids while elemental analysis showed traces of iron, lead, sodium, and potassium in concentrations of 0.23, 0.03, 0.82 and 9.5 mg/g respectively. Statistical analysis of Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT), Alkaline Phosphatase (ALP), Albumin and protein showed no

significant changes at P<0.05. Changes in Packed Cell Volume (PCV), White Blood Cells (WBC), Haemoglobin (Hb), Platelets, and Differential Leucocyte Count (Lymphocytes, Monocytes, Eosinophils, Heterophils/Neutrophils and Basophils) were equally statistically insignificant at P<0.05. However, gross and histopathological examination of some organs and tissues (heart, liver, kidney, small intestine and lungs) revealed lesions. Conclusion: It was concluded that the extract had no significant effect on blood parameters when administered orally at tolerable doses since controls were also affected but have lethal effects at higher doses since the effect was found to be dose-dependent.

RA027

DIFFERENTIAL GENE EXPRESSION AND GENE NETWORKS INDUCED WITH GENOTOXIC AND NON-GENOTOXIC HEPATOCARCINOGENS IN MOUSE LIVER EXAMINED BY QUANTITATIVE REAL-TIME PCR

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It would be invaluable to determine whether toxicogenomics is useful to examine the carcinogenic potential of chemicals. In the present study, we examined the patterns of gene expression by quantitative real-time PCR (qPCR) in liver in 8 genotoxic hepatocarcinogens (GT), 5 non-genotoxic hepatocarcinogens (NGT) and a non-carcinogenic hepatotoxin 4 and 48 h after their administration to B6C3F1 mouse. Firstly 4 GT [2,4-diaminotoluene (DAT), 4-dimethyl(amino)azobenzene (DMAA), N-nitrosomorpholine (NNM) and quinoline (QN)] and 2 NGT [1,4-dichlolobenzene (DCB) and clofibrate (CL)] as a test set were given intraperitoneally to groups of five 9-week-old male mice. Total RNA from each individual liver was reverse-transcribed to cDNA and the levels of 40 genes selected based on our previous DNA microarray study were quantified by qPCR. The results were analyzed by Dunnett's test, hierarchical cluster analysis (HCA), principal component analysis (PCA), and Ingenuity Pathways Analysis (IPA) for gene networks. The gene expression was classified into groups with or without increase or decrease at 4 and 48 h compared to control animals. At 4 and 48 h, 4 GT exhibited differential gene expression compared to 2 NGT by HCA and PCA. Three gene networks related to cancer, cell cycle and cell death were extracted by IPA. Secondly 4 GT [2-acetylaminofluorene (2AAF), diisopropanolnitrosamine (DIPN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NKK), urethane (U)], 3 NGT [dichloro-diphenyl-trichloroethane (DDT), di(2-ethylhexyl)phthalate (DEHP), furan] and a hepatotoxin [phenacetin (PNT)] as a validation set were examined similarly for 32 genes among 40 aforementioned genes. At 4 and 48 h, 4 GT exhibited differential gene expression compared to 3 NGT and a hepatotoxin by HCA and PCA. Similar three gene networks as in test set chemicals related to cancer, cell cycle and cell death were extracted by IPA. Five genes (Ccng1, Hspb1, Jun, Ly6a and Plk2) exhibited great effect to PCA at 4 h in both test and validation sets. Hmox1 showed high effect to PCA at 48 h in both test and validation sets. As a conclusion the present results suggest that qPCR analysis in dozens of selected genes 4 and 48 h after administration is useful to examine the carcinogenic potential of chemicals.

RA028

XRCC1 AND XRCC3 VARIANTS AND RISK OF GLIOMA AND MENINGIOMA

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Background: Despite decades of research, the etiology of human brain tumors is poorly understood. In recent years, it has been shown that variability in DNA repair capacity plays a role as a modifier of cancer risk. Increased risks of various cancer types have been asso-ciated with single nucleotide polymorphisms (SNPs) within the DNA repair genes. There-fore, we evaluated the association of SNPs in the DNA repair genes with the risk of pri-mary adult brain tumors. Methods: Our Caucasian study population consisted of 701 glioma (including 320 glioblastoma) cases, 524 meningioma cases, and 1,560 controls in a prospective popula-tion-based case-control study conducted in Denmark, Finland, Sweden, and the UK. The investigations were conducted on SNPs that have been associated with cancer predisposi-tion in the X-ray cross-complementing group 1 (XRCC1) and X-ray cross-complementing group 3 (XRCC3) genes. We included SNPs Arg194Trp (rs1799782), Arg280His (rs25489), and Arg399Gln (rs25487) in the XRCC1 and Thr241Met (rs861539) in the XRCC3. The chosen SNPs were examined in a 96-well format using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) based method. Results: The studied SNPs were not significantly associated with the risk of brain tumors. The highest odds ratios (ORs) for the associations were observed between the homozygous variant genotype XRCC1 Gln399Gln and the risk of glioma (OR = 1.32; 95% confidence interval, CI, 0.97-1.81), glioblastoma (OR = 1.48; 95% CI, 0.98-2.24), and meningioma (OR =1.34; 95% CI, 0.96-1.86). However, in pair-wise comparisons a few SNP combina-tions were associated with the risk of brain tumors: Among others, carriers of both homo-zygous variant genotypes, i.e., XRCC1 Gln399Gln and XRCC3 Met241Met, were associ-ated with a three-fold increased risk of glioma (OR = 3.18; 95% CI, 1.26-8.04) and men-ingioma (OR = 2.99; 95% CI, 1.16-7.72). Conclusions: No significant association with brain tumors was found for any of the poly-morphisms, when examined one by one. However, our results indicated possible associa-tions between combinations of XRCC1 and XRCC3 SNPs and the risk of brain tumors.

RA029

REDUCTION OF MISLEADING ("FALSE") POSITIVE RESULTS IN MAMMALIAN CELL GENOTOXICITY ASSAYS. I: CHOICE OF CELL TYPE

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Current in vitro genetic toxicology assays have a high rate of reported positive results, when compared with negative rodent carcinogenicity data. Moreover, the rate of misleading positive results with a combination of assays was found to be at least 80%1. Poor predictivity was expected to be worst in p53-deficient cell lines of rodent origin, particularly long established and widely used Chinese hamster cell lines. Since in vivo models have been banned by the EU Cosmetics Directive since March 2009, in vitro models need to be more predictive for the risk assessment of cosmetic ingredients. As part of a larger framework for improvement of in vitro genetic toxicology assays the performance of currently used cell lines is being investigated and compared with p53-competent cells2. Comparisons have been made between Chinese hamster Lung, Chinese hamster Ovary, V79, TK6, HepG2 and Human peripheral blood lymphocytes. These comparisons were made using the in vitro micronucleus assay to evaluate clastogenic potential and highlight any differences in sensitivity between cell lines, with a selection of compounds that are accepted as producing misleading positive results in in vitro clastogenicity assays3. Micronucleus assay data from these comparisons show similar patterns of responses between different cell lines; however sensitivity differs markedly particularly when comparing levels of toxicity and when compared with p53-competent cells.

1Kirkland et al. Mutat. Res. 2005, 584(1-2):1-256; 2Kirkland et al. Mutat Res. 2007, 628(1):31-55; 3Kirkland et al. Mutat. Res. 2008, 653(1-2):99-108.

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RA030

REDUCTION OF MISLEADING (“FALSE”) POSITIVE RESULTS IN MAMMALIAN CELL GENOTOXICITY ASSAYS. II: IMPORTANCE OF ACCURATE TOXICITY MEASUREMENT.

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Current in vitro genetic toxicology assays have a high rate of reported positive results, when compared with negative rodent carcinogenicity data. A recent analysis of published data highlighted the inaccuracy of current in vitro assays, the rate of misleading positive results with a combination of assays was found to be at least 80%1. As a result of the 7th Amendment to the Cosmetics Directive which came into force in March, positive results for cosmetic ingredients can no longer be followed-up by in vivo testing. Therefore, in vitro assays must be more predictive in order to avoid attrition of promising chemicals. One focus of a European funded project is to determine the optimal measurement of cytotoxicity used alongside measurements of genotoxicity. Comparisons have been made between different measures of toxicity after exposure to previously identified chemicals giving rise to misleading positive results in the in vitro micronucleus assay. Emphasis has been placed on chemicals that have a steep toxicity profile and a maximum testing concentration limited by toxicity. Comparisons were performed between cell counts, mitotic index and replication index measurements, intracellular toxicity endpoints were also investigated, including apoptosis. Our results demonstrate that certain measures have the potential to seriously overestimate toxicity, the implication of which is a higher maximum testing concentration which may contribute to the generation of misleading positive results with in vitro genotoxicity assays.

1Kirkland et al. Mutat. Res. 2005, 584(1-2):1-256.

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RA031

POLYMORPHIC MICRO-RNA TARGETS WITHIN CD86 AND INSR AND RISK OF COLORECTAL CANCER.

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Recent evidence indicate that small non-coding RNA molecules, called micro-RNAs (miRNA), can bind to the 3'UTRs of mRNAs and affect with their translation, thereby regulating cell growth, differentiation, apoptosis, and tumorigenesis. Genetic polymorphisms (SNPs) can reside on miRNA binding sites. Thus, miRNA regulation may be affected by SNPs on the 3' UTRs. Since gene de-regulation is one of the key mechanisms by which cells can progress to cancer, we hypothesized that common polymorphisms within miRNA target binding sites could play a role in the individual risk of cancer. In the present study, we selected the 3'UTR regions of 129 genes candidate for colorectal cancer (CRC) and we identified putative miRNA binding sites by specialized algorithms (PicTar, DianaMicroT, miRBase, miRanda, TargetScan, and microInspector). We evaluated the SNPs for their ability to affect the binding of the miRNA with its target, by assessing the variation of Gibbs free energy between the two alleles of each SNP. We found 15 common polymorphisms. We added to this list 8 SNPs in miRNA sequences. All the polymorphisms were further investigated by a case-control association studies. The study was carried out on a series of cases and controls from Czech Republic, a population with the highest worldwide incidence of CRC. We found statistically significant associations between risk of CRC and variant alleles of CD86 (OR=2.74 95%CI=1.24-6.04, for the variant homozygotes) and INSR genes (OR=1.94; 95%CI=1.03-3.66, for the variant homozygotes). Then, these two polymorphisms were genotyped in three different populations: Spanish, Italians, and Germans. The statistical analyses for all the samples (Czech, Spanish, Italian, and German) confirmed the association between risk of CRC and the SNPs in CD86 and INSR. These results are the first reporting positive association between miRNA-binding SNPs sequences and cancer risk. We are undertaking the cloning of the 3′-UTR of the target gene downstream of the luciferase (with the wild type allele and with the variant allele in the polymorphic site) and transfected these recombinant plasmids and a miRNA of interest into a host cell.

RA032

ENVIRONMENTAL PROTEOMICS ASSESSMENT AROUND DOÑANA NATIONAL PARK (SW SPAIN)

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The utility of the Environmental Proteomic approach was assessed by comparing protein expression in organisms from sites within Doñana National Park (DNP, SW Spain) with those from Doñana surroundings, exposed to metals and pesticides of agricultural origin, and animals from the Domingo Rubio Stream, exposed to pyritic metals, contaminants fromHuelva Industrial Area, and agrochemicals. A bivalve mollusk (Scrobicularia plana), two decapod crustaceans (Carcinus maenas, Procambarus clarkii), and a free-living rodent (Mus spretus) were used as bioindicator organisms in different habitats. Proteins significantly altered were identified by de novo nESI-MS/MS sequencing in C. maenas and S. plana, and by MALDI-TOF-PMF in M. spretus, due to its homology to model M. musculus species, fully sequenced. They pertained to cytosqueletal dynamics, membrane transport , proteolysis, biotransformation, oxidative stress adaptation , metabolism, and transcription. The utility of a quantitative Protein Expression Signature (PES) approach was also shown in non-model species from different sites of DNP and its surroundings. In P. clarkii, four protein expression

patterns were established based in the fold-number of up-/down regulation of 35 differentially expressed proteins. Sites located within Doñana Biological Reserve (DBR) were essentially free of contaminants and those near DNP limits were only slightly polluted. The higher proteomic responses found at the upper "Rocina" and "Partido" courses indicate that non-persistent agrochemicals are mainly used in Doñana surroundings. The highest responses corresponded to rice growing areas placed between the Guadiamar stream and the Guadalquivir River, according to the extended and intensive use of agrochemicals in such areas. In M. musculus, again, DBR sites were essentially clean, while the more altered PES were found at the lower "Rocina" and "Partido" courses and at the rice growing areas placed East of Guadiamar stream. Grants CTM2006-08960 (Spanish Ministry of Education and Science), RNM-523 and P08-CVI-03829 (Andalusian Agency of Innovation and Science)

RA033

EFFECTS OF pH ON BACTERIAL REVERSE MUTATION ASSAY – POSSIBLE FEASIBILITY ON MUTAGENESIS STUDIES OF ACID AND BASIC COMPOUNDS

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Genotoxic responses of chemical compounds containing acidic or basic functional groups can be affected by pH even on the well-standardized assays. As example the mutagenesis of several polyphenols are inhibited in acidic pH when detected by mitotic gene conversion and chromosomal aberrations assays. Usually mutagenesis is evaluated using Salmonella/microsome assay at pH 7.2. This assay is widely recognized to evaluate mutagenesis of several environmental or consumer compounds. We propose that a systematical evaluation of the pH effects on its response would be recommendable to evaluate the mutagenesis mechanisms of several acidic or basic compounds. In the present work, Salmonella/microsome assay using Salmonella typhimurium TA98 and TA100 strains and plate incorporation procedure were performed for negative and positive controls in triplicate following OECD protocol without exogenous metabolizing system. However, the pH buffers of the bacterial suspension were varied at 3.5, 4.5, 7.4 (standard) and 8.5, prior procedures of pre-incubation and plate pouring. Negative and positive (at 0.5 microg/assay) controls were, respectively, dimethyl sulfoxide and 4-nitroquinoline 1-oxide for TA98 and water and sodium azide for TA100. Concomitantly to evaluate cell viability, aliquots (10 microL) of the bacterial suspension were diluted and further poured and incubated in nutrient media plates. Data were analyzed by two-way ANOVA. Independently of pH, positive controls did not differ significantly ($P > 0.05$) and their mutation induction indexes were higher than 2, as expected. Independently of the pH, the spontaneous reversions of the negative controls were found among the historical registrations. The preliminary results reported herein indicate a possible feasibility of the Salmonella/microsome assay at pH range from 3.5 to 8.5 allowing to improve mutagenesis risk evaluations of several products formulated at unphysiological pH, such some cosmetic and food products. Support: CNPq, SR2-Uerj, FAPERJ.

RA034

BIODISTRIBUTION AND SUB-CHRONIC TOXICITY OF MULTI-WALLED CARBON NANOTUBES

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Background:Multi-walled carbon nanotubes (MWNTs) have attracted great attention not only in electrical, optical and mechanical applications but also in biological and pharmaceutical applications. Thus, it is important to examine the biodistribution and potential toxicity of MWNTs when they are introduced into living systems. Methods:The biodistribution of MWNTs in mice were investigated following three different administrations by 125I labeled radioisotope tracing method. Then, acute and sub-chronic toxicity of phosphoryl choline-grafted multi-walled carbon nanotubes (MWNTs-PC) were detected. Mice were treated with daily injection of MWNTs-PC for 28 days at 10, 50, and 250 mg/kg by the intraperitoneal administration. Furthermore, the cellular bioeffects of MWNTs-PC were evaluated. 16-HBE cells were incubated with various concentrations of MWNTs-PC, the effects of cell proliferation, cell apoptosis, cell cycle and DNA damage were detected by methyl thiazolyl tetrazolium assay, flow cytometry, single cell gel electrophoresis assay and micronucleus assay, respectively. Results:MWNTs was quickly delivered around the whole mice body and accumulated mainly in lung and also slightly in spleen and liver after mice were exposed by intravenous injection for 24h. Repeat exposure of MWNTs-PC did not lead to obvious systemic toxicity effects in mice. The body weight of high-dose group was significantly lower than that of the control group during the period of initial 3 weeks in male mice. The tissue coefficients of liver, spleen and lung rose obviously with the increase of exposure dose of MWNTs-PC and there were significantly different between high-dose exposure group and control group. Accumulation of carbon nanotubes and gentle inflammation response in liver, spleen and lung were observed in high-dose exposure group. No systemic toxicity and histopathological changes were induced in low-dose exposure group. Compared with the control group, there were no significant differences in the changes of cell proliferation, cell apoptosis, cell cycle and DNA damage. Conclusions:The date supports the low toxicity of the MWNTs in vivo and in vitro, but MWNTs accumulate easily in liver, spleen and lung. The long-term deposit effects must be carefully considered in further research.

RA035

DETECTION OF HERBICIDE IN ENVIRONMENTAL SAMPLES

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Paraquat (1,1'-ethylene-2,2'-bypyridylium ion) is widely used as herbicide in India. It is toxic to human, animals and others. A new spectrophotmetric method for rapid detection of paraquat has been developed. It is based on alkaline hydrolysis of paraquat, and subsequent reduction with ferrous ions. The blue colored species formed exhibit absorption maximum at 500 nm. The value of molar absorptivity of the species is $(4.00)x10^4$ l $mole^{-1}$ cm^{-1}. The method followed Beer's law up to 7.0 mg l^{-1} paraquat. The absorbance of the species is stable at room temperature (30±2 ^oC) for > 24 hrs. The interferences of other inorganic and organic species present in environmental samples are discussed. The method was tested for the detection of paraquat in a variety of environmental samples.

RA036

SENSITIVE DETECTION OF INSECTICIDE IN AGRICULTURAL SAMPLES

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Insecticide such as cypermethrine {(2, 2'-dichlorovinyl)-2,2-dimethyl-cyclopropanecar boxylate} is frequently used to control pests in a variety of agricultural crops. A new and sensitive spectrophotometric method for rapid detection of cypermethrine, based on alkaline hydrolysis into cyanide ions and subsequent condensation with p-aminoacetophenone to produce azo dye, crystal violet (CV) is described. The value of molar absorptivity of the CV in the term of cypermethrine is

$(6.10)x10^5$ l $mole^{-1}$ cm^{-1} at l_{max}, 400 nm. The calibration curve followed Beer's law up to 2.4 mg l^{-1} cypermethrine. The interferences of common ions and compound present in environmental samples are discussed. The method was tested for the detection of cypermethrine in a variety of agricultural samples.

RA037
INVESTIGATING THE GENOTOXICITY AND DOSE-RESPONSE CURVES OF THE AZO DYES SUDAN-1 AND PARA RED

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Background: Sudan-I and para red are industrial dyes that have been illegally added to some foodstuffs, leading to withdrawal of the adulterated products throughout the UK since 2003. This resulted in international concern that arose because Sudan-1 is classified by IARC as a category 3 carcinogen. However, little is known about the dose-response of this chemical at low, more biologically relevant, doses. Aims: The study therefore aimed to characterise the dose-response for gene mutation and chromosomal damage induced by two azo dyes, namely Sudan-1 and para red. Methods: Gene mutations were analysed using the HPRT forward mutation assay and chromosomal damage was measured using the cytokinesis-blocked micronucleus assay. Two cell lines were used in these investigations. These were the AHH-1 cell line which inducibly expresses CYP1A1, and also the MCL-5 cell line that is derived from a subpopulation of AHH-1 cells and expresses a particularly high level of CYP1A1 activity. The MCL-5 cell line has also been transfected with 2 plasmids that stably express CYP1A2, CYP2A6 and CYP3A4 and all four of these CYP enzymes are known to metabolically activate Sudan-I. AHH-1 cells were used to investigate the dose response of the azo dyes, and MCL-5 cells were used to see if the dose response changed with increased metabolism. Results: Sudan-I induced a non-linear dose response curve for gene mutation (NOEL, 1.5µg/ml) and chromosomal damage (NOEL, 2.5µg/ml) in AHH-1 cells. The genotoxic activity of Sudan-1 was greatly increased in MCL-5 cells (NOELs, 0.5µg/ml and 1.25µg/ml respectively). This indicated that the oxidation metabolites from Sudan-I were both more mutagenic and more clastogenic than the parent compound. Para red also demonstrated a non-linear dose response for both gene mutation (NOEL, 6µg/ml) and chromosome mutation (NOEL, 7.5µg/ml) in AHH-1 cells, and an increase in MN induction was observed after increased oxidative metabolism in MCL-5 cells (NOEL, 2.5µg/ml). Conclusions: Sudan-I and para red are genotoxic chemicals with non-linear dose responses, and oxidative metabolism increases the genotoxic effect of both compounds. However, the data are not yet clarified as to the mechanisms of interaction with the genetic material and further data are necessary.

