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

International Workgroup on Genetic Toxicology (IWGT) Quantitative WorkGroup (QWG) - Foz do Iguassu, Brazil. November, 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**.

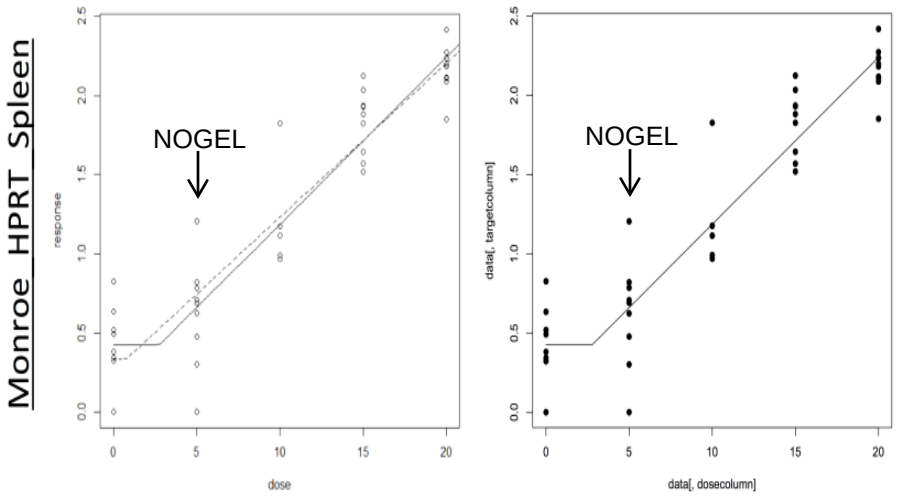
Point of Departure Preference

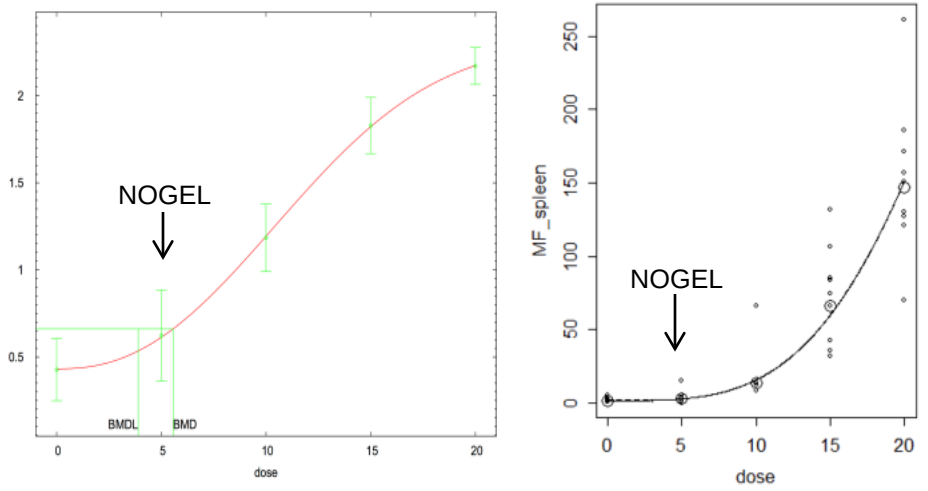
1. The working group critically examined and considered numerous **PoD metrics**.

