Summary Presentation

IWGT Working Group on Quantitative Approaches to Genetic Toxicology Risk Assessment

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On behalf of the Quantitative Work-group of the 6th IWGT Iguaçu Falls, Brazil, 2013
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Topics addressed:

1. **The need** for quantitative dose-response analyses.

2. **Methods** to analyze exposure-response relationships & derive point of departure (PoD) metrics.

3. Points of departure (PoD) and **mechanistic** considerations regarding **“thresholds”**.

4. Approaches to define exposure-related risks and **“regulatory limits”** (e.g., PDE, TDI, ADI etc).

5. Empirical **relationships** between **Genotoxic Potency** (Mutation or Chromosomal Damage) and **Carcinogenic Potency**.

6. Issues pertaining to **extrapolations** across test systems and species.
Working Premise

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

2. Quantitative analyses of genetic toxicology results can provide metrics for improved risk characterization.
Point of Departure Preference

1. The working group critically examined and considered numerous PoD metrics.

2. Detailed examination of the benchmark dose (BMD), the NOGEL, and estimation of a PoD from a bilinear model → preference for the BMD method.
Breakpoint Dose and Benchmark Dose modelling - MNU gene mutation dataset

<table>
<thead>
<tr>
<th>Bilinear Models</th>
<th>Benchmark Dose Approaches</th>
</tr>
</thead>
<tbody>
<tr>
<td>L&amp;L</td>
<td>BMD_{15D} (BMDS)</td>
</tr>
<tr>
<td>segmented</td>
<td>BMD_{10} (PROAST)</td>
</tr>
</tbody>
</table>

**Monroe HPRT, Spleen**

- NOGEL

**Break-Point Dose (BPD, BPDL)**

**Bench-Mark Dose (BMD, BMDL)**

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*Gollapudi, Johnson et al., (2014) EMM, DOI: 10.1002/em.21727*

*Johnson, Soeteman-Hernández, Gollapudi et al., (2014) EMM, DOI: 10.1002/em.21870, FREE*
## Breakpoint Dose and Benchmark Dose modelling - MNU gene mutation dataset

<table>
<thead>
<tr>
<th>Study</th>
<th>Type</th>
<th>Endpoint</th>
<th>Response transformation</th>
<th>Trend test</th>
<th>Slope &lt; NOGEL</th>
<th>Linear &lt; NOGEL</th>
<th>NOGEL Test</th>
<th>L &amp; L BPDL</th>
<th>mgcv</th>
<th>segmented</th>
<th>BMDS</th>
<th>BMDS&lt;sub&gt;1SD&lt;/sub&gt;</th>
<th>PROAST BMDL&lt;sub&gt;10&lt;/sub&gt;/BMDL&lt;sub&gt;10&lt;/sub&gt;</th>
<th>Units</th>
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<tbody>
<tr>
<td>Monroe (1998) HPRT_Spleen Mouse</td>
<td>vv</td>
<td>GM</td>
<td>LogR</td>
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<td>0</td>
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<td>GM</td>
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<td>LogR&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>0.26</td>
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<tr>
<td>Bryce (2010) TK6_Human Expt 2</td>
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<td>NA</td>
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<td>no BPDL</td>
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<td>0.008</td>
<td>2.80</td>
<td>0.80</td>
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</tr>
</tbody>
</table>

**v v, in vivo; vt, in vitro; NA, not applicable; ID, insufficient doses; GM, gene mutation; MN, micronucleus; No DR, no dose response, BMS, Bristol-Myers Squibb unpublished data; SI, small intestine; +, positive gradient; NOGEL, no observed genotoxic effect level; BPDL, breakpoint dose; BMDS, breakpoint dose lower confidence interval; STD, slope transition dose; STDL, slope transition dose lower confidence interval; BMDL<sub>1SD</sub>, benchmark dose 1 standard deviation lower confidence interval; BMDL<sub>10</sub>, benchmark dose 10 lower confidence interval, BMDU<sub>10</sub>, benchmark dose 10 upper confidence interval; L&L, Lutz and Lutz, 2009.**

*Underlined PoD values were obtained after dropping high dose(s).*

*Poor fit for benchmark dose model, P < 0.05.*

*Doses log transformed as well.*

Response Transformation, same number added to ‘R’ to ensure all responses were above the value of 1 before transformation with Log or Sqrt.

