Summary Presentation

Working Group on Quantitative Approaches to Genetic Toxicology Risk Assessment

Foz do Iguassu, Brazil
November 1, 2013

Jim MacGregor, Chair
Roland Frötschl, Co-Chair
Paul White, Rapporteur

IWGT Working Group on Quantitative Approaches to Genetic Toxicology Risk Assessment (QWG)

James T. MacGregor (Chair)
Toxicology Consulting Services, USA

Roland Frötschl (Co-Chair)
Bundesinstitut für Arzneimittel und Medizinprodukte, Germany

Paul A. White, PhD (Rapporteur)
Health Canada, Canada

Kenny S. Crump
Consultant, USA

David A. Eastmond
University of California – Riverside, USA

Shoji Fukushima
Japan Bioassay Research Center, Japan

Melanie Guérard
F. Hoffmann-La Roche Ltd., Switzerland

Makoto Hayashi
Biosafety Research Center, Foods, Drugs and Pesticides, Japan

Lya Soeteman-Hernandez
RIVM - National Institute for Public Health and the Environment , Netherlands

George E. Johnson
Swansea University School of Medicine
United Kingdom

Toshio Kasamatsu
Kao Corporation, Japan

Dan Levy
Washington, D.C., USA(or)

Takeshi Morita
National Institute of Health Sciences, Japan

Lutz Müller
Hoffmann-La Roche Inc., Switzerland

Rita Schoeny
U.S. Environmental Protection Agency
USA

Maik J. Schuler
Pfizer, Inc., USA

Veronique Thybaud
Sanofi-aventis, France

Topics addressed:

- **The need for quantitative dose-response analysis.**
- **Methods to analyze exposure-response relationships & derive point of departure (PoD) metrics**
- **Points of departure (PoD) and mechanistic threshold considerations.**
- **Approaches to define exposure-related risks**
- **Empirical relationships between Genetic Damage (Mutation) and Cancer**
- **Extrapolations across test systems and species**

Working Premise

- **Genetic toxicity assays have typically been used for hazard identification (i.e., qualitative +- classifications).**

- **Quantitative analyses of genetic toxicology results can provide metrics for improved risk characterization.**

METHOD	BMD	NOGEL	Threshold using a bi-linear model
Measurement	Benchmark dose - dose associated with a specific benchmark response (BMR)	Highest dose with no statistically signif. response	Estimate of Threshold (Breakpoint)
Advantages	- Has proper incentives* - Uses data efficiently & fits a model to all dose-response data - Currently used by many regulatory agencies	- Easy to apply - Does not require dose response modeling	- Has proper incentives*
Disadvantages	- Continuous & quantal data modeled differently - Requires consensus on appropriate, biologically relevant, benchmark response (BMR) for each endpoint.	- Statistical assumptions must be met - Highly dependent on exptl design - Does not have proper incentives*	- Based on 1 model - Unstable metric - Cannot account for other functions - Mechanistic support required to justify.

*Lower power tends to provide smaller PODs

General preference lies in this order BMD>NOGEL>bilinear

GM = Gene Mutation; MN = Micronuclei,
**Not enough data below NOEL to satisfy threshold analysis