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

Bilinear Models	Benchmark Dose Approaches
L&L segmented	BMD_{1SD} (BMDS) BMD_{10} (PROAST)
Break-Point Dose (BPD, BPDL)	Bench-Mark Dose (BMD, BMDL)





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

Study	Type	Endpoint	Response transformation	Trend test	Slope < NOGEL	Linear < NOGEL	NOGEL Test	NOGEL	L&L BPDL	mgcv STDL	segmented BPDL	BMDS	$BMDS_{1SD}$	PROAST $BMDL_{10}$	PROAST $BMDU_{10}$/ $BMDL_{10}$	Units
Monroe (1998) HPRT_Spleen Mouse	*vv*	GM	LogR	2.80E+13	0	ID	Dunnett's	5	0.78	1.40	0.98	3.90		2.06	2.32	mg/kg
Monroe (1998) LacI_Spleen Mouse	*vv*	GM	LogR	0.0005	0	yes	Dunnett's	15	8.22	no STD	10.62	11.53		5.26	3.09	mg/kg
Lynch (2011) *Pig-a*_RET Rat	*vv*	GM	Raw	0.048	NA	NA	Dunnett's	2.5	no BPD	no STD	no BPD	0.77		0.10	4.54	mg/kg
Lynch (2011) *Pig-a*_RBC Rat	*vv*	GM	SqrtR	0.02	0	yes	Dunnett's	1.25	no BPD	no STD	0.19	0.77		0.11	2.61	mg/kg
BMS *Pig-a*_RBC Rat Day 4	*vv*	GM	LogR	0.26	NA	NA	Dunn's	5	NA	no STD	NA	3.75		No DR	No DR	mg/kg
BMS *Pig-a*_RBC Rat Day 15	*vv*	GM	LogR	3.00E-06	+	yes	Dunn's	2.5	NA	no STD	NA	1.57		0.20	1.51	mg/kg
BMS *Pig-a*_RBC Rat Day 29	*vv*	GM	LogR	8.50E-10	NA	NA	Dunnett's	None	no BPD	no STD	no BPD	0.43		0.015	8.71	mg/kg
BMS *Pig-a*_RET Rat Day 4	*vv*	GM	LogR	0.53	NA	NA	Dunn's	5	NA	no STD	NA	4.33		No DR	No DR	mg/kg
BMS *Pig-a*_RET Rat Day 15	*vv*	GM	LogR	1.70E-07	0	ID	Dunnett's	0.9	no BPD	no STD	no BPD	0.74[a]		0.10	1.64	mg/kg
BMS *Pig-a*_RET Rat Day 29	*vv*	GM	LogR	3.50E-08	NA	NA	Dunnett's	None	no BPD	no STD	no BPD	0.69		0.0007	75.01	mg/kg
LeBaron (2009) PCE Rat	*vv*	MN	LogR[b]	9.20E-09	+	yes	Dunn's	1	NA	0.60	NA	0.08		0.02	2.42	mg/kg
LeBaron (2009) NCE Rat	*vv*	MN	LogR[b]	0.75	NA	NA	Dunn's	50	NA	no STD	NA	42.2[a]		No DR	No DR	mg/kg
Lynch (2011) PCE_Event 1 Rat	*vv*	MN	Raw	4.50E-06	+	yes	Dunnett's	0.9	0.20	no STD	0.27	0.16		0.10	1.37	mg/kg
Lynch (2011) PCE Event 2 Rat	*vv*	MN	LogR	6.10E-07	+	yes	Dunnett's	0.6	0.15	no STD	no BPD	0.16		0.13	3.03	mg/kg
Lynch (2011) NCE_Event 1 Rat	*vv*	MN	Raw	0.36	NA	NA	Dunnett's	2.5	no BPD	1.17	0.30	1.30		0.73	3.12	mg/kg
Lynch (2011) NCE_Event 2 Rat	*vv*	MN	Raw	0.002	+	yes	Dunnett's	1.25	no BPD	no STD	no BPD	0.43		0.13	2.22	mg/kg
BMS Day 4 Rat	*vv*	MN	LogR	8.80E-09	+	ID	Dunn's	1.25	NA	0.56	NA	0.42		0.30	2.66	mg/kg
BMS Day 29 Rat	*vv*	MN	LogR	3.60E-09	NA	NA	Dunnett's	None	no BPD	no STD	no BPD	0.40		0.18	3.55	mg/kg
Doak (2007) AHH1_Human *HPRT*	*vt*	GM	SqrtR	1.00E-14	+	ID	Dunnett's	0.005	no BPD	no STD	no BPD	0.004		0.0006	1.51	µg/mL
Pottenger (2009) L5178Y_ Mouse *Tk*	*vt*	GM	LogR[b]	2.00E-08	0	yes	Dunnett's	0.69	1.01	0.49	1.03	0.83		0.61	2.03	µM
Thomas (2013) AHH1_Human *HPRT*	*vt*	GM	SqrtR	0.0004	0	no	Dunnett's	0.0075	0.002	no STD	no BPD	0.008[a]		0.006	1.33	µg/mL
Bryce (2010) TK6_Human Expt 1	*vt*	MN	LogR[b]	0	+	no	T3	1.25	NA	0.07	0.08	0.47		0.26	2.10	µg/mL
Bryce (2010) TK6_Human Expt 2	*vt*	MN	LogR[b]	4.50E-14	+	no	Dunn's	0.23	NA	no STD	NA	0.20		0.066	1.09	µg/mL
Doak (2007) AHH1_ Human	*vt*	MN	Raw	2.50E-09	+	yes	Dunnett's	0.025	no BPD	no STD	no BPD	0.008		0.003	2.80	µg/mL