'Slope<NOGEL' tests whether slope up to and including the NOGEL differs significantly from zero.

'Linear<NOGEL' tests whether slope up to and including the NOGEL is fit better by linear or nonlinear model (i.e., smoothing regression spline).*
<table>
<thead>
<tr>
<th>PoD</th>
<th>Full Name</th>
<th>Definition</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOGEL</td>
<td>No Observed Genotoxic Effect Level</td>
<td>Highest dose with no statistically significant response.</td>
<td>Easy to determine, analogous to NO(A)EL.</td>
<td>Dependent on study design, low power tends to provide larger PoDs.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Td or</td>
<td>Threshold or Breakpoint Dose</td>
<td>Estimate of threshold dose.</td>
<td>Lower power tends to provide smaller PoDs (conservative), appropriate for some MOAs.</td>
<td>Single functional form, ability to define BPD highly dependant on study design.</td>
</tr>
<tr>
<td>BPD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Benchmark Dose (e.g., BMD$<em>{10}$ or BMD$</em>{15D}$)</td>
<td>Dose associated with a specific response known as Benchmark Response (BMR)</td>
<td>Lower power tends to provide smaller PoDs (conservative), flexible methodology and functions, comparable to analyses for other endpoints, requires fewer doses.</td>
<td>Requires consensus on appropriate BMR for each endpoint. Continuous &amp; quantal data modelled differently.</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
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 and also with **low-dose sublinear** 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 & Mode of Action (MoA) Considerations

• **Mutations** generally considered to be **deleterious** and establishment of **exposure limits** will **minimise** the **risk** of adverse effects associated with genetic damage.

• **Quantitative approaches**, including dose-response modelling, should proceed incrementally and be **consistent** with available **MoA** information.

• A **number of factors** need to be **considered** when scrutinising the nature of the dose-response (e.g., linear, sub-linear, etc.).

  – Factors include (i) **non-DNA targets**, (ii) **DNA repair capacity**, (iii) **detoxification capacity**, (iv) **disruption of DNA synthesis and cell cycle progression**, (v) **ADME limiting target exposure**, (vi) **nucleotide pool disturbance**, (vii) **structural similarities to well-documented compounds**, **observation of collateral damage**). Examples provided in 2nd manuscript.

• Subsequently – **use PoD** to estimate a **level** of exposure associated with **negligible risk**.
Extrapolations – Experimental Observations of Genetic Toxicity to Human Risk

- For *in vivo* results – comprehensive ADME/PK analyses can reduce the need for uncertainty factors.
- For *in vitro* results – “acceptable” quantitative extrapolation is considerably more complex and problematic.
  - Bacterial (e.g., Salmonella) mutagenicity can be useful for MoA determination and potency ranking within a structural class of chemicals.
  - Mammalian assay results can be used to support *in vivo* results with respect to MoA and provide evidence to support a “practical threshold”.
  - Exogenous metabolic activation systems *in vitro* – predominately facilitate cytochrome P450-mediated oxidative reactions. Limitations complicate the use of *in vitro* data for quantitative assessments of agents that require metabolic activation.
Factors to Consider Regarding Effect Uncertainty/Relevance

- Human Epidemiology
- **In Vivo** Animal Studies
- **In Vitro** Mammalian Cell Studies
- In Silico

**Endpoint**
- Mutations/MN
- DNA damage

Uncertainty when extrapolating: High

Uncertainties Regarding Exposure Assessment For Risk Estimation

- Human Exposure Data
- Exposure Data From Animal Studies
- Exposure Data From **In Vitro** Studies

**Target organ exposure**
- Plasma exposure
- Dose levels

Low

Uncertainty when extrapolating: High

Modified from a figure developed by Roland Frötschl, BfArM
Empirical Relationships Between Genotoxic Potency and Carcinogenic Potency

DMH = 5.8x
NDA = 2.8x
TCE = 1.3x

88% (23/26) of compounds had a Genetox BMDL_{10} < Tumor BMDL_{10}
Lowest Genotoxicity $\text{BMD}_{10}$ (MN or TG) versus Tumor $\text{BMD}_{10}$

Only the red points are tissue-matched
Almost half have different route of exposure

