SOT FDA Colloquia on Emerging Toxicological Science Challenges in Food and Ingredient Safety

Role of Mode of Action in Dose-Response Assessment for Carcinogens

January 25, 2016

Presentation title

- The Mutagenic Mode of Action and the Choices for the Dose-Response Analysis
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Conflict of Interest Statement

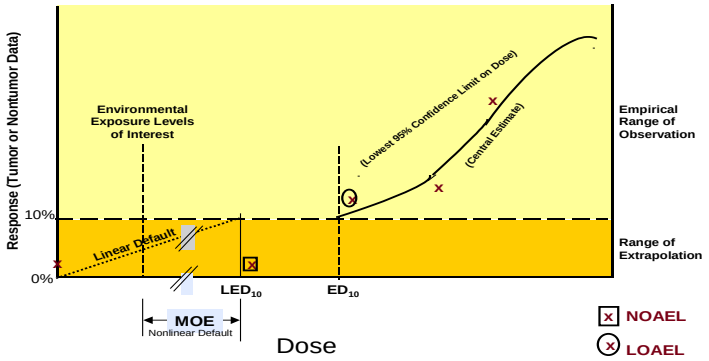
- Neither I nor members of my immediate family, have any financial interest or affiliation with a commercial organization that has a direct or indirect interest in the subject matter of my presentation.
- Furthermore, I am no longer from the Government, albeit still here to help you.
- And these are my opinions, unless otherwise stated.

So What Is EPA Policy?

- Science Policy
  - Defaults, methods, Guidelines
  - Used when there are data or methodology gaps
  - Peer reviewed
  - Lots of documentation, which is publicly available
- Policy based on science
  - May be set by EPA Executive Level
  - Generally involves regulations or other risk management choices; science is peer reviewed, action involves public comment; May be subject to Federal Advisory Committee Act
  - Lots of documentation; may be docket; publicly available

Why Do You Care about MOA ?

- MOA is key in Hazard Identification
  - Helps describe circumstances under which agent is carcinogenic (High dose? Route?)
  - Relevance of data for humans
- MOA determines choice of Low Dose Extrapolation
- Life stage risk
  - Mutagenic MOA

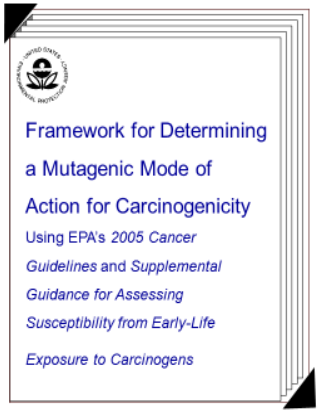


Supplemental Guidance for Cancer

- Use age-specific values for exposure and potency
- When data permit, develop separate potency estimates for childhood exposure
- In risk characterization, mutagenic MOA risk is increased by age-dependent adjustment factor (used with exposure info for age group)
  - <2 yrs old, 10 fold
  - 2 to < 16yrs, 3 fold
- No MOA, linear extrapolation without ADAF; non-linear MOA, no ADAF

Other Policies Are Different

- EU makes distinction between genotoxic and non-genotoxic chemicals
  - May be evolving.
- Several U.S. environmental agencies apply ADAF to all putative carcinogens



- Public Comment completed 12/07
- External peer review completed 05/08

Major Points of 12/13 MMOA Draft

- There is no default MOA for cancer
- Defined Genotoxic and Mutagenic
- MMOA determination is a WOE with a hierarchy of data preference
  - Human data > animal
  - *In vivo* > *in vitro*
  - Target site > indicator
  - Data on mutation > other tests

Definitions

- Mutagenicity

is the induction of permanent, transmissible changes in the amount, chemical properties, or structure of the genetic material. These changes may involve a single gene or gene segment, a block of genes, parts of chromosomes, or whole chromosomes. Effects on whole chromosomes may be structural and/or numeric (e.g., aberrations and/or aneuploidy). In most cases, mutations involve changes in DNA structure that either have no effect or cause harm.

- Genotoxicity

is the induction of alterations to genetic material. It is a broader term than mutagenicity in that genotoxicity refers to potentially harmful effects on genetic material, which are not necessarily persistent and transmissible. Genotoxicity may be mediated directly or indirectly by chemical or physical agents, and may or may not be associated with mutagenicity. Tests for genotoxicity include tests for mutagenicity, as well as other tests which provide an indication of induced damage to DNA. For example, such tests may include unscheduled DNA synthesis (UDS), sister chromatid exchange (SCE), mitotic recombination, DNA adduct formation, or DNA strand breaks.