vv, *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; BPD, breakpoint dose; BPDL, breakpoint dose lower confidence interval; STD, slope transition dose; STDL, slope transition dose lower confidence interval; $BMDL_{1SD}$, benchmark dose 1 standard deviation lower confidence interval; $BMDL_{10}$, benchmark dose 10 lower confidence interval, $BMDU_{10}$, benchmark dose 10 upper confidence interval; L&L, Lutz and Lutz, 2009.

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

[a]Poor fit for benchmark dose model, $P < 0.05$.

[b]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).

Johnson, Soeteman-Hernández, Gollapudi et al., (2014) EMM, DOI: 10.1002/em.21870, FREE

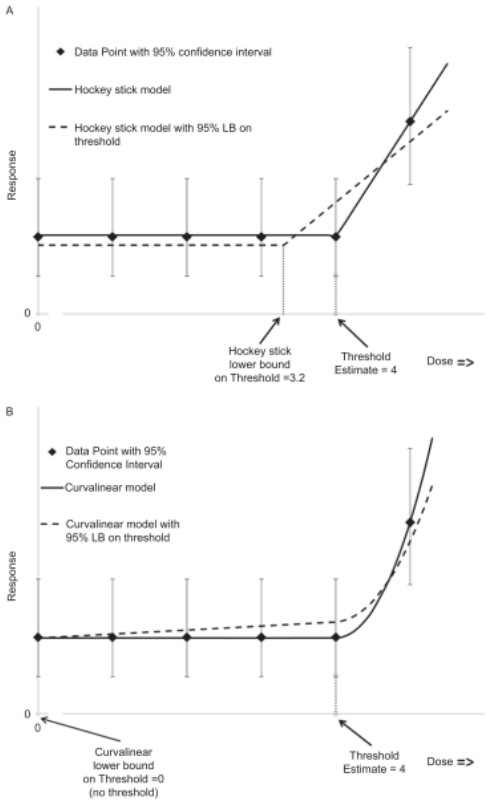
PoD	Full Name	Definition	Advantages	Disadvantages
NOGEL	No Observed Genotoxic Effect Level	Highest dose with no statistically significant response.	Easy to determine, analogous to NO(A)EL.	Dependent on study design, low power tends to provide larger PoDs.
Td or BPD	Threshold or Breakpoint Dose	Estimate of threshold dose.	Lower power tends to provide smaller PoDs (conservative), appropriate for some MOAs.	Single functional form, ability to define BPD highly dependant on study design.
BMD	Benchmark Dose (e.g., BMD_{10} or BMD_{1SD})	Dose associated with a specific response known as Benchmark Response (BMR)	Lower power tends to provide smaller PoDs (conservative), flexible methodology and functions, comparable to analyses for other endpoints, requires fewer doses.	Requires consensus on appropriate BMR for each endpoint. Continuous & quantal data modelled differently.