RA038
IMPACT OF CIGARETTE AND WATER PIPE SMOKING ON ADULT MORTALITIES IN EGYPT

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Background Smoking is associated with an increased risk of total and cause-specific death rates. Prevalence of cigarette and water pipe smoking is progressing in Egypt. Objectives To measure the mortality risks of cigarette smokers and water pipe smokers versus non smokers. Methods Death certificates of 38,918 males (according to ICD-10) whose smoking status was reported during 2003-2005 were analyzed. The excess risk of dying due to smoking for smoking-related diseases and smoking-related cancers were compared with the non-smokers' risk of dying by calculating the proportionate mortality ratio (PMRi), and the mortality odds ratio (MORi). Results Cigarette smokers had a significantly increased risk of dying from all smoking related diseases (MORi =1.25,CI=1.21,1.44), and from all cancers (MORi=1.31, CI=1.21,1.46) compared to non-smokers. Water pipe smokers had a significantly increased risk of dying from all smoking related diseases (MORi=1.11,CI=1.02,1.32) but not all cancers compared to non-smokers. Cigarette smoking compared to non-smoking increases the risk of dying almost 7 times from tuberculous (TB) lung, 4 times from lung cancer, 2 times from laryngeal cancer or stomach cancer, 1.7 times from coronary heart disease, 1.5 times from chronic obstructive pulmonary disease (COPD) and liver cancer, and 1.3 times from pneumonia, other neoplasm and cerberovascular diseases. Water pipe smoking compared to non-smoking increases the risk of dying from TB lung (not confirmed bacteriological) by 3.7 times, from stomach cancer by nearly 3 times, and from cerebrovascular diseases by 1.3 times. The mortality risk for stomach cancer was higher among water-pipe smokers than cigarette smokers. No significant increased risk of mortality among either types of smokers (cigarettes or water pipe) compared to non-smokers were observed for atherosclerosis, hypertension, bladder cancer, esophageal cancer, kidney cancer, leukemia, oral cancer, and pancreatic cancer. Conclusion Smoking makes a significant contribution to the mortality experienced among Egyptians for many diseases and reflects the excess mortality attributable to smoking. Continuous reporting of the deceased individual's smoking status in the death certificate is essential for quantifying the mortality risks

RA039
GENETIC BIOMONITORING OF INHABITANTS EXPOSED TO URANIUM IN THE MUNICIPALITIES OF MONTE ALEGRE, PRAINHA AND ALENQUER IN THE STATE OF PARÁ, BRAZIL

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Uranium is a natural radioactive metal, whose effects in organisms are cumulative and chronic exposition to this element may induce carcinogenesis. Three cities of the Amazon region – Monte Alegre, Prainha and Alenquer – in the north of Brazil are located in one of the world's largest uranium mineralization area. The aim of this study was to assess the exposed populations' susceptibility to ionizing radiation emitted from uranium, evaluating the frequencies of mutations in the DNA repair genes XRCC1 and XRCC3 and in the metabolic gene GSTM1. The XRCC1 allele frequencies for the 194Trp polymorphism in Monte Alegre, Prainha and Alenquer were 0.12, 0.13 and 0.07, and for 399Gln polymorphism were 0.28, 0.30 and 0.32, respectively. These frequencies were comparable with those described for Brazilian individuals from other regions of the country. The allele frequencies of the XRCC3 241Met polymorphism were 0.28 and 0.33, respectively to Monte Alegre and Alenquer populations and were comparable to allelic frequencies in Brazilian subjects. The 241Met allele frequency for Prainha population was 0.13 and differed statistically from Monte Alegre and Alenquer ($p < 0.05$). The population frequencies of the GSTM1 gene deletion homozygotes were 0.36, 0.31 and 0.40, respectively to Monte Alegre, Prainha and Alenquer and were also similar to those previously obtained for Brazilian individuals. These results for the polymorphic allelic frequencies in XRCC1 and XRCC3 genes and for the GSTM1 gene deletion frequencies sum with our research group's previous results concerning the assessment of genotoxic and carcinogenic effects due to uranium exposition in these same populations, in which DNA damage and micronuclei and chromosomal aberrations frequencies were not increased in those three populations compared to the negative control group. Both cytogenetic biomonitoring and assessment of polymorphisms in DNA repair and metabolizing genes in Monte Alegre, Prainha and Alenquer populations indicate that the emission of uranium in the studied region is probably not sufficient to induce genetic alterations and that the frequencies of important polymorphic features of cellular DNA repair and metabolic apparatus are not different from populations from other regions of Brazil.

RA040
CORRELATION BETWEEN THE RESULTS OF IN VITRO AND IN VIVO CHROMOSOME DAMAGE TESTS
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In vitro chromosomal aberration and in vitro micronucleus tests are widely used for genotoxicity assessment of chemicals. However, it was recently reported that these tests induce a remarkably high rate of "irrelevant" positive results when compared with in vivo tests. In this study, we examined the correlation of results between in vitro and in vivo chromosome damage tests using in-house data, and quantitatively compared the lowest effective concentration (LEC) in in vitro test with the plasma concentration in in vivo test. Furthermore, in order to explore the factors involved in "irrelevant" positive results, several parameters in the in vivo and in vitro tests were analyzed. Of 33 compounds showing in vitro positive results, 5 compounds were positive in vivo. For all of these 5 compounds, the maximum plasma concentrations (Cmax) on in vivo tests were almost equal or higher than the LEC on in vitro tests. In 28 compounds that were negative in in vivo, Cmaxs of 9 compounds were higher than the in vitro LECs. These cases that were negative in in vivo even at exposure levels higher than the LEC are considered to show "irrelevant" positive results on in vitro tests. There were no obvious differences in plasma protein binding and the fold increase of the chromosomal damage between cases showing "irrelevant" and relevant positive findings. However, AUC, the treatment method on in vitro test, and the cell survival ratio at LEC on in vitro test might be factors that cause irrelevant positive findings in vitro. These results suggest that in vivo positive results require exposure levels comparable to those in the in vitro positive condition, and excess cytotoxicity and continuous exposure in in vitro might be factors associated with irrelevant positive results.

RA041
EXPLORATION OF STUDENT'S OPINION ON ANTICIPATED PENALTIES TO REDUCE SMOKING INSIDE AIN SHAMS UNIVERSITY CAMPUS
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Background: Smoke free policy inside universities is very important to ban smoking. The prevalence of smoking in Egypt is very high. Smoking among adult males in Egypt was estimated from a national survey in 2002 focusing on hypertension, obesity and diabetes prevalence to be 47%. This epidemic is reaching pre adolescent students in schools where the prevalence reached 19% in some areas in Cairo. Smoking inside medical campus reached 31% in previous studies. Aim: This study aimed at measuring the prevalence of smoking inside Ain Shams University. Also to explore the student's opinion on the anticipated penalties that should be applied to ban smoking in the University campus. Subjects and methods: 400 Medical and non medical students were randomly selected. A self administered questionnaire was distributed to the students. It consisted of three sections Section A: smoking status of the student with previous history of his smoking habit. Section B explored the student's opinion on the penalties to ban smoking inside the university. Section C was designed to identify the antismoking health education activities given to students inside the university Results: This study revealed a higher prevalence of smoking among non medical students 30.7% compared to 17.8% and the difference is highly significant statistically. Male students had a significant higher prevalence rate of smoking compared to female students. Almost 50% of the students foresee application of the fine penalty on smoker students is an effective method of banning smoking. The study showed that only 28% of the students attended antismoking workshops inside the university. Conclusion: Urgent law enforcement of banning of smoking should be strictly applied inside University campus. The proposed penalties of this study should be integrated in the policies of the university to ban smoking. Also improvement and increase of the health education campaigns and activities to ban smoking should be considered.

RA042
QUANTITATIVE ANALYSIS OF Nε-ETHYLLYSINE IN HUMAN PLASMA PROTEINS BY NCI-GC/MS AS A BIOMARKER FOR ACETALDEHYDE EXPOSURE
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Worldwide, approximately 3.6% of all cancer cases are related to alcohol consumption. Acetaldehyde, the first metabolite of ethanol, may mediate alcohol-induced carcinogenesis. It is also one of the most prevalent carcinogens in cigarette smoke and is found widely in the environment. Nε-Ethyllysine (NEL) has been reported to be one of the major products formed by the reaction of acetaldehyde with proteins, although its role in carcinogenesis is not fully evaluated due to lack of analytical methods. For these reasons, we have developed a sensitive and specific method to determine NEL in human plasma proteins by negative ion chemical ionization gas chromatography-mass spectrometry (NCI-GC/MS). Proteins were separated from free amino acid by gel filtration using Sephadex G-15 and hydrolyzed with pronase E. NEL and lysine in the hydrolysates, after adding corresponding internal standards containing stable isotopes, were derivatized with pentafluorobenzyl (PFB) bromide to form PFB derivatives, which were then quantified by NCI-GC/MS. The detection limit for PFB-NEL was 30 fmol/injection. The mean and SD of recovery rates of NEL added into protein samples was 110.4 ± 3.6%. Using this method, we analyzed NEL in human plasma from 20 healthy volunteers. NEL was detected in all samples with the mean ± SD being 0.235 ± 0.06 NEL/103 lysines. However, the levels of NEL in human plasma proteins were not significantly correlated with smoking or drinking habits. We are currently investigating possible associations between levels of NEL and polymorphisms of the genes encoding for alcohol dehydrogenase (ADH) and aldehyde dehyderogenase2 (ALDH2).

RA043
DEVELOPMENT OF A MONONUCLEATE IN VITRO MICRONUCLEUS SCREENING METHOD USING THE CELLOMICS ARRAYSCAN VTI HIGH CONTENT SCREENING (HCS) READER
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The Micronucleus Bioapplication for the Cellomics ArrayScan VTI HCS Reader is a multichannel High Content Screening software module that enables automated scoring of micronucleus formation in single cells in a multiwell plate format. It has advantages over conventional techniques for quantifying micronucleus formation by reducing compound requirements and analysis times. The Cellomics Micronucleus reagent kit utilises the cytokinesis-block method of measuring micronucleus induction in binucleate cells. However the assay can be adjusted to enable mononucleated cells to be assessed for micronuclei, eliminating the requirement for cytochalasin B within the assay. The aim of this study was to develop and validate a mononucleate in vitro micronucleus method based on the Cellomics Micronucleus Bioapplication to enable rapid screening of test compounds. CHO-K1 cells were treated with ten chemicals with a range of known mechanisms of action (3 non-genotoxins, 5 direct acting genotoxins and 2 pro-mutagens). Two exposure protocols were employed; a 4 hour treatment in the presence and absence of metabolic activation followed by 24 hours recovery, and a 20 hour treatment in the absence of metabolic activation followed by 28 hours recovery. Duplicate wells were prepared for each treatment condition. Positive and negative controls were included in each experiment. Cytotoxicity was assessed based on the percentage of cells present following treatment compared to concurrent controls. The highest scoreable dose did not exceed 60% cytotoxicity. A test

chemical was considered positive if it induced a biologically significant increase in the proportion of cells with micronuclei, i.e. at least 2-fold higher than concurrent negative controls at one or more concentrations. The results for each of the ten chemicals correlated with their expected result but with two exceptions; the non-genotoxin sodium chloride gave a weakly positive response, possibly due to osmotic effects, and the promutagen benzo[a]pyrene was positive only in the absence of S9. We found that S9-related cytotoxicity appeared to reduce the sensitivity when detecting pro-mutagens. Overall the assay was an effective in vitro micronucleus screen, providing that the experimental conditions were closely monitored.

RA044

EVALUATION OF THE GREENSCREEN HC (HUMAN CELLS) ASSAY USING 40 CODED CHEMICALS

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The GreenScreen HC assay was developed by Gentronix as a high throughput screen for genotoxicity. The assay employs TK6 human lymphoblastoid cells that are modified to incorporate a green fluorescent protein (GFP) reporter system linked to the GADD45a gene. The GADD45a gene mediates the adaptive response to genotoxic stress, therefore exposure to genotoxic chemicals results in the expression of GFP, which is measured by increased cellular fluorescence. The assay is conducted in multi-well plate format and the GFP fluorescence is detected using a microplate reader. Until recently, there were difficulties in incorporating an S9 fraction into the assay to provide metabolic activation of pro-mutagens due to interference with the GFP signal. This has been overcome by adapting the methodology to measure the GFP fluorescence by flow cytometry. The performance of the GreenScreen HC assay using the microplate reader in the absence of S9 and flow cytometry in the presence of S9 was evaluated in a blind study using 40 coded compounds. The chemicals, selected by Unilever, comprised of direct and indirect acting rodent carcinogens, non-carcinogens that were negative in standard in vitro genotoxicity assays and non-carcinogens that have produced irrelevant positive responses in standard in vitro genotoxicity assays. The experimental work was carried out by Gentronix. When the results for the chemicals were assessed up to a maximum concentration of 10 mM, 18/21 carcinogens were positive, 11/12 non-carcinogens and 5/7 'irrelevant positive' non-carcinogens were negative in the GreenScreen HC assay. Four of the ten direct acting carcinogens were positive in the flow cytometry assay but negative when using the microplate reader. This suggests that the flow cytometric method of detecting the GFP signal may be more sensitive than the microplate reader method, probably because it is less prone to interference from intense auto-fluorescence, high colouration or precipitation of the test chemicals. Overall the results showed good performance of the GreenScreen HC assay in predicting rodent genotoxic carcinogens / non-carcinogens and suggest that incorporation of flow cytometric detection may improve performance.

RA045

DEVELOPMENT OF AN ASSAY FOR DETECTING PIG-A GENE MUTATION IN HUMAN RED BLOOD CELLS

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The endogenous X-linked PIG-A gene is involved in the synthesis of glycosyl phosphatidyl inositol (GPI) anchors that tether specific protein markers to the exterior of mammalian cell cytoplasmic membranes. A single inactivating mutation in the PIG-A gene can result in the disruption of GPI synthesis and a deficiency in multiple GPI-anchored proteins (GPI-APs) at the cell surface. In humans, peripheral blood mononuclear cells and red blood cells (RBCs) may become GPI-AP deficient due to PIG-A mutation in bone marrow precursors, as occurs in the rare acquired hematologic disorder, paroxysmal nocturnal hemaglobinuria (PNH). Earlier studies in rodent models indicate that Pig-A mutant lymphocytes and RBCs can be induced in animals treated with genotoxic agents, and that high throughput flow cytometry can be used to identify RBCs deficient in the GPI-AP, CD59, as a marker of Pig-A mutation. We are investigating if a similar approach can be used for monitoring genotoxicity in humans, especially during clinical trials of new drugs. In this initial study, we determined the frequency of spontaneous CD59-deficient RBCs (presumed PIG-A mutants) in approx. 75 healthy volunteers, recruited through a donor services agency. For most subjects, the frequency of CD59-deficient RBCs was low ($5.1+/-4.9\times10(-6)$) and displayed no correlation with the age, gender, ethnic origin, or smoking status of the subject. Two individuals, however, had markedly increased CD59-deficient RBC frequencies of ~300 and $\sim100\times10(-6)$; correspondingly elevated frequencies of PIG-A mutant T-cells also were detected for one of these subjects. In addition, the high frequencies of PIG-A mutant RBCs in these donors were confirmed in repeat assays on blood samples collected at least 1 month apart. These results indicate that the RBC PIG-A assay may be useful for monitoring genotoxicity in humans, especially if a baseline (pre-exposure) mutant frequency can be established. The following stage of the study will involve monitoring mutation in human oncology patients undergoing chemotherapy with DNA-reactive antineoplastic drugs.

RA046

REGULATORY AUTOMATED MICRONUCLEUS ASSAY SCORING FOR COMPOUND SCREENING IN PHARMACEUTICAL INDUSTRY

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The micronucleus assay scoring demands both time and expertise. To increase the scoring throughput and decrease operator-dependence of results, we implemented an automated scoring process using the automated IMSTAR PathfinderTM imaging system to be used in further regulatory in vitro micronucleus studies compliant with Good Laboratory Practice1. We present here the validation work designed to assess the capability of the automated imaging system to detect micronucleated cells as accurately as visual scoring. Mouse lymphoma L5178Y cells were exposed to known genotoxic compounds at various concentrations according to different treatments conditions (short and long treatments, with or without metabolic activation). The scores of micronucleated cells were compared between visual and automated scoring in terms of time-saving, repeatability, reproducibility and accuracy. The results showed that, despite minor explainable differences, the IMSTAR PathfinderTM imaging system was concluded to be able to detect micronucleated cells in the in vitro micronucleus assay using L5178Y cells as accurately as visual scoring. 1: OECD Principles of Good Laboratory Practice [C(97)186/Final].

RA047

ASSOCIATION BETWEEN GENETIC POLYMORPHISMS IN THE CYP3A4, CYP3A5 AND GSTO GENES AND BREAST CANCER SUSCEPTIBILITY

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Individual differences in susceptibility to carcinogens play an essential role in the development of sporadic tumors. The biochemical basis for this susceptibility is related to genetic polymorphisms that normally occur in the general population regarding genes involved in predisposition to a specific cancer, in the metabolic activation or detoxification of environmental genotoxins, and in controlling DNA repair or cellular damage. The aim of this study was to determine the frequency of the CYP3A4*1B, CYP3A5*1 e GSTO1*A140D polymorphisms in the Brazilian population and to assess the relationship between this polymorphisms and its possible role in the development of breast cancer. Polymorphisms were determined in 75 women with breast cancer diagnosed between 25 and 70 years and 88 age-matched healthy controls. DNA was obtained from blood samples and was genotyped by PCR-RFLP. The variant allele frequencies for patients with breast cancer and healthy controls, respectively, were: CYP3A4*1B, 0% and 0% (P = 1.00); CYP3A5*3, 25% and 21% (P = 0.61), GSTO*A140D, 19% and 32% (P = 0.05). Therefore, only GSTO variant frequencies differ between breast cancer patients and healthy controls. No differences between variant alleles were observed with regard to age, age at menarche, age at menopause, hormone use (contraceptive, hormone therapy), number of gestations, family history of cancer and tumor characteristics. This study suggests that CYP3A4*1B, CYP3A5*1 e GSTO1*A140D polymorphisms is not associated with breast cancer risk in Brazilian population.

RA048

XRCC3241 POLYMORPHISM INFLUENCE ON MICRONUCLEI FREQUENCY IN WORKERS OCCUPATIONALLY EXPOSED TO FORMALDEHYDE

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Formaldehyde (FA) has been deemed carcinogenic to humans on the basis of manifold *in vitro* studies that indicated that FA is genotoxic. The most sensitive genetic endpoints for the detection of FA mutagenicity appear to be chromosomal aberrations and micronucleus (MN). The goal of this study is to determine whether there is an *in vivo* association between genetic polymorphism of the enzyme *XRCC3* and the frequency of MN in occupationally workers exposed to formaldehyde. *XRCC3* is involved in the homologous recombination repair of cross-links and chromosomal double-strand breaks and at least one polymorphism has been reported in codon 241 of *XRCC3*. We compare a sample of 56 workers exposed to FA in pathological anatomy laboratories with 85 controls, in order to investigate whether exposure to FA and of genetic polymorphism of *XRCC3* is associated with the frequency of MN in human peripheral blood lymphocytes, as measured by *cytokinesis-block micronucleus assay*.The mean of micronucleated binucleated lymphocytes in the occupationally exposed workers was 3,96, which compares with 0,81 in the controls, a statistically significant difference (Mann-Whitney test, p<0.001). The exposed workers carrying the Thr/Met *XRCC3*^{241} genotype were found to have higher MN mean (5,05) than Met/Met (2,92) and Thr/Thr (3,72) *XRCC3* ^{241} genotypes. Moreover, the values were higher when compared with their control counterparts (0,73; 1,15 and 0,75 respectively). Multiple regression analysis indicated that the exposure to formaldehyde was an important variable affecting the genotoxic response, but the polymorphisms of XRCC3 at codon 241 were not found statistically significant. Understanding the complexity of the relationships between exposure, DNA repair and MN frequencies probably require larger scale studies and complementary biomarkers.

RA049

NUCLEOPLASMIC BRIDGES AND NUCLEAR BUDS FREQUENCIES IN HUMAN LYMPHOCYTES IN VIVO

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The cytokinesis-block micronucleus (CBMN) assay was originally developed as an ideal system for measuring micronucleus (MN), however it can also be used to measure nucleoplasmic bridges (NBP) and nuclear buds (NBUD). NBPs are originate from dicentric chromosomes in which the centromeres have been pulled to the opposite poles of the cell at anaphase and are therefore indicative of DNA mis-repair, chromosomes rearrangement or telomere end-fusions. NBUDS is considered as a marker of gene amplification and/or altered gene dosage because the nuclear budding process is the mechanism by which cells removed amplified and/excess DNA. The International Agency for Research on Cancer (IARC) classifies FA as carcinogenic to humans (group 1), on the basis of sufficient evidence in humans and sufficient evidence in experimental animals. Manifold in vitro studies clearly indicated that FA is genotoxic. FA induced various genotoxic effects in proliferating cultured mammalian cells. The aim of this study is to determine the association between NPB and NBUDs frequencies and formaldehyde exposure in peripheral blood lymphocytes. A study on 56 workers exposed to formaldehyde and 85 referents was performed in order to examine the influence of the exposure to formaldehyde and the frequency of NPB and NBUDS in human peripheral blood lymphocytes, as measured by *CBMN*.The mean of NPB in exposed was 3,04 and of NBUDS was 0,98 and in the referents 0,18 and 0,07 respectively. Differences between means of NPB and NBUDS and the exposition to formaldehyde in the two groups were Z = 6,637 (p<0,001) to NPB and Z = 4,882 (p<0,001), both evaluated using Mann-Whitney U test. The importance of scoring NPBs should not be underestimated because it provides direct evidence of genome damage resulting from misrepaired DNA breaks which is otherwise not possible to deduce by scoring MN only which could originate from acentric fragments or chromosome loss. NBUDs should be considered as a genotoxic biomarker, with an origin comparable to that of MN. It appears to originate from interstitial or terminal acentric fragments, possibly representing nuclear membrane entrapment of DNA that has been left in cytoplasm after nuclear division, or excess of DNA that is being extruded from the nucleus.