					NOEL	TD	BMD	Reference
EMS	GM (HPRT)	*in vitro*	AHH-1	µg/ml	1	0.95	1.08	Doak et al 2007; Johnson
	MN (Flow Cyto.)	*in vitro*	TK6	µg/ml	1.17	4.76	0.54	Bryce et al 2010
	MN (CB-MN)	*in vitro*	AHH-1	µg/ml	1.3	0.87	1.29	Doak et al 2007; Johnson et al 20
	GM (PigA)	***in vivo***	**Blood**	**mg/kg/day**	**25**	**21.9**		**Dobo et al., 2011**
	GM (MutaMouse)	***in vivo***	**Bone Marrow**	**mg/kg/day**	**50**	**21.46**	**9.29**	**Gocke and Wall, 2009**
	GM (MutaMouse)	***in vivo***	**Liver**	**mg/kg/day**	**50**	**25.67**	**41.00**	**Gocke and Wall, 2009**
	MN	***in vivo***	**GI Tract**	**mg/kg/day**	**25**	**12.97**	**12.23**	**Gocke and Wall, 2009**
	MN	***in vivo***	**Bone Marrow**	**mg/kg/day**	**80**	**56.66**	**58.68**	**Gocke and Wall, 2009**
MMS	GM (HPRT)	*in vitro*	AHH-1	µg/ml	1	0.86	0.56	Doak et al 2007; Johnson et al 20
	GM (MLA-TK)	*in vitro*	L5178Y	µg/ml	1.1	0.519	0.52	Pottenger *et al.*, 2009
	MN (CB-MN)	*in vitro*	AHH-1	µg/ml	0.8	0.14	0.54	Doak et al 2007; Johnson et al 20
	MN (Flow Cyto.)	*in vitro*	TK6	µg/ml	0.63	0.26	0.19	Bryce et al 2010
	MN	***in vivo***	**Blood**	**mg/kg/day**	**5**	**14.07**	**1.47**	**Dow Chemical 2012**
MNU	GM (MLA-TK)	*in vitro*	L5178Y	µg/ml	0.07	0.021	0.0093	Pottenger *et al.*, 2009
	MN (Flow Cyto.)	*in vitro*	TK6	µg/ml	0.31	0.19	0.023	Bryce et al 2010
	MN (CBMN)	*in vitro*	AHH-1	µg/ml	0.01	**	0.003	Doak et al., 2007
	GM (Pig-A)	***In vivo***	**RBC, RET**	**mg/kg/day**	**1.5**	**2.17/3,18**		**Lynch et al 2011. GSK**
	MN (Flow)	***in vivo***	**Blood**	**mg/kg/day**	**0.60**	**0.20**	**0.27**	**GlaxoSmithKline**
ENU	GM (HPRT)	*in vitro*	AHH-1	µg/ml	0.2	0.22**	0.11	Doak et al., 2007
	MN (Flow Cyto.)	*in vitro*	TK6	µg/ml	2.344	1.32	0.87	Bryce et al., 2010
	GM (PigA – Flow)	***In vivo***	**Blood (PigA)**	**mg/kg/day**	**0.25**	**0.64**		**Dobo et al., 2011**
	GM (MutaMouse)	***in vivo***	**Bone Marrow**	**mg/kg/day**	**	**	**0.00018**	**Gocke and Wall 2009**
	GM (MutaMouse)	***in vivo***	**Liver**	**mg/kg/day**	**1.39**	**	**0.145**	**Gocke and Wall 2009**
	MN	***in vivo***	**Bone Marrow**	**mg/kg/day**	**1.11**	**	**0.098**	**Gocke and Wall 2009**
	MN (Flow Cyto.)	***in vivo***	**Blood**	**mg/kg/day**	**5**	**2.54**	**0.120**	**Litron 2011**

Gollapudi, Johnson *et al.*, 2013, EMM

Johnson *et al.*, 2014. In Preparation

Point of Departure Preference

- **The working group critically examined and considered numerous PoD metrics.**

- **Detailed examination of the benchmark dose (BMD), the NOGEL, and estimation of a PoD from a bilinear model $\longrightarrow$ preference for the BMD method.**

Key Issue: Are there thresholds for Genotoxic Substances?

- **General consensus that some genotoxic agents, acting by indirect non-DNA-reactive mechanisms, mechanistic information indicates that there would be no effect below a defined exposure threshold.**
  - ***e.g.*, many aneugens, disturbance of nucleotide pools, glutathione depletion, DNA synthesis inhibitors (Thybaud et al., Mutat. Res. 627: 41-58, 2007)**

Regarding thresholds for DNA-reactive genotoxic substances -

- **At low doses, it is not possible to experimentally determine whether a small incremental risk is within the normal range of the (ever-present) spontaneous background.**
- **Any data set – no matter how extensive -- will be consistent with both threshold and low-dose linear responses (Crump, Crit. Rev. Toxicol. 41: 637-650, 2011)**
- **Evaluations should be made on a *case-by-case* basis taking into account all known mechanistic information regarding Mode-of-Action (e.g., metabolism, DNA-repair, etc.).**

Pragmatic Approach to Genetic Toxicity Risk Assessment

- **Since mutations are generally considered deleterious, protection against mutagenesis should minimize the risk of adverse health effects.**

- **PoDs, when combined with safety or uncertainty factors, can be used to examine risk of genetic damage, and identify an exposure associated with negligible risk.**

  - **Although it is desirable to directly relate genetic damage to specific health outcomes.**

    - ✓ **Many factors can influence the consequences of genetic damage (i.e., apical disease).**

Mode of Action (MoA) Considerations

- **Quantitative approaches, including appropriate dose-response models, should be consistent with MoA understanding.**

- **A number of factors need to be considered to determine the nature of the dose-response relationship (e.g., linear, sub-linear, other), subsequently a meaningful PoD can be used to estimate a level at which the risk of deleterious effect is negligible.**

  - **Non-DNA targets, exceeding detox capacity, disruption of DNA replication or cell-cycle progression, ADME/PK, nucleotide pool disruption, etc.**