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.).



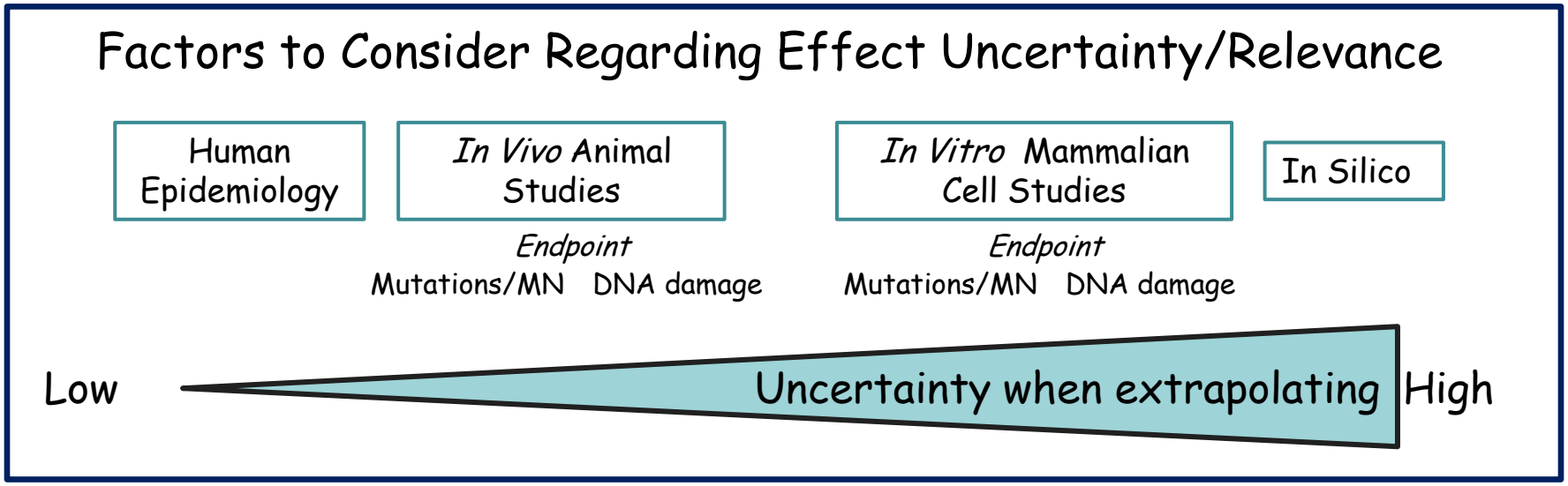
Pragmatic Approach & Mode of Action (MoA) Considerations