Red = tissue-matched (4/18 compounds)
Rest = lowest genotoxicity $\text{BMD}_{10}$ (TG or MN) vs. Lowest tumor $\text{BMD}_{10}$

MeIQx: Results of analysis

**DNA adduct**
- MeIQx-DNA adduct liver
  - Fukushima *et al.* (2002)
  - BMD$_{10}$: 6.41e-05 ppm
  - BMDL$_{10}$: 2e-05 ppm
  - BMDU$_{10}$: 0.00018

**Mutation**
- Mutant frequency
  - Hoshi *et al.* (2004)
  - 4 weeks: 4 weeks
  - 16 weeks: 0.41

**Preneoplastic lesion**
- GST-P $^+$ ve Foci in liver
  - Fukushima *et al.* (2002)
  - 16 weeks: 0.24
  - 32 weeks: 0.39

**Tumor**
- Liver Hepatocellular adenoma and carcinoma
  - Kushida *et al.* (1994)
  - 56 weeks corrected for less than lifetime exposure
  - Cancer: 11.4

**BMDL$_{10}$ ranking:**
- DNA adduct$_{4\text{ weeks}}$ << Mutation$_{16\text{ weeks}}$ < Foci$_{16\text{ weeks}}$ < Foci$_{32\text{ weeks}}$ < Cancer$_{56\text{ weeks}}$
Potency Comparisons – Conclusions & Limitations

1. **Encouraging** results to date, but more (good!!) data required. Need for tissue-specific and/or tissue-matched analyses?

2. **MeIQx Data** - suggests that exposure limits to protect against key genotoxic events in a cancer AOP may protect against the adverse outcome (i.e., cancer).

3. Limitations: **NEED MORE DATA!**
   1. Few compounds with good dose-response data, and often not matched for species, strain & exposure regimen.
   2. Often tissue(s) where tumor(s) was/were observed do not correspond to tissue(s) examined for genotoxicity.
   3. Few genetic toxicity endpoints with good dose-response data (micronucleus & transgenic rodent mutation assays).
   4. Rarely any supporting information on mechanism/mode of action.
Consensus Statements Manuscript #1-

Methods and metrics for defining exposure-response relationships and points of departure (PoDs). Submitted to Steering Committee March 6, 2014.

1. Generally not possible to definitely establish the existence of a true threshold for mutagenic substances.

2. For some non-DNA-reactive agents a mechanism supports a practical threshold is well accepted.

3. General preference of the QWG for BMD>NOGEL>Td (BPD). The BMDL is robust, and thus recommended for general use.

4. BMD Advantages-
   i. Analysis can be performed on studies with minimal data,
   ii. Uses the entire data set to derive BMD estimate,
   iii. Effect size (BMR) is defined in advance, and always > than zero,
   iv. Covariate analyses can be performed,
   v. Within limits, PoD minimally affected by experimental design, dose selection and dose spacing.
   vi. Confidence limits on BMDs can be derived.

5. General agreement that the NOGEL can be a suitable for genetic toxicity dose-response data.

6. General agreement that the bi-linear model could be used when mechanistic data supports the existence of a “Breakpoint” dose.
1. **General acceptance** regarding the use of *in vivo genetic toxicity* dose-response data to determine PoDs, and via the use of appropriate uncertainty factors, establish “regulatory” exposure limits below which effect is negligible to assess and manage risk.

2. Among genetic toxicity endpoints, those associated, either empirically or mechanistically, to known human diseases, should be given the most weight (i.e., MN, mutations and stable bulky adducts).

3. Endpoints should be consistent with recognized Key Events in AOPs of human diseases (where available).

4. Uncertainty factors must consider (1) species ADME differences and allometric scaling, (2) differences in study duration, (3) inter-individual variability, (4) endpoint severity, (5) PoD uncertainty.

5. MoA information can be crucial for selection of appropriate endpoints and extrapolation methods. May reduce uncertainty for extrapolation.

6. Carcinogenic potency is correlated with genotoxic potency (MN and mutation) across a range of compounds. Strength of relationship expected to improve if data are matched by species, strain, route of exposure, and cancer target tissue.

7. Quantitative extrapolation from *in vitro results* is complex and challenging - most useful for potency ranking and MoA determination.