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Quantitative Analyses of Genetic Toxicity Dose-Response Data – From Potency Determination to Risk Assessment

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YOUR HEALTH AND SAFETY... OUR PRIORITY.

Acknowledgements (Co-investigators)

Health Canada Santé Canada

Swansea University
Prifysgol Abertawe

Ali Long

John Wills

George Johnson - Swansea

CHEMICALS MANAGEMENT PLAN

PLAN DE GESTION DES PRODUITS CHIMIQUES

Roche

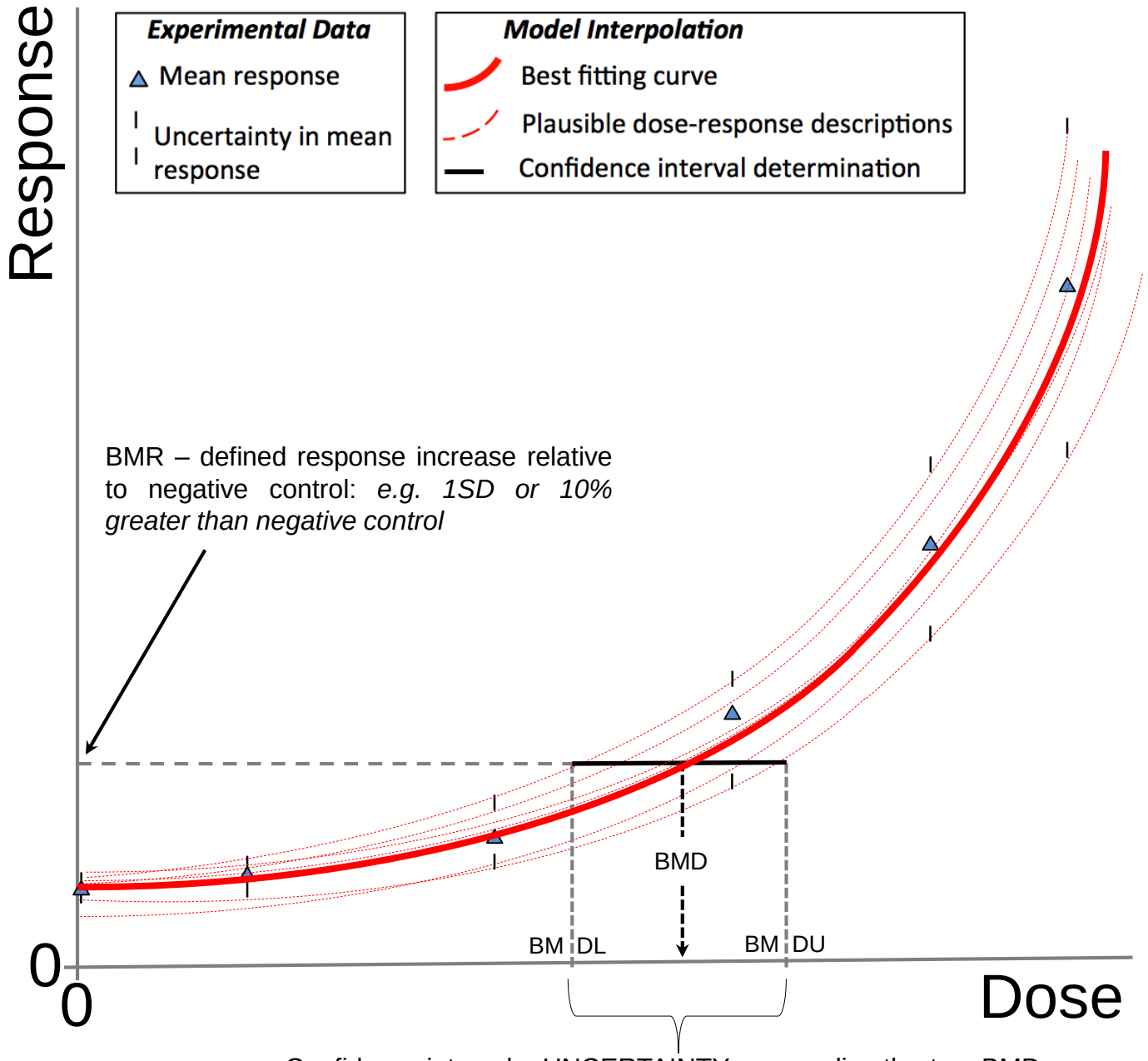
Wout Slob RIVM

National Institute for Public Health and the Environment
Ministry of Health, Welfare and Sport

If we recognize that

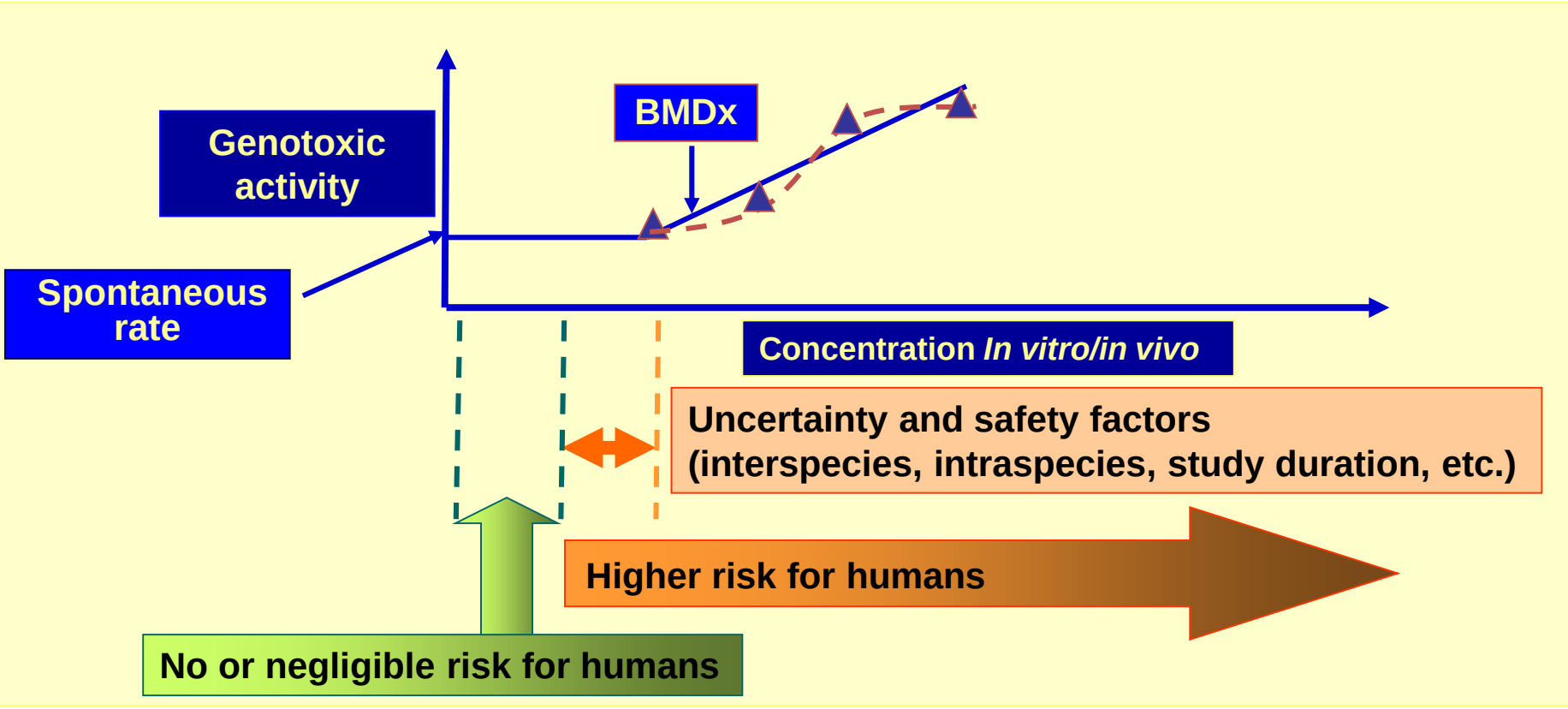
(1) Mutation is a Relevant Toxicological Endpoint;
(2) Dose-response Patterns for Genotoxic Substances are Distinctly Non-linear ("Thresholded")

Wouldn't it make sense to develop quantitative methods to calculate dose-response Reference Points (PoD) that can be used to determine human exposure limits, and/or Margin of Exposure (MOE) values (i.e., HBGVs), that can in turn be used for risk assessment and regulatory decision-making?



Confidence interval = UNCERTAINTY surrounding the *true* BMD:
e.g. in the dose that causes a response 10% greater than negative control

Conceptual Framework

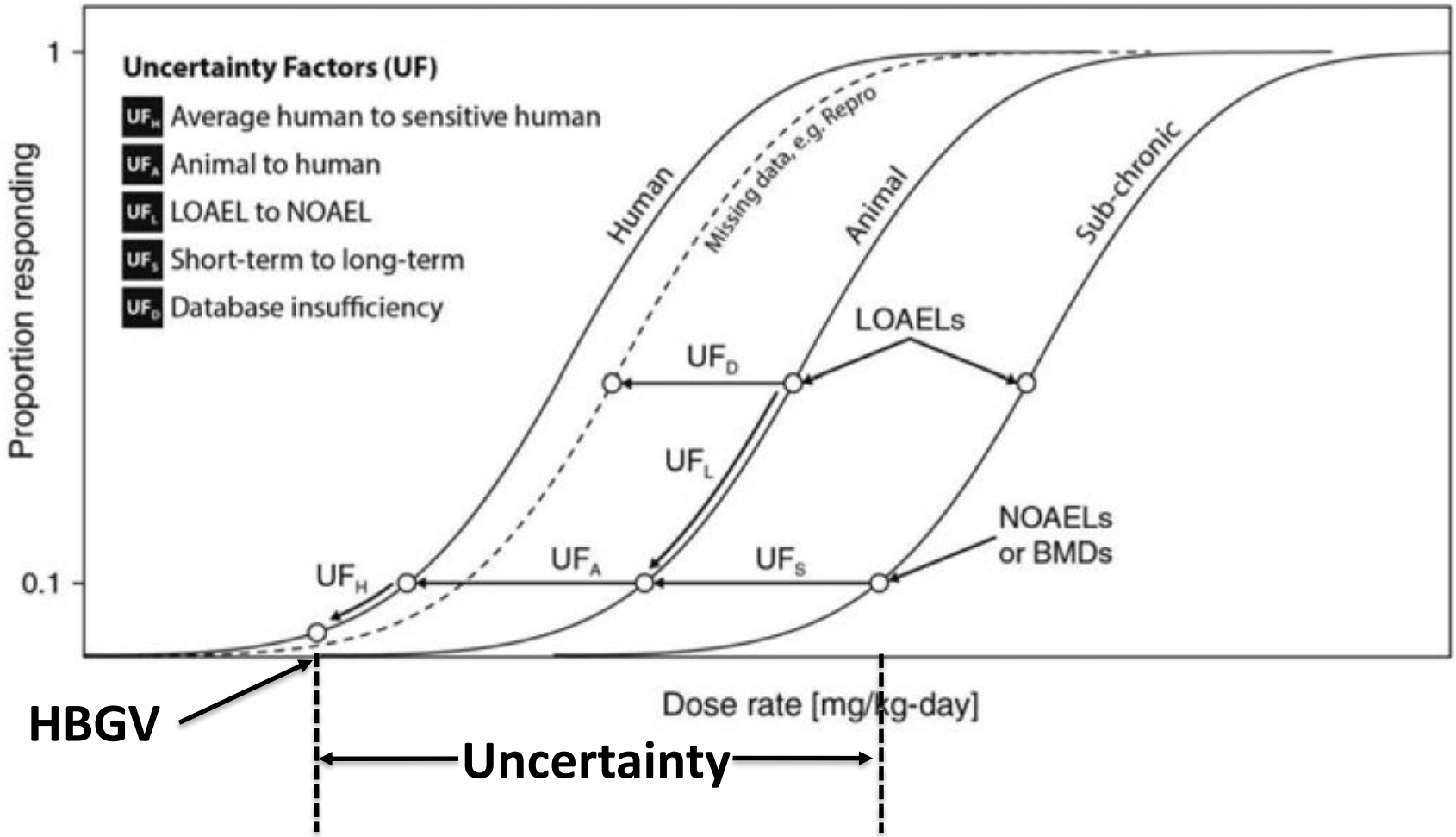


- **Assumption: At low doses cellular protection mechanisms are efficient and not saturated; response indistinguishable from spontaneous/background.**

Adapted from a figure developed by Veronique Thybaud.