RA050

PERFORMANCE VALIDATION OF THE PERIPHERAL BLOOD HUMAN LYMPHOCYTE MICRONUCLEUS ASSAY USING THE DRAFT OECD 487 GUIDELINE REFERENCE CHEMICAL LIST

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Following issue of the latest draft OECD test guideline (487 – February 2008) on the conduct of the in vitro Mammalian Cell Micronucleus Test we have assessed the performance of the assay in human peripheral blood lymphocytes using the reference chemicals (as listed in Annex 3 of the test guideline). These included a total of ten chemicals, of which six are active via a variety of differing modes of action (including clastogens requiring metabolic activation, direct acting clastogens and aneugenic agents). Four negative compounds (known to be non-DNA reactive) were also included. All compounds were tested using the standard recommended two experiment design and included both possible options (A and B) for extended exposure in the absence of S-9 (treatment for 1.5-2.0 normal cell cycles with either zero recovery or a further 1.5-2.0 cell cycles recovery - as detailed in OECD 487) to allow comparison and possible recommendation as to which could

be the most suitable. All compounds were treated up to 10 mM or 5000 μg/mL (whichever was the lower), unless limited by cytotoxicity or precipitation. The results confirmed the acceptable performance of the assay using the reference chemicals. Furthermore, from this limited data set we would recommend the use of Option B for the continuous treatment in the absence of S-9 as being the more sensitive (treatment for 1.5-2.0 normal cell cycles in the absence of Cyto-B with recovery with Cyto-B for a further 1.5-2.0 cell cycles). This was the only treatment regime where the clastogenic agent Cytosine arabinoside was found positive and which also provided a more consistent response with the aneugenic compound vinblastine.

RA051

CUMULATIVE PATIENT EFFECTIVE DOSE AND ACUTE RADIATION-INDUCED CHROMOSOMAL DNA DAMAGE IN CHILDREN WITH CONGENITAL HEART DISEASE

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BACKGROUND: The seventh Biologic Effects of Ionizing Radiation (BEIR VII) report underlines "the need of studies of infants who are exposed to diagnostic radiation because catheters have been placed in their hearts". The aim was to determine the Lifetime Attributable Risk (LAR) of cancer associated with the cumulative medical radiation exposure, and to assess acute chromosomal DNA damage after cardiac catheterization procedures. METHODS: We calculated the cumulative effective dose in milliSievert (mSv) in 59 children (42 males, age=2.8±3.2 years) with congenital heart disease (CHD). LAR cancer was determined in accordance with BEIR VII estimates. In a subset of 18 patients, micronucleus (MN), biomarker of DNA damage and long-term risk predictor of cancer, was assayed before and 2 hours after procedures. Dose–area product (DAP; Gy cm2) was assessed as measure of patient dose. RESULTS: Median cumulative effective dose was 7.7 mSv per patient (range 4.6-41.2 mSv). For a 1-year-old child, the LAR cancer was 1 in 382 (25th-75th percentiles 1 in 531-1 in 187) and 1 in 156 (25th-75th percentiles 1 in 239-1 in 83) for male and female patients, respectively. The median of DAP values was 20 Gy cm2 (range of 1-277 Gy cm2). MN levels increased significantly when compared to baseline (pre= 7.4±5.4‰ vs post=10.5±5.3, p=0.02). CONCLUSION: CHD children are exposed to a significant cumulative dose. Both indirect cancer risk estimations and direct DNA data emphasize the need for strict radiation dose optimization in children.

RA052

MATERNAL TOXICANT EXPOSURE, GLUTATHIONE S-TRANSFERASE GENETIC POLYMORPHISMS AND RISK FOR CONGENITAL HEART DEFECTS

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Background. Congenital heart defects (CHD) result from a combination of genetic predisposition and environmental factors. Genetic variations in the Glutathione S-transferases (GST) detoxification enzymes (GSTM1 and GSTT1) may modify the teratogenicity of maternal toxicant exposure. The aim was to investigate whether the CHD risk is greater among offspring who lack the genetic capacity to produce GSTM1 and GSTT1 relevant to detoxification of toxicant agents. Methods. Using a case-only design, we genotyped 121 children (75 male; age=6.5±7.5 years) with CHD for null variants of the GSTT1 and GSTM1 genes. A detailed questionnaire was used to collect data from mothers during interviews at the hospital about their demographics, peri-conceptional and lifestyle exposures, including items concerning smoking habits, environmental and occupational exposure, and other potential harmful exposures (e.g. radiation diagnostic exposure). Genetic polymorphisms were analyzed by PCR/RFLP analysis. Results. Maternal smoking tended to increase CHD risk in children carrying GSTM1 null genotype (OR=1.3, 95% CI=0.6-2.9). Maternal exposure to environmental and occupational exposure among the offspring with absence of GSTM1 was associated with a 2.4-fold increased CHD risk (95% CI=1.1-5.2). No significant interactions were observed for absence of GSTT1. Conclusions. These results suggest significant joint effects of the GSTM1 deletion and maternal exposure on CHD risk.

RA053

ASSESSMENT OF DAMAGE INDUCED IN MOUSE LIVER BY ORAL EXPOSURE TO FURAN

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Furan is highly toxic and carcinogenic in rodents liver. The mechanisms behind furan hepatocarcinogenicity is not well understood, and both genotoxic and non-genotoxic processes have been hypothesized. In this study the effects induced in mouse liver by oral exposure to furan were investigated. The compound was given by daily gavage for 4 weeks to male B6C3F1 mice, at dose levels carcinogenic in long-term bioassays (2, 4, 8 or 15 mg/kg b.w.). Twenty-four hrs after the last administration animals were sacrificed, liver excised and the following parameters evaluated: induction of hystological alterations, apoptosis, cell proliferation, polyploidy, changes in overall DNA methylation, and genotoxicity by alkaline comet assay. Furthermore, as furan metabolites include multifunctional electrophilic intermediates, a modified comet protocol was also applied to specifically detect the presence of DNA cross-links by evaluating the decrease of gamma rays induced DNA migration. The results obtained indicate that, at the dose levels applied, repeated oral administration of furan was moderately toxic to liver, which showed mild hystological alterations with necrotic figures, apoptosis and regenerative cell proliferation at the highest doses. A preliminary evaluation of overall DNA methylation did not show treatment related changes. The flow cytometric analysis of DNA content revealed a low but statistically significant increase of the fraction of 8N nuclei at 15 mg/kg b.w. Comet assay did not highlight the presence of DNA strand breaks or cross-links after four weeks of oral exposure to furan. However, since comet assay could fail to detect furan induced short-lived lesions, which could be quickly repaired and not accumulate during the 4 weeks of exposure, a further experiment was carried out administering furan as single high dose (15, 100 or 250 mg/kg b.w.) three hours before sacrifice. The analysis of DNA damage by alkaline comet assay showed a significant increase of comet parameters in liver of animals treated with the highest dose, which was associated with evident cellular toxicity. In the same animals, a distinct decrease of gamma ray-induced DNA migration was observed when applying the modified comet protocol. These results suggests that DNA cross-links may play a major role in furan induced (geno)toxicity in mouse liver.

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RA054

GENOTOXIC EFFECT OF REPEATED ORAL ADMINISTRATION OF FURAN IN MOUSE SPLEEN

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Furan, a liver toxicant and carcinogen in laboratory mice and rats, occurs in a broad variety of heat-processed foods. The health risk posed by the presence of low doses of furan in food is not elucidated. To this

aim, further data on exposure and toxicity, particularly on the mechanism of tumor induction, have been considered necessary (EFSA, 2004). Furan oxidization by cytochrome P4502E1 leads to cis 2–butene-1,4-dial, a a,b-unsaturated dialdeyde. DNA-protein and DNA-DNA cross-links can be formed as consequence of the direct reaction of cis 2–butene-1,4-dial, or from secondary etheno adducts. Despite so, contradictory data on the genotoxicity of furan have been reported. In this study the genotoxic activity of furan in vivo has been investigated in B6C3F1 mice under the same exposure conditions applied in the NTP long term study, viz. daily gavage of furan dissolved in corn oil at 2, 4, 8, and 15 mg/kg b.w. After four weeks of treatment, DNA damage was evaluated in splenocytes, selected as a suitable cellular target for the detection of cumulative damage. The following biomarkers were investigated: a) micronuclei in cytokinesis-blocked cells stimulated with concavalin A; b) foci of phosphorylated histone γ-H2AX, in both quiescent and proliferating cells; c) DNA strand breaks, detected by the standard alkaline comet assay in quiescent cells, and d) cross-links, using a gamma radiation-modified comet protocol. The results obtained show a dose related, statistically significant increases of micronucleated splenocytes ($p<0.001$ Mann-Whitney U test) in furan treated mice. The immunohistochemical detection of g-H2AX highlighted a significant increase of foci in mitogen stimulated splenocytes of animals treated with 8 and 15 mg furan/kg b.w., while no increase over control was observed in freshly isolated, resting cells. In these cells no treatment related increase in DNA strand breaks was shown by the standard alkaline comet assay, nor a modification of gamma radiation-induced DNA migration was observed with the modified comet protocol. These results indicate that the in vivo exposure to furan may give rise to premutagenic DNA damage in resting splenocytes which is converted in frank lesions during the S-phase upon mitogen stimulation. The resulting DNA strand breaks are visualized by the increase in g-H2AX signals in interphase, and may originate micronuclei at the subsequent mitosis. Acknowledgements: European Commission 6thFP, project SSPE-CT-2006-44393

RA055

FURTHER EVALUATION OF A FLOW CYTOMETRIC IN VITRO MICRONUCLEUS ASSAY IN CHO-K1 CELLS: A RELIABLE PLATFORM THAT DETECTS MICRONUCLEUS AND DISCRIMINATES APOPTOTIC BODIES

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The in vitro micronucleus (MN) assay has been widely used as an alternative/replacement of the in vitro chromosome aberration (CA) assay as part of several recommended regulatory test batteries for genotoxicity testing. Traditionally, evaluation of MN events has been conducted using microscopy. In recent years, a flow cytometric method for enumeration of micronuclei in mammalian cell culture has been developed, which allows automated scoring thus greatly shortens analysis time and enhances assay throughput. However, there has been a concern that the MN results obtained from flow cytometry can be impacted by chromatin bodies produced during apoptosis or necrosis. In this work, we further evaluated this flow cytometry based in vitro MN assay with CHO-K1 cells in a 24-well platform. A total of 21 compounds were used in this evaluation, including direct- or indirect-clastogens, aneugens and non-genotoxic chemicals. More importantly, several apoptosis inducers or cytotoxins which have been previously reported to produce "artificial positives" in various in vitro genotoxicity tests were also evaluated in this system. The results showed that the MN ratio determined using the flow cytometric method was highly correlated with the microscopy results. A sensitivity of 83.3% and a specificity of 100% were obtained from the 21 compounds we tested. Moreover, the apoptosis-inducers such as staurosporine, dexamethasone did not produce "false positive" results in the flow cytometric system with CHO-K1 cells when cytotoxicity is above 50 ± 10%. Finally, significant increase of incidents in the hipodiploid region, an aneugenic signature, was confirmed in our evaluation. In conclusion, the flow cytometric in vitro MN assay is a reliable method that can be used to detect clastogenic or aneugenic potentials of the test substances in CHO-K1 cells.

RA056

WHAT MAKES AN IN VITRO POSITIVE GENETIC TOXICITY RESULT IRRELEVANT? A CASE STUDY WITH EUGENOL AND THE ALLYL BENZENES

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In a three test battery of short term genetic toxicity tests, many compounds are found positive only in the mammalian cell assays but not in the *in vivo* micronucleus or bacterial mutagenesis assays. Because many of these compounds are not rodent carcinogens, they are sometimes labeled "irrelevant" positives. Eugenol was recently classified as an "irrelevant positive" at a workshop sponsored by the European Centre for the Validation of Alternate Methods. Eugenol, methyleugenol, isoeugenol, safrole, and estragol are allyl benzenes, a class of compounds found in many plants used for food. All are positive or equivocal carcinogens in rodent bioassays, although eugenol and isoeugenol were negative in early, short-exposure assays. They are generally Ames negative and positive or equivocal in mammalian *in vitro* assays. The detection of unscheduled DNA synthesis or adducts in liver tends to correlate with carcinogenic potency. Allyl benzenes form mutagenic DNA adducts via a multistep metabolic activation starting with cytochrome P450 oxidation followed by conjugation by sulfate which in turn leaves resulting in a DNA-reactive carbonium intermediate. Thus, atypically, "phase II" metabolism is responsible for activation to the mutagenic intermediate. Elimination of these compounds *in vivo* is relatively rapid. Addition of sulfate or glucuronate to the free hydroxyl group on eugenol tends to accelerate elimination via non-mutagenic metabolites. Published risk assessments of allyl benzenes rely heavily on low carcinogenic potency, low levels of exposure and the quantitative and qualitative metabolism determined in humans and in rodents. Detection of genetic damage by eugenol *in vitro*, rather than being "irrelevant", is a useful signal of genetic hazard which can be addressed using the appropriate *in vivo* assays.

RA057

DNA REPAIR GENE XRCC1 AND hOGG1 POLYMORPHISMS AND RISK OF PROSTATE CANCER IN A COLOMBIAN POPULATION

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X-ray cross-complementing group1 (*XRCC1*) and human oxoguanine glycosylase 1 (*hOGG1*) genes are involved in DNA base excision repair (BER) pathway. Numerous SNPs in different DNA repair genes have been related with human cancer susceptibility. Polymorphisms in *XRCC1* and *hOGG1* genes have been associated with prostate cancer risk. To determine whether the *XRCC1* (codon *Arg194Trp*, *Arg*399*Gln*) or *hOGG1* (codon *Ser326Cys*) polymorphisms are associated with prostate cancer susceptibility, we genotyped these polymorphisms in 310 prostate cancer patients and 153 healthy donors. Subjects were genotyped with a PCR-RFLP assay. For statistical analysis a chi-square test was used to determine if the control population was in Hardy-Weinberg Equilibrium for the polymorphisms studied. Odds ratios were estimated by logistic regression model and adjusted for age and prostate specific antigen (PSA) level. *XRCC1* (codon Arg399Gln) and *hOGG1* (codon *Ser326Cys*) were associated with an increased risk of prostate cancer (OR ad= 1.52; 95 CI= 3.18-6.67 and OR ad= 1.98; 95 CI= 1.03-3.81 respectively). The variant at codon 194 was not associated with prostate cancer risk (OR ad= 0.3; 95 CI= 0.07-1.36). In summary, our results suggest that the *XRCC1* (codon *Arg399Gln*), and *hOGG1* (codon *Ser326Cys*) genotypes have a positive effect on

prostate cancer risk, presumably through a defective function of the *XRCC1* and *hOGG1* DNA repair genes.

RA058

GSTM1 AND GSTT1 POLYMORPHISMS AND THE RISK OF PROSTATE CANCER IN A COLOMBIAN POPULATION.

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Prostate cancer is the most common cancer among men in many countries. The etiology of prostate cancer is not clear, both genetic and environmental factors may be involved. Age, androgen metabolism, ethnicity, and genetic background have been reported as possible risk factors. Glutathione-S-transferases (GSTs) are detoxification enzymes of a wide variety of endogenous or exogenous carcinogens. The genetic polymorphisms of GSTM1 and GSTT1 genes have been studied to evaluate the prostate cancer risk. In the present study, we examined the association of the GSTM1 and GSTT1 gene polymorphisms with sporadic prostate cancer patients in a Colombian population. It was a case control study including 310 prostate cancer patients and 153 controls. The GSTM1 and GSTT1 genotypes were identified by multiplex PCR in peripheral blood DNA samples. Odds ratios were estimated by logistic regression model and adjusted for patient's age and Prostate Specific Antigen (PSA) level. Frequencies of null genotypes for GSTM1 and GSTT1 genes in prostate cancer patients were 39.7% (123/310) and 19% (59/310) respectively. The frequencies in control group for GSTM1 and GSTT1 null genotypes were 49% (75/153) and 25.5% (39/153) respectively. No association between null genotype and prostate cancer risk for GSTM1 (OR ad= 0.48; 95% CI = 0.25- 0.92), and for GSTT1 (OR ad= 0.68; 95% CI = 0.46- 1.01) was observed. The frequency of non-deleted GSTM1 genotype in the case group was 60.3% (187/310) and 51% (78/110) in control group. The non-deleted GSTM1 genotype was associated with an increased risk of prostate cancer (OR ad= 2.07; 95% CI = 1.08- 3.98). Genetic risk has been associated with the null genotype GSTM1, in several studies. In contrast our results suggest the importance of considering GSTM1 non deleted genotype as a biomarker for risk of prostate cancer in Colombia.

RA059

OCCUPATIONAL RISK ASSESSMENT OF BRAZIL PAINT INDUSTRY WORKERS

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Background and Aim: Thousands of chemical compounds are used in paint products as pigments, extenders, binders, additives and solvents. Workers in paint manufacture are potentially exposed to the chemicals that are found in paint products, although the patterns and levels of exposure to individual agents may differ from those of painters. Considering that, to our knowledge, there is no published cytogenetic data concern paint-industry workers in Brazil, the objective of this study was to evaluate the genotoxic risk of these workers using the Comet assay in peripheral blood leukocytes and oral mucosa cells and the MN test in oral mucosa cells. Methods and Results: The study involved 58 male paint-industry workers who were employed in the sectors where they were occupationally exposed to solutions containing organic mixtures. The control group consisted of 17 healthy males with no occupational exposure that worked at administrative section of the industry. Blood and urine samples were obtained from individuals in the two groups on the same day. As a biomarker of toluene exposure, 50mL of urine samples were collected in the end of the working day and analyzed for hippuric acid (HA). The alkaline Comet assay in blood lymphocytes and mucosa oral cells and the Micronucleus test in mucosa oral cells were employed for genotoxicity evaluations. The comparison of the mean values of urine HA level of the control and exposed group show a significant increase in HA levels observed in the exposed group relative to the controls ($P \leq 0.05$, Mann–Whitney *U*-test). For the micronucleus test in buccal exfoliated cells, no significant difference was detected between the control and paint industry workers. The Comet assay in epithelia buccal cells showed that the Damage Index (DI) and Damage Frequency (DF) observed in the exposed group were significantly higher relative to the control group ($P \leq 0.05$, Mann–Whitney *U*-test). In the same way, the Comet assay data in peripheral blood leukocytes showed that both analysis parameters of this assay (DI and DF) were significantly greater than that for the control group ($P \leq 0.05$, Mann–Whitney *U*-test). Conclusion: Chronic occupational exposure to paints may lead to a slightly increased risk of genetic damage among paint industry workers.

RA060

INTERNATIONAL VALIDATION STUDY OF THE IN VITRO ALKALINE COMET ASSAY

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The Comet assay is widely used for detecting initial DNA damage in individual cells. The *in vitro* alkaline Comet assay, especially, might serve as a simple and sensitive test for identifying the genotoxic hazard of chemicals. The performance of this assay, however, depends on the procedures used and published results for the same chemical greatly vary. To develop a standardized protocol for the *in vitro* alkaline Comet assay and to evaluate assay reliability and relevance, JaCVAM is supporting an international validation study. In this study, TK6 human lymphoblastoid cells are treated with a chemical for 4 h with or without metabolic activation (i.e., S9 mix). As cytotoxicity parameters, trypan blue dye exclusion (TBDE), non-detectable cell nuclei (NDCN; hedgehog), and cell growth after the treatment are measured. The recommended top concentration is one with 80% TBDE, 20% NDCN, or no cell growth. To date, 5 laboratories have examined 11 genotoxic or non-genotoxic chemicals using a standardized protocol. In the absence of S9, appropriate positive responses for ethylmethanesulfonate, 9-aminoacridine, camptothecin, and etoposide were obtained, while a significant increase in DNA migration was not detected for mitomycin C, a cross-linking agent. Also, the non-genotoxic chemicals (cycloheximide, triton-X, mannitol) were appropriately negative. However, the results from S9 studies with genotoxic chemicals requiring metabolic activation (2-aminoanthracene, cyclophosphamide, diethylnitrosamine) were inconsistent, indicating that additional optimization of the protocol is needed. The results from the completed phases and the overall design of the validation study are presented. According to the validation study, we would like to propose a standard protocol, criteria for data acceptance, and a statistical method to judge the results in the *in vitro* Comet assay for regulatory use.