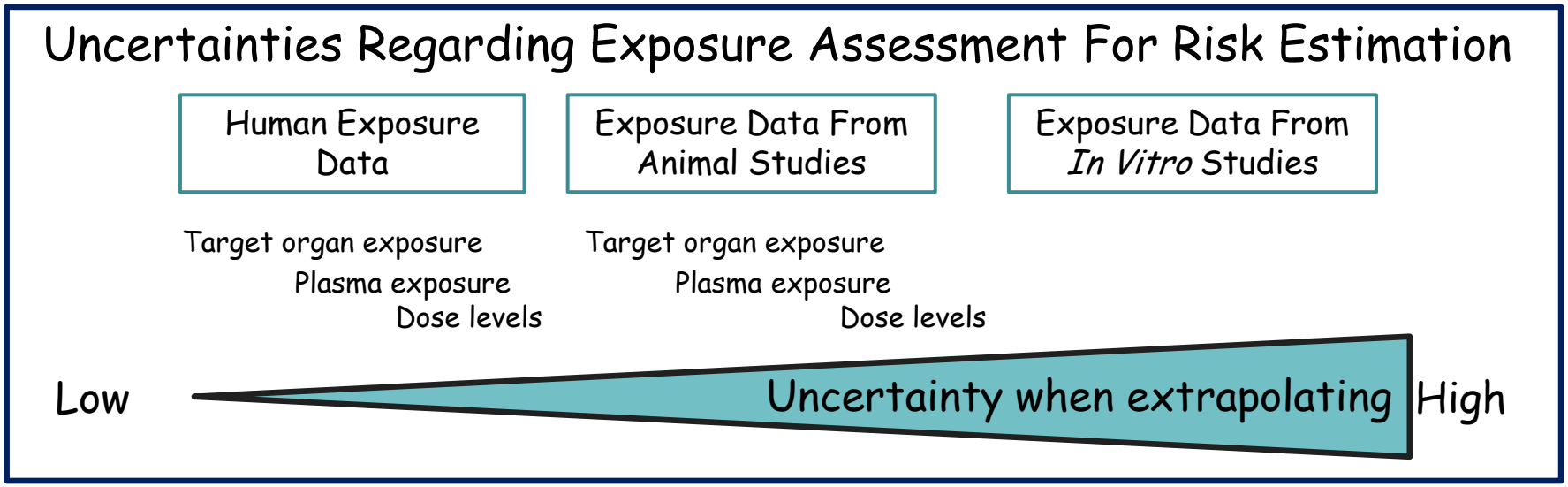
- **Mutations** generally considered to be **deleterious** and establishment of **exposure limits** will **minimise** the **risk** of adverse effects associated with genetic damage.
- **Quantitative approaches**, including dose-response modelling, should proceed incrementally