Factors that Contribute to Uncertainty: Animal to Human Extrapolation

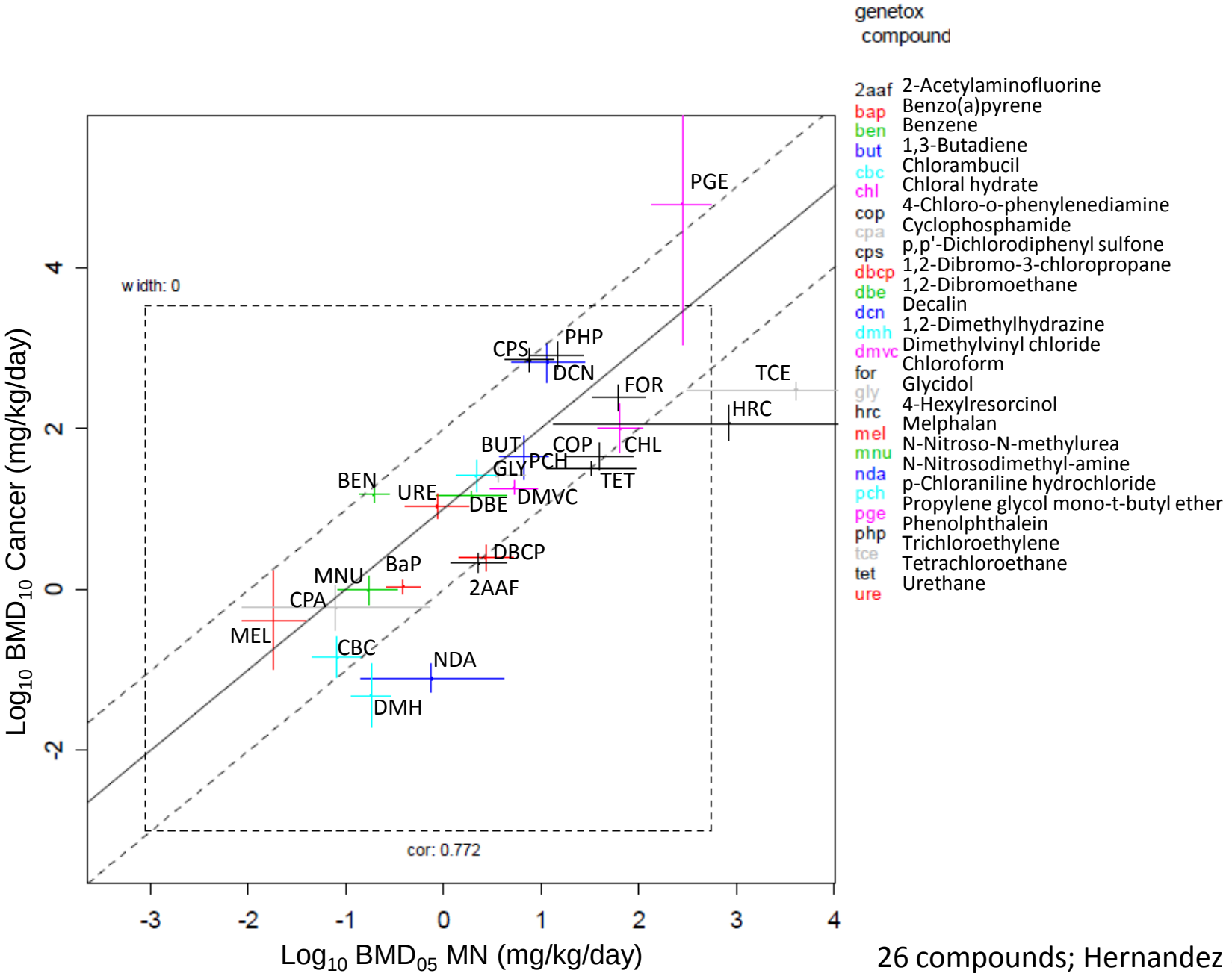
- Scaling to account for allometric differences between rodents and humans;
- Severity of effect
- Inter-individual variability
- Differences in repair capacity
- Study endpoint & duration
- Metabolic and PK differences

Extrapolation - Experimental Genetic Toxicity to Human Risk

- **In vivo factors -**
  - **Comprehensive exposure, ADME/PK assessments reduce need for uncertainty factors**
  - **Viracept: quantitative analysis and extrapolation of can be accepted by regulators**