Use of Genetic Toxicity BMDs for Calculation of Health-based Guidance Values (HBGVs), i.e., human exposure limit values such as TDI, ADI, RfD, OEL, PDE

The Benzo[*a*]pyrene Case Study



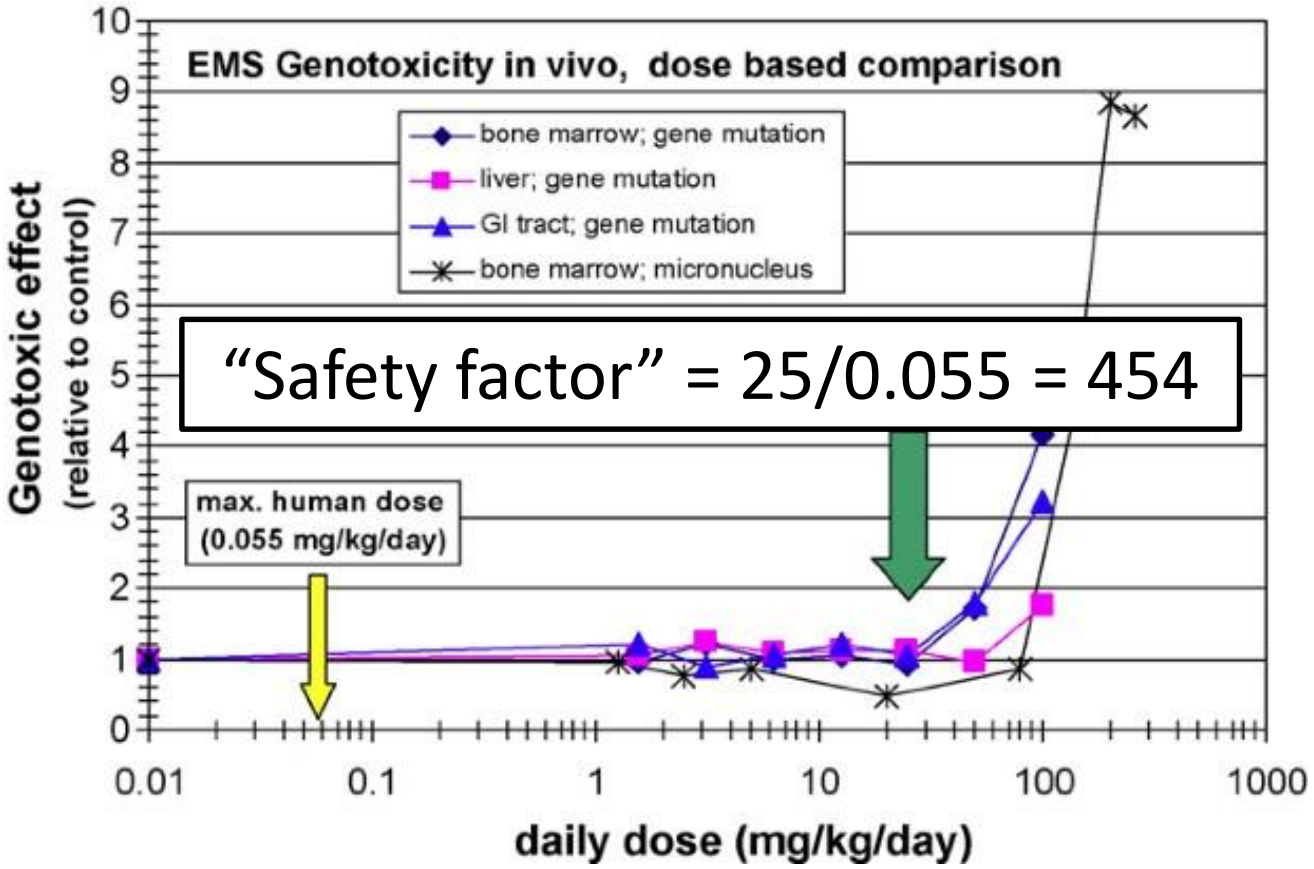
UF_A – Animal to Human, UF_H – Inter-individual human,
UF_L – Absence of NOAEL, UF_S – study duration, UF_D – Database insufficiency

HBGV (Health-based Guidance Value) – TDI, ADI, RfD, PDE, OEL

Source: Dankovic et al. (2015) *J Occup Environ Hyg* **12**:S55-S68.

Low-Dose *In Vivo* Mutagenicity of EMS

MutaMouse 28-day oral



Source: Gocke and Muller, *Mutat Res* **678**:101-107, 2009

Calculation of an "Exposure Limit" for Regulatory Decision-making (e.g., the PDE)

PDE (Permissible Daily Exposure)- detailed in ICH Harmonised Guideline Q3C(R5) "acceptable amounts of residual solvents and other impurities in pharmaceuticals" (conceptually similar to ADI, TDI)

Safety factors, Modifying factors, Uncertainty factors

F1: Extrapolation between species	**(2-12 allometric scaling)**
F2: Interindividual variability	**(10 humans)**
F3: Study duration	**(1= ≥half lifetime, 10=short)**
F4: Severe toxicity	**(10 genotoxic)**
F5: Variable factor	**(NOEL = 1, only LOEL reached = 10)**

$$PDE = \frac{NOEL \times Weight\ Adjustment}{F1 \times F2 \times F3 \times F4 \times F5}$$

From G. Johnson, based on info provided Andreas Zeller (Roche).

Muta™Mouse TGR *lacZ* assay GI Tract results (i.e., small intestine) used to determine "Safety Factor" and PDE (Permitted Daily Exposure).

Gocke et al., 2009. *Tox Lett*. 678:101-107.

EMS *In Vivo* Genetic Toxicity (Muta™Mouse, 4 week, oral)

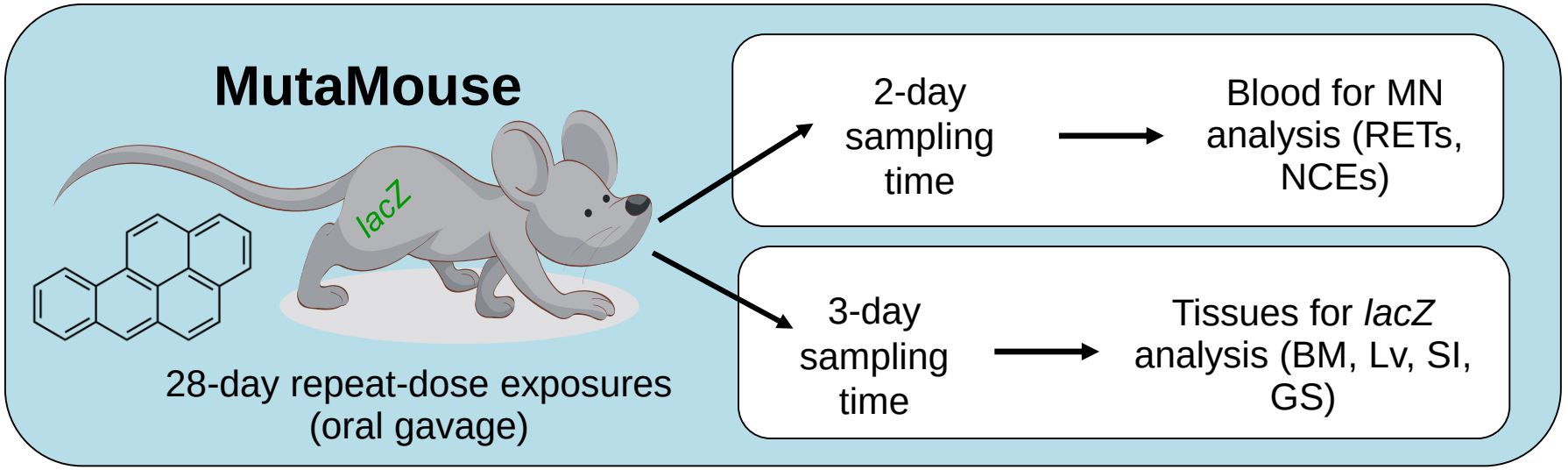
- **$NOGEL_{Mouse}$ GI Tract/Maximum Human Exposure (Viracept®) =**

  25 mg/kg / 0.055 mg/kg = 454-fold safety factor

- **$NOGEL_{mouse}$ GI tract = 25 mg/kg**

$$PDE = \frac{25\ {}^{mg}/_{kg} \times 50\ kg}{12 \times 10 \times 10 \times 10 \times 1} = 104\ \mu g/person/d = 2.1\ \mu g/kg/d$$