RA061

CURRENT PROGRESS ON A NOVEL IN VIVO GENE MUTATION ASSAY USING THE PIG-A GENE AS AN ENDOGENOUS REPORTER

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The phosphatidylinositol glycan complementation group A gene (Pig-A), which is located on the X chromosome, codes for an enzyme involved in the synthesis of glycosylphosphatidylinositol (GPI) cell-surface anchors. Mutation in Pig-A could abolish GPI synthesis, which, in turn, would cause a deficiency in GPI anchored protein markers (e.g., CD59 or CD24) on the cell surface. We have used this reasoning to develop a rapid in vivo gene mutation assay. In our studies, SD rats or CD-1 mice were treated with genotoxic chemicals, and peripheral blood (PB) samples were collected from rats and mice or bone marrow (BM) samples from mice. The samples were stained with antibodies that react with a marker of white blood cells (e.g., CD45), an erythroid marker, or a GPI-anchored protein, and analyzed by flow cytometry (FCM). Pig-A mutant frequencies were calculated as the ratio of red blood cells (RBCs) negative for the GPI-linked marker (CD59 for rats, CD24 for mice) to total RBCs. In studies conducted with rats, we are evaluating the sensitivity of the assay to relatively weak in vivo mutagens, like ethylmethanesulfonate (EMS). To reduce the background Pig-A mutant frequency, we developed a new assay employing antibody clone HIS49, which binds to rat erythroid cells, Data from this HIS49/CD59 assay was compared to that of our conventional CD45/CD59 method (Miura et al., Environ Mol. Mutagen 49:614). We found that the HIS49/CD59 method produces lower background frequencies than the CD45/CD59 assay. In addition, the HIS49/CD59 method detected weak, but positive increases in CD59-negative RBCs in rats treated with EMS. In studies conducted with mice, we compared the time courses for CD24-negative RBC manifestation in PB and bone marrow from mice treated with N-ethyl-N-nitosourea (ENU). While the Pig-A mutant frequency (MF) in PB was increased at 2- and 4-weeks after ENU dosing, the MF in BM was increased starting at 1-week, with the elevated MF persisting for at least 4 weeks after dosing. These data suggest that Pig-A mutants are fixed, proliferate, and differentiate in the BM, and that erythrocytes derived from these BM Pig-A mutants may transit from the BM and accumulate in PB. Sequencing of the Pig-A gene from CD24-negative nucleated erythroids is ongoing.

RA062

IN VITRO EVALUATION OF PROPOXUR INDUCED OXIDATIVE STRESS, IMMUNOSUPPRESSION AND APOPTOSIS IN AVAIN LYMPHOCYTES

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Today chemical pesticides are playing vital role in controlling agricultural, industrial, home garden and public health pests globally. At the same time these pesticides are constantly contaminating environment and adversely affecting animal and human health. Several studies have reported immunosuppressive effects of carbamate group of pesticides in animals. But few such reports are available employing in vitro system of avian splenocytes. Propoxur (2-isopropoxyphenyl N-methylcarbamate) is a carbamate insecticide of moderate mammalian toxicity. It is used in homes, hospitals, factories and stables as potent insecticide. Present communication reports propoxur induced immunosuppression, oxidative stress and apoptosis in avian lymphocyte cell culture system employing cytokine assay, annexin V assay, oxidative stress assay, DNA fragmentation assay and electron microscopy. Cytokine assay revealed significant reduction in IL1 and IL2 levels. There was significant increase in oxidative stress in propoxur treated cells as compared to controls cells as revealed by nitric oxide estimation. Annexin V assay showed increased number of cells under going apoptosis which was further confirmed by DNA agarose gel electrophoresis. Both TEM and SEM showed prominent ultrastructural changes in the cells exposed to propoxur which showed distinct features of apoptosis, further strengthening the evidence of propoxur induced immunotoxicity in avian lymphocytes. Thus, from our findings it is revealed that low level dose of propoxur induces significant immunosuppressive effects, higher oxidative stress and apoptosis in avian lymphocytes. Further, it is suggested that in vitro lymphocytes cell culture system can prove helpful in preliminary screening of low level pesticide exposure and reduce experimental animal testing. Key Words: propoxur; immunosupression; oxidative stress; apoptosis; cytokines; avian lymphocytes; electron microscopy.

RA063

INHIBITORY EFFECT OF DIMETHYL SULFOXIDE ON THE MUTAGENICITY OF PROMUTAGENS IN THE AMES TEST

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The preincubation version of the Ames test (preincubation test) is often used to detect the mutagenicity of chemicals. Although dimethyl sulfoxide (DMSO) is believed to be a superior organic solvent for the Ames test due to its relatively low cytotoxicity and high ability to dissolve many test articles, it has been reported to inhibit CYP enzymes, which are involved in metabolic activation or detoxification of chemicals. In this study, we examined the effect of DMSO concentration on the mutagenicity of 14 promutagens that require metabolic activation in the preincubation test. Fourteen promutagens that require CYP enzymes or nitroreductase for metabolic activation were examined for mutagenicity in the TA100 or WP2uvrA(pKM101) strains at 1% and 14% DMSO (corresponding to the concentration often used; the presence of 0.1 mL DMSO in 0.7 mL of reaction mixture) in the presence (for 12 promutagens that require CYP enzymes, benzo[a]pyrene, 3-methylchoranthrene, quinoline, 2-aminoanthracene, 2-aminofluorene, 2-acetylaminofluorene, 4-acetylaminofluorene, N,N'-dimethyl-4-aminoazobenzene, TrpP-2, N-nitrosodimethylamine, N-nitrosopyrollidine and cyclophosphamide) or absence (for 2 promutagens that require nitroreductase, 4-nitroquinoline-N-oxide and AF-2) of S9 mix in the preincubation test. Many of the promutagens (10 chemicals) showed more potent mutagenicity (2- to 5-fold increases with 9 chemicals, 10-fold increase with N-nitrosodimethylamine) in 1 % DMSO compared to 14% DMSO. Four chemicals had equal mutagenicity in 1% DMSO compared to that found in 14% DMSO. The dose showing the highest revertant colonies per plate and the appearance of bacterial background lawn, the two markers of lethality, demonstrated an equal or slightly stronger effect at 1% DMSO compared to that found at 14% DMSO. The reduction in the yield of revertant colonies at the 14% DMSO concentration was confirmed not to be derived from higher cytotoxicity at the 14% compared to the 1% DMSO concentrations in other experiments. These findings suggest that DMSO has an inhibitory effect on the drug metabolizing enzymes and/or alters the balance between metabolic activation and detoxification (preference for detoxification over activation).

RA064

EVALUATION OF GENOTOXIC EFFECTS OF OCCUPATIONAL EXPOSURE TO LEAD BY MEANS OF THE COMET ASSAY

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Lead and lead compounds are still present in many industrial procedures. Although many studies have pointed out the interaction of lead with biological processes that result in DNA damage, literature data with regard to its genotoxic properties are contradictory and biochemical and molecular mechanisms still remain poorly understood. The aim of this study was to evaluate the genotoxic effects of occupational exposure to lead in 98 males from two different factories, in relation to 35 male controls. Lead content in whole blood was estimated using atomic absorption spectroscopy. Comet assay was used in order to evaluate the DNA strand breaks, whereas a modified protocol which introduces an incubation step with hOGG1 enzyme was applied to detect the oxidative DNA damage. Exposed individuals showed significantly higher blood lead levels (BLL) than the controls whereas no

significant differences were detected in the DNA and oxidative damage between these two groups. Individuals from factory 2 had significantly lower BLL but higher oxidative damage than those from factory 2, suggesting that chemicals other than lead are related to the higher oxidative damage observed. A positive correlation was obtained between age and BLL in control and exposed individuals, and also increase in the time of exposure was related to higher BLL. Data analysis according to tobacco consumption showed significantly lower DNA damage in the group of exposed smokers with regard to exposed non-smokers and control smokers, pointing to a lower susceptibility of that group as a consequence of the strengthening of repair mechanisms by the chronic contact with tobacco compounds and the occupational environment. The results obtained showed that, although occupational exposure is related to higher BLL, no increase in the genotoxic parameters analysed could be observed in the exposed individuals. Further research including other genotoxic parameters and a larger control group is being undertaken. Research partly supported by Xunta de Galicia (INCITE08PXIB106155PR) and Portuguese Fundação da Ciência e Tecnologia (PDCT/SAU-OBS/59821/2004).

RA065

A STEP FORWARD ON THE ROAD TO PREVENTING CERVICAL CARCINOMA: A NUTRIGENETIC APPROACH

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BACKGROUND/AIMS Population studies have described the frequency of common *MTHFR* SNPs in many populations and conditions. Controversy exists as to their role in cervical neoplasia (CIN). Aim of our research was to investigate the role of *MTHFR* SNPs on CIN risk and the nutritional status of women of childbearing age, particularly folate intake, in relation to SNPs, adopting a nutrigenetic approach. METHODS Two cross sectional surveys were designed: in the cancer survey 232 cervical swabs were collected from women referred to a Service of Colposcopy; and in the nutritional survey 204 blood samples were collected from healthy women of childbearing age in Catania, Italy. Folate intake was estimated by a validated semiquantitative food frequency questionnaire. Determination of C677T and A1298C polymorphisms was performed using the TaqMan allelic discrimination Assay. RESULTS A decreased risk of CIN for individuals homozygous for the T-allele was shown (OR 0.221; CI95%: 0.055 – 0.880). After multiple logistic analysis, the presence of the T-allele was the best explaining protective factor against cervical carcinogenesis. In the nutritional survey, the mean folate intakes were 212.0 µg/d, 222.5 µg/d and 197.9 µg/d, respectively for the overall sample, for pregnant and non-pregnant women. Inadequate folate intake was shown in 94.6%, 97.4% and 90.8%, respectively of the overall sample, of pregnant and non-pregnant women. A higher TT genotype frequency was observed in 24-28 years group of women when compared with older groups. The allelic distribution followed the Hardy-Weinberg equilibrium expectations. CONCLUSIONS Data generated by observational and metabolic studies suggest that women with the 677TT genotype have higher folate requirements than the others. It has been reported a diminished DNA methylation in TT women but only when folate status is low. Thus, given the high prevalence of the 677TT genotype, women in Catania may have higher folate requirements and increased susceptibility to cervical cancer as well as obstetric complications under conditions of folate inadequacy compared with other population groups. Cancer prevention strategies should take into account such epidemiologic *scenario* in order to identify priorities and address nutritional interventions.

RA066

AN ALTERNATIVE LINEAR TREND ANALYSIS IN ASSESSING BIOLOGICAL SIGNIFICANCE OF THE MOUSE LYMPHOMA ASSAY RESULTS

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According to the recommendations of the Mouse Lymphoma Assay (MLA) Workgroup of the International Workshop on Genotoxicity Testing (2005), a trend test should be performed if an induced mutant frequency (IMF) of at least 126 x 10^{-6} (the global evaluation factor, GEF, based on historical control data from a number of laboratories, corresponding to a 2-3 fold increase in MF) is achieved at one or more test concentrations. Only those responses positive for both the GEF and the trend analysis are biologically relevant. In the UKEMS recommended trend test (1989, applied in Mutant V2.40), MF averaged (pooled) from replicate cultures following a consistency test are used in a variance-weighted least-squares linear regression (with χ^2 model). It returns significant results in virtually all cases positive for the GEF, including those with no apparent dose-response. We have proposed an alternative method where log-transformed MF and its variance are estimated for each culture separately and all individual replicate ln(MF) are used in an ordinary weighted least-squares linear regression (F model). A log transformation of MF is preferred as the variance is estimated for ln(MF), not for MF, and it tends to normalize the distribution of MF. The heterogeneity between replicates is taken into account in the linear regression as each dose now has more than one outcome value. Ordinary weighted linear regression is chosen due to the sensitivity of t-test to the number of data points. Using different reference cases, all positive for the GEF, our method is shown to be sensitive to changes in the number of replicates, the shape and magnitude of mutant induction, in contrast to Mutant V2.40. Cases with no apparent dose-response and thereby questionable biological significance are tested positive by Mutant V2.40, but negative by our method. It is concluded that the ordinary weighted linear regression of log-transformed replicate MFs provides a meaningful complement to the GEF in assessing the biological significance of the MLA results. The method has been implemented in an in-house developed program (MoLy) with great success.

RA067

USE OF GENETIC TOXICITY TEST BATTERY IN HAZARD IDENTIFICATION FOR POTENTIAL CARCINOGENICITY OF VETERINARY DRUGS AND FEED INGREDIENTS.

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The DES (diethylstilbestrol) proviso of the Delaney Clause of the FFD&C prohibits the use in food-producing animals of any compound that induces cancer in animals or humans unless it can be determined by an FDA approved analytical method that no residue of that compound will be found in edible tissues of these animals (21 CFR 500.80). Therefore, carcinogenicty assessment is one of the key areas in the human food safety assessment of veterinary drug residues. The need for chronic cancer bioassay to address carcinogenicity of veterinary drugs or feed ingredients is determined by threshold assessment, in which the results of three standard genotoxicity tests (VICH Guideline 23) are used for predicting carcinogenic potential. Authors are presenting a draft decision tree for using genotoxicity testing in cancinogenicity risk assessment of veterinary drugs or feed ingredients. In general, chronic bioassays are recommended if a feed ingredient or a veterinary drug induces positive response in any one of the three genetic toxicology test battery or in any combination of the test battery. In situations where an equivocal result is obtained, authors propose that a second in vivo test be conducted in a different species and tissue, to determine the genotoxicity of that veterinary drug or feed additive. Authors also propose that a positive response in an in vitro mammalian cell assay be evaluated, on a case by case basis, to determine whether a second in vivo test needs to be conducted before recommending a cancer bioassay. The second in vivo test, if recommended, may be conducted along with the VICH-recommended one year chronic toxicity study. CVM regulates both genotoxic and non-genotoxic carcinogens using sensi-

tivity-of-method (SOM) procedures. For a genotoxic carcinogen, a default 1-in-1-million linear low dose extrapolation approach is used to determine acceptable tissue residues in food-producing animals. For a non-genotoxic carcinogen, alternative approaches or the default approach may be used in deriving the acceptable tissue residues.
Disclaimer: The opinions and information in this poster are those of the authors, and do not represent the views and/or policies of the U.S. Food and Drug Administration.

RA068
MICRONUCLEUS INDUCTION IN THE BONE-MARROW OF RATS AFTER ACUTE AND 28 DAY DOSING WITH CYCLOPHOPHAMIDE, HEXAMETHYLPHOSPHORAMIDE AND GEMIFLOXACIN

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The Step 2 revision of the ICH S2 guidelines for genotoxicity testing allows integration of the in vivo micronucleus test into toxicology studies. Whilst investigating the practicability of integrated studies for routine use at AstraZeneca, the humerus was found to be a suitable source of bone-marrow that did not interfere with the requirements for the histopathological and clinical pathological examinations also required in these studies. Therefore, to establish historical control ranges, micronucleus frequencies were measured in the humeri from control animals from 9 28-day studies performed from 2008 to 2009. The mean and reference limits were found to be directly comparable with those for the acute studies performed in this laboratory with the same strain of rat. In addition, to compare the sensitivity of the integrated method, 3 studies were performed using positive control compounds, cyclophosphamide (CPA), hexamethylphosphoramide (HMPA) and gemifloxacin (GF), in which groups 7 Han Wistar rats orally administered for 1 or 2 days and 28 days. CPA was given at 0.5, 1.67, 5, 10 & 20 mg/kg as a single dose and 0.5, 1.67 and 5 mg/kg/day for 28 days. Statistically significant results were seen at 5, 10 and 20 mg/kg following a single dose and at 0.5, 1.67 and 5 mg/kg/day following 28 days of dosing. HMPA was given at 120 and 240 mg/kg/day for 14 days and at doses and doses of 50, 100 and 200 mg/kg/day for 28 days. Statistically significant results were seen at 240 mg/kg following 14 days exposure and 100 mg/kg/day following 28 days dosing. GF was given at doses of 160, 600, and 1200 mg/kg/day for 2 days and doses of 160, 300 and 600 mg/kg/day for 28 days. Statistically significant results were seen at 160 and 600 mg/kg/day following two doses but were not seen following 28 days of dosing.

RA069
CHROMOSOME ABERRATIONS IN PERIPHERAL LYMPHOCYTES FROM RATS DOSED FOR 28 DAYS WITH HEXAMETHYLPHOSPHORAMIDE (HMPA)

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The FDA Guidance on the Recommended Approaches to Integration of Genetic Toxicology Study Results (FDA 2006) identifies metaphase analysis of peripheral blood lymphocytes from repeat-dose studies in rats as an additional in vivo assay that can be useful in clarifying in vitro positive results. The premise of the assay is that peripheral lymphocytes are exposed to blood levels of drug and metabolites and, as they are not dividing and have limited repair capacity, any genetic damage incurred may be accumulated and expressed in the first metaphase when they are stimulated to divide ex vivo. In this study, hexamethylphosphoramide (HMPA) was used to investigate sensitivity of the assay with increased duration of treatment. HMPA was orally administered to groups of 7 male Han Wistar rats at doses of 50, 100 and 200 mg/kg/day for 28 days. Blood samples were taken from the tail vein pre-dose and on days 2, 15 and 29 of treatment. Duplicate cultures were initiated for each animal in RPMI 1640 and stimulated with concanavalin A and PHA and incubated for 72 hours. Metaphase slides were prepared and 700 cells per dose group were analysed for chromosome aberrations. At doses of 50 and 100 mg/kg/day of HMPA, no increases in chromosome aberrations were seen after 2 days of dosing. However, statistically significant increases were observed in the samples following 15 and 28 days of dosing. At a dose of 200 mg/kg/day of HMPA, statistically significant increases in chromosome aberrations were observed after 2 days of dosing. This dose was reduced to 150 mg/kg/day on day 16, due to toxicity, and the group taken off dose completely on day 19 and maintained until day 29. Blood samples taken from this group on day 29 showed statistically significant increases in chromosome aberrations. In conclusion, statistically significant increases in chromosome aberrations were observed in ex vivo cultured rat lymphocytes following doses of 50 and 100 mg/kg/day of HMPA after 15 and 28 days, showing that damage had accumulated over this time, as it was not seen at day 2. Animals previously given 200 mg/kg/day for up to 15 days expressed significant levels of chromosome aberrations after 29 days, demonstrating that damage incurred during exposure to HMPA had persisted.

RA070
IDENTIFICATION OF MIRNA WITH TOXICOLOGICAL POTENTIAL AFTER BENZO[a]PYRENE EXPOSITION

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Benzo[a]pyrene is one of the Polycyclic Aromatic Hydrocarbons (PAH) compounds. PAHs are chemicals with carcinogenic and mutagenic potential and are mainly derived from incomplete combustion processes from industries, incineration, heating and vehicular emissions. Importantly, PAHs are associated with increased incidence of several human cancers. Toxicological studies designed to assess safety and possible toxicity of compounds in human populations frequently rely on the use of *in vitro* systems. The application of *in vitro* models and microarrays technology to toxicology has spawned the discipline of toxicogenomics. Although much useful data from studies of gene expression profiling in response to toxins and toxicants has been performed, the understanding of these responses is limited. Profiling microRNA in response to toxic compounds may increase our understanding of toxicant-induced effects at the cellular level. MicroRNAs are short non-coding RNAs, approximately 22 nucleotides in length MicroRNAs which have emerged as powerful negative regulators of mRNA levels could be responsible for mRNA downregulation or upregulation of important genes implicated in PAHs toxicity. The aim of this study is to obtain microRNA profiles as a signature of PAH toxicity in humans. In order to reveal which part of the transcriptomic changes may be due to miRNA regulation, we study in HepG2 cells (a liver cell line competent in biotransformation of BaP) the effects of BaP on miRNA profiles. Eventually, the microRNA-mRNA signatures following BaP exposure may contribute to enhanced risk assessments and chemical safety predictions in humans for PAHs.

RA071
MOLECULAR GENETIC ANALYSIS OF C677T AND A1298C POLYMORPHISMS OF THE GENE OF METHYL TETRA HYDRO FOLATE REDUCTASE AS A RISK FACTOR FOR DEVELOPMENT OF CHRONIC MALNUTRITION IN CHILDREN UNDER 3 YEARS OF THE MUNICIPALITY OF LURIBAY, EXPOSED TO PESTICIDES.

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Objectives. To determine whether the C677T and A1298T polymorphisms of the gene of Tetra Methyl Hidrofolato Reductase (MTHFR) are risk factors of chronic malnutrition in children under 3 years exposed to agrochemicals in the county of Luribay in La Paz, Bolivia. Methods. We present a case-control study, our universe is 154 children. Cases were defined as children under 3 years with chronic malnutrition and exposure to agrochemicals (n = 41), controls were children without malnutrition, with the same inclusion criteria (n = 113). We have also considered mothers of these children leaving in this region in at least the last 5 years. The bioethics and ethics issues were considered. We have identified the following genotypes of MTHFR: the polymorphism 677 (CC, CT and TT), and 1298 (AA, AC and CC). Results. Among the preliminary findings in the study population, was found a frequency of polymorphisms: 677C 37.01%, 48.70% CT 677, 677T, and 14.29% (p = 0.542), no significant differences were found in the association between MTHFR polymorphism and chronic malnutrition. Conclusion. Our preliminary data are not significant in the analysis of MTHFR polymorphism as a genetic risk factor for chronic malnutrition; these must be correlated with other tests (MN in oral mucosa and polymorphisms of CYP2E1). The results will identify the etiology of non-response to nutritional intervention of the Program for Growth and Development of Save the Children. Based on nutrigenomics principles to intervene on an individual treatment (based on nutritional requirements, nutritional status and genotype) to prevent, mitigate or treatment of chronic diseases.