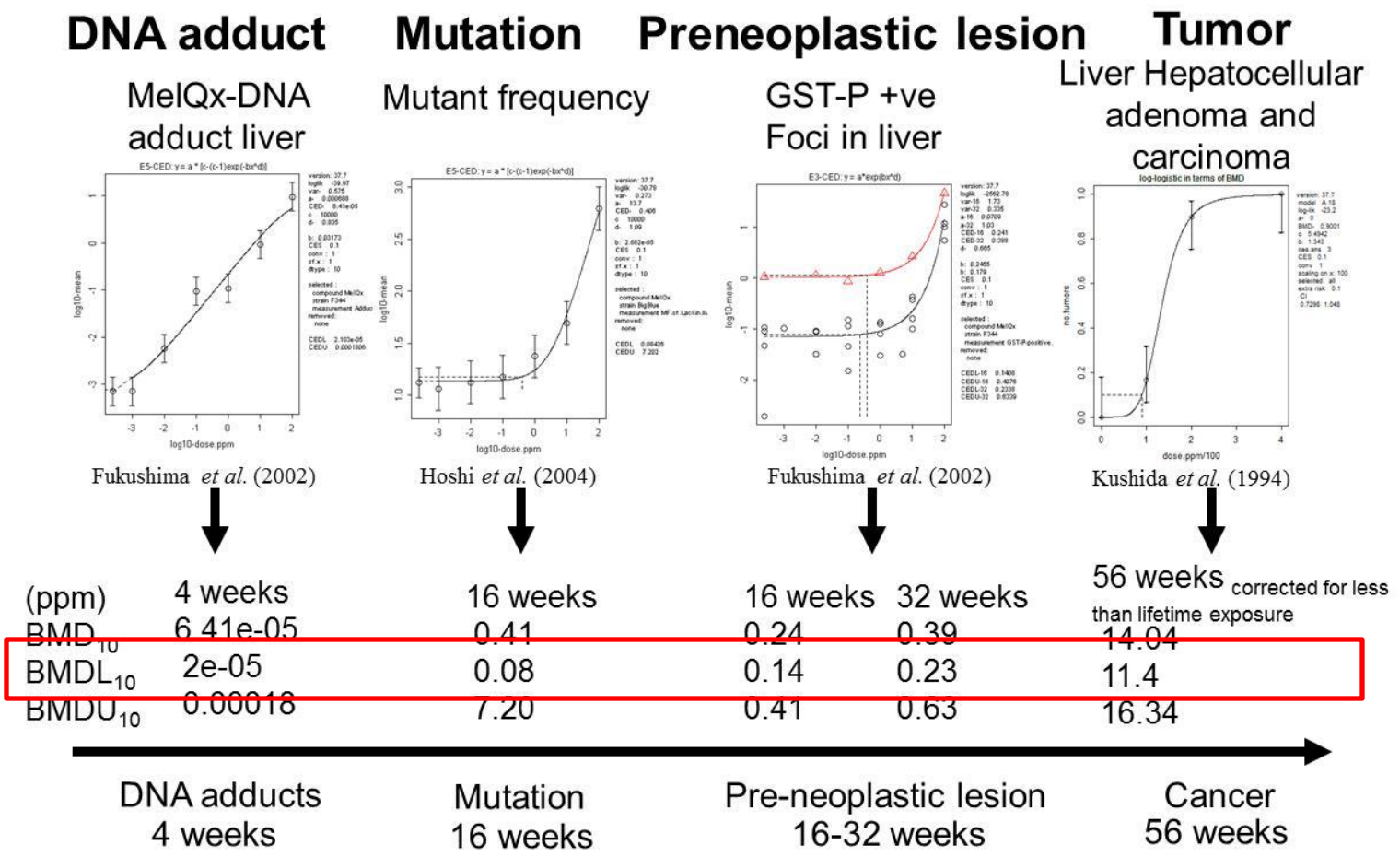
- **In vitro -**
  - **Bacterial mutagenicity data can be useful (MOA determination, potency ranking within structural classes).**
  - **Data from mammalian assays can be used to support the data package (incl. in vivo data) for quantitative extrapolation**
  - **Quantitative extrapolation from Ames data, especially for compounds requiring metabolic activation, is in most cases extremely complex**

Can we use genotoxicity data to refine cancer risk assessment?



26 compounds; Hernandez *et al.* (2013)

MeIQx: Results of analysis



$BMDL_{10}$ ranking:

$DNA\ adduct_{4weeks}$ << $Mutation_{16weeks}$ < $Foci_{16weeks}$ < $Foci_{32weeks}$ < $Cancer_{56weeks}$

Conclusions from Mutation-Cancer Correlations

- Need to calculate PoDs for induction of different types of cancerous lesions in different organ tissues.
- MelQx: suggests that protection against key genetic events in cancer induction may protect against cancer induction
- Only a few compounds with genotoxicity data matched with carcinogenicity (tissue, species, etc).
- Analyses to date indicated that these all may be achievable but more (good!!) data required.