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Data Preference for MMOA

- Cancer-relevant oncogene or tumor suppressor gene mutations can be detected in the target tissue following chemical exposure.
- Surrogate gene mutations and/or chromosome aberrations or numerical changes can be detected in the target tissue following chemical exposure.
- Chemical-specific DNA adducts (known to be mutagenic adducts) can be detected in the target tissue following chemical exposure.
- Primary DNA damage can be detected in the target tissue following chemical exposure.
- Gene mutations or chromosome aberrations can be detected in surrogate tissues after *in vivo* exposure.
- DNA adducts or other measures of DNA damage and/ repair or can be detected in surrogate tissues after *in vivo* exposure.
- The chemical can induce mutations, cytogenetic damage, DNA adducts and/or primary DNA damage *in vitro*.

Modified from Moore (2008).

Steps in WOE

- 1. Assemble the Relevant Data
  - Assume there are tumor data
  - Many data types
- 2. Evaluate the Data against Current Acceptance and Quality Criteria
- 3. Apply the Cancer Guidelines (or IPCS or OECD or other) MOA framework
  - Genetox ≠ Mutagenic
  - Mutagenic *in vitro* ≠ Mutagenic in humans
  - Mutagenic ≠ Mutagenic MOA

Gene- tox Tests Measure Different Events

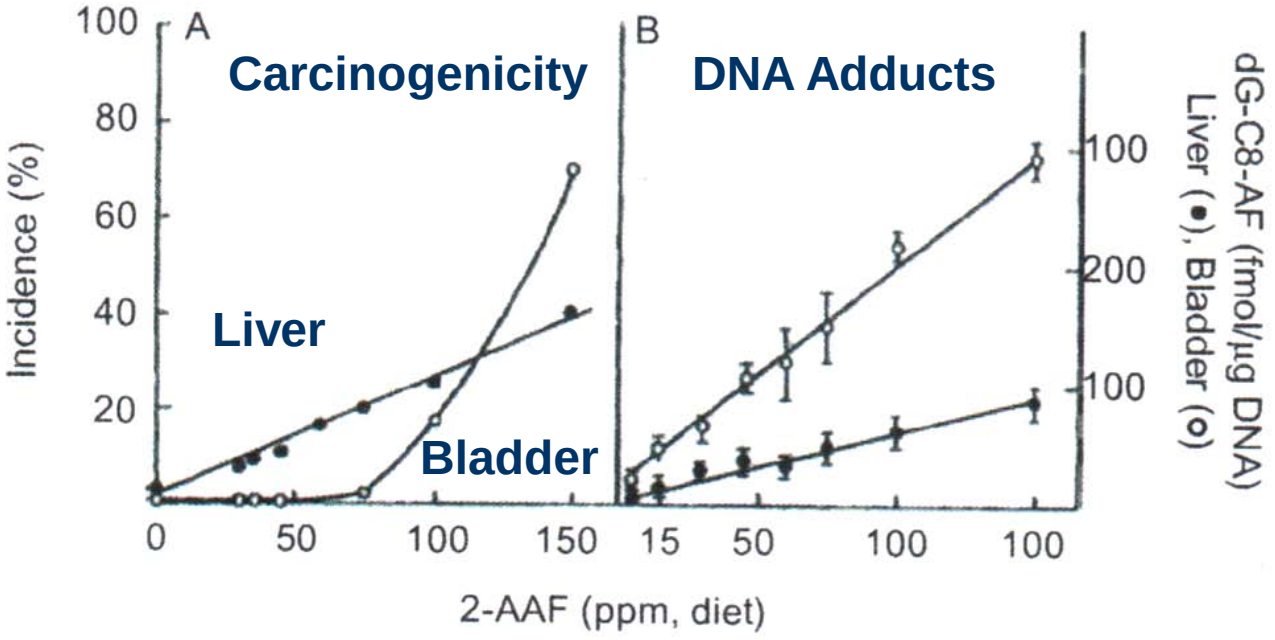
	Genotoxicity Assays		
Type of Damage	**Mouse Lymphoma**	**Chromosome Aberrations CHO cells**	**Ames Bacterial Mutagenicity**
Point mutation	Yes	No	Yes
Oligonucleotide insertion or deletion	Yes	No	Yes
Allele Loss	Yes	No	No
Small Chromosome alteration	Yes	?	No
Large Chromosome alteration	Yes	Yes	No
Aneuploidy	?	Yes	No

Adapted from M. Moore (2004)