RA072

HOMOEOPATHY – ARISTOLOCHIA ACID CONTAINING PLANT EXTRACTS ARE A GENOTOXIC RISK?

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For therapy of arthritis, gout, rheumatism and festering wounds Aristolochia plant extracts have been used in medicine because of their anti-inflammatory properties. Among other constituents, the active components are aristolochic acids (AAI + AAII). In the 1980s all pharmaceutical preparations containing AAs were withdrawn from the market in Germany due to the genotoxicity of AAs. However, homoeopathic preparations of dilution D11 or above using Aristolochia plant extracts are still available. Currently it is discussed to prohibit all homoeopathic preparations containing aristolochic acids. Hence there is a high interest to determine a threshold value. For this purpose the effect of aristolochic acid I (AAI) on micronuclei frequency, a parameter of chromosome aberrations, was analysed using the cytokinesis-block micronucleus assay (CBMN assay). Furthermore, the alkaline unwinding technique was applied to determine the induction of DNA strand breaks. The two methods were applied to the human hepatoma cell line HepG2 with and without addition of an exogenous metabolising system (S9). According to the OECD Guideline 487 in vitro micronucleus test (draft proposal) cytotoxicity was determined using the replication index. In the CBMN assay AAI did not cause an increase in micronuclei frequency at non-cytotoxic concentrations (up to 10 µg/ml) after extended exposure (24h). At cytotoxic concentrations (20 µg/ml) after 24 h incubation an increase of micronuclei frequency was observed. After short exposure (6 h) at cytotoxic concentrations (from 30 µg/ml) AAI showed a rising frequency of micronuclei also in the presence of S9. By means of alkaline unwinding the induction of DNA strand breaks at non-cytotoxic concentrations were not detectable. The first results did not indicate DNA damage at concentrations relevant for homoeopathic preparations of dilution D11 or above. However further investigations are required.

RA073

ASSOCIATION BETWEEN GENETIC POLYMORPHISMS IN THE DNA REPAIR GENES XRCC1 AND XRCC3 AND BREAST CANCER SUSCEPTIBILITY.

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Background: Breast cancer is the most common malignancy affecting women worldwide. Population-based studies have reported significant associations between specific genetic polymorphisms and breast cancer susceptibility. A number of studies have demonstrated that common variants of genes involved in the DNA repair pathways act as low penetrance breast cancer susceptibility alleles. We aimed to investigate the association of single nucleotide polymorphisms (SNPs) in the DNA repair genes *XRCC1* and *XRCC3* and breast cancer risk. *Methods:* The association was analyzed in Brazilian women in a case-control study of 107 women with breast cancer diagnosed between 25 and 70 years and 175 age-matched healthy controls. DNA was obtained from blood samples and was genotyped by PCR-RFLP. *Results:* Heterozygous *XRCC3* 241Met carriers had an increased risk of breast cancer (odds ratio 2.23; 95% CI 1.32-3.76; P = 0.002). The association of the genotypes of 241Met allele (Thr/Met + Met/Met) was associated to increased risk of breast cancer (odds ratio 2.11; 95% CI 1.29-3.45; P = 0.003). No significant associations were observed between the *XRCC1* polymorphism Arg399Gln and breast cancer. *Conclusions:* This study suggests that *XRCC3* 241Met allele may influence breast cancer susceptibility, while *XRCC1* polymorphism is not associated with breast cancer risk.

RA074

ANALYSIS AND IDENTIFICATION OF METHYL VINYL KETONE HEMOGLOBIN ADDUCTS

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Hemoglobin (Hb) adducts are useful as biomarkers of exposure to electrophilic compounds/metabolites and could also be used to measure the “internal dose” of such potentially genotoxic compounds (1). The new adduct FI*R*E*-procedure has high potential to be used as tool in search for previously unidentified “unknown” adducts to N-terminal valine in Hb. The principles for preparation of samples and analysis with LC/MS is less discriminating with regard to the chemical properties of the adduct and give a broad application range (2). The fragmentation pattern of different reference analytes, the fluorescein thiohydantoins (FTHs), both as unsubstituted and deuterium substituted, have been interpreted and compared. An unknown peak was identified from the fragmentation pattern to correspond to the Hb-adduct formed from methyl vinyl ketone (MVK, CH_2=CH-$COCH_3$). The MVK-adduct was confirmed by incubation of blood with MVK, and the structure of the FTH-MVK-Val was further verified by synthesized FTH-MVK-Val, both unsubstituted and deuterium substituted. Maternal and cord blood samples have then been analysed for the MVK Hb-adduct. This study is done within the EU-project “NewGeneris” (3). The results indicate similar background adduct levels as for acrylamide and also a similar Fetal/Maternal adduct ratio (~0.4) as for acrylamide (from food intake). The source for the MVK Hb-adducts is not yet known. The MVK Hb-adduct is present in human blood samples but not present in blood from cow or sheep. Our preliminary data indicate that it has higher electrophilic reactivity than acrylamide. The results show that it is possible to use this method for screening of previously unknown adducts from electrophilic compounds in human blood samples.

*The abbreviation "FIRE" derives from "the FITC reagent for measurements of adducts (R) by a modified Edman procedure".

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(2) Rydberg, P. PCT Int. Appl. (2005) WO/2005101020
(3) http://www.newgeneris.org

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RA075

ASSESSMENT OF THE PERFORMANCE OF THE GADD45A-GFP (GREENSCREEN HC) GENOTOXICITY ASSAY USING ECVAM RECOMMENDED GENOTOXIC AND NON-GENOTOXIC CHEMICALS

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A recent ECVAM workshop considered how to reduce falsely predictive positive results when undertaking in vitro genotoxicity testing, thus avoiding unnecessary follow-up animal tests. It was anticipated that new assays might contribute to a solution so a panel of experts were asked to identify a list of chemicals which could be used in their evaluation. Three categories of test chemicals were proposed. Here we present test results from the GreenScreen HC assay. All compounds were tested to a maximum of 10 mM where possible. Each was tested in triplicate, on separate days, with and without S9 (1%). Data were collected using either a microplate spectrophotometer or flow cytometer. The results can be summarised as follows: Group 1 (chemicals which should be detected as positive in in vitro mammalian cell genotoxicity tests): 18/20 (90%) were reproducibly positive in GreenScreen HC. Group 2 (chemicals which should give negative results in in vitro genotoxicity tests): 22/23 (96%) were reproducibly negative in GreenScreen HC. The expert panel noted that the similar numbers of carcinogens/genotoxins and non-carcinogens/non-genotoxins in Groups 1 & 2 provide a good balance for concordance calculations. Overall concordance from groups 1 and 2 for this assay was 40/43 (93%). Group 3 (chemicals which should give negative results in in vitro mammalian genotoxicity tests but have been reported to induce chromosomal aberrations (or tk mutations in mouse lymphoma cells), often at high concentrations or high levels of cytotoxicity): 13/18 (72%) were reproducibly negative in GreenScreen HC. The exceptional compounds from each group will be discussed, including follow-up experiments with 3 compounds from Group 3 that are all antioxidants which may be acting as pro-oxidants in the hyperoxic conditions of cell culture. Overall the predictive figures from these lists are similar to those generated in other studies with the GreenScreen HC assay. It has sensitivity comparable with the current in vitro mammalian assays and very much higher specificity. Its routine use would reduce falsely predictive positive results and avoid unnecessary subsequent animal tests.

RA076

HOW DOES INCREASING CYTOTOXICITY AFFECT THE ACCURACY OF THE GADD45A-GFP (GREENSCREEN HC) GENOTOXICITY ASSAY: A COMPARISON OF DIFFERENT METHODS TO ESTIMATE CYTOTOXICITY.

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Recent evaluations of the in vitro mammalian genotoxicity assays have suggested that increased levels of cytotoxicity may increase the prevalence of uniquely positive genotoxicity results. Retrospective studies have shown these assays to be misleading in their prediction of in vivo genotoxicity and carcinogenicity results at high levels of cytotoxicity. These findings have focused attention on the methods used for toxicity assessment in the in vitro mammalian genotoxicity assays. The GreenScreen HC genotoxicity assay has sensitivity comparable to other in vitro mammalian tests but higher specificity. In the assay validation, relative cell density (RCD), was used as a measure of toxicity and a limit for data inclusion was set at 30% RCD. The experiments presented here have assessed other measures of cytotoxicity in cells exposed to toxins which reduced RCD to 30% or below. The aim of this work was to investigate whether the use of RCD in the GADD45a-GFP assay contributes to the assays high levels of specificity. Initially, cytotoxicity data were collected using intracellular ATP levels, dye exclusion, fluorescent DNA staining and RCD. 16 chemicals were tested at 9 different concentrations up to the current ICH test limits for in vitro genotoxicity assays. This set of chemicals included compounds which have produced misleading positive predictions in other assays. Further work, with a subset of the compounds, focussed on the comparison of RCD with methods used to estimate cytotoxicity in the regulatory in vitro genotoxicity assays. RCD was shown to underestimate cytotoxicity compared to other methods: 30% RCD for some compounds corresponded to less than 10% viability when measured by dye exclusion or intracellular ATP levels. These compounds still generated negative results for genotoxicity in the GreenScreen HC assay even when tested to these highly cytotoxic doses. As no increase in GADD45a-GFP induction was observed with highly cytotoxic non-carcinogenic compounds, this suggests that cytotoxicity alone does not induce the GADD45a-GFP reporter. From these data it would appear that the GreenScreen HC assay is not as susceptible to erroneous genotoxicity results seen in other in vitro mammalian genotoxicity assays at highly cytotoxic doses.

RA077

LUC HERE: A BRIGHTER ENDPOINT FOR HIGH THROUGHPUT GENOTOXICITY SCREENING

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The GADD45a-GFP GreenScreen HC genotoxicity assay provides high sensitivity and high specificity together with the ability to screen several compounds in a microplate format. The stable signal from GFP provides a lasting signature of GADD45a expression which is induced by all classes of genotoxin. Here we describe a new generation of high throughput genotoxicity assessment. BlueScreen HC (GADD45a-GLuc) is a new genotoxicity screening assay coupling the established GADD45a reporter with a luciferase gene from the marine copepod Gaussia princeps (GLuc from Nanolight Technology). GLuc shares the high stability of GFP and allows data collection at a single time point. Initial validation data suggest that the performance of BlueScreen HC is similar to GreenScreen HC. To date two validation exercises have been carried out, one with S9 metabolic activation (46 compounds) and one without S9 (53 compounds). All assessments were performed in triplicate and the assay demonstrates excellent reproducibility. Example data and the derivation of thresholds to define a positive result will be presented. The higher signal-to-noise ratio from GLuc makes it more effective in assessing optically interfering coloured and fluorescent compounds. This expands the scope of chemicals which can be tested with a GADD45a assay and further data will be shown to illustrate this. One major advantage of the new BlueScreen HC assay is the potential compatibility with much higher throughput than previously achieved with GreenScreen HC. This will appeal to screening facilities where the use of 384-well microplates is more commonplace. Indeed preliminary data suggest that, combined with a fluorescent assessment of cell number, it is a relatively simple procedure to miniaturise the assay on to 384-well microplates. Industrial collaborators are currently undertaking proof-of-concept experiments with a view to implement the assay within their laboratories. The arrival of BlueScreen HC signifies an exciting new era in genotoxicity screening. Multiple industrial sectors previously unable to utilise GreenScreen HC will now be able to undertake early stage genotoxicity assessment, allowing key decisions to be made at an earlier point during compound discovery.

RA078

IN VIVO DOSES OF ACRYLAMIDE AND GLYCIDAMIDE IN HUMANS AFTER DEFINED EXPOSURES OF ACRYLAMIDE VIA FOOD

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The discovery that acrylamide (AA) is formed at high temperatures in common food stuffs initiated a discussion on possible health risks associated with AA exposure via diet. For the purpose of health risk assessment, quantitative intake assessments are necessary. AA is metabolised via the cytochrome P450 2EI (CYP 2E1) to the epoxide glycidamide (GA), known to be genotoxic and to form DNA-adducts and hence considered as the genotoxic agent in AA exposure. It is therefore important to investigate the relation between AA exposure via food in humans and the corresponding *in vivo* dose of GA. This present study concerns controlled intake of AA at low doses through 1) total diet and 2) potato chips added to a baseline diet. *In vivo* doses of AA and GA along with other toxicokinetic parameters are calculated based on measured haemoglobin (Hb) -adducts of AA and GA. Group 1) in the study was on average exposed to approximately 11 µg AA/kg bw and day, through carbohydrate rich foods prepared at high temperatures. After exposure during 4 days, AA-adduct levels increased by 57 (range 39 to 83) pmol/g globin and GA-adduct levels by 24 (-8 to 66) pmol/g globin. Group 2) was exposed to AA through potato chips added to the participants' baseline diets during 28 days, giving an "extra" amount of 2.4 µg AA/kg bw and day. The AA-adduct levels in this group increased by 40 (0 to 85) pmol/g globin and GA-adduct levels by 24 (1 to 51) pmol/g globin. The *in vivo* doses for AA and GA were calculated from the adduct levels, by using the reaction rate constants for AA and GA to the *N*-terminal valine in hemoglobin determined *in vitro.* The in vivo doses could then be used for extrapolation from studies of effects in animal experiments etc.

RA079

THE GADD45A-GFP (GREENSCREEN HC) ASSAY AS A VALIDATED ALTERNATIVE METHOD: A CASE STUDY

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Organisations such as ECVAM, ICCVAM and JaCVAM provide a formal system of internationally agreed principles for the assessment of the validity of test methods. Method validity is defined by its relevance and reliability for a specific purpose. Validation and acceptance of test methods that impact on the 3Rs is high on the political agenda and the validating bodies have a critical role to play in expediting the roll-out of such methods. The predictive value of results from the ICH's regulatory battery of in vitro genotoxicity tests has been widely discussed. Evidence published by several authors illustrates the high sensitivity but low specificity of some assays in the battery. In particular, positive genotoxicity findings from the in vitro mammalian tests can provide misleading predictions of in vivo genotoxicity and hence trigger follow-up investigations in vivo. Greater accuracy in the in vitro assays would help to reduce unnecessary usage of lab animals. Workshops set up by ECVAM, ILSI HESI and others, have reached common conclusions and proposed 2 main strategies as solutions: (1) amend/improve the current testing strategy and guidelines, (2) investigate new methods with suitable attributes. The GreenScreen HC in vitro mammalian genotoxicity assay meets all of the proposed requirements of a new test method. Published data for the assay reveal it to exhibit both high sensitivity and high specificity, leading to a straightforward prediction model: GreenScreen HC positive compounds have a high likelihood of being in vivo genotoxins and/or genotoxic carcinogens, whilst GreenScreen HC negative compounds are likely to be non-carcinogens and non-genotoxic in vivo. Important facets of the ECVAM modular approach to validation have been performed independently of ECVAM. This presentation will provide a summary of the studies performed to assess GreenScreen HC assay reliability: method transfer studies, as well as within- and between- laboratory reproducibility studies. Data from GreenScreen HC are currently used by more than 60 companies worldwide, either within their own labs or through contract testing. The body of evidence thrusts GreenScreen HC forwards as a strong candidate as an alternative method.

RA080

CHILDREN EXPOSURE TO HEAVY METALS CONTAMINATION AT ELECTRONIC WASTE RECYCLING SITE RELATED TO TEMPERAMENT ASSESSMENT

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Background Guiyu, a town in south China, is one of the biggest electronic waste (e-waste) centers of the world. E-waste is disassembled and recycled by the local with archaic and uncontrolled methods that produce extensive environmental pollutants. To assess whether such recycling activities in Guiyu have been associated with alterations in child behavior, we investigated the blood lead levels (BLLs) and blood cadmium levels (BCLs) of children and evaluate the dose-response effect of heavy metal exposure related to their temperament alterations. Methods We analyzed two cohorts of children that were followed from 3 to 7 years of age in Guiyu (n=151) and Chendian (n=150). BLLs and BCLs were measured and investigation of temperament were performed. After detection of BLLs, all the children were divided into two groups of high blood lead (BLLs≥100µg/L, HBL) group and low blood lead (BLLs<100µg/L, LBL) group to test whether the BLLs of children had additional effect on temperament. Results Both mean BLLs and the proportion of BLLs more than 100 µg/L in Guiyu group were significantly higher than control group (P<0.01). The median BCLs of children was also significantly higher than control group (P<0.01). Activity level, approach-withdrawal, and adaptability dimensions were significantly different between Guiyu and the control (P<0.01). We found significant differences in activity level and adaptability dimensions in boys and girls between the two groups (all P<0.05), and significant differences in activity level dimension in 3 and 5 years old children, and approach-withdrawal and adaptability dimensions in 4 and 6 years old children between the two groups (P<0.05 or P<0.01). There were difference in intensity dimension in 4 years old children and in approach-withdrawal and threshold of responsiveness dimensions in 5 years old children between the HBL and LBL groups (all P<0.05). Correlation and regression analysis showed that elevated BLLs and children lived in Guiyu increased the risk of high scores in activity level, and low scores in approach-withdrawal and adaptability dimensions. Conclusion Children's temperament alterations are significantly associated with the elevated BLLs and exposure to primitive e-waste recycling activities in Guiyu.

RA081

INORGANIC CALCIUM (CA2+) INDUCES DNA DAMAGE IN VITRO AND IN VIVO

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Investigations into the molecular and physiological consequences of controlling calcium metabolism and regulation have been a common research theme in many therapeutic areas. Ca^{2+} regulation is a common target for pharmaceuticals developed to treat various cardiovascular conditions and, at the same time, is one of the most widespread sec-

ond messengers used in signal transduction. Recent studies have focussed on the toxicological implications of manipulating intracellular Ca^{2+} with the most recent and remarkable finding being the potential of inorganic calcium to induce DNA damage, both *in vitro* and *in vivo*. When testing high concentrations of drugs with this molecular action for genotoxic effects *in vitro* and *in vivo*, the effects on intracellular Ca^{2+} concentration are potentially very dramatic and far in excess of likely changes under therapeutic conditions. However, there are several publications demonstrating that disruption of the content and movement of various cations, including Ca^{2+}, can lead to inhibition of DNA synthesis, production of reactive oxygen species, DNA damage and ultimately apoptosis. Consequently, induction of DNA damage at high concentrations of a drug manipulating intracellular Ca^{2+} levels could be an extreme manifestation of pharmacology. In order to investigate this possibility, stomach cells harvested from rats orally dosed with $CaCO_3$ and three $CaCl_2$-treated CHO K1 cell lines[1] were assessed for statistical changes to both tail intensity and moment relative to vehicle/control in Comet assays. Both compounds induced significant increases in comet damage at high doses solely in cells expressing a functional CaSR. These results suggest that care should be taken when interpreting comet data from experiments using high doses of Ca^{2+}-regulating agents that act on Ca^{2+}–dependent signal transduction.

[1]CHO K1 cell lines: *wild-type*, one cell line expressing human Ca^{2+}-sensing receptor (CaSR) and one cell line expressing a non-functional CaSR mutant.

RA082

DIRECTING GENOTOXICITY TESTING TO ASSIST IN THE DEVELOPMENT OF IN SILICO MODELS

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In silico systems are becoming increasingly important for the assessment of genotoxicity. Irrespective of the modelling approach used, e.g. QSARs or structural alerts, they share a common requirement for sufficient, high-quality data. However, even in large data sets it can be difficult to model areas of chemical space that are sparsely populated or contain conflicting data. This work describes the generation of additional data for in silico model development by suggesting candidates for an in vitro chromosomal aberration testing strategy. Previously, approximately 100 prototype structural alerts for the chromosome damage endpoint were derived from several collections of in vitro chromosomal aberration test data. These were further developed into completed structural alerts if sufficient supporting evidence was available in either the data sets or the published literature. In several cases, development was not possible because of insufficient or conflicting data. To address this problem, databases of commercially available chemicals were interrogated to find relevant, untested compounds which it was reasoned would allow completion of the development process. Where such compounds were identified, their activity in the in vitro chromosomal aberration test was evaluated. The results were then used in the development of structural alerts. Overall, 10 compounds were assessed for activity in the in vitro chromosomal aberration test. The positive results for several compounds, including allyl ethyl ether, azoxybenzene and thiobenzamide, were used to extend existing alerts and support a new alert for chromosome damage. However, not all positive results were immediately useful for model development since, for example, in some cases the new test results were indicative of a different mechanism of action to structurally related chemicals for which data were already available. Negative results were also informative, e.g. the inactivity of allylbenzene itself was a useful addition to a structure-activity relationship for the allylbenzene class of compounds. In conclusion, this work has shown that a closer integration of in silico modellers and genetic toxicologists can further knowledge of genotoxicity and improve the performance of in silico systems for this endpoint.

