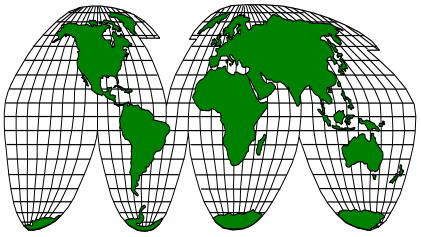

International Workshops on Genotoxicity Testing
(IWGT)

6TH INTERNATIONAL WORKSHOP ON GENOTOXICITY TESTING

Bourbon Cataratas Hotel,
Foz do Iguaçu, Brazil

October 31 – November 2, 2013

IWGT 2013 – detailed program

1. Welcome and Introduction (Iguazu I, II, III)
David Kirkland & Hans-Jörg Martus

2A. Liver micronucleus group (Iguazu I, II, III)

Chair: Yoshifumi Uno
Co-Chair: Takeshi Morita
Rapporteur: Mirjam Luijten
Working group members: Carol Beevers,Shuichi Hamada, Satoru Itoh, Wakako Ohyama, Hironao Takasawa

Discussion topics
Agree recommendations on how to conduct the test technically and reliably in order to identify the relevant agents.

Discuss the pros and cons of the different variations of the test, in order to arrive to defendable and robust recommendations.

Discuss when and where to use this test strategically:
1) liver (or other tissues) MNT
2) bm/blood MNT
3) strategic considerations (pharma, chemicals, others).

2B. Germ cell assays (Ipe I)

Chair: George Douglas
Co-chair: Carole Yauk
Rapporteur: Jan van Benthem
Working group members: Marilyn Aardema, Jack Bishop, Kerry Dearfield, David DeMarini, Yuri Dubrova, Masamitsu Honma, James Lupski, Francesco Marchetti, Marvin Meistrich, Francesca Pacchierotti, Jane Stewart, Michael Waters

Topics for discussion

- Why do we need germ cell tests? (brief intro)
  - More than cancer
  - Heritable induced genetic diseases – offspring of exposed people
- What are the regulatory requirements for germ cell tests in different countries and international organizations?
  - Regulations and recommendations in the USA, EU, Japan, and Canada
  - IPCS
- When do you do germ cell tests and when do you NOT do them?
  - Do somatic tests protect germ cells? Are there germ-cell specific effects?
  - Are there instances where germ cells more susceptible than somatic cells?
  - When you have a complete battery of somatic cell assays that are negative, do you need to do a germ cell mutation assay?

    - When somatic cell assays are positive but you have no evidence of exposure of germ cells (DMPK, tissue distribution), do you need to do a germ cell assay?
    - How do you do risk assessment with germ cell and heritable effects assays?
        - (Review of Favor et al 199X) – 4 reports applying Favour or other similar methods. How can we improve on this?
- What are the current assays that are being used? (Advantages and Disadvantages)
    - Dominant Lethal
    - Spermatogonial chromosomal aberrations
    - Transgenic rodent sperm mutations
    - Others? E.g., comet assay on sperm.
- What new assays are in the pipeline and what's missing?
    - Tandem repeats (germ and somatic cells?)
    - Sperm micronucleus
    - Next generation sequencing
    - Copy number variants
- Can germ cell effects be extrapolated to inherited effects?
    - Germ cell assays versus offspring and pedigree assays
- Pharmacokinetics and pharmacodynamics for germ cell effects
    - How to integrate germ cell tests with standard regulatory guidelines? Capturing all types of genome-level effects. Related endpoints – should reproductive toxicity assays trigger further germ cell testing?
    - TGR
    - High throughput assays – micronucleus
    - Comet assays
    - TR – somatic and germ cells
- How will the new approaches and endpoints as outlined contribute to the 3 Rs?
    - How would new tests reduce the use of animals over current tests?
    - Germ cells versus pedigrees
- AOPs for germ cells
    - Identifying gaps
    - Establishing modes of action for risk assessment
    - Guiding assay development and identifying needs
- Providing context to data produced from high-throughput and omics platforms
- **Wrap-up:**
    - Why do we need germ cell tests?
    - What tests are needed?

2C. Pig-A working group (Ipe II)
Chair: Bhaskar Gollapudi
Co-chair: Anthony Lynch
Rapporteur: Bob Heflich
Working group members: Steve Dertinger*, Vasily Dobrovolsky*, Roland Frötschl, Karsuyoshi Horibata, David Jacobson-Kram*, Michelle Kenyon*, Takafumi Kimoto, David Lovell, Leon Stankowski*, Paul White, Kristine Witt*

Detailed List of Topics for Discussion:
1. Scope of the assay:
    a. Principle of the test method
    b. Summary of validation studies
    c. Genetic confirmation of the mutant phenotype
    d. Advantages of the assay over other in vivo mutation assays.
    e. Limitations of the assay compared to other in vivo mutation assays.

f. Strategic placement of the assay in genetic toxicology battery
g. Regulatory need
h. Pig-a as a translational biomarker and its clinical significance.