and be **consistent** with **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) **mutagenicit**y 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.**

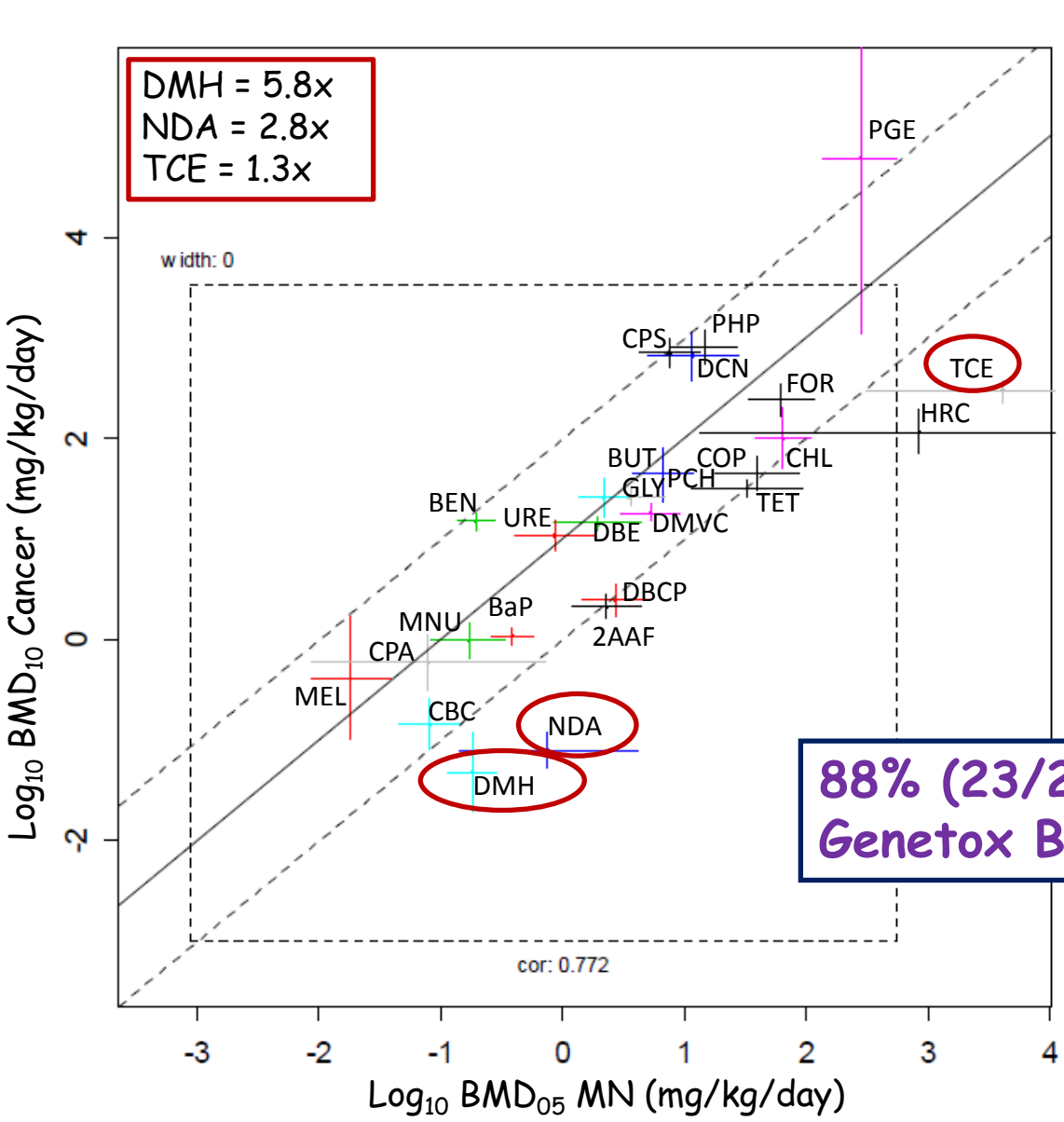
Graphical Illustration of Increasing Uncertainties Along a Phylogenetic Spectrum from Cells to Humans