Muta™Mouse - Benzo[*a*]pyrene 28-Day Repeat Dose Oral

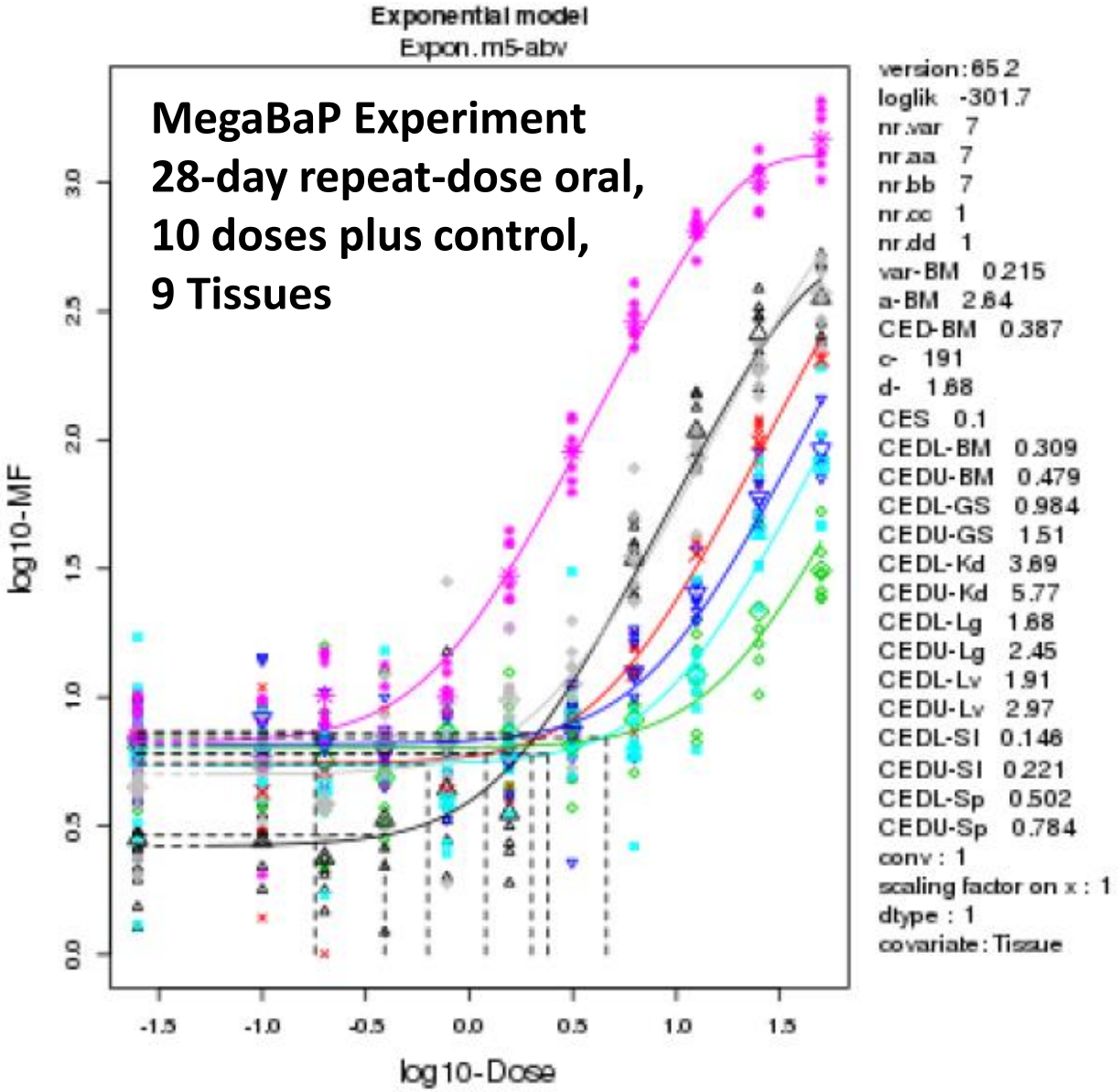


- Endpoints examined
  - *lacZ* mutations in SI, GS, BM, Liv, Lung, Kid, Spleen
  - Micronuclei and Pig-a mutations in peripheral blood.
  - DNA adducts in selected tissues (SI, BM, GS, Liv, Lung).
  - Serum chemistry and hepatic enzyme profile.
  - Immunohistochemical analyses (e.g., Ki-67, Caspase III).

Long et al., 2017. Benchmark dose analyses of multiple genetic toxicity endpoints permit robust, cross-tissue comparisons of MutaMouse responses to orally delivered benzo[a]pyrene. *Arch Toxicol.*, in press

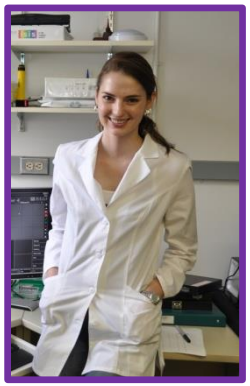
LacZ Mutant Frequency Dose-Response Analysis

BMD Combined Covariate Method in PROAST, BMR=10%

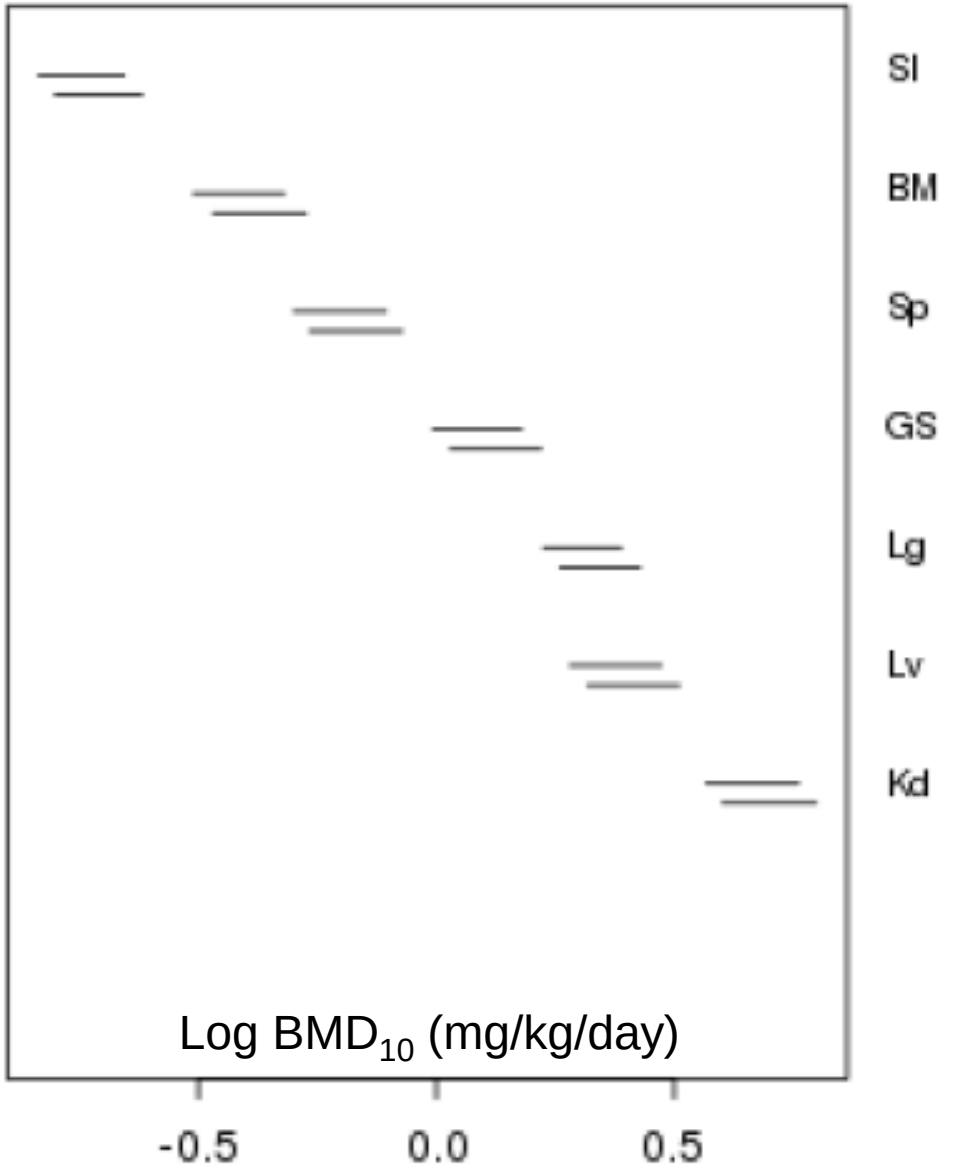


MutaMouse MegaBaP Study – Tissue Comparisons

10-dose plus control, 7 animals per dose-group, 7 tissues



Ali Long
Health Canada



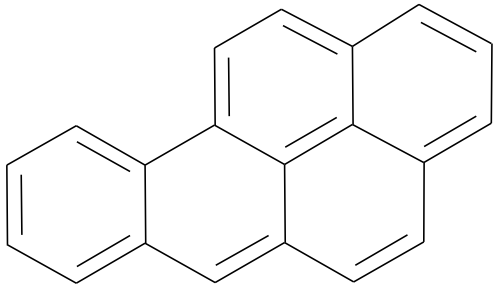
- **BMD_{10} values across tissues vary ~1.5 orders of magnitude**
- Which value is appropriate for human health risk assessment and calculation of HBGV (MOE, PDE etc.)?

Determining Human Exposure Limits for BaP Based on Muta™Mouse *In Vivo* Mutagenicity Study

- Lowest BMD_{10} (small intestine) – 0.26 mg/kg/day
  $BMDL_{10}$ = 0.20, $BMDU_{10}$ 0.34

- Allometric Scaling Factor (FDA, 2005) = 0.081 for mouse
- Human-equivalent dose, assuming 60kg = 0.97 – 1.65 mg/person/day

- Additional Uncertainty/Adjustment Factors
  = 10 interindividual x 10 study duration
  x 10 Effect Severity = 1000
- Could be argued that it should be, for example, ~300



Benzo[*a*]pyene

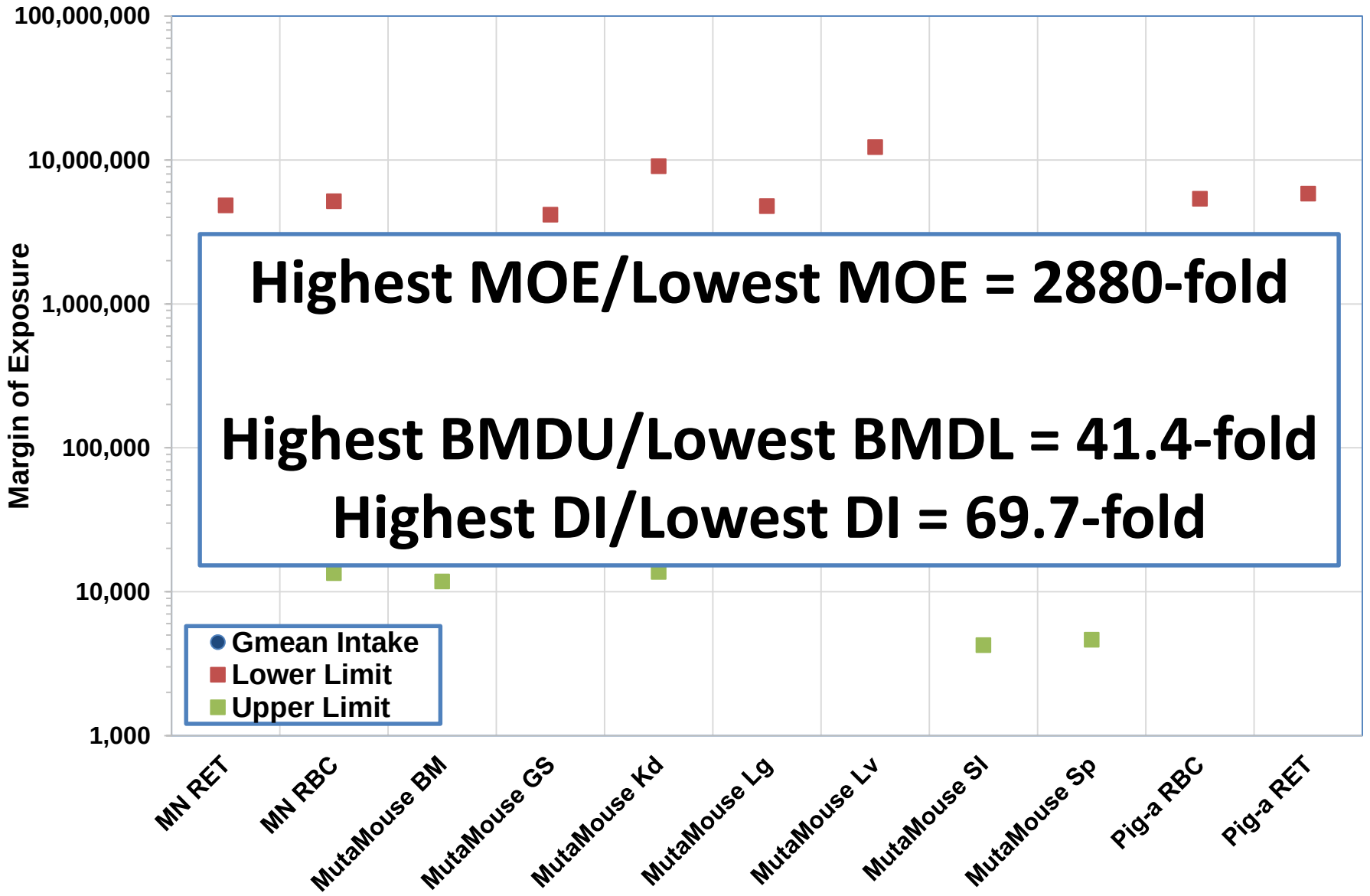
- Tolerable Daily Intake Estimate = 0.97 – 1.65 µg/person/day