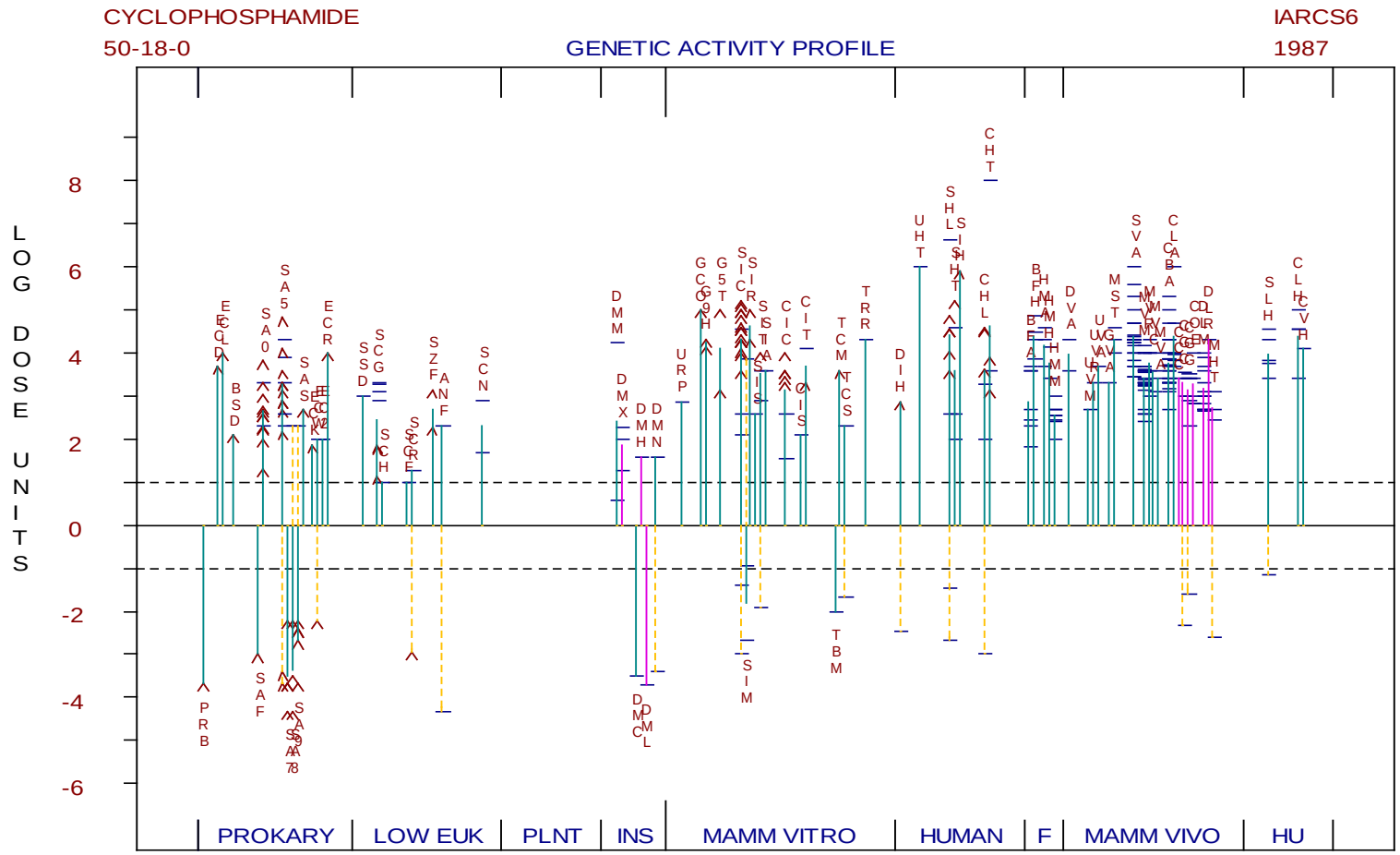
DNA Damage ≠ Mutation ≠ Cancer

Induction of Liver and Bladder Tumors and DNA Adducts by 2-AAF in 25,000 Mice

DW Gaylor, J Environ Pathol Toxicol 3:179, 1979
MC Poirier et al., Carcinogenesis 12:895, 1991