RA083

INFLUENCE OF THE GLUTATHIONE S-TRANSFERASE GENETIC POLYMORPHISM ON THE EXCRETION LEVELS OF URINARY BENZENE METABOLITES.

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The identification of specific and sensitive biological markers is critical for the definition of exposure to low benzene level and the assessment of the health risk. SPMA is generally considered a very specific biomarker of benzene, successfully applied to distinguish exposed and nonexposed subjects. This study was planned to investigate the modifying effect of genetic polymorphisms of the glutathione S-transferases enzymes (GSTP1, GSTT1 and GSTM1) involved in benzene detoxification on the levels of examined biomarkers (SPMA and t,t-MA). A total of 227 workers at a petrochemical factory benzene 137 exposed and 90 controls (administrative employees) were enrolled. We measured end-shift urinary levels of SPMA and t,t-MA for each worker and calculated the t,t-MA/SPMA ratio. To determine the GSTM1 and GSTT1 genotypes, a multiplex PCR was used; for GSTP1 gene the detection method used was based on real-time polymerase chain reaction (PCR). The observed genotypes agree with allele frequencies reported previously for other European populations: GSTT1 (178) 78.4% positive genotype, (49) 21.6% null genotype; GSTM1 positive genotype (106) 46.7%, (121) 53.3% null genotype; GSTP1 (106) 46.7% ile/ile, (100) 44% ile/val, (21)9.3% val/val. Our results are in agreement with the latest literature data, that benzene-exposed workers who carried GSTT1-deficient genotype excreted lower levels of SPMA than GSTT1-positive genotype subjects (higher t,t-MA/SPMA ratio). Besides our data show that for GSTM1 deficient genotype subjects the t,tMA/SPMA ratio is slightly higher than GSTM1 positive subjects. GSTP1 genotype seems not to influence the amount of excreted SPMA. Urinary levels of t,t-MA are comparable for all subjects and are not influenced by the polymorphism of these genes.

RA084

COMPARATIVE STUDIES ON GENOTOXICITY AND CYTOTOXICITY OF POLYMERIC GENE CARRIER POLYETHYLENIMINE (PEI) AND POLYAMIDOAMINE (PAMAM) G4 IN JURKAT T CELLS

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Background The thiopurine antimetabolite azathioprine (Aza) is used in the treatment of cancer, inflammatory conditions and as an immunosuppressant. Systemic treatment with Aza results in high levels of DNA 6-thioguanine (6-TG). Exposure of cells containing DNA 6-TG to low doses of UVA produces reactive oxygen species (ROS) and photochemical oxidation of DNA 6-TG into cytotoxic and mutagenic lesions.*Aims* We examined the role of the Base Excision Repair DNA glycosylase Mutyh and the Mismatch repair (MMR) protein Msh2 in the cellular response to 6-TG/UVA-induced DNA damage. The contribution of an oxidized dNTP pool to 6-TG/UVA cytotoxicity was also investigated. *Methods* Mutyh- or Msh2-defective mouse embryo fibroblasts (MEFs) were exposed to a non-toxic concentration of 6-TG and irradiated with a low dose of UVA. Survival was determined by clonal assays. DNA 6-TG and 8-hydroxydeoxyguanosine (8-oxodG) were measured by HPLC and HPLC/EC, respectively. *Results* 6-TG and UVA were synergistically toxic to wild-type MEFs - neither 6-TG or UVA alone detectably affected survival. Mutyh- or Msh2-defective cells were more resistant than wild-type MEFs to killing by 6-TG/UVA. The combined treatment significantly increased the levels of

DNA 8-oxodG. The dNTP pool contributed to both the increased levels of DNA 8-oxodG and to the enhanced toxicity of combined 6-TG/UVA. Wild-type cells grown for 24hrs in 6-TG and UVA irradiated immediately contained more DNA 8-oxodG and were more sensitive than the same cells irradiated after a further 24h growth in medium without 6-TG to deplete the pool of 6-TG nucleotides. *Conclusions* The combination of 6-TG and UVA introduces DNA 8-oxodG and causes cell death. Surprisingly, the absence of either Mutyh or Msh2 confers tolerance to 6-TG/UVA. The molecular mechanism underlying this phenotype is currently under investigation.

RA085

VALIDATION OF THE (Q)SAR COMBINATION APROACH FOR MUTAGENISITY PREDICTION OF FLAVOUR CHEMICALS

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Many flavour chemicals in current food use have been evaluated under the TTC concept at JECFA. The concept was referred as the establishment of human exposure thresholds for chemicals, below which would cause no appreciable risk to health. The concept had evolved from the historical knowledge, in order to develop the assessment methods for large group of chemicals with unknown toxicity. Most exposure levels of flavour in food are considered to be extremely low. In such case, genotoxic properties should be mainly taken account of safety evaluation in addition to the empirical thresholds. One of the limitations of the TTC could be compensated by the (Q) SAR system. We have recently established the (Q)SAR combination system of mutagenicity prediction for industrial chemicals. The (Q)SAR system is composed of three individual models (DEREK for Windows, MCASE/MC4PC and ADMEWORKS). The definitive judgement of mutagenicity is defined by the combination of predictive results from the three models. We have tried to validate our (Q)SAR system for the assessment of 375 flavour chemicals, which had been evaluated by JECFA, and the Ames test results of which were accessible. We judged 10 of the 375 chemicals as positive by our (Q)SAR system, when two or more models evaluated positive. Only five chemicals were misjudged as false positive for 331 Ames-negative chemicals, and the specificity was 98.5%. However, only five chemicals were judged positive for 44 Ames-positive chemicals (which were defined as positive in this study, if at least one positive result was reported), and the sensitivity was extremely low. In contrast, we judged 66 chemicals as positive, if one or more models evaluate positive. The sensitivity increased to 45.5%. This increase of the sensitivity was mainly owing to the prediction by the DEREK model (knowledge-base model). Although half of Ames-positive chemicals remained false negative, most chemicals possessed some kinds of common sub-structures. These results suggested that the approach of improving the sub-structural alerts could effectively contribute to increasing the predictability of mutagenicity for flavour chemicals. It is because that most flavours possess categorically functional sub-structures or are composed of some series of derivatives.

RA086

ENVIRONMENTAL RISK INDEX FOR A COMPLETE RISK ASSESSMENT (ERICA): APPROACH AND CASE STUDIES WITH ATTENTION ON MUTAGENS DESCRIPTORS

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Environmental risk assessment is a complex procedure to evaluate the health status of an investigated area. Such procedure is often expensive and rarely takes into account both ecological and human targets. ERICA (Environmental Risk Index for a Complete Assessment)'s task is to create a complete and valid tool giving a detailed analysis of the global risk for the area under observation in a simple, clear output. ERICA merges human toxicology, ecotoxicology and environmental fate parameters of compounds in a single value. Nineteen priority compounds (e.g. dioxins, PCBs, PAHs, metals, etc.) are the minimum scenario used to perform ERICA. These compounds are chosen keeping into account their toxicological profile, with particular attentions for carcinogenic and mutagenic ones. In this way it is possible to easily compare different sites. Also other pollutants (i.e. VOC, pesticides) can be added to the priority set to better fit assessment on investigated areas and to perform a more complete evaluation. Data on priority compounds came from peer review literature, international databases and predictive reliable methods. New validated and predictive methodologies (e.g. EPISuite, CAESAR models, etc.) were used in the event of missing experimental physico-chemicals data or toxicological values. A particular attention is dedicated at this stage to the process of carcinogenesis: most of the 19 priority compounds can induce cancer by any route of exposure but carcinogenic potential and potency may depend on the conditions of exposure (e.g. route, level, pattern and duration of exposure). Carcinogenic chemicals have conventionally been divided into two categories according to the presumed mode of action: genotoxic or non-genotoxic. ERICA can be used to determine environmental quality of various territories such us area surrounding industries, incinerators and landfills, but also urban regions. Some case studies designed on real investigated sites and Italian legal limit case were used to evaluate ERICA and compared to a detailed risk assessment analysis performed by our laboratory. ERICA is a concise and transparent method; researchers and assessors can compare critical areas interested by different chemical charges, also along time and space dimensions.

RA087

PUBLIC ATTITUDE AND RISK PERCEPTION OF NANOTECHNOLOGY IN TAIWAN

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Background and Objective: Nanotechnology is widely used in many kinds of products and researches in nowadays world, but the health and environmental impacts of nanotechnology are not well known in the current state of public understanding. Public attitude is a key to technology development. To avoid controversies in gene technology such as GMO, there is a must to realize the public attitude and risk perception toward nanotechnology. Thus, in this study we'll explore the public attitude perception of knowledge, benefit, risk, and trust of nanotechnology in Taiwan. Method: From 2007 August to September, we conducted a telephone survey with the computer-assisted Telephone Interviewing system (CSTI) via two-stage geographically stratified systematic sampling. Sampling population was based on people who were older than 18 and using telephone at home. A total of 1,251 persons successfully completed the telephone interviews, with a completion rate of 17.8%, and a rejection rate of 35.4%. The sample was representative in the distribution of age and gender. Result: There were a significantly higher percentage of women (89.9%) than men (78.4%) who had heard of nanotechnology. Those who had heard of nanotechnology were younger and with higher education. However there were higher percentages of men (39.4%) than women (24.95%) who said they knew what nanotechnology was. Discussion: There is risk-benefit trade-off in the perception on nanotechnology. Other than sex and age, trust play an important role in determining risk and benefit perception of nanotechnology while knowledge plays a minor role in this study.

RA088

IN VIVO GENOTOXIC EFFECTS OF CITRIC ACID IN MOUSE BONE MARROW CELLS

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Citric acid has been used as a food additive in many factory made foods. It is also a chemical that plays an important role in the physiological oxidation of glucose and other carbohydrates, as well as fats and proteins. The citric acid concentration controls several metabolite pathways and in higher organisms it regulates the utilization of calcium contained in foods (Gautier-luneau et al. 2007). Citrate is present in blood plasma in submillimolar concentration and promotes the bioavailability of dietary iron (Gautier-luneau et al. 2007, Parkes et al. 1991). Owing to fact that the mobilization of iron may lead to the formation of highly toxic hydrogen radicals (Pierre and Fontecave 1999, Gautier-luneau et al. 2007), one can question concerning the innocence of adding citric acid in large amounts in foods or drinks (Gautier-luneau et al. 2007). In this research, in vivo genotoxic effects of citric acid were investigated in mouse bone marrow cells. Animals were treated with four doses (50, 100, 200 and 400 mg/kg bw) of citric acid for 24h. Citric acid significantly increased the frequency of chromosomal aberrations at all doses compared with the control. Chromatid and choromosome breaks were the most common aberrations in bone marrow cells of treated animals. Based on the results we concluded that citric acid is a genotoxic agent in bone marrow cells of mice.

RA089

APPLICATION OF HIGH SENSITIVITY, HIGH-THROUGHPUT IMMUNOCHEMICAL ASSAYS FOR DNA ADDUCTS FOR USE IN MOLECULAR EPIDEMIOLOGY

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The need for sensitive and high-throughput methods to measure DNA damage is widely recognised, especially in view of the prospect of large-scale molecular epidemiology studies. We have recently developed improved immunochemical methodology satisfying these criteria (45th Eurotox 2008 Congress, Rhodos, Greece). A limit of detection of <1 adduct/109 nucleotides, using 10 μg DNA per well, was achieved for the assay of O6-methylguanine and benzo[a]pyrene diol epoxide DNA adducts. The method has been validated extensively using different approaches, including comparison of the outcome with adduct analysis using (GC-MS/MS). Analysis of human samples showed that both adducts were detectable in human leucocytes. Preliminary results were obtained from the analysis of DNA adducts in leukocyte DNA samples obtained from maternal and cord blood from the Rhea cohort which participates in the NewGeneris project. The objective of the latter project is to investigate the role of prenatal and early-life exposure to genotoxic chemicals present in food and the environment in the development of childhood cancer and immune disorders. 17 mother-child pairs have been analyzed for O6-methylguanine and 12 mothers for benzo[a]pyrene diol epoxide like DNA adducts so far. Four mother's and 6 children's leukocyte DNA show undetectable levels for O6-methylguanine. The levels in the positive samples are very low ranging from 5 adducts /1010 nucleotides to 20 adducts/1010 nucleotides. All twelve samples were positive for benzo[a]pyrene diol epoxide like DNA adducts ranging from 5 adducts/109 nucleotides to 57 adducts/109 nucleotides. Examination of further human samples from the same and other cohorts which participate within the framework of the NewGeneris project, is ongoing and association between DNA adducts, exposure and early biological effects (i.e. Micronuclei) will be assessed. Supported by the EU ECNIS (Environmental Cancer, Nutrition and Individual Susceptibility) Network of Excellence (contract no. FOOD-CT-2005-513943) and the EU Integrated Project NewGeneris (Newborns and genotoxic exposure risks) (contract no. FOOD-CT-2005-016320)

RA090

INCORPORATION OF THE GADD45-GFP GREENSCREEN HC ASSAY INTO PRE-CLINICAL DRUG DEVELOPMENT SCREENING TO REDUCE COMPOUND ATTRITION.

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Genotoxicity findings often result in termination of individual drugs, and sometimes whole projects. The current genotoxicity early screening assays used at GlaxoSmithKline (GSK), the Ames test (bacterial mutagenicity) and Mouse lymphoma assay (mammalian genotoxicity) are cut-down versions of *in vitro* GLP regulatory assays. These are relatively low throughput, time consuming and compound hungry. They do not present screening solutions that can be applied earlier in drug development and before significant investment in time, money & emotional attachment is committed to a compound or project. To address attrition due to genotoxic safety earlier in the drug development process, GSK has introduced the "GreenScreen HC" assay [1] into a panel of screens performed during optimisation of lead chemical series of potential candidate drug compounds. This assay utilizes the transcriptional response of human GADD45a gene [2] to genotoxic stress to drive a reporter gene, green fluorescent protein (GFP). The initial validation studies of GreenScreen HC at GSK using 75 compounds [1] has been extended to include 75 marketed pharmaceuticals [3]. Results revealed high sensitivity to *in vivo* genotoxins and genotoxic carcinogens coupled with high specificity. Data from an additional 10 proprietary GSK compounds with known genotoxic liabilities were 100% concordant with the results of the standard regulatory genotoxicity tests. Based on these data GreenScreen HC has been implemented for "genotoxic liability hit triage" following high throughput pharmacology screening and key chemical optimization milestones. Here we review the performance of the initial GreenScreen HC data from its inclusion in the GSK screening panel, assess the impact of impurities and show how results may be used to inform decision making prior to pre-candidate selection.

[1]. Hastwell et al. (2006), Mutat. Res. 607, 160-175; [2] Zhan et al. (2005), Mutat. Res. 569, 133-143; [3]. Hastwell et al. (2009), Mutagenesis. *In press*.

RA091

DEVELOPMENT AND VALIDATION OF AN IN VIVO PHOTO-MICRONUCLEUS ASSAY IN RATS FOR USE IN PRECLINICAL DRUG DEVELOPMENT

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For pharmaceuticals, current regulatory guidance for photosafety testing state that studies are warranted for drug candidates that both absorb light in the range of 290 - 700 nm, and that are either applied locally/topically, or 'reach' the skin or eyes. In contrast to standard genotoxicity evaluations, where a positive (or equivocal) result in vitro can be placed into context with additional testing in vivo, there are no equivalent short term in vivo photo-genotoxicity assays in the current photosafety test battery. Therefore, a short-term *in vivo* assay for the evaluation of a photo-genotoxic potential in the skin, the target organ for photo-carcinogenicity, was developed in rats. After oral administration of the potent photocarcinogen, 8-Methoxypsoralen (8-MOP), rats were exposed to UV and sacrificed 3 days after treatment to isolate epidermal cells for subsequent micronucleus (MN) evaluation. Once a

robust protocol had been established, six compounds from the same chemical class (fluoroquinolones) with varying photosafety liabilities were given orally; subsequently, rats were dorsally irradiated and the MN frequencies in epidermal skin cells were determined. Photo-clastogenicity was detectable following treatment with photo-activated Sparfloxacin and Lomefloxacin. Conversely, no significant increases in MN induction were evident following photo-activated Ciprofloxacin, Levofloxacin, Gemifloxacin or Gatifloxacin treatment. These results were encouraging and correlated with their reported photo-carcinogenicity. Therefore, the *in vivo* rat skin MN test is a promising tool for the early prediction of photo-carcinogenic liability. Additional work will be required using different chemical classes to further validate the assay.

RA092

PHOTOTOXICITY ASSESSMENT DURING THE VALIDATION OF AN IN VIVO PHOTO-MICRONUCLEUS ASSAY IN RATS FOR USE IN PRECLINICAL DRUG DEVELOPMENT

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Chemical photo-activation following the absorption of solar ultraviolet radiation (UVR) may result in the formation of reactive intermediates, or the release of various reactive oxygen species. These can interact with biological systems and ultimately lead to cellular cytotoxicity and/or DNA damage (i.e. phototoxicity and/or photo-genotoxicity), and if prolonged, photo-induced ageing and/or photo-carcinogenicity. As part of the validation of an in vivo photo-micronucleus assay in rat skin, the phototoxicity of orally administered, 8-Methoxypsoralen (8-MOP, a potent photo-carcinogen) was characterised. Parameters included in-life clinical observations, dorsal erythema measurements using a mexameter, water and food consumption, ophthalmoscopy, macroscopic observations at necropsy and histopathological evaluation of dorsal skin and eyes. Once a robust protocol had been established, six compounds from the same chemical class (fluoroquinolones) with varying photosafety liabilities were given orally; subsequently, rats were dorsally irradiated and the phototoxicity and MN frequencies in epidermal skin cells were determined. The individual results will be discussed. Importantly, in the absence of gross phototoxicity, no significant increases in photo-genotoxicity were observed (photo-micronucleus evaluations are presented separately). Results of the present study indicate that the *in vivo* rat skin MN test allows the simultaneous measurement of the phototoxicity and photogenotoxicity potential of a test compound in the same animal. The in-life phototoxicity observations showed good concordance with the microscopic changes observed histopathologically and were consistent with the relative potencies observed for photo-genotoxicity. Additional work will be required using different chemical classes to further validate the assay.

RA093

CHROMOSOME ABERRATION ASSAY IN SEPARATED HUMAN LYMPHOCYTES – TOXICITY DETERMINATION USING POPULATION DOUBLING

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Current guidelines for the chromosome aberration (CA) assay limit testing to concentrations that induce ≥50% mitotic inhibition. Recently, population doubling (PD) is regarded as a more accurate estimation of cytotoxicity, taking into account cell growth and stasis. This is important as more accurate estimates of cytotoxicity may reduce the incidence of false positives associated with mammalian cell genotoxicity assays. To validate the PD method in human lymphocytes we compared separated lymphocytes with whole blood. Average generation times and control CA levels showed no difference between methods. To characterise cell types & dynamics in separated lymphocytes over the 24-70h culture period typical of the CA assay, individual cells were identified by cell surface markers: CD3, CD4 and CD8 (for T-lymphocytes), CD25 (T-cell activation), CD19 and CD56 (for B and NK-cells), CD13 and CD14 (for monocytes and granulocytes), and monitored using flow cytometry (FC) or Advia® 120. FC showed an increase in $CD3^+$ cells (and associated increases in $CD4^+$ and $CD8^+$ subpopulations) from 24-70h with ~70% of cells co-expressing CD25 from 24 hours, indicating good T-cell activation. All other cell types (~20% of total on day 0) remained quiescent so that < 9% of non-T-cells were present between 48-70h of cultures. Advia® data confirmed growth of lymphoid-type cells (2.2 fold increase from 48-70 h) with no proliferation of other cell types. Thus, T-cell lymphocytes are the only active cell population under the conditions of the chromosome aberration assay. Advia data was then collected for separated lymphocytes treated with various tool compounds (Cyclophosphamide 3hr+S9, Mitomycin-C 3hr-S9, Methyl methanesulfonate 20hr-S9, 2,4-Dichlorophenol 20hr-S9) and analysed for CA. All treatments induced concentration dependent decreases in PD of lymphoid cell numbers, which were highly reproducible. Non-lymphoid cells were unaffected by compound treatments and remained low during culture. Haematological identification of cell types confirmed the Advia data and showed that ~97% of cells analysed were T-cell lymphocytes. Based on these studies, we are confident that PD measurements reflect only lymphocyte growth and therefore present a reliable and reproducible measure of toxicity for the chromosome aberration assay.