2. Study Design:
i. Selection of species and strains
j. Sex and Age of the animals
k. Number of animals/dose group
l. Treatment period
m. Dose levels including the limit dose
n. Positive and negative controls
o. Route of administration
p. Tissue sampling and storage – tissues other than blood and bone marrow that are amenable for analysis.
q. Tissue sampling time
r. Sample preparation
s. Flow cytometric evaluation
t. Verification of laboratory proficiency
u. Practical consideration in integrating the end point on repeat dose toxicology studies

3. Data evaluation and reporting:
v. Treatment of results
w. Statistical evaluation of data
x. Inter- and intra-animal variability
y. Influence of age and gender
z. Influence of toxicity on the end point (bone marrow toxicity, exceeding MTD, etc.)

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3. Comet assay (Iguazu I, II, III)

Chair: Günter Speit
Co-chair: Hajime Kojima
Rapporteur: Dan Levy
Working group members: Brian Burlinson, Carol Beevers, Andrew Collins, Marlies De Boeck, Patricia Escobar, Peter Kasper, Sachiko Kitamoto, Kamala Pant, Stefan Pfuhler, Ulla Plappert-Helbig, Jin Tanaka, Yoshifumi Uno, Marie Vasquez

Discussion topics

- Towards an OECD guideline – critical issues. Introductory speaker - **Yoshifumi Uno**
- How much standardization and calibration is needed? Introductory speaker - **Andrew Collins**
- Cytotoxicity – measures and impact on test results. Introductory speaker - **Marie Vasquez**
- Intra- and inter-laboratory reproducibility of comet assay results. Introductory speaker - **Brian Burlinson**
- The comet assay in current test strategies: combination and integration. Introductory speaker - **Ulla Plappert-Helbig**
- What makes comet assay acceptable to regulating agencies? Introductory speaker - **Peter Kasper**

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4. Quantitative workgroup (Iguazu I, II, III)

Chair: Jim MacGregor
Co-chair: Roland Frötschl
Rapporteur: Paul White
Working group members: Kenny Crump, David Eastmond, Shoji Fukushima, Melanie Guerard, Lya Hernandez*, Makoto Hayashi*, George Johnson, Toshio Kasamatsu, Dan Levy, Takeshi Morita, Lutz Müller*, Rita Schoeny, Maik Schuler*, Veronique Thybaud*

Discussion topics

Need for quantitative assessment of genotoxic risk *vs.* hazard assessment

-When and why is it necessary to quantify risk *vs.* hazard i.d.
-Need for *in vivo* data to estimate risk of genetic damage
- Need for (anticipated) ADME data in animals and human
-Need for data to link risk of genetic damage to risk of disease and/or pathology
-Definition of *in vivo* target sites of concern (germ vs. somatic vs. specific organs, tissues, or cell populations) and types of damage (primary damage, mutations, chromosome damage, direct versus indirect mechanisms)

Acceptable Risk *versus* precautionary principle depending on human use and population

-*In vivo* risk *vs.* hazard i.d.
-Risk relative to spontaneous background
-Disease risk (adverse effects) vs. risk of genetic damage (hits)
-Human risk vs. laboratory model risk

Methods to analyze exposure-response relationship of genotoxic responses and best descriptors

-BMD modelling
-NOGEL determination (statistical power)
-Threshold determination
-Linear extrapolation
-Other approaches
- Minimal amount of data needed
-Margins of safety: how determine and when applicable
-Appropriate *vs*. inappropriate statistics

Consensus on thresholds/points of departure

-Define what we mean and what are the expectations/criteria?
- Discuss the rationale for non-linear dose-responses and underlying mechanisms (protection mechanisms, saturation, compound specific versus global mechanisms, inter- and intra-species variability)

Parameters and data necessary to define exposure-related risk

-Extrapolation from *in vitro* data
-Extrapolation from *in vivo* to human data
-*In vivo* NOGEL relative to spontaneous rates (for each type of damage)
-Minimal increment above spontaneous
-Uncertainty factors to be applied to the descriptors
- Link with statistical power of the different assays
-Margins of safety *versus* margin of exposure
-Define criteria to determine, or define acceptable safety margin below a minimal increase

Interspecies extrapolation

-Define factors to be taken into account

-Dose extrapolation when human data unavailable
-Interspecies uncertainty factor

Overall recommendations

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5. Final plenary session - Reports from each working group (Iguazu I, II, III)

Discussion on Next Generation Test Strategies
Chair: Kerry Dearfield
Co-chair: Bhaskar Gollapudi
Rapporteur: Mirjam Luijten

- Overview talk and initial thoughts on strategy formation – Kerry Dearfield
- *In vitro* extrapolation to *in vivo* - Rusty Thomas
- Epigenetic considerations - Bhaskar Gollapudi
- Genomic plasticity - Anthony Lynch
- Regulatory perspective from Japan's point of view - Masa Honma
- Open discussion facilitated by Kerry, Bhaskar, and Mirjam.

Closing remarks

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* Will probably join by teleconference and webinar.