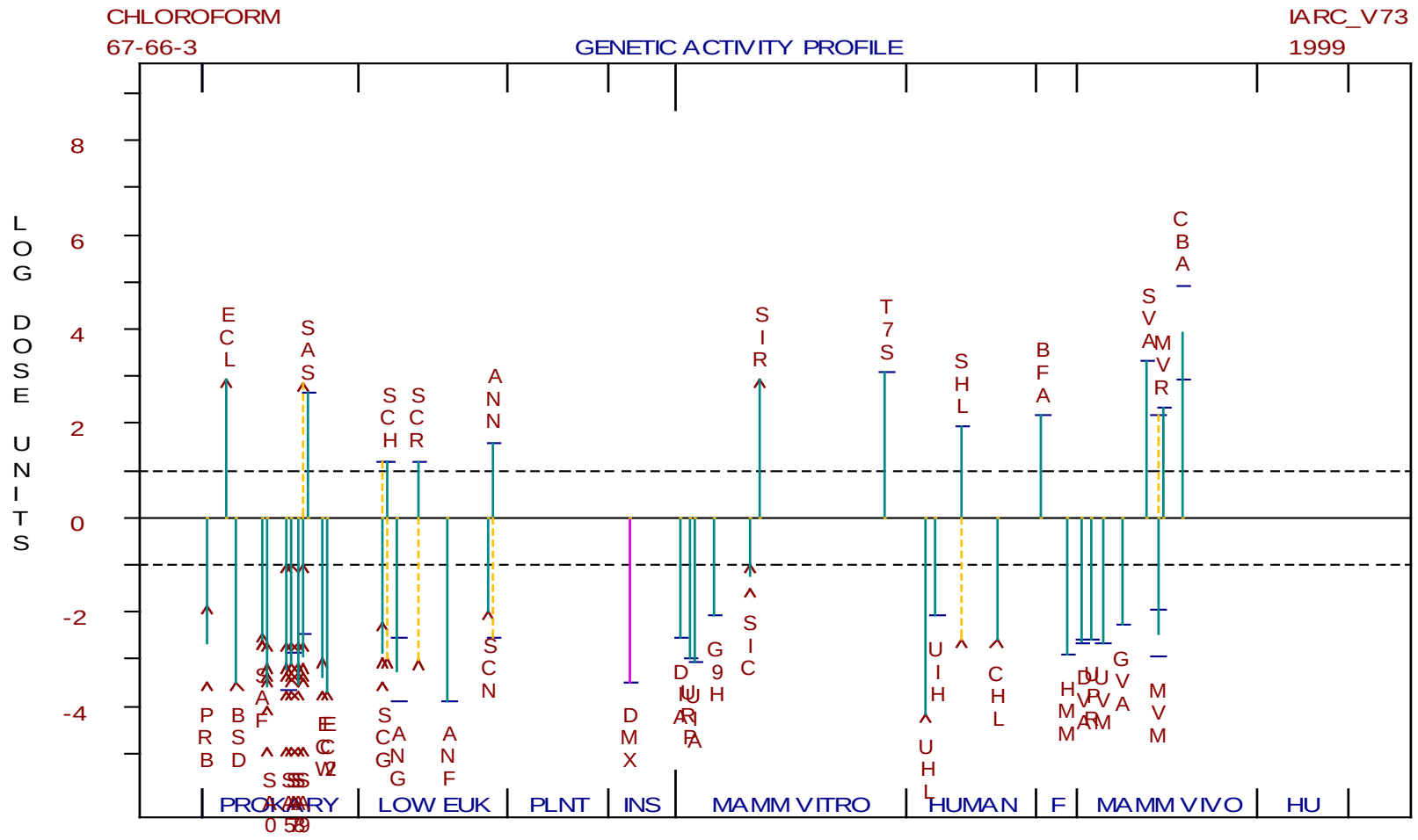


Cyclophosphamide GAP



IARC human carcinogen (group 1: human - sufficient, animal - sufficient)

CCl_3 Genetic Activity Profile



IARC possible human carcinogen (group 2B: human - inadequate, animal - sufficient)

WOE vs. Strength of Evidence

In vivo Mammalian CCl_4 Genotoxicity Results

Liver	Positive	Negative/ equivocal	Notes
DNA strand breakage	4	9	TUNEL assay, toxicity
UDS	1	7	Questionable
Chrom. Abs Micronuclei	2	5	Reproducible, toxicity
Mutations	0	3	Reproducible

In vivo Mammalian CCl_4 Genotoxicity Results

Liver	Positive	Negative/ equivocal	Notes
Radiolabeled CCl_4 binding to DNA	3	6	Weak. many equivocal, no confirmation
ROS or lipid perox.-derived DNA adducts	8	4	Mass Spect. confirmation, toxicity
Methylation changes	2	0	Toxicity

Draft framework suggested tabular presentation and consistent descriptors for assay conclusions and overall WOE

Eastmond
SRA 2015

In vitro Assays Test System	Concen-trations	Cytotoxicity observed	Duration of Exposure	Results With metabolic activation (+ S9)	Results Without metabolic activation (- S9)	Conform to relevant guideline	Ref
Gene Mutation							
Bacterial							
Salmonella, reverse mutation							
E. coli, reverse mutation							
Mammalian							
CHO gene mutation, hprt locus							
Mouse L5178Y, tk locus							
Chromosome Mutation							
Micronucleus assay							
Chromosomal aberrations							
DNA Effects							
Mammalian							
Unscheduled DNA synthesis							
Sister chromatid exchanges							
Comet assay							
DNA adduct analysis							
Lower Eukaryote							
Saccharomyces cerevisiae, gene conversion							

Mode of Action Frameworks

U.S. EPA

- Hypothesized MOA: summary description and identification of key events
- Experimental support:
  - Strength, consistency, specificity of association
  - Dose-response concordance
  - Temporal relationship
  - Biological plausibility and coherence
- Consideration of the possibility of other MOAs
- Relevance to humans

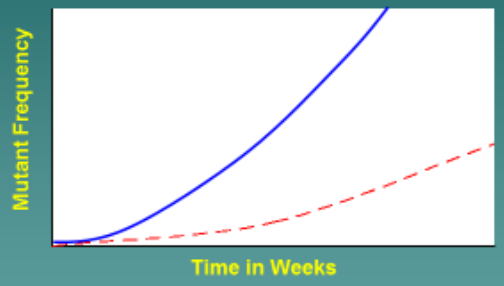
IPCS

- Postulated mode of action (theory of the case)
- Key events
- Concordance of dose-response relationships
- Temporal association
- Strength, consistency and specificity of association of tumour response with key events
- Biological plausibility and coherence
- Other modes of action
- Uncertainties, inconsistencies, and data gaps
- Assessment of postulated mode of action