Modified from a figure developed by Roland Frötschl, BfArM

Empirical Relationships Between Genotxic Potency and Carcinogenic Potency



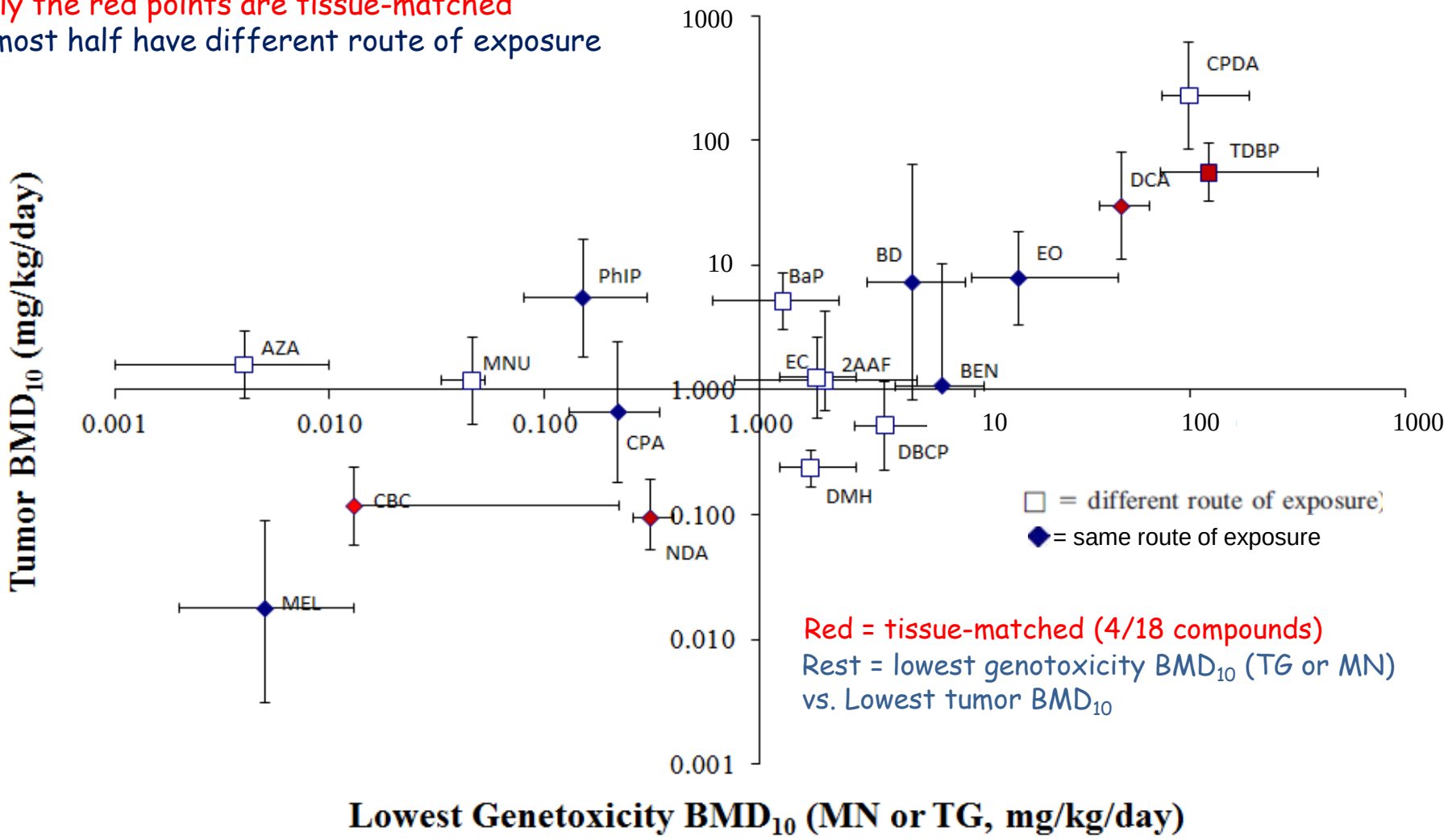
2aaf	2-Acetylaminofluorine
bap	Benzo(a)pyrene
ben	Benzene
but	1,3-Butadiene
cbc	Chlorambucil
chl	Chloral hydrate
cop	4-Chloro-o-phenylenediamine
cpa	Cyclophosphamide
cps	p,p'-Dichlorodiphenyl sulfone
dbcp	1,2-Dibromo-3-chloropropane
dbe	1,2-Dibromoethane
dcn	Decalin
dmh	1,2-Dimethylhydrazine
dmvc	Dimethylvinyl chloride
for	Chloroform
gly	Glycidol
hrc	4-Hexylresorcinol
mel	Melphalan
mnu	N-Nitroso-N-methylurea
nda	N-Nitrosodimethyl-amine
pch	p-Chloraniline hydrochloride
pge	Propylene glycol mono-t-butyl ether
php	Phenolphthalein
tce	Trichloroethylene
tet	Tetrachloroethane
ure	Urethane

88% (23/26) of compounds had a Genetox $BMDL_{10}$ < Tumor $BMDL_{10}$

26 compounds; Hernández et al. (2013)