- USA Dietary Intake for BaP (IARC Monograph 92, etc., 5 studies) =
- 0.04 – 2.8 µg/person/day (Geom. Mean = 0.29 µg/person/day)

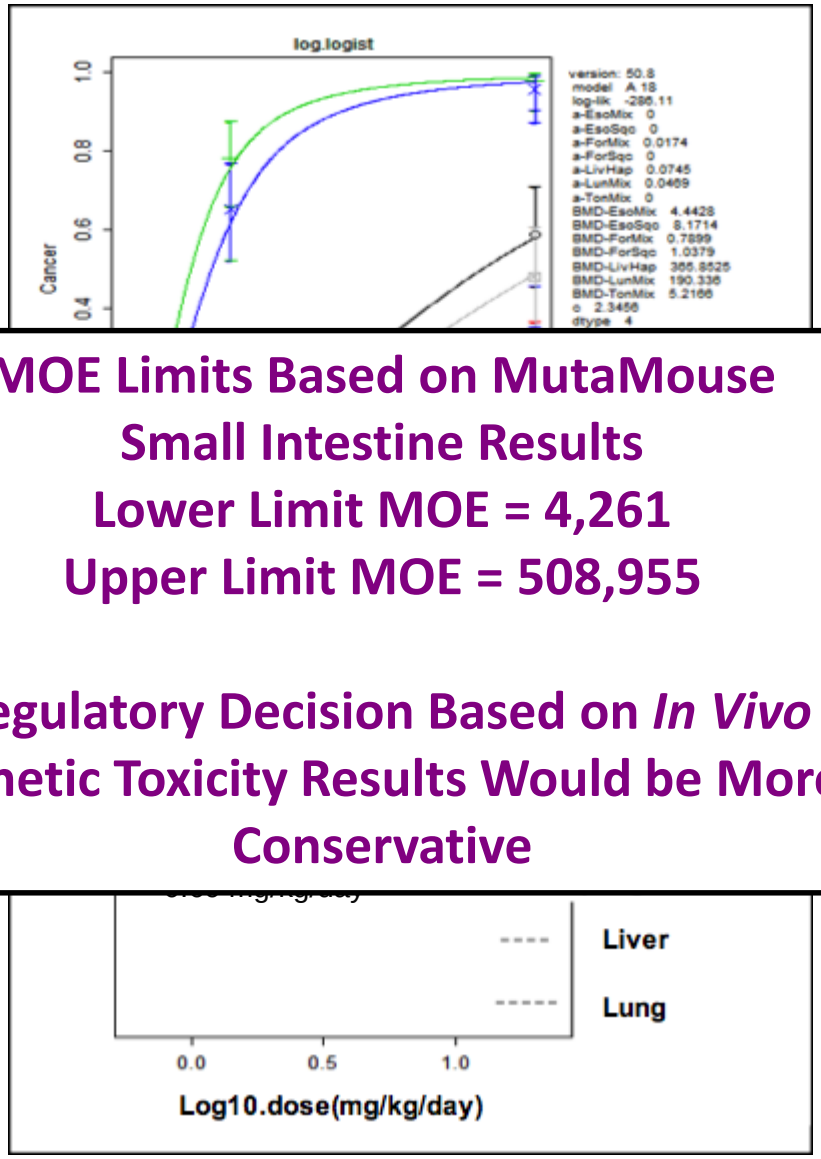
- MOE Limits (BMDL/upper DI to BMDU/lower DI) = 4,261 – 508,955

Long et al., 2017. Benchmark dose analyses of multiple genetic toxicity endpoints permit robust, cross-tissue comparisons of MutaMouse responses to orally delivered benzo[a]pyrene. *Arch Toxicol*., in press

Benzo[*a*]pyrene MOE Values Calculated Using BMD_{10}
(10% Above Study Control)



Comparison with MOE Values Based on Cancer $BMDL_{10}$ Values



MOE Limits Based on MutaMouse Small Intestine Results
Lower Limit MOE = 4,261
Upper Limit MOE = 508,955

Regulatory Decision Based on *In Vivo* Genetic Toxicity Results Would be More Conservative

Data from Gold Carcinogenic Potency database (CPDB)

Log logistic modelling with BMR = 10% extra risk

BMD-Covariate (PROAST) modelling across tissues

MOE based on Forestomach (most sensitive tissue)

$BMDL_{10}$ sqc = 0.69 mg/kg/day

$BMDU_{10}$ sqc = 1.41 mg/kg/day

Lower limit MOE = 12,321

Upper limit MOE = 1,762,500

BMD analysis courtesy of George Johnson, Swansea University College of Medicine

Routine Use of Genetic Toxicity BMDs for Human Health Risk Assessment (HHRA)

Two Tough Nuts to Crack!

1. Definition of Endpoint-specific CES Values.
2. Identification of Suitable UFs.

Approaches for Selecting a Benchmark Response (BMR)

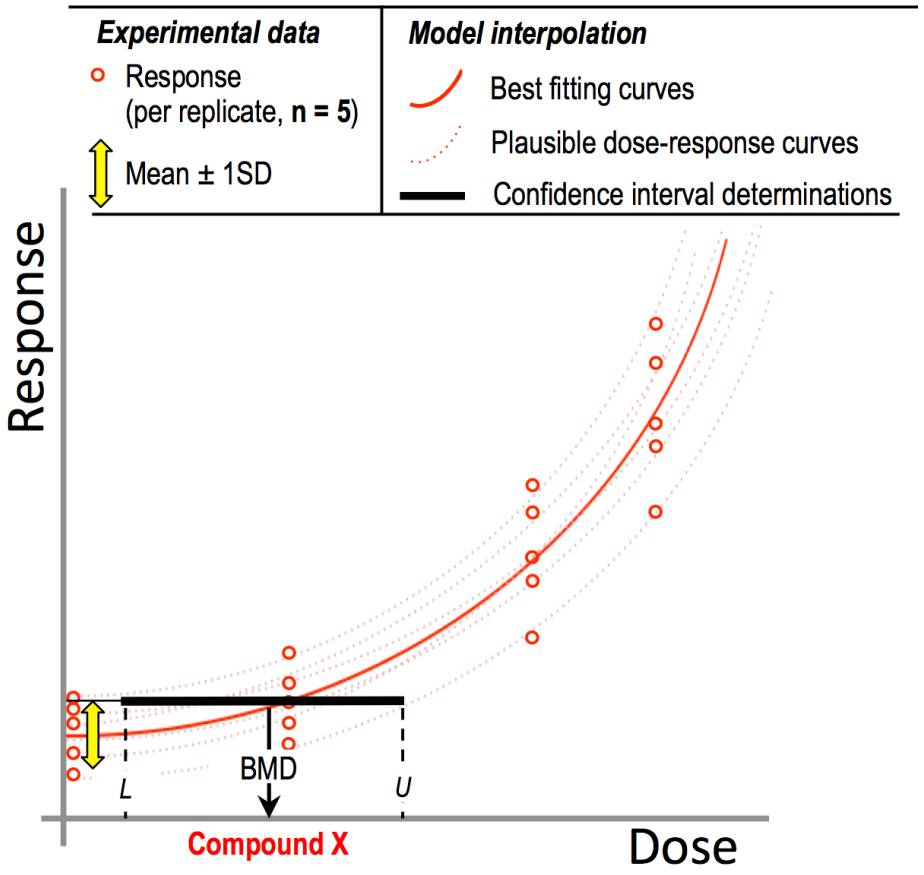
1. **Percentage increase** relative to control group mean ($BMD_{\%}$).
2. Control group mean plus one control group **standard deviation** (BMD_{1SD}).
3. Other approaches (e.g., Zeller et al., 2017; Slob, 2016).

Percentage increase – well-suited for comparisons across compounds or other covariates.

- Choice of BMR percentage is unimportant since comparisons across covariates (e.g., compound, cell type, sampling time, etc.) remain stable across different BMR percentages.

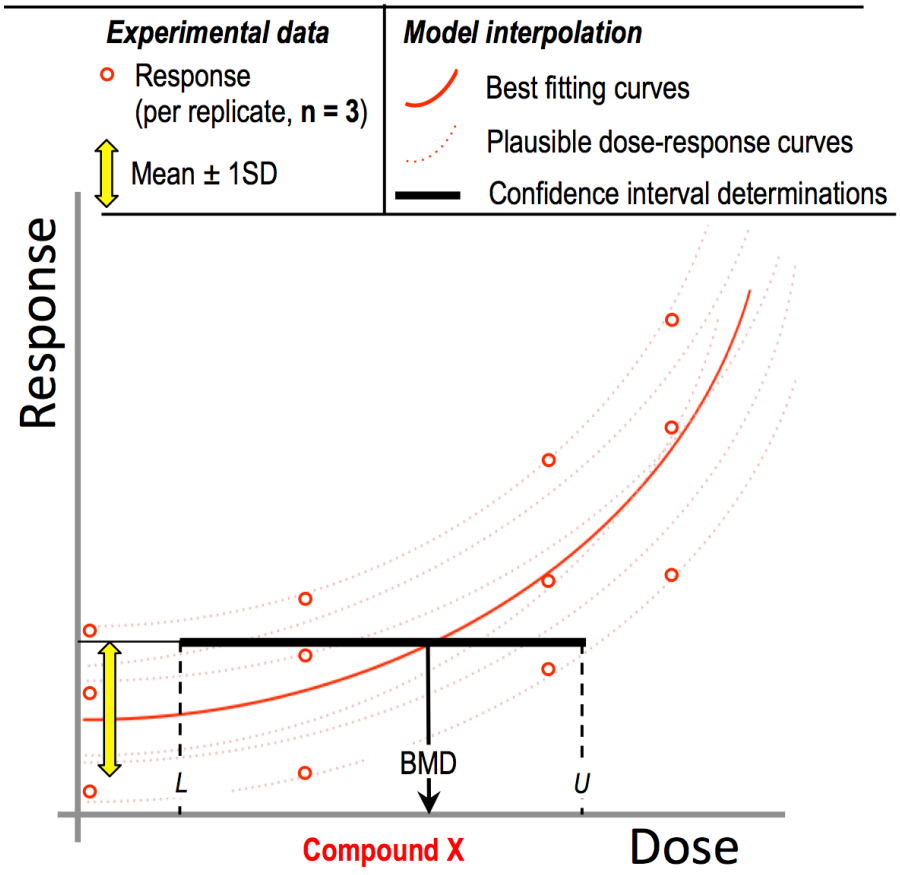
Scrutiny of the Study-specific BMD_{1SD} Approach

Compound X - 'high quality' DR data



Compound X - 'Poorer quality' DR data

Doubled control-group SD size



Dangerous precedent - poor dose-response data yields a *less conservative* (i.e., larger) BMD

Table 1 Formulas used to estimate CES

Nr	Name	Formula	Data
1	$CESISD_{study}$	$\frac{\bar{x}+sd}{\bar{x}} - 1$	Mean and SD of the concurrent study
2	$CESISD_{hc}$	$\frac{\bar{x}_{hc}+sd_{hc}}{\bar{x}_{hc}} - 1$	Mean and SD of all historical control (hc) data in a lab

Arch Toxicol (2017) 91:3799–3807
https://doi.org/10.1007/s00204-017-2037-3

REGULATORY TOXICOLOGY

An appraisal of critical effect sizes for the benchmark dose approach to assess dose–response relationships in genetic toxicology