RA094

THE LEGAL ISSUES OF APPLYING RISK MANAGEMENT MECHANISM TO THE REGULATORY SCHEME OF TAIWAN ENVIRONMENTAL LAWS

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Background: The Twenty-First Century is popularly known as an epoch full of potential risks in various aspects. To mitigate the impact that could be caused on social systems (especially on legal system) because of such potential risks, many countries regard risk management as a major consideration in enacting their laws and regulations. However, the issue here is the gap between the regulatory measure and the actual situations. To be specific, when the administrative agency is taking responsive measures to potential risks, there should be applicable legislative foundations for such measures. This paper aims to analyze the legal issues of applying risk management mechanism to the regulatory scheme of Taiwan environmental laws Methods: The comparative legal analysis and the case studies. Results: To modern countries, when the government is confronting the risks, they need to adopt effective ways to deal with their uncertainty and unpredictability. In generally, most countries are used to order, approve or ratify police actions against risks with high certainty and material scale. Therefore, it is worthwhile to explore the issues related to the law and regulation on risk management, such as the scope of restriction and the measures of enforcement. In this connection, the US courts usually will study feasibility and cost on the enforcement measures so as to ensure that such actions are in compliance with the principle of equalization and proportion. Conclusions of the Study: To comply with the law principles of Constitutional Law and Administrative Procedural Act, a legal basis for the administrative agency to exercise its rights will be required, both on traditional risk management or modern risk management. In addition, the U.S. courts' decisions regarding the judicial reviews of risk regulations have illustrated several rules. For example, in the case of *American Textile Mfrs. Inst.*, the courts recognized that "unreasonable risk" statutes require a "generalized balancing of costs and benefits". And in the *Lead Industries* case involving a CAA regulatory standard: "adequate margin of safety" as well as in the *Vinyl Chloride* case involving ample margin of safety standard, the courts refused to consider feasibility or cost. Those are all important factors to consider in the applications of risk mechanism in environmental laws.

RA095
AGRICULTURAL EXPOSURE AND THE MOLECULAR CONNECTION TO LYMPHOMAGENESIS

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Non-Hodgkin lymphoma (NHL) is a heterogeneous group of malignancies of largely unknown etiology. Increased risks of NHL have been reported among farmers, regularly but not firmly associated with pesticide use. The follicular lymphoma-associated translocation t(14;18)(*Bcl-2/JH*), and the mantle cell lymphoma-associated one t(11;14)(*CCND1/JH*), are early events in lymphomagenesis. t(14;18)+ clones are present in the peripheral blood of most healthy individuals, where they can persist for years[1]. We showed that long-lived t(14;18)+ cells are mainly constituted by an atypical population of incompletely maturated B-cells, closer to follicular lymphoma than was hitherto suspected[2]. To investigate the potential causative link between agricultural exposures and the risk of NHL, we measured t(14;18) and t(11;14) translocations among farmers from a prospective cohort, and among non-farmers from the same geographical area. The evolution of t(14;18) frequency was followed over 9 years in a sample of 128 farmers selected as pesticide users on crops at enrolment, and in non-farmers. Whereas frequency slowly increased in the latter (+87%), a dramatic increase was observed for farmers (+253%). Contrary to non-farmers, neither age nor ageing effects were found in farmers at enrolment or follow-up. Extensive clonal expansions of activated t(14;18)+ B-cells, evocative of an immunogenic effect, was observed in most farmers, while some of them displayed an accumulation of distinct t(14;18) translocations. Some t(14;18)+ clones showed aberrant AID activity linked to malignant progression, compatible with a follicular lymphoma-like phenotype[3]. t(11;14)+ cells were present in the peripheral blood in 7% (2/28) of the non-farmers and in 6 % (3/48) of pesticide users on crops. t(11;14)-positive clones with identical *CCND1/JH* junction persisted over nine years. In two farmers, an expansion of clonotypic B cells in the circulation, without appearance of new clones was observed[4]. Altogether, these data provide a molecular connection between agricultural exposures, translocation frequencies in blood, and clonal progression.

[1]*Roulland et al. Leukemia, 2006, 20,158–162;* [2]*Roulland et al. J Exp Med, 2006, 203, 2425–31*
[3]Agopian et al. J Exp Med, 2009, in press; [4] Lecluse et al Leukemia, 2009, Feb 26

RA096
COMPARISON AND VALIDATION OF THE MICRONUCLEUS ASSAY WITH AND WITHOUT CYTOCHALASIN B IN MAMMALIAN CELL LINES.

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As a consequence of the reduced, refined and ultimate replacement of animal usage in genetic toxicology, more emphasis has been placed on *in vitro* assays. The *in vitro* micronucleus assay is one such test system, and we are working with a number of European laboratories to standardise and validate the assay, as recommended in the late 2007 version of the draft OECD Test Guideline 487. Cytochalasin B (CytoB) is used in the cytokinesis block micronucleus (CBMN) assay is widely used to assess chromosomal damage in cells that have undergone one cellular division. The micronucleus assay can also be carried out without CytoB in the non-CBMN assay, although this is less widely conducted due to lack of control of mitotic kinetics. In this communication we compare these two methodologies by testing the known spindle poisons, vinblastine and diethylstilboestrol (DES) in both test systems in CHO cells. Relative population doubling (RPD), relative increase in cell count (RICC) and relative cell counts (RCC) were used in the non-CBMN assay and the replicative index (RI) was used in the CBMN assay. These measures were then compared and their implications for dose selection are highlighted. Notably, RI exaggerated toxicity and lead to a lower dose being selected to give 50% toxicity (vinblastine 0.7µg/ml; DES 4µg/ml), possibly due to temporary mitotic block. RCC underestimated toxicity and lead to higher doses selected (vinblastine >2µg/ml; DES >6µg/ml). Failure to detect known genotoxins (so called false negatives) at doses selected using RPD is its main criticism for use, however, in the following report, I have shown this is not the case. This work supports the premise that RPD and RICC are appropriate measures for the non-CBMN assay *in vitro*. We are also using this information to help in our validation of both the CBMN and non-CBMN assays using the Metasystems Metafer automated system.

RA097
DNA DAMAGE AFTER EXPOSURE TO DIFFERENT "NANOCLAYS": COMPARISON OF FULLY AND SEMI-AUTOMATED COMET ASSAY SCORING.

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The comet assay is a well established genotoxicity test and has been used for many years for both in vitro and in vivo genotoxicity testing of chemical compounds and mixtures. Nanoclays have a huge commercial potential as reinforcing fillers to strengthen biopolymers. However, the potential use of nanoclays in biopolymers cannot be fully appreciated yet because of lack of knowledge about the potential health effects of nanoclays. In this project two types of nanoclays, a natural montmorillonite (Cloisite® Na+) and a modified montmorillonite (Cloisite® 30B) were tested for genotoxic potential by using the comet assay on Caco-2 cells. Cloisite® 30B but not Cloisite® Na+ was genotoxic in Caco-2 cells. In addition, we have synthesized the modifier in Cloisite® 30B and it is being tested for genotoxic potential on Caco-2 cells. In order to get faster and more reliable comet assay results by eliminating subjectivity, a fully automatic system was developed in cooperation with Imstar. With this system it is also possible to increase the number comets scored per sample, which will increase the statistical power of the assay. The IMSTAR Pathfinder automatic scoring system was validated by comparing the results with a semiautomatic system (Kinetic Imaging 5.0). This project (NanoPack) is a national funded project by NABIIT under The Danish Research and Innovations Agency.

RA098
IN VITRO MICRONUCLEUS SCORING BY FLOW CYTOMETRY: A HIGH THROUGHPUT APPROACH PROVIDING SIMULTANEOUS MODE OF ACTION

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The in vitro micronucleus assay is becoming an important component of genotoxicity testing batteries. Factors that have contributed to its increased use include the simplicity of the endpoint, and its ability to detect clastogens and aneugens. A further advantage of the assay is its compatibility with automated scoring. Experiments herein were performed to further evaluate the performance of the In Vitro MicroFlow® flow cytometry-based method. Initial studies were performed utilizing human TK6 cells which were treated for 27-30 hrs with 74 diverse chemicals in a 96 well plate format. In the case of negative results, a short term treatment in the presence of a rat liver activation system was performed. The results correlated well with expected in vitro cytogenetics data: positive predictivity was 85.0%; negative predictivity was 90.6%. Further experimentation was performed using the attachment cell line CHO-K1. For these experiments, 36 chemicals were treated

for 24-27 hrs continuously in a 96 well plates. The positive and negative predictivity were 82.6% and 92.3% respectively. In addition to MN frequency, mode of action signatures were evident in CHO-K1 cells. Specifically, aneugens were observed to consistently i) generate larger MN, as evidenced by increased DNA-associated fluorescence, and ii) increase the frequency of hypodiploid nuclei. These data indicate that flow cytometric scoring of micronuclei can be objectively accomplished for suspension or attachment cell lines in a high throughput format—(96 well plates and autoloader). Furthermore the method provides several concurrent measures of cytotoxicity, and appears capable of discriminating between aneugenic and clastogenic modes of action.

RA099

A FIRST INTERLABORATORY TRIAL OF THE HEN'S EGG MICRONUCLEUS (HET-MN) ASSAY AS A POTENTIAL REPLACEMENT FOR THE IN VIVO MICRONUCLEUS TEST (MNT)

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Existing *in vitro* genotoxicity tests exhibit a very low specificity. Therefore, *in vivo* assays must be often performed to clarify the biological relevance of the *in vitro* experiments. However, due to the EU 7th Amendment to the Cosmetic Directive i*n vivo* tests for cosmetic ingredients are banned since March 2009. In addition, due to the new chemical legislation REACH the number of *in vivo* mutagenicity assays will increase by an estimated factor of 3 (Van der Jagt *et al.*, 2004). Here we investigate whether the Hen's Egg Micronucleus (HET-MN) has the potential to serve as a replacement method for the *in vivo* MNT. The developing egg is studied since a long time as an alternative test system to animal tests (Wolf *et al.*, 1997, 2002, 2003, 2008). Since it is not protected by a maternal organism it has to be already competent in metabolising xenobiotics, like (pre)mutagens. In order to investigate the predictability of the test system, the mutagenic potential of ten compounds were tested in two independent laboratories: non-mutagens: isopropylmyristate, ampicillin, L-ephedrin; mutagens: p-chloroanilin, malathion, cyclophosphamide, dimethylbenzanthracene; presumably false-positive: isophorone, dichlorophenol; aneugen: carbendazim. The mutagenic, non-mutagenic compounds and the aneugen, were correctly predicted by the HET-MN. The presumably false-positive compounds were evaluated as non-mutagens in both laboratories. The results are in line with i*n vivo* MNT results. Their relevance should be supported by testing additional compounds. In summary, the HET-MN is a promising candidate for a prevalidation and might aid in the replacement of *in vivo* testing.

RA100

***PIG-A* MUTATION AND MICRONUCLEUS SCORING BY FLOW CYTOMETRY: INTEGRATION INTO AN ACUTE DOSING SCHEDULE**

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The integration of genotoxicity endpoints into toxicology studies has the potential to significantly reduce animal usage. This laboratory has developed techniques for monitoring two blood-based genotoxicity endpoints (MN-RETs and gene mutation at the *pig-a* locus) using a flow cytometric platform. The kinetics of the *pig-a* response to known mutagens was characterized using *N*-ethyl *N*-nitrosourea (ENU), 7,12-dimethyl-1,2-benz[*a*]anthracene (DMBA), 4-nitroquinoline-1-oxide, benzo(a)pyrene, and *N*-methyl-*N*-nitrosourea, while concurrently monitoring micronucleus induction. For all studies, treatment of Wistar Han rats or CD-1 mice occurred on three consecutive days via oral gavage (i.e., Days 1-3), with blood samples collected on Days -1 (rat only), 4, 15, 30, 45 and 90. For MN-RET measurements, blood was stained and analyzed according to MicroFlow® Kit instructions. Mutant phenotype erythrocytes and reticulocytes were measured following staining with SYTO 13 in combination with either anti-CD59-PE (for rats) or anti-CD24-PE (mice). Statistically significant increases in %MN-RET were observed for all chemicals on Day 4. Mutant phenotype cells were not evident on Day 4, but significant increases were observed on Day 15 for each chemical. The persistence of the responses were markedly different for these chemicals, which presumably relates to the degree to which mutation is occurring in long-lived hematopoietic stem cells versus cells with limited self-renewal capacity. These data support the integration of these complementary endpoints into toxicology studies to provide valuable genotoxicity data while reducing animal usage.

RA101

INDUCTION OF OXIDATIVE DNA DAMAGE AND MICRONUCLEI IN MICE CHRONICALLY EXPOSED TO FURAN

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Furan is used as a solvent as well as an intermediate in the production of organic-based compounds. It is also present as a contaminant of several types of food and beverages. Chronic exposure to Furan induces hepatocellular carcinomas and cholangiocarcinomas in mice and rats. Because of contradictory results on Furan genotoxicity, both genotoxic and non-genotoxic mechanisms have been proposed to underlie the carcinogenicity of this compound. Here we addressed the possible role of oxidative DNA damage in liver toxicity as well as in genotoxicity associated with Furan exposure. In a first approach this has been studied in a mouse strain (B6C3F1) where carcinogenicity of Furan was previously investigated. Following daily oral administration of furan to male mice for 5 weeks (5 days/week), DNA was isolated from several organs and analyzed for the presence of 8-hydroxyguanine (8-oxoG) by HPLC-EC. DNA 8-oxoG levels were unaffected by treatment in brain, kidney, spleen and bone marrow, while the lung and the liver showed significant increases when compared to controls (1.9-fold and 1.5-fold, respectively). A trend of increased levels of this oxidized purine was also observed in small intestine DNA (1.6-fold). Although no treatment related increase in 8-oxoG levels was identified in the spleen, a significant increase in micronuclei was observed in splenocytes of Furan-treated mice. We also investigated whether oxidative DNA damage associated with furan exposure in a different strain of mice (C57Black) or in animals defective in the repair of this lesion (*Ogg1*$^{-/-}$). In wild-type C57Black mice, Furan induced a 2.3-fold and 1.6-fold increase in 8-oxoG levels respectively in lung and small intestine. No further increases were observed in any organs of *ogg1*$^{-/-}$ mice. A clear genotoxic effects was again observed in splenocytes of Furan treated C57Black mice, with no increases in DNA 8-oxoG levels of this organ. These results suggest that *in vivo* exposure to Furan is associated with a very limited amount of oxidative stress to DNA and Furan-associated chromosomal damage is independent from oxidative DNA damage. The relationship between liver toxicity and accumulation of 8-oxoG is currently under investigation. Acknowledgements: European Commission 6th FP, project SSPE-CT-2006-44393.

RA102

GENETIC VARIATION AS A PREDICTOR OF SMOKING CESSATION SUCCESS USING ANTIDEPRESSANT THERAPY.

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Recent research strongly suggests that variation in genetic background is an important determinant of smoking behaviour and addiction, and might also influence efficacy of pharmacotherapies used for smoking cessation. Generally, important risk factors related to smoking are variants in genes that influence the response to nicotine (e.g. nicotine

metabolism and nicotinic receptors) and genes that may predispose to addictive behaviour due to their effects on key neurotransmitter pathways (e.g. dopamine, serotonin, acetylcholine and β-endorphin). Additionally, treatment efficacy might also be influenced by variants in genes that influence the medication levels of smoking cessation therapies (e.g. metabolism and secretion). The aim of the present study is to investigate which genetic variants in these genes are associated with the individual variance in smoking cessation success using the antidepressant drugs bupropion SR and nortriptyline. DNA samples were collected from 217/255 adults participating in a randomized, double-dummy, placebo-controlled trial on the efficacy of bupropion SR and nortriptyline in smokers at risk for or with existing COPD. The primary outcome measures are the level of nicotine dependence (determined by the Fagerström Test of Nicotine Dependence; FTND), and prolonged abstinence from smoking from weeks 4-12 (end-of-treatment), 4-26 and 4-52 after the target quitting date. A total of 36 single nucleotide polymorphisms (SNPs) have been determined using the iPLEX™ Assay (Sequenom®). Preliminary analyses show that several of these genetic variants seem to influence the level of nicotine dependence and prolonged abstinence rates using bupropion and nortriptyline. Thus, smoking-related genetic variants seem to influence nicotine dependence levels and treatment outcome of antidepressant therapy used for smoking cessation and may therefore have therapeutic implications.

RA103

THE CYTOKINESIS BLOCKED MICRONUCLEUS CYTOME ASSAY AND RISK PREDICTION OF LUNG CANCER

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Tobacco smoke carcinogens and individual susceptibility play key roles in determining risk of lung cancer. In a case-control study of 250 lung cancer cases and 250 matched healthy controls, we used the cytokinesis-block micronucleus (CBMN) assay, an established biomarker for DNA damage and genomic instability, to evaluate individual susceptibility to the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). We measured the frequency of baseline and NNK-induced chromosomal damage endpoints (micronuclei [MN] and nucleoplasmic bridges [NPBs])/1000 binucleated cells. Our results showed significantly higher levels of both baseline and NNK-induced chromosomal damage in cases compared to controls ($p<0.001$). We then extended our investigation to test the hypothesis that NNK-induced CBMN assay is a sensitive predictor of lung cancer risk due to involvement of specific regions in the genome that are critical for lung carcinogenesis. Using Fisher linear discriminant analysis (FLDA) we constructed a linear model involving NNK-induced MN and NPB outcomes. We used Spectral imaging (SKY) to identify the specific chromosomes involved in the formation of baseline and NNK-induced MN and NPBs in cases and controls that comprise the upper two quintiles of the distribution in the FLDA model. Our results indicate that chromosomes 1, 2, 3 and 4 are the main chromosomes involved in formation of baseline MN in presence of NPBs in the cases. Chromosomes 9, 13 and 16 are the main source of baseline MN, while NNK induced MN at chromosomes 8, 15 and 21. Interestingly, NNK induced NPBs formation at chromosomes 8,11,15,19 and 21. These preliminary results suggest that NNK may potentially induce damage at specific regions in the genome. Recently chromosome 15 has been identified as the top hit for genomic association with lung cancer through genome wide association studies and harbors genes that encode subunits of the nicotine acetylcholine receptor which have affinity to nicotine. Additionally, association between lung cancer development and chromosomes 8 and 21 have been reported. Our results suggest that using this approach will allow for identification of high-risk individuals. Supported by CA98549, CA 129050, ES07784

RA104

INVESTIGATION OF PARAMETERS BY IMAGE ANALYZING SYSTEMS IN THE ALKALINE COMET ASSAY.

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The alkaline Comet Assay (Single cell gel electrophoresis, SCGE, assay) is a widely used to detect DNA damage and expected as an effective in vivo genotoxicity test. For the comet assay, there, however, are various parameters to determine of the damage and several systems (softwares) to analyze, and each software gives a different numerical value even when the same comet image is analyzed. Therefore, we examined the differences in each parameter from softwares and effects on decision of results. Method: TK6, human lymphoblastoid cells, were treated with several concentrations of methyl methanesulfonate. Comet slides were prepared by an alkaline condition (>pH 13.0), and stained by Cyber gold. The same slides were analyzed by three image analyzing systems (S1, S2, and S3) for Comet assay, what have own camera and software. Results: Tail length, Tail intensity (% DNA in tail) and Tail moment what are recommended parameters to assess a DNA damage in the comet assay showed positive response by statistical analysis in all system, however, each system showed characteristic value in each parameter as follows. Tail length: Each system showed most characteristic values for this parameter, and there were large difference between each system. The difference were constant in all concentrations between one and others, values by S2 were longer than that by S1 and values by S3 were longer than that by S2 with about 10 μm difference. Tail intensity (% DNA in tail): S1 and S2 showed similar values but values by S3 were about 20% higher than that by S1 and S2.Tail moment: All systems showed similar values and manner. Conclusion: Each analyzing system for the comet assay showed characteristic numerical values for some comet parameters with significantly difference from other systems, the parameters, however, could detect a positive response in all systems. Therefore, difference in absolute numerical value in the parameters from other systems is not a problem when background value is well controlled. The tail moment was the best parameter, because increasing ratio in positive response was maximum in all system used. Additionally, all systems showed similar values of the tail moment, therefore, it is useful when we compare among data by different analyzing systems.

RA105

AN INVESTIGATION INTO THE EFFECTS OF ANEUGENS IN THE MOUSE LYMPHOMA ASSAY

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The detection of aneugenic compounds in genetic toxicology assays is essential due to their potential to cause cancer. The Mouse Lymphoma Assay (MLA) is a mammalian gene mutation assay that is capable of detecting a wide range of genetic damage ranging from point mutations to large scale chromosomal events [Clive *et al.* Mut Res 59:61; Combes *et al.* Mutagen 10:403], recombination [Liechty *et al.* Mutagen 13:461] and possibly aneuploidy [for example: Honma *et al.* Mut Res 493:101]. To investigate the effects of aneugens on MLA cells, multiple endpoints were assessed. L5178Y $Tk^{+/-}$ cells were treated (with carbendazim or colchicine) in accordance with OECD TG 476, coupled with a modified *in vitro* micronucleus assay (IVM) including antikinetochore staining. Resultant $Tk^{-/-}$ clones from the MLA were analysed for microsatellite loss of heterozygosity (LOH) and whole chromosome 11 presence by fluorescent *in situ* hybridisation (FISH). Cell cycle parameters, necrosis and apoptosis were also assessed. MLA was positive for carbendazim. For colchicine, mutant frequencies > Global Evaluation Factor (GEF) were only observed at high toxicity levels (i.e. <10% RTG). For both aneugens:

1) high % of micronuclei present at end of treatment were centromere-positive
2) LOH analysis revealed chromosomal damage but not potential aneuploidy in clones from treated cultures
3) cell cycle disruption was observed with some recovery seen over a 72 hour period
4) caspase-3 (apoptosis marker) showed dose-related increases across the 72 hour (extended) recovery.