Dose and Time Concordance

Temporality: Evaluate time-to-mutation

Mutagenic carcinogens would be expected to show a positive mutation response after relatively short treatment periods



Nonmutagenic carcinogens would be expected to be negative after long chronic treatment, or show a positive response only after long chronic treatment

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Tumor Induction: Time-related Accumulation of Events

Mutagenic Carcinogen

Initiating Mutation → Multiple events → Tumor

Nonmutagenic Carcinogen

Toxicity
Altered Gene Expression
Cell Proliferation

→ Initiating Mutation → Multiple events → Tumor

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Dose and Time Concordance 2

https://aopkb.org/aopwiki/index.php/Aop:46

AFB1: Ideal Dose- and Time-Concordance

↑Time

↑AFB1 Dose

Dose	Pre-MIE: Activation to exo-epoxide by hepatic metabolism	MIE: Formation of pro-mutagenic DNA adducts	KE1: Insufficient repair or mis-repair of pro-mutagenic DNA adducts	KE2: Induction of mutation in critical gene(s)	KE3: Proliferation/ clonal expansion of mutant cells (AHF)	AO: Hepatocellular carcinoma (HCC)	Time to effect
0 ppb in diet	–	–	–	–	–	–	
1 ppb	+	+	–	–	–	–	Pre-MIE: short-term
5 ppb	++	++	+	+	+/–	–	MIE: short-term KE1: short-term KE2: short -term
15 ppb	++	++	+	+	+/–	+/–	
50 ppb	+++	+++	++	++	+	+	KE3: mid-term
100 ppb	++++	++++	+++	+++	++	++	AO: long-term
Reversibility							
Oltipraz/ Chlorphyllin / CDDO-Im	+ (?)	++	+ (?)	+/– (??)	–	–	

Pottenger *et al.*, 2015

PO: Dose- and Time-Concordance

↑Time

↑ PO Dose

Dose	MIE: Sustained, severe GSH depletion	AE: Biomarkers: DNA & protein adducts	KE1: Increased cell proliferation	KE2: Inflammation	KE3: Hyperplasia of nasal epithelium	AO: Rodent tumors in nasal cavity	Time to effect
0 ppm	–	–	–	–	–	–	
<50 ppm	+/–	+	–	–	–	–	AE: short-term
100 ppm	+	++	+	+	–	–	MIE: short-term KE1: mid-term KE2: long-term
200 ppm	++	+++	++	++	+	–	KE3: long-term
≥ 300 ppm	+++	++++	+++	+++	++	+	AO: long-term
Reversibility							
30-d stop-exposure	+(?)	+(?)	+/–(?)	++	+	–	
Supporting MOA data: reversibility	+(?)	+(?)	+/–(?)	+ (Wistar rat chronic data)	+	–	

Klapacz *et al.*, in preparation

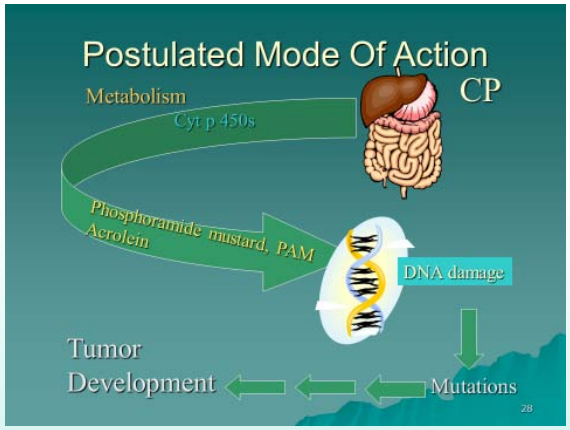
MOA and AOP

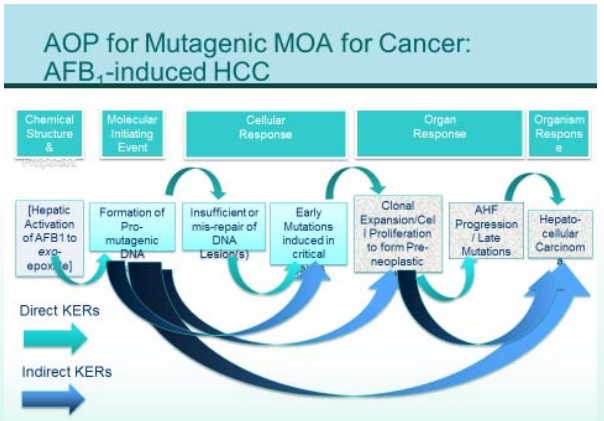
MOA

- Chemical specific
- Used to inform Hazard ID, Dose Response, Life Stage, Grouping chemicals in mixed exposure
- Uses “modified” Hill Criteria