Andreas Zeller[1] · Gonzalo Duran-Pacheco[1] · Melanie Guérard[1]

Nr of Samples with study id info	1207	359	253	113	128	–	–	77	41	1010	1010	202
Nr of Studies	195	54	44	19	21	–	–	13	7	33	33	18
$CESISD_{study}$												
Min	0.07	0.02	0.10	0.11	0.10	–	–	0.17	0.14	0.52	0.66	0.15
Max	1.73	1.25	1.06	0.55	0.50	–	–	0.84	0.40	3.62	3.94	0.87
Mean	0.50	0.09	0.31	0.30	0.30	–	–	0.52	0.25	1.44	1.74	0.39
$CESISD_{hc}$ [b]	0.56	0.70	0.88	0.44	0.51	0.84	0.75	0.73	0.55	2.13	2.48	0.53
$CESISD_{thc}$ [b]	0.49	0.34	0.75	0.39	0.40	0.66	0.66	0.57	0.55	0.76	1.17	0.42
$CESIMAD_{hc}$ [b]	0.64	0.35	0.77	0.42	0.45	0.85	0.80	0.55	0.65	0.67	1.24	0.45
$CESISD_{WBhc}$ [b]	0.61	0.72	0.64	0.47	0.54	–	–	0.76	0.61	2.14	2.53	0.53
ICC[a]												
Min	0.03	0.10	0.53	0.41	0.61	–	–	0.26	0.79	0.04	0.01	0.38
Max	0.46	0.78	0.75	0.59	0.61	–	–	0.26	0.79	0.07	0.35	0.38

Transgenic Rodent (TGR) *In Vivo* Gene Mutation Assays

CES Determined Using Mean and Standard Deviation of Trimmed Historical Control Values

Transgenic Rodent	Transgene	N	HC-trimmed
MutaMouse	cII	105	0.49
MutaMouse	lacZ	1408	0.47
BigBlue Mouse	cII	327	0.71
BigBlue Mouse	lacI	435	0.74
BigBlue Rat	cII	216	0.31
BigBlue Rat	lacI	262	0.74
lacZ Plasmid Mouse	lacZ	222	0.26
Arithmetic Mean			**0.53**

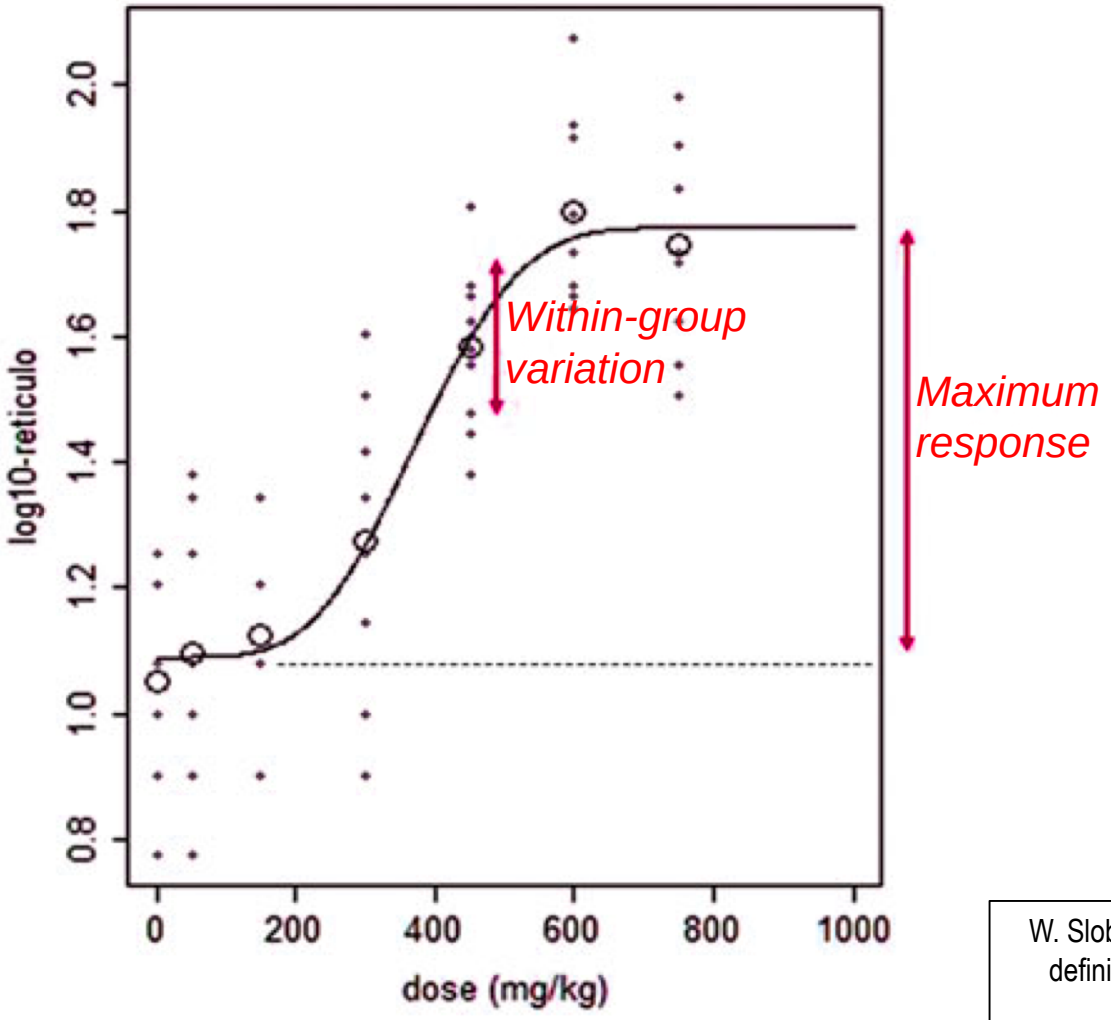
Transgenic Rodent (TGR) *In Vivo* Gene Mutation Assays

Tissue-specific CES Determined Using Mean & Standard Deviation of Trimmed Historical Control Values

Transgenic Rodent	Transgene	Tissue	N	HC-trimmed
BigBlue Mouse	cII	Liver	76	0.61
BigBlue Mouse	cII	Lung	79	0.60
BigBlue Mouse	lacI	Liver	167	0.51
BigBlue Mouse	lacI	Spleen	50	0.87
BigBlue Ra				43
BigBlue Ra				73
BigBlue Ra				60
Plasmid Mouse	lacZ	Liver	95	0.23
MutaMouse	lacZ	Bone Marrow	285	0.42
MutaMouse	lacZ	Liver	384	0.39
MutaMouse	lacZ	Lung	92	0.24
MutaMouse	lacZ	Small Intestine	92	0.22
MutaMouse	lacZ	Spleen	52	0.41
MutaMouse	lacZ	Stomach	54	0.28

Mean CES for TGR Liver (across assay variants) = 0.48

Defining Endpoint-specific Benchmark Response (BMR) Values
Scaling According to Maximum Response of Each Endpoint



Consideration of 27 (geno)toxicity endpoints across ~450 studies demonstrated a relationship between **within-group** variation and **maximum response**

Requires knowledge of **typical within-group variance** – *estimated across large numbers of studies*

W. Slob. 2016. A general theory of effect size, and its consequence for defining the benchmark response for continuous endpoints. *Critical Reviews in Toxicology* **47(4)**:342-351.

Table 1. Estimated maximum response (*M*) and within-group standard deviation (*s*) in 27 biological parameters. See Supplementary Material for more details on the underlying data.

Endpoint	Number of studies	*s**	*s*, LB†	*s*, UB†	*M*	*M*, LB†	*M*, UB†
AChE (acetylcholinesterase)	32‡	0.186	0.179	0.192	6.06	5.65	6.51
ALT	4	0.480	0.447	0.520	42	30	69
ASAT	1	0.164	0.145	0.190	2.36	1.69	Inf
BW							
Ce							
Ce							
Ch							
Cy							7
DI							
DI							
Fe							
He							
IF							
Ki							
La							
Li							
Li							
MN (micronucleus) counts	139‡	0.640	0.616	0.665	24.57	18.91	33.75
Neutrophils	2	0.726	0.598	0.922	490	110	579
PCO (palmitoyl CoA oxidase)	1	0.190	0.179	0.202	9.67	5.30	153
Red blood cells (counts)	5	0.063	0.058	0.070	1.45	1.28	4.17
RBC mutants	7	0.566	0.520	0.608	94	64	138
Reticulocytes	1	0.490	0.436	0.566	5.77	4.43	12.3
Spleen.weight	1	0.112	0.095	0.135	2.59	2.13	Inf
Spleen weight/BW	1	0.118	0.105	0.138	5.20	1.80	Inf
Thymus weight/BW	1	0.313	0.272	0.367	6.25	4.48	19.23
Urinary volume	1	0.200	0.176	0.230	5.52	2.10	156

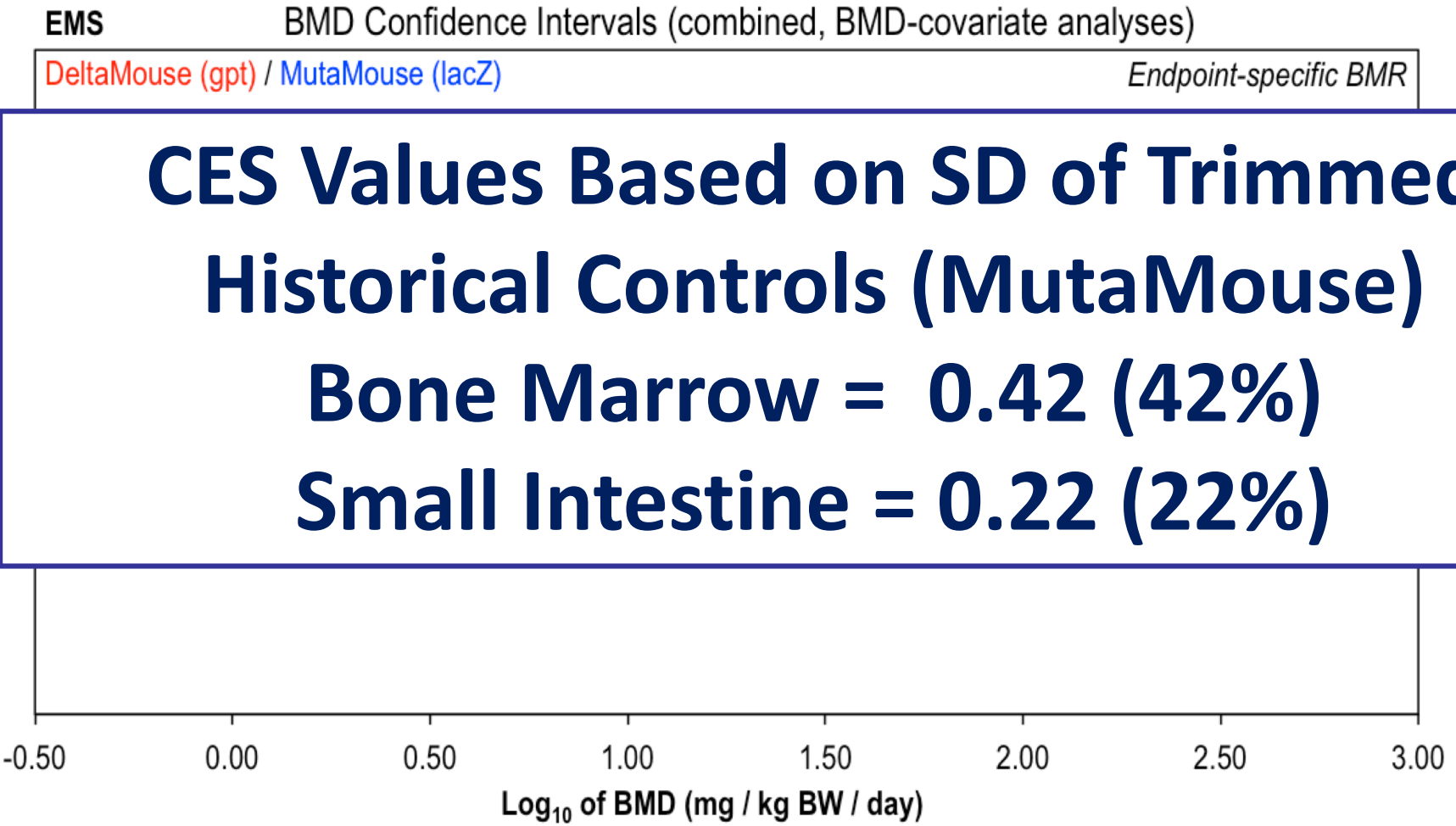
CES Value for MN Endpoint (based on 139 studies)