Further experiments with colchicine revealed increased levels of p53 at doses showing elevated apoptosis. Preliminary results from p53-target-

ed RNA interference experiments indicate that knockdown of p53 results in a decreased apoptotic response. These data show that aneugens can cause cell cycle delay and apoptosis (which appears to be p53-mediated) in MLA cells. The probability of the MLA to detect loss of $Tk1_b$ could be greatly reduced due to the apoptosis-mediated clearance of irregular / damaged cells during the expression period. Therefore, caution should be exercised when interpreting data from this compound class in the MLA.

RA106

PRACTICAL THRESHOLD FOR MICRONUCLEATED RETICULOCYTE INDUCTION OBSERVED FOR LOW DOSES OF ACRYL AMIDE AND POTASIUM BROMATE BY ACRYDINE ORANGE SURPRAVITAL STAINING AND FLOW CYTOMETER METHODS

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Micronucleus induction was studied for the clastogens acryl amide (AA) as contaminant in fried food and Potassium bromate (KBrO3) used as a flour improver in order to evaluate the dose–response relationship at very low dose levels. We selected 100mg/kg as a high dose level for both AA and KBrO3. The six dose levels were spaced by the square root 10 for AA and by the 2 for KBrO3 with 7-week healthy CD1 male mice. Solvent control was assigned to each experiment as reference. The acridine orange (AO) supravital staining method was used for fluorescence microscopy and the anti-CD71-FITC based method was used for flow cytometric analysis. In the AO method, 2000 reticulocytes were analysed as commonly advised, but in the flow cytometric method, 2000, 20 000, and 200 000 reticulocytes for AA and 2000, 20000, 200000, and 1 000 000 reticulocytes for KBrO3 were analysed for each sample to increase the detecting power (i.e. sensitivity) of the assay. The present data show that increasing the number of cells scored increases the statistical power of the assay when the cell was considered as a statistical unit. When the animal was considered as a statistical unit, only the top dose group for each chemical showed significant increase of micronucleated reticulocytes frequency. As non-linear dose–response curves were obtained for each of the two chemicals studied, these observations provide evidence for the existence of a practical threshold for the DNA target clastogens studied.

RA107

TOWARDS STRATEGY FOR RISK ASSESSMENT OF GENOTOXIC AND CARCINOGENIC TRACE SUBSTANCES IN FOOD: IN VIVO GENOTOXICITY OF MYOSMINE

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Rapidly increasing rates of esophageal carcinoma in EU population raised questions on health risk assessment of myosmine, 3-(1-pyrroline-2-yl)pyridine,a naturally occuring minor alkaloid in widely consumed foods (cereals, fruits, vegetables, milk).Endogenously formed dihydroxypyridylbutatone-releasing DNA and protein adducts of dietary myosmine are the same as the ones of the human esophageal carcinogen N-nitrosonornicotine. Myosmine is genotoxic in in vitro COMET assay to human esophageal adenocarcinoma cell lines, human lymphocytes and upper aerodigestive tract epithelial cells.There is no published data on in vivo genotoxicity of myosmine. The present mouse bone marrow micronucleus (MN) studies on myosmine are carried out using the alternative protocol of the in vivo MN assay (OECD TG 474)in BALB/C male mice.A range of single-1000,500,250 and 125 mg.kg b.w, and repeated oral doses of myosmine- 200,100 and 50 mg.kg b.w.(3 consecutive daily treatments at 24 h)were tested and bone marrow was sampled at 24h and 48 h after single dosing and 24h after the last subacute oral dose. Myosmine is established as active in the mouse bone marrow MN assay at 24h and 48 h sampling times following oral exposure to highly toxic doses of 1000 (MTD) and 500 mg.kg b.w. as well as non-toxic dose-levels of 250 and 125 mg.kg b.w., inducing significant 2-4 times increased MN frequencies as compared to the negative control groups. The most pronounced cumulative MN activity of myosmine was obtained in the subacute treatment regimen with no signs of myelotoxicity.Myosmine induced 1.4-3.6 times increased MN frequencies at all test doses of myosmine with dose-effect relationship. Our results proved that in vivo MN assay is appropriate when follow-up testing is needed for risk assessment with potential genotoxic trace substances in food. The financial support of the Bulgarian Ministry of Sciences is acknowledged(Grant TKL 1608-06)

RA108

HEPARG® CELL LINE: A NEW MODEL FOR THE DETECTION OF REACTIVE CHEMICALS AND FOR THE POSSIBLE IDENTIFICATION OF CYTOTOXIC AND GENOTOXIC COMPOUNDS

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The human HepaRG® cells represent the first human hepatoma cell line able to differentiate *in vitro* into hepatocyte-like cells and display several hepatocyte like functions at levels comparable to those measured in primary human hepatocyte cultures. Several lines of evidence have demonstrated that HepaRG ® cells exhibit (i) a hepatocyte-like morphology; (ii) a greater metabolic competence for phases I and II enzyme activities; (iii) a concomitant expression of hepatic influx and efflux transporters; (iv) a good inducibility on drug metabolizing enzymes *(Aninat et al, 2006; Le Vee et al, 2007; Kanebratt KP et al, 2008).* Recent results suggest that HepaRG® cell line could be used as a promising *in vitro* and long-term hepatocyte model for:

- investigation of enzyme induction in drug discovery
- evaluation of hepatic drug transport function
- characterization of hepatotoxic effects of cholestatic and steatotic molecules
- identification of cytotoxic and genotoxic compounds

RA109

STATISTICAL ANALYSIS OF ENVIRONMENTAL FACTORS ON THE INCIDENCE OF GASTRIC CANCER IN COSTA RICA.

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Costa Rica from 1983 to 1997 showed the highest mortality from gastric cancer in the world in 30 countries including Japan and China, although, it has fallen remains one of the countries with the highest incidence and mortality in gastric cancer the world. It is now accepted that *Helicobacter pylori´s* infection plays a causal role, this bacterial infection is acquired in childhood and persists for many decades unless people are treated with antibiotics, is also classified by the International Agency for Research on Cancer (IARC) as carcinogenic to humans. Costarican incidence is totally geographical, the provinces in the center of the country have highest rate than the provinces of the coast, this pathology is considered multicausa. Research of Sierra showed that in a zone of high incidence Cartago province, the 90% of the people have positive antibodies against *H pylori.* Aims: Establish statistical correlations between rates of high and low incidence of gastric cancer with geophysical environmental factors related to survival and acquisition of Helicobacter pylori in water consumption population. Methods: It visit either the 10 cantons with the highest incidence and 10 cantons with lower incidence of gastric cancer and collects the following information: altitude, temperature, soil type, type of water used and

Operator of the Aqueduct Authority. Searched statistically significant correlations between variables. RESULTS: Determines that there are important and significant correlations by Pearson Correlation Coefficient between gastric cancer incidence and altitude of 0.867, temperature 0, 853, and the source of water 0.651, while the Linear Regression Coefficient between incidence and temperature was R^2 = 0.728. It was conducted a Fisher exact test to assess the level of independence between incidence and Operator of the Aqueduct Authority of water, and concluded that a relationship exists between variables. Conclusions: From the above it is clear that for the Costa Rica´s case, it is possible to prevent this pathology and to reduce effectively the incidence of gastric cancer. Water consumption should be avoided without proper treatment chlorination, prevents the transmission of H. pylori under conditions given for his specific development.

RA110

FUTURE APPLICATION OF GENETIC TOXICITY ASSAYS

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With the advent of new technologies (e.g. genomics), new regulations (e.g. the European changes required by REACH), and new or revised genotoxicity tests and standards, the field of regulatory genetic toxicology is in a state of transition. We offer an analysis of current methods for evaluating mutagenic risk using the standard genotoxicity test battery, and summarize information on new assays that may be valuable additions to genetic toxicity testing schemes. We point out that the standard genotoxicity test battery, including *in vitro* mammalian cell assays, was developed for hazard identification, that is, to detect the largest number of mutagenic and carcinogenic substances, both known and new. Given that large numbers of new molecular structures are being produced via high-throughput technologies, this hazard-identification approach would still appear to be valid. A summary of the uses of genotoxicity data in different U.S. regulatory centers/agencies is provided as a framework for this perspective. Issues related to the role and value of *in vitro* mammalian cell genetic toxicity assays were discussed recently (Elespuru et al., *Toxicological Sciences* 2009). These included the validity of test results that are (1) weak positives, (2) associated only with considerable toxicity, (3) seen only at high concentrations, (4) not accompanied by positive results in the other tests of the standard test battery, and/or (5) not correlating well with rodent carcinogenicity tests. While highly valuable, long-term bioassays have their own limitations as surrogates for human cancer risk. Thus we believe concordance with rodent carcinogenicity is not the only standard against which short term genetic toxicity tests should be measured. Until the advent of effective direct human biomonitoring to assess mutagenic and carcinogenic potential, we suggest that a consideration of mechanisms is appropriate and feasible, given the development of new analytical technologies in genetics and genomics.

*The views and opinions expressed are solely those of the authors and are not official policy of the affiliated institutions.

RA111

PERIPHERAL BLOOD LYMPHOCYTE ASSESSMENT OF CHROMOSOME ABERRATIONS IN HUMAN VOLUNTEERS DOSED WITH SUMATRIPTAN 85MG FORMULATED WITH RT TECHNOLOGY/NAPROXEN SODIUM 500MG (SUMART/NAP)

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A study was performed to assess the production of metaphase chromosome aberrations (CA) in peripheral blood lymphocytes (PBL) in human volunteers after dosage of SumaRT/Nap or naproxen sodium 500mg versus placebo. Non-smoking healthy volunteers (22 male, 20 female) aged 19-35 were randomized to SumaRT/Nap, naproxen, or placebo BID for 7 days. Study dosing reflected the maximum therapeutic dose allowed in any previous SumaRT/Nap study whilst the duration of dosing was used to mimic chronic dosing conditions. Consistent with OECD guidelines for CA in non-clinical safety studies, 10 subjects (5 per gender) were selected per treatment arm for the primary analysis. Pre-and post-dosing PL CA frequency (PLCAF), average generation time (AGT) and mitotic index (MI) were determined. Blood samples were collected on days 8 (determination of MI and PLCAF), and 9 (determination of AGT) and cultured. Metaphase cells (200) were analysed per subject. Positive control slides were randomly inserted to confirm the ability to identify a positive response and showed unequivocal increases in aberrant cells. Neither pre-dose nor 24 hour (day 8) comparisons between SumaRT/Nap or naproxen sodium with placebo demonstrated statistically significant differences in mean CA frequencies (- gaps). This is consistent with a lack of treatment-related clastogenicity. In all cases, both the pre-dose and 24 hour (day 8) post-dose CA frequencies (- gaps), for all treatment groups, fell within the laboratory historical control range (observed range) for standard in vitro CA assays using human PBL [(0-5% males, 0-4% females for aberrant cells (-gaps)]. Therefore, it was concluded that no treatment-related clastogenic effects were detected with SumaRT/nap or naproxen, as compared to placebo, following 7 days of treatment in healthy human volunteers.

RA112

CHROMOSOME ABERRATIONS IN WORKERS EXPOSED TO ORGANIC SOLVENTS: INFLUENCE OF POLYMORPHISMS IN XENOBIOTIC-METABOLISM AND DNA REPAIR GENES

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Organic solvents are widely used as diluents or thinners for oil-paints, gasoline and other organic mixtures. We evaluated chromosome aberrations (CAs) in lymphocytes of 200workers exposed to organic solvents and 200 referents and the influence of polymorphisms in xenobiotic-metabolism (CYP2E1, GSTM1 and GSTT1) and in DNA repair genes (XRCC1194 Arg/Trp, XRCC1280 Arg/His, XRCC1399 Arg/Gln and XRCC3241 Thr/Met). Polymorphisms were determined by PCR–RFLP. Poisson regression analysis indicates a significant CA frequency increase in exposed workers, representing a higher risk in relation to the matched referent (RR 2.15, 95% CI 1.21–1.53, $p < 0.001$). The CA frequency in exposed workers was influenced by the polymorphic genotypes: GSTM1 null (RR 1.33, 95% CI 1.31–1.69, $p < 0.001$), XRCC1194 Arg/Trp, Trp/Trp (RR 1.23, 95% CI 1.08–1.40, $p < 0.001$) and by the wild genotypes CYP2E1 C1/C1 (RR 1.20, 95% CI 1.05–1.37, $p < 0.001$), GSTT1 positive (RR 1.49, 95% CI 1.31–1.69, $p < 0.001$), XRCC1280 Arg/Arg (RR 1.44, 95% CI 1.26–1.64, $p < 0.001$) and XRCC1241 Thr/Thr (RR 1.54, 95% CI 1.34–1.76, $p = 0.001$).We contribute to the follow-up predictive value of individual susceptibility biomarkers and their CA frequency influence during occupational organic solvent exposure. We provide tools for surveillance and prevention strategies to reduce potential health risks in countries with a large population of car painters not using protection devices and limited organic solvents use control.

RA113

AN INTEGRATED GENOMICS AND METABONOMICS APPROACH TO DEVELOPING *IN VITRO* METHODS FOR ASSESSING THE CARCINOGENIC POTENTIAL OF COMPOUNDS TO THE KIDNEY

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This work is part of a Carcinogenomics Project funded by the European Union which aims to develop genome-wide transcriptomics and metabonomics *in vitro* models for testing and prediction of the carcinogenic potential of chemical compounds. Initially NRK-52E cells (normal rat kidney cell line) were characterized. Giemsa karyotyping analysis showed no structural abnormalities and normal number of chromosomes. However, cytochrome P450 activity using a range of substrates showed no xenobiotic metabolizing capability in NRK-52E cells. Next the cytotoxicity of several genotoxic and non-genotoxic carcinogens using two assays MTT and LDH release were determined. NRK-52E cells were grown in 96 well-plates in Dulbecco's Modified Eagle's Medium without serum and then exposed to genotoxic [dimethylnitrosamine (DMN), benzo(a)pyrene (BaP), 2-nitrofluorene (2NF), potassium bromate ($KBrO_3$), streptozotocin (STZ)] and non-genotoxic [ochratoxin A (OTA), monuron (M), chlorothalonil (CT), bromodichloromethane (BDCM) and S-(1,2-dichlorovinyl-L-cysteine) (DCVC)] compounds that cause an increased incidence of renal tubule tumours in rats. Non-carcinogenic compounds- negative controls (nifedipine, tolbutamide, D-mannitol and sodium diclofenac) were also examined. The concentration that caused 10% toxicity (IC_{10}) after 72h exposure with the most sensitive assay was established (n=3) and will be used for transcriptomics and metabonomics. IC_{10} at 72h for genotoxic compounds was obtained for 3 out of 5 compounds and was 20µM for 2NF, 40µM for BaP and 500µM for $KBrO_3$. IC_{10} for 72 h for non-genotoxic compounds was 0.75µM for OTA, 0.8µM for CT, 5µM for DCVC, 250µM for BDCM and 400□M for M. Additionally an *in vitro* micronucleus assay (MN) was conducted in NRK-52E cells at the IC_{10} for 24h to assess any DNA damage caused by these compounds. No increase in micronuclei was seen with BaP or 2NF suggesting lack of bioactivation, in contrast to *in vivo / in vitro* experiments. In summary, we have determined doses and MN data with some of these carcinogens to enable us to start transcriptomics and metabonomics, however for some genotoxic carcinogens we need to increase the metabolic capability of NRK-52E cells in order to try and obtain a MN response.

ABSTRACTS

Authors' index

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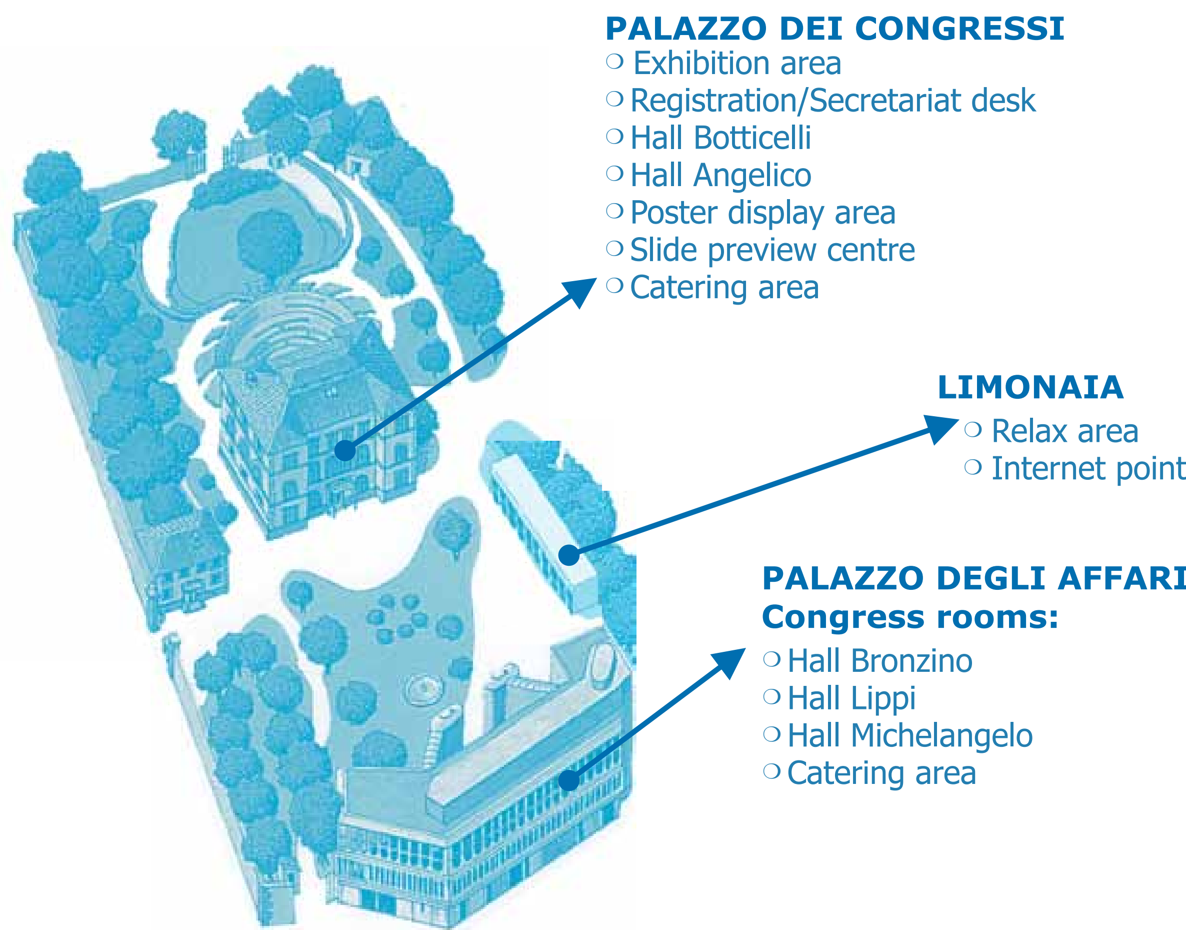

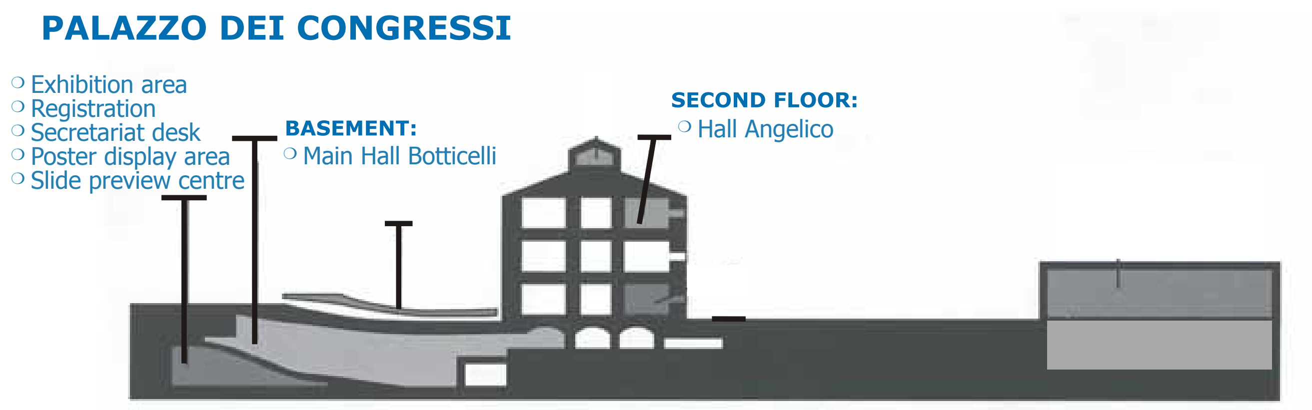

PALAZZO DEI CONGRESSI - Basement