Lowest Genotoxicity BMD_{10} (MN or TG) versus Tumor BMD_{10}

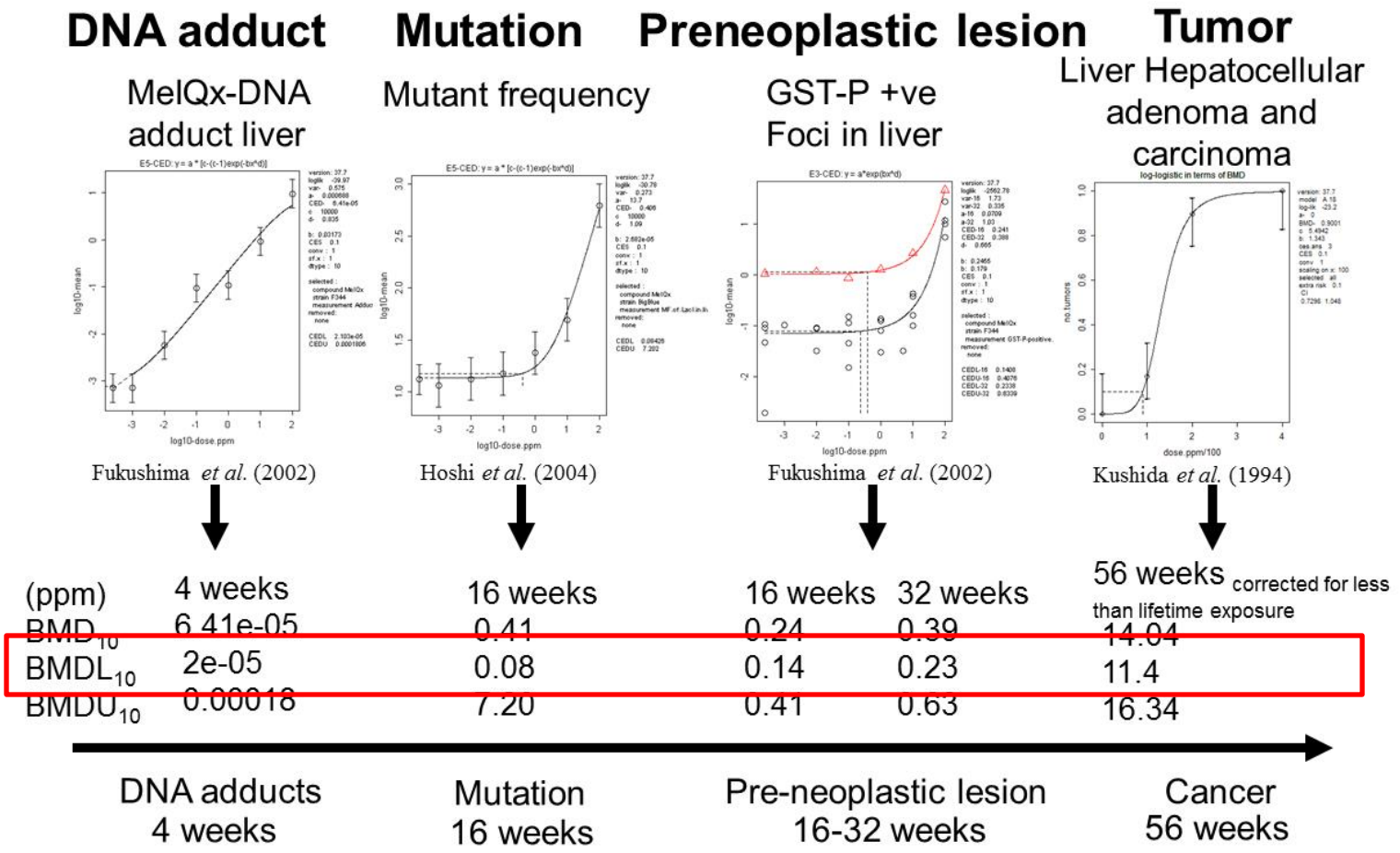
Only the red points are tissue-matched
Almost half have different route of exposure



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

18 compounds; Hernández et al. 2011. *Environ Molec Mutagen* 52:518-528.

MeIQx: Results of analysis



$BMDL_{10}$ ranking:

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

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.

Consensus Statements Manuscript #2-

Use of Point-of-Departure (PoD) metrics in defining acceptable exposure limits and assessing human risk. Submitted to SC June 26, 2014.

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 is 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.