=

$M^{1/8} = 24.57^{1/8} = 1.49$ (i.e., 49%)

Need to have data for a large number of studies

Endpoint-specific Effect Sizes to Compare *gpt* delta Mouse and Muta™Mouse (*lacZ*) EMS Dose-response Data Across Tissues



CES Values Based on SD of Trimmed Historical Controls (MutaMouse)

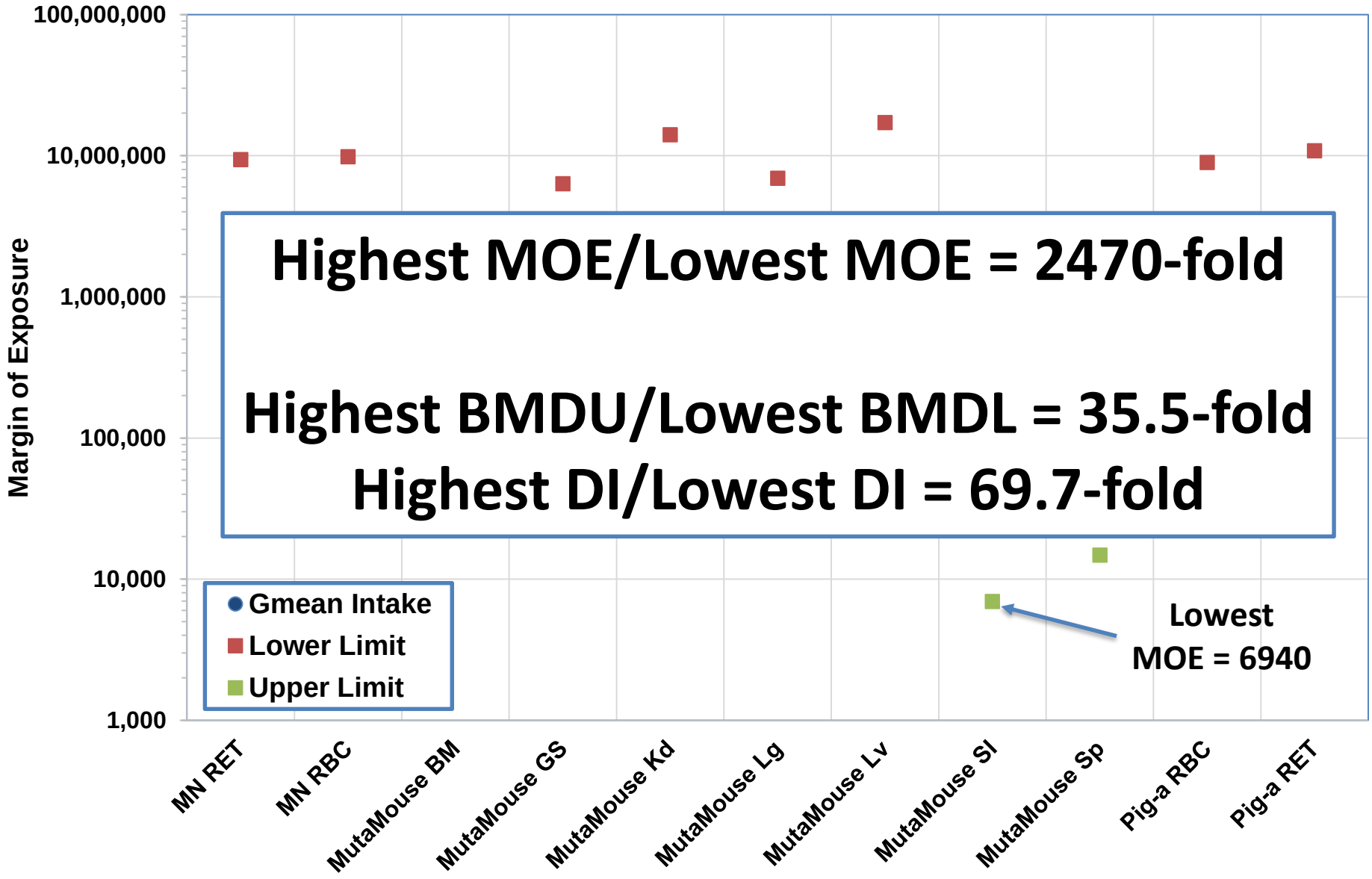
Bone Marrow = 0.42 (42%)

Small Intestine = 0.22 (22%)

*** BMRs based on a small number of studies – preliminary estimates***

Source: Wills et al. (2017) *Environ. Molec Mutagen*. 58:632-643.

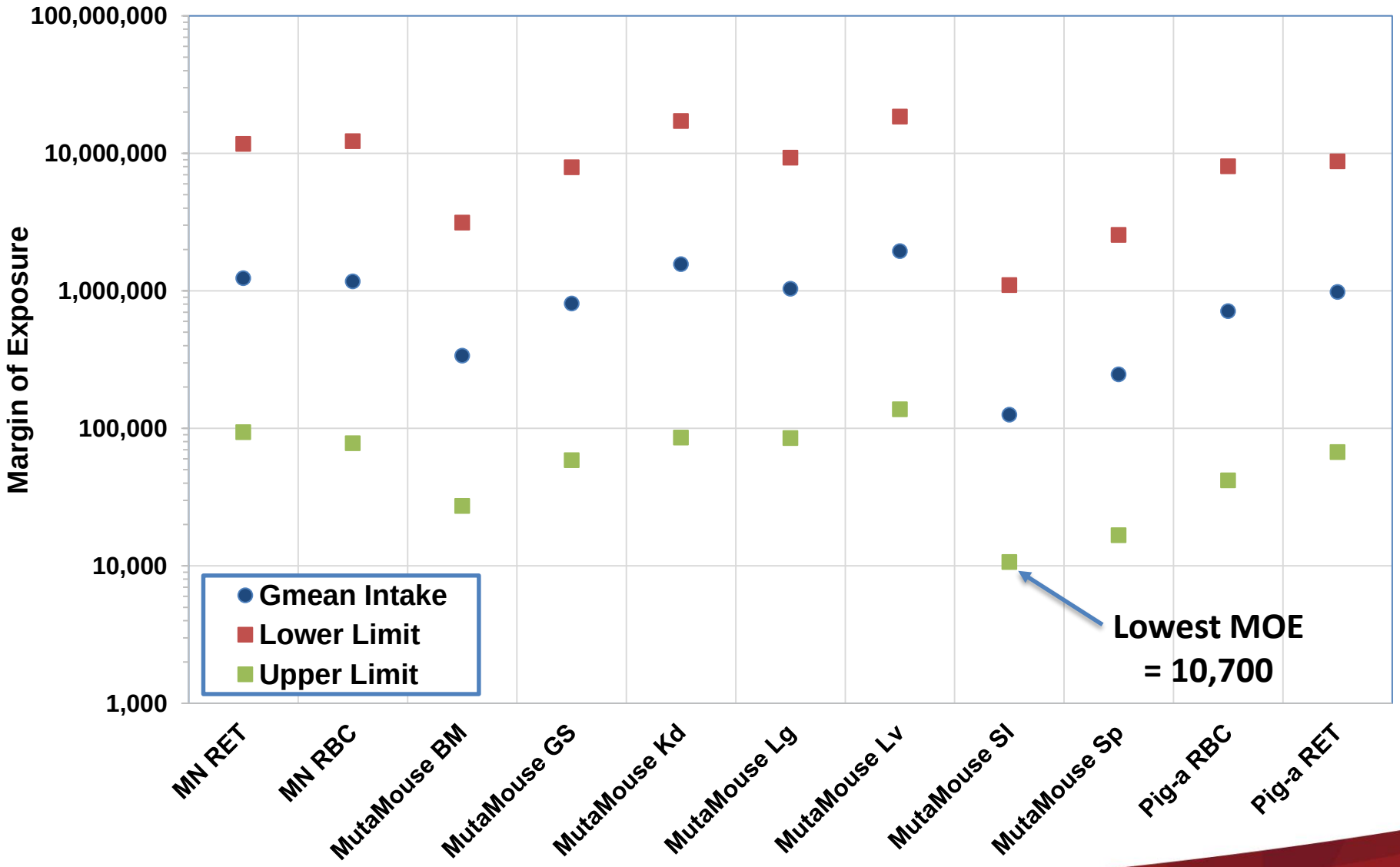
Benzo[*a*]pyrene MOE Values Calculated Using BMD_{THC}
(One Trimmed Historical Control Standard Deviation Above Study Control)



Critical Effect Size Values for Genetic Toxicity Endpoints TAKE-HOME MESSAGE

Endpoint	Average CES	Data Source
Compare with other CES values calculated using the data presented in Slob (2016) **Body weight (N=112) = 0.087 = 8.7%** **Kidney weight (N=44) = 0.10 = 10%** **Liver weight (N=93) = 0.12 = 12%**		
Arithmetic mean CES across all endpoints	**0.50**	

Benzo[*a*]pyrene MOE Values Calculated Using BMD_{50}
(50% Above Study Control)



Summary of Uncertainty Factors (UFs) Commonly Employed in HHRA (Human Health Risk Assessment)