AOP

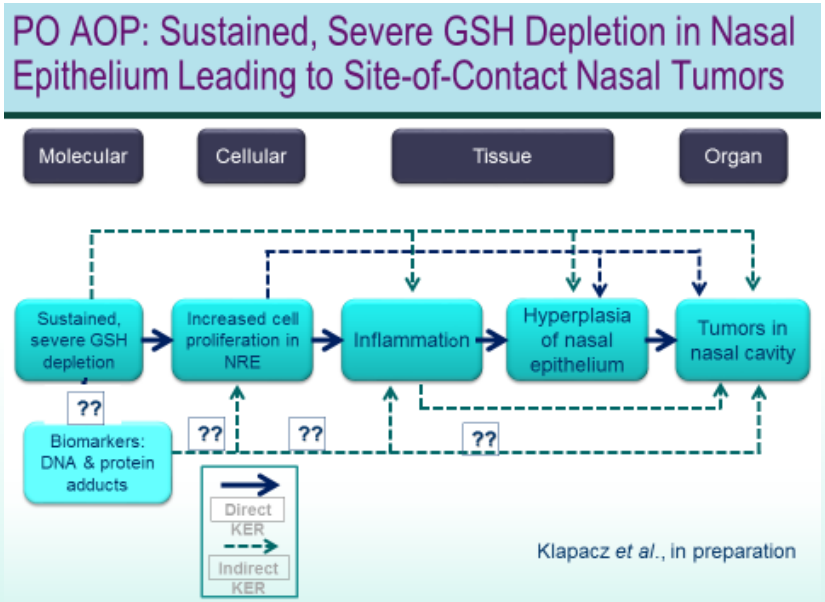
- Chemical agnostic
- Used to inform Hazard ID, Dose Response, Life Stage, Grouping chemicals in mixed exposure
- Uses “evolved” Hill Criteria





A Couple More Big Conclusions

- Mutagenic (Genotoxic) and carcinogenic ≠ Mutagenic MOA
  - Tamoxifen
    - Hormonally active agent
    - Causes endometrial cancer in humans
  - Pesticide
    - Produces only thyroid tumors
  - Others?



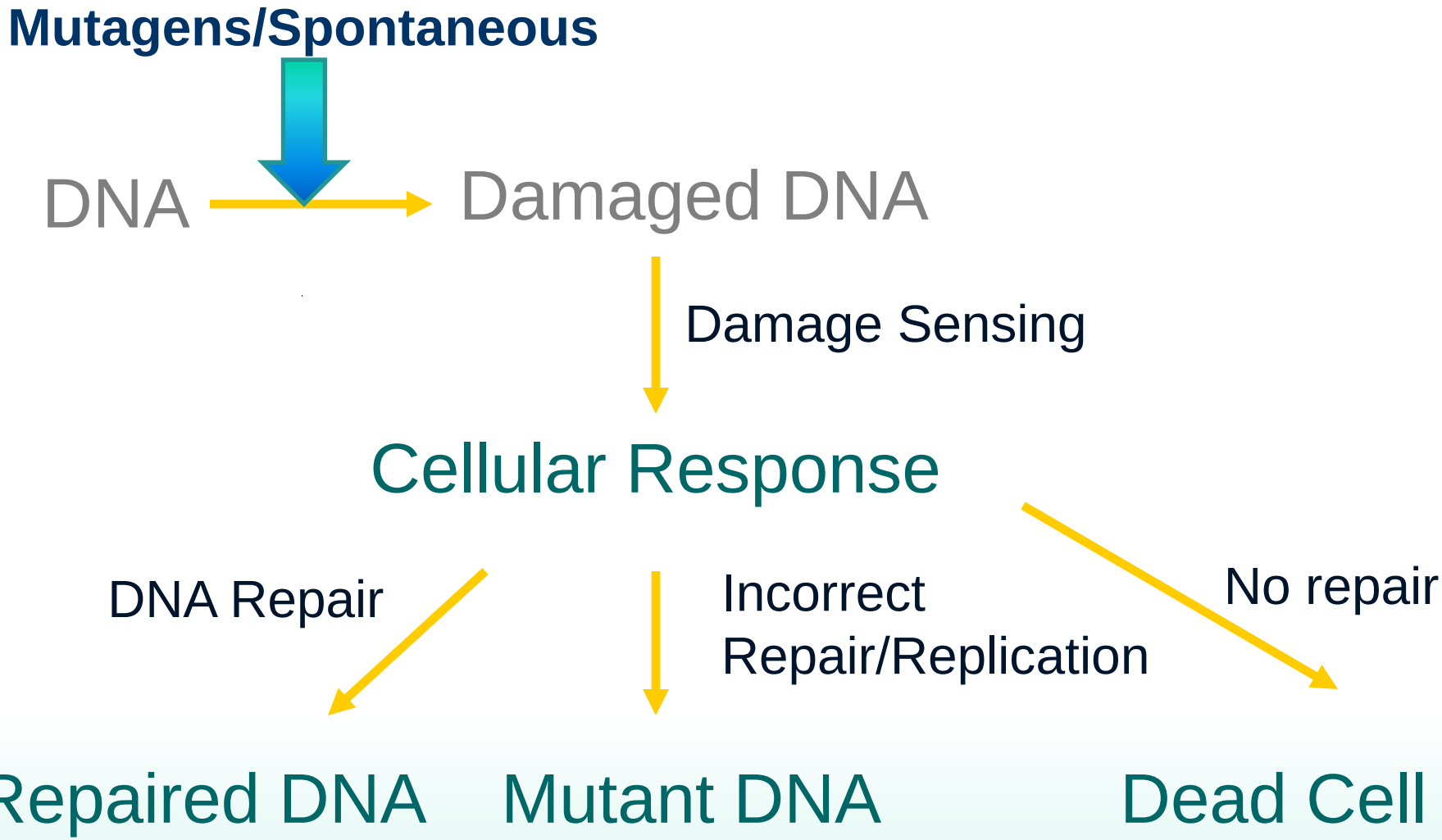
Mutagenic MOA Does Not Mean Low Dose Linear