Generally 5 or 6, or 5 plus Allometric Body Weight-based Dose Scaling

TABLE 7. UFs Applied by WQHB, PMRA, and Under CEPA

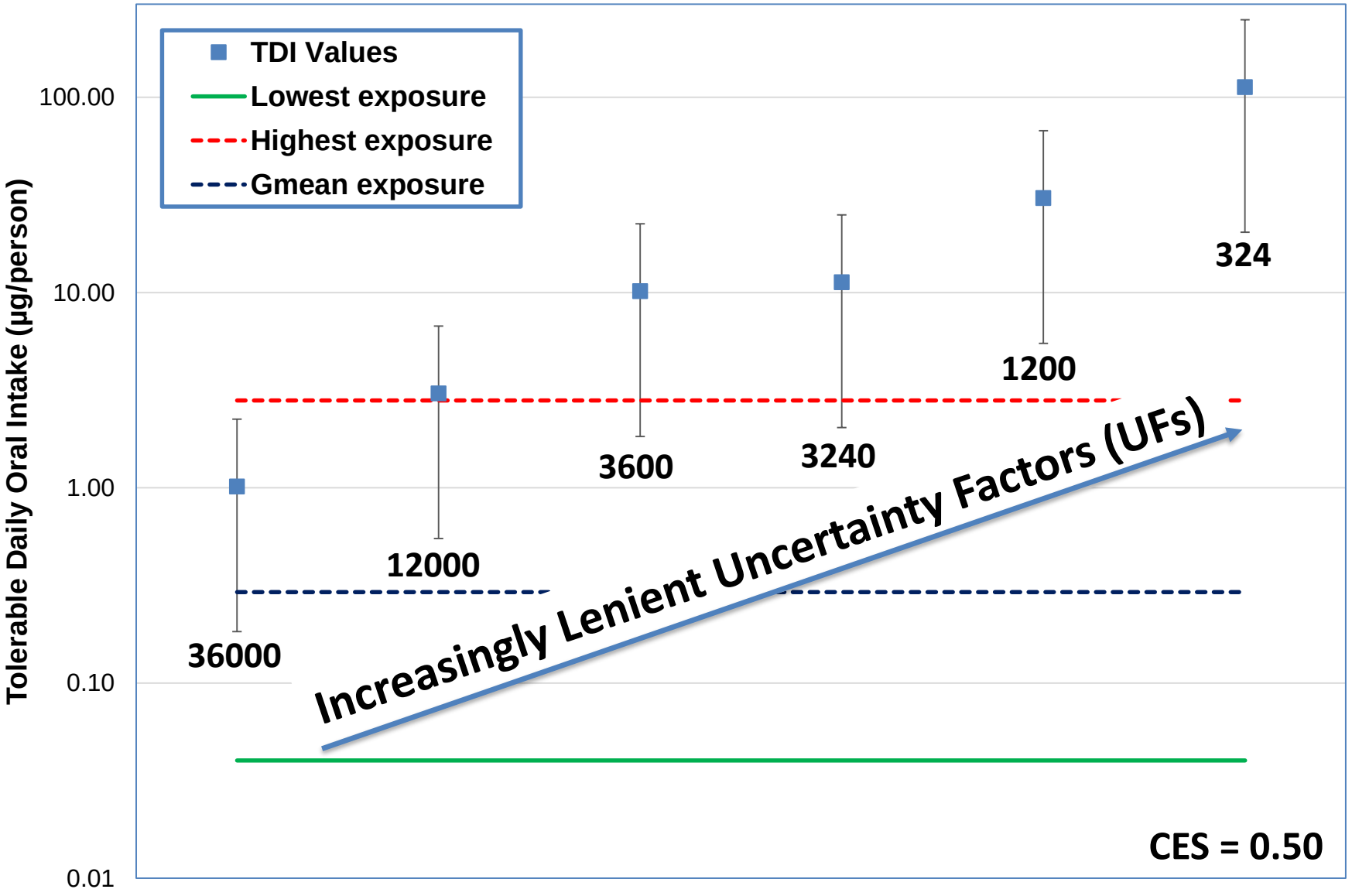
Uncertainty/safety factor	WQHB	PMRA	CEPA
Interspecies variation **2, UF_A, Not ICH**	1–10	10	1–10
Intraspecies variation **1, UF_H, ICH F2**	1–10	10	1–10
Database/studies deficiency **UF_D, EPA MF, Not ICH**	1–10	3–10	1–100
LOAEL instead of NOAEL **4, UF_L, ICH F5**	1–10	3–10	Subset of database deficiency
Subchronic to chronic extrapolation **3, UF_S, ICH F3**		Subset of database deficiency	
Nature and severity of effect **ICH F4**	1–10	1–10	1–10
Potential interaction with other chemicals	1–5		1–5
Protection of children		1–10	

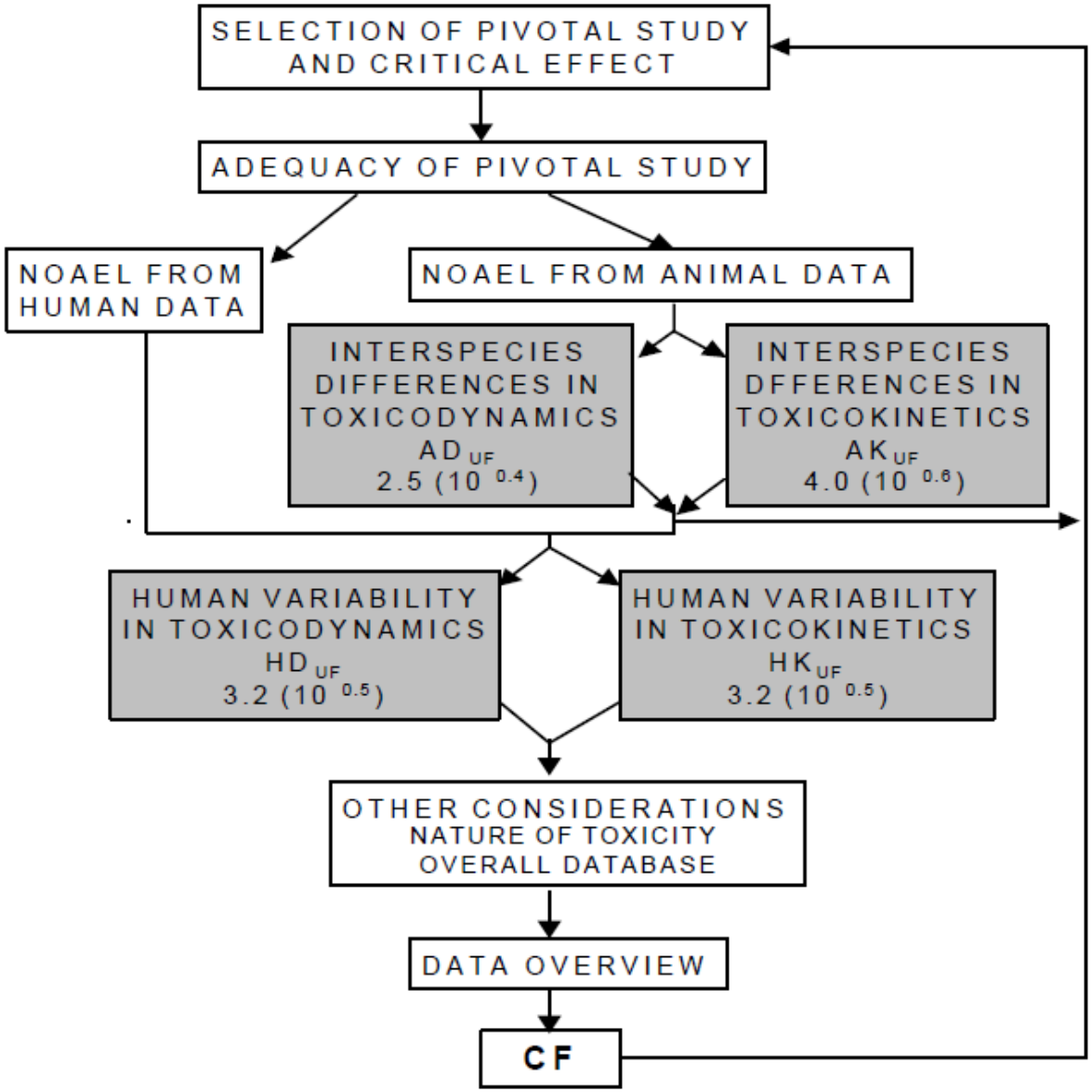
After calculating HED (human equivalent dose), some jurisdictions recommend an additional uncertainty factor (e.g., $10^{0.5}$ = 3.16) for "any remaining TK/TD differences between species"(IPCS, 2014)

Sources: Ritter et al. (2009). *J Toxicol Envir Health Part B* **10**:527-557; USEPA (1994) *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry*. EPA/600/8-90/066F; Nielsen et al (2008) *Toxicological Risk Assessment of Chemicals: A Practical Guide*. CRC Press; IPCS (2014) *Guidance Document on Evaluating and Expressing Uncertainty in Hazard Characterization*.

TDI (Tolerable Daily Intake) Values for Benzo[*a*]pyrene

Comparison Across Increasingly Strict UF Values





Framework for the Introduction of Quantitative Toxicokinetic & Toxicodynamic Data into Dose/concentration–Response Assessment (from IPCS, 1994)

Calculation of CF (Composite Factor) by replacement of UFs with AFs; CF is composite of CSAFs (Chemical-specific Adjustment Factors)

AD_{UF} = animal-to-human TD
AK_{UF} = animal-to-human TK
HD_{UF} = inter-individual TD
HK_{UF} = inter-individual TK

Source: WHO/IPCS (2001) *Guidance Document for the Use of Data in Development of Chemical-Specific Adjustment Factors (CSAFs) for Interspecies Differences and Human Variability in Dose/Concentration–Response Assessment*.

Variability in Terminal Elimination Rate of 1-OH-Pyrene (i.e., tissue to urine)

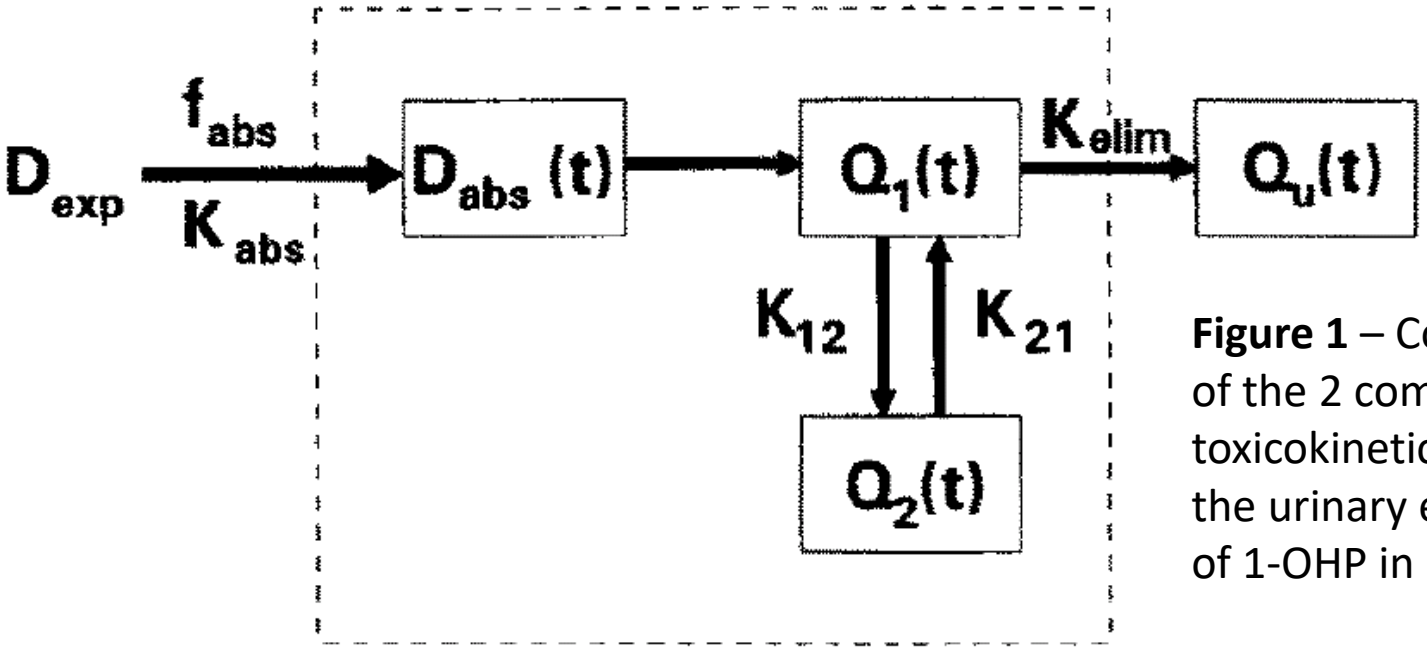


Figure 1 – Components of the 2 compartment toxicokinetic model for the urinary elimination of 1-OHP in humans.

$$EF_{AK} = \frac{D_A}{D_H} \text{ OR } \frac{Cl_A}{Cl_H}$$

Human K_{elim} = 0.012 min^{-1}, $T_{1/2}$ = 58 mins
Animal K_{elim} = 0.032-0.059 min^{-1}, $T_{1/2}$ = 11 = 22 mins

Therefore, human elimination rate of absorbed pyrene is 2.6- to 5.3-fold slower than rat. In line with IPCS 4-fold default (i.e., $10^{0.6}$) for Animal TK Uncertainty.