Mechanisms Associated with Non-linear Dose Response Determinations

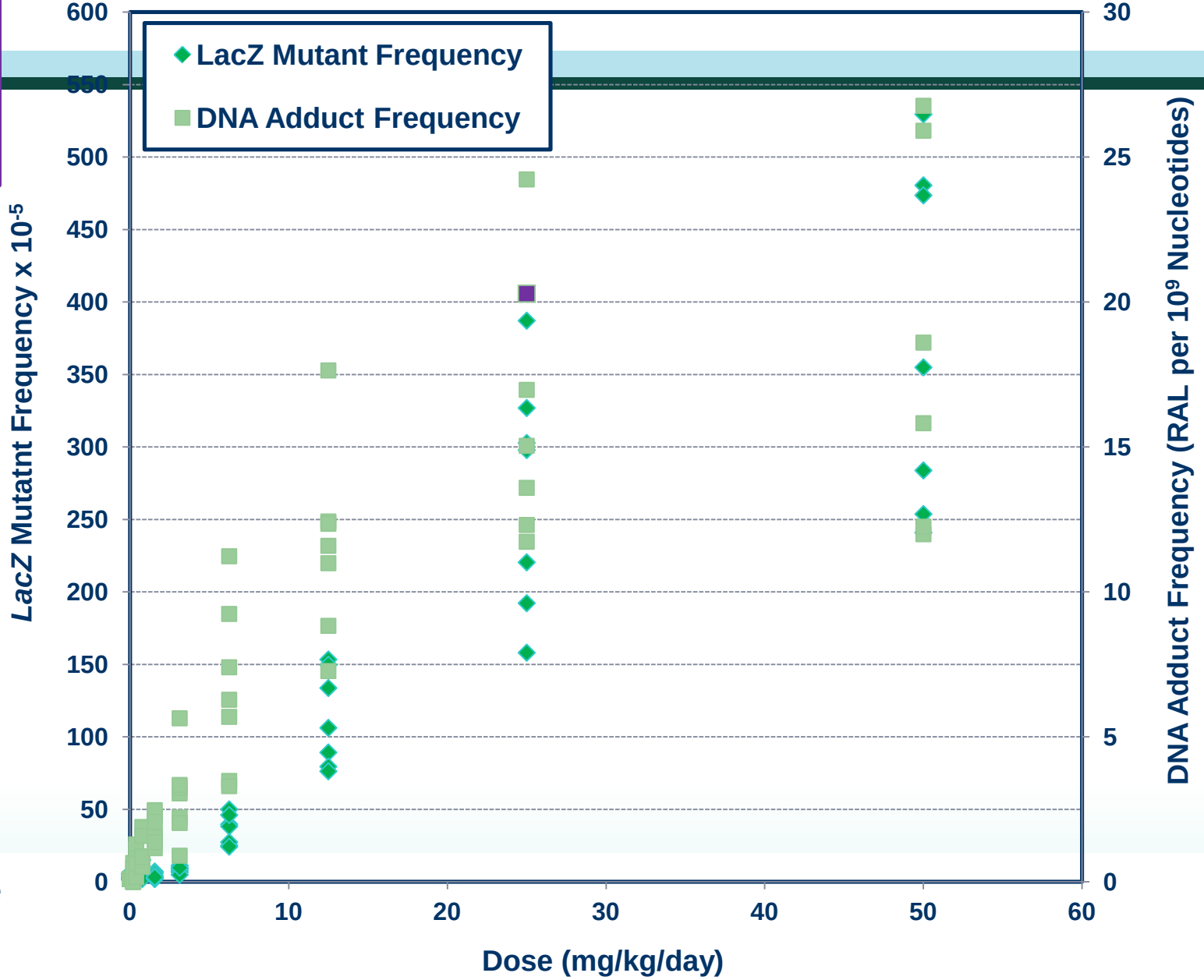
- Critical involvement of non-DNA targets
- Contribution of DNA repair mechanisms
- Detoxication capacity exceeded
- Disruption of DNA synthesis or replication enzymes
- Mutational spectrum in tumor genes similar to that in untreated animals
- Chemical reactivity or properties unlikely to occur in vivo
- Inadequate uptake or distribution to target
- Structural similarities to threshold-acting agent
- Indirect origin of damage
- Species and tumor-specific non-genotoxic MOA