Source: Viau et al (1995) *Sci Total Environ* **163**:1979-186.

Variability in Reported Human Half-life of 1-Hydroxypyrene

Table 3. Reported 1-Hydroxypyrene Half-Lives (h) in Published Studies on Human Populations[a]

study design	no. person, smoking status	exposure source	average $t_{1/2}$ (range)	ref
Ingestion Exposure				
3-day sampling from office workers	9, NS	barbecued chicken	3.9 [3.0–5.7][b]	this study
7-day sampling from college students	9, NS	barbecued meat	5.7 (3.0–9.9)	15
3-day sampling from male adults	2, NS	500 μg pyrene in olive oil	12	17
6-day sampling (8-h composite urine) from male adults	5, NS	grilled beef	4.4 (3.1–5.9)	16
Inhalation Exposure				
3-day sampling from subjects exposed at an aluminum plant	5, n/a	6-h aluminum plant air	9.8 [7.9–11.7][b]	12
4-day samples from shooting target factory workers	7, n/a	petroleum pitch	6.1 (1.9–12.5)	13
4-day pre and post samples from locomotive plant workers	17, NS,S	diesel exhaust	29 (6.4–128)	32
10-day sampling from smokers	8,S	cigarette smoke	6.0 (3.7–9.9)	14
Dermal Exposure				
3-day sampling from 1 psoriasis patient and 2 volunteers	3, NS	creosote or 500 μg pyrene	12.8 (11.5–15)	17 and 18
Inhalation and Dermal Occupational Exposure				
3-day pre/post/bedtime samples from asphalt pavers	20,NS,S	asphalt	13.3 [7.8–46][b]	20
3-day of 5 composite urine/day from creosote workers	2, S	coal tar creosote	5–6 h; 22–24 h[c]	36
5-day pre and post samples from needle coke plant workers	16, NS,S	workplace	10.4 (3.9–26.7)	21
4-day pre and post samples from coke oven and graphite electrode workers	15, NS,S	workplace	18 (13.4–26.3)	19
3-day pre and post samples from coke oven workers	18, NS,S	workplace	n/a (6–35)	33

[a]Abbreviations: NS, nonsmoker; S, smoker; n/a, not available. [b]Calculated $t_{1/2}$ with 95% confidence interval. [c]Half-lives in two-phase excretion.

Half-life range for ingestion exposure of 25 individuals = 12/3.0 = 4.0

Half-life range for occupational exposures of 71 individuals = 9.0

Source: Li et al (2012) *Chem Res Toxicol* **25**:1452-1461.

Variability in Human Cell Sensitivity to UV Light Normal Genotype versus XP Mutations

"Understanding MOA for the agent of interest ensures that TD responses used to derive DDEFs are relevant to the adverse outcome of interest. These responses could include receptor affinity, enzyme inhibition, and molecular changes……Repair of DNA or tissue damage….are considered."

USEPA (2014) ***Guidance for Applying Quantitative Data to Develop Data-derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation*****. EPA/100/R-14/002**

Lymphoid Cell UV survival curves show that XP mutants are 5.5- to 12-fold more sensitive than wild type cells.

Source: Cleaver et al (2007) *J Invest Dermatol* **127**:493-496.

Table 2; TK6 mutants (Feb. 2017)

No.	Gene name	Ref.	Laboratory
1	53BP1		KYOTO
2	53BP1, BRCA1		KYOTO
3	53BP1, MRE11,		KYOTO
4	ALC1		KYOTO, TOKYO
5	ALC1, PARP1		KYOTO
6	APLF		
7	APRF, XRCC1		
8	ATRX		
9	BLM		
10	BLM, EXO1		KYOTO
11	BLM, MLH1		KYOTO
12	BLM, MLH3		KYOTO
13	BLM, MUS81		KYOTO
14	BLM, SMARCAL1		KYOTO
15	BLM, XPF		KYOTO
16	BRCA1		KYOTO
17	BRCA1, REV7		KYOTO
18	CtIP		KYOTO
19	CtIP, MRE11		KYOTO
20	DNA2 +/-		KYOTO
21	DNA-PKcs		KYOTO
22	DNA-PKcs, SMARC		KYOTO
23	ERCC6		NIHS
24	EXO1		KYOTO
25	EXO1, FAN1		KYOTO
26	FAN1		KYOTO
27	FANCC		KYOTO
28	FANCD2		KYOTO
29	GEN1		KYOTO
30	GEN1, MLH3		KYOTO
31	GEN1, MLH3, PMS2		KYOTO
32	GEN1, MUS81		KYOTO
33	GEN1, RAD54		KYOTO
34	LIG4		KYOTO
35	LIG4, POLQ		KYOTO
36	LIG4, POLQ, RAD5		KYOTO
37	LIG4, RAD54		KYOTO
38	LIG4, RNF8		KYOTO
39	LIG4, SMARCAL1		KYOTO
40	MLH1		
41	MLH1, MLH3		
42	MLH1, MLH3, PMS2		
43	MLH1, MUS81		
44	MLH1, PMS2		
45	MLH3		
46	MLH3, PMS2		
47	MRE11		
48	MRE11, P53		
49	MRE11, TDP1		
50	MRE11, TDP2		
51	MRE11, TIR		
52	MSH2		
53	MSH6		
54	MUS81		
55	MUS81, PMS2		
56	NEK1		
57	NEK11		
58	NEK8		
59	OGG1		
60	P53		
61	P53, PDIP38		
62	P53, POLH		
63	P53, RAD54B		
64	PARP1		
65	PARP1, XRCC1		
66	PARP2 +/-		
67	PDIP38		
68	PDIP38, POLH		
69	PDIP38, POLH, XPA,		KYOTO
70	PDIP38, PrimPol		TOKYO
71	PDIP38, XPA		KYOTO
72	PIAS1		KYOTO
73	PIAS1, PIAS4		KYOTO
74	PIAS1, PIAS4		KYOTO
75	PIAS1, PIAS4, POLH		KYOTO
76	PIAS4		KYOTO
77	PMS2		
78	POLB		NIHS
79	POLD1		KYOTO
80	POLD3		TOKYO
81	POLE		KYOTO
82	POLH		KYOTO
83	POLH, PrimPol		KYOTO, TOKYO
84	POLH, PrimPol, RAD54		KYOTO, TOKYO
85	POLH, RAD18, XPA		KYOTO, TOKYO
86	POLH, RAD54		KYOTO, TOKYO
87	POLH, XPA		KYOTO
88	POLL		KYOTO
89	POLQ		KYOTO, TOKYO
90	PrimPol		KYOTO, TOKYO
91	RAD18		KYOTO
92	RAD18, XPA		KYOTO
93	RAD51AP1		KYOTO
94	RAD51AP1, RAD54		KYOTO
95	RAD51AP1, RAD54, RAD54B		KYOTO
96	RAD54	8, 9, 10	KYOTO
97	RAD54B		KYOTO
98	RECQL5		HIROSHIMA
99	REV3		KYOTO, TOKYO
100	REV7		KYOTO
101	RNASEH2A		KYOTO
102	RNF8		KYOTO
103	RPA, SMARCAL1		KYOTO
104	SLX1		KYOTO
105	SLX4		KYOTO
106	SMARCAL1		KYOTO

* The original TK6 cell line is avilable from JCRB (http://cellbank.nibiohn.go.jp/english/) and ECACC (https://www.phe-culturecollections.org.uk/collections/ecacc.aspx)

Table 1; Members of TK6 Mutants Consortium

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Source: http://www.nihs.go.jp/dgm/tk6.html

Conclusions & Take-home Messages

1. Genetox community increasingly accepts/recognises quantitative analysis of genetic toxicity dose-response data; moreover, extrapolation from RPs to HBGVs. The Benchmark Dose approach is well accepted for robust dose-response analysis and determination of RPs for HBGV calculation.
2. CES is debated. Detailed examinations of various options for CES determination revealing that 10% is not appropriate for genetic toxicity endpoints. The trimmed historical control approach yields TGR assay CES values in the 0.22-0.87 range, with a mean of 0.53. For the MN assay, values are in the 0.34-0.49 range. Detailed analyses indicating that 50% is a pragmatic choice for endpoints other than Pig-a. Detailed analyses of Pig-a data currently underway.
3. The BaP case study revealed that a regulatory evaluation based on genetic toxicity data is well aligned with an evaluation based on carcinogenicity data. TDI associated with $BMDL_{10}$ small intestine equates to 3.7×10^{-5} risk; $BMDL_{50}$ derived value equates to 9.3×10^{-5} risk. 22 similar case studies underway.
4. About one-third of the range in calculated MOEs for BaP can be attributed to variability in BMD across endpoints and tissues; two-thirds is attributable to the range in oral daily intake (i.e., CES and endpoint have a limited impact on HBGVs).
5. Jurisdictional guidelines provide options for the use of UFs to calculate HBGVs (e.g., human exposure limits), but little agreement on most appropriate deterministic values. Use of typically-recommended UFs yields BaP TDI value (CES=50%) greater than even the upper limit of daily intake. Not clear which UFs, and which UF values, are appropriate for routine risk assessment of genotoxic substances. Need entire workshop to address this topic.
6. Following IPCS/USEPA paradigms, it should be possible (necessary?) to use MOA information to determine DDEF/CSAF values for effective risk assessment of genotoxic substances.

William Thomson, 1st Lord Kelvin

Library of Congress

"When you can measure what you are speaking about, and express it in numbers, you know something about it, when you cannot express it in numbers, your knowledge is of a meager and unsatisfactory kind; it may be the beginning of knowledge, but you have scarely, in your thoughts advanced to the stage of science."

Guided Discussion – Quantitative approaches

1. If the regulatory community is not willing to accept the use of dose-response Reference Point (PoD) metrics for risk assessment of DNA-reactive substances, should we be spending so much time developing quantitative approaches? If we build it, will anyone come?

2. Do we need to derive CES values for each genetox endpoint (e.g., MN, Pig-a, TGR, Comet)? How should we define an endpoint, e.g., all TGRs, each TGR-transgene combination, each TGR-transgene-tissue combination, etc?

3. We are currently using two methods to determine the most appropriate CES values for in vivo genetic toxicity endpoints (i.e., method based on trimmed historical controls and method based on geometric mean within-group variance). How should we reconcile any differences?

4. How should we reconcile deviations from the standard 5% or 10% CES values recommended by EFSA and EPA, respectively? Will this conflict with the use of dose-response data for other toxicity endpoints (i.e., HBGVs for severe genetic effects could be comparatively high)?

5. Which genetic toxicity endpoint(s) should be used to calculate HBGVs? The most sensitive? Of how many? How do we define database sufficiency?

6. With respect to Uncertainty Factors (UFs), should we, as genetic toxicologists, be determining the most appropriate deterministic values for genotoxic substances? Is this a task for risk managers? If yes, should we consider more complex probabilistic approaches? For example, the “approximate probabilistic” approach (i.e., IPCS, 2014)?