From Dave Eastmond SRA 2015

Mutagenesis Paradigm

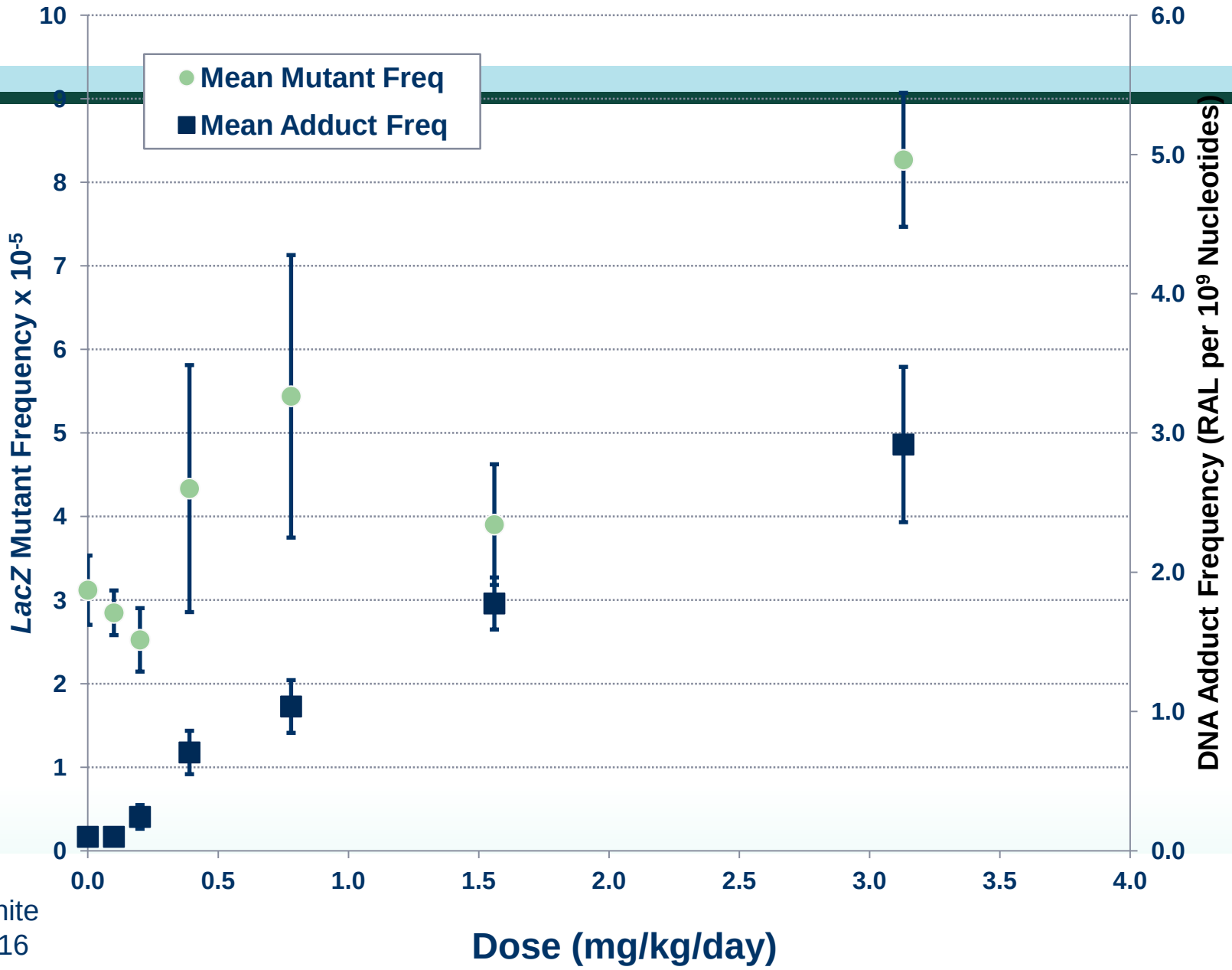


Benzo[*a*]pyrene Extended Dose Experiment – Muta™Mouse, 28-day Repeat Dose Oral, 10 doses plus control, Bone Marrow



Paul White
SRA 2016

Closer Look at Responses Below 5 mg/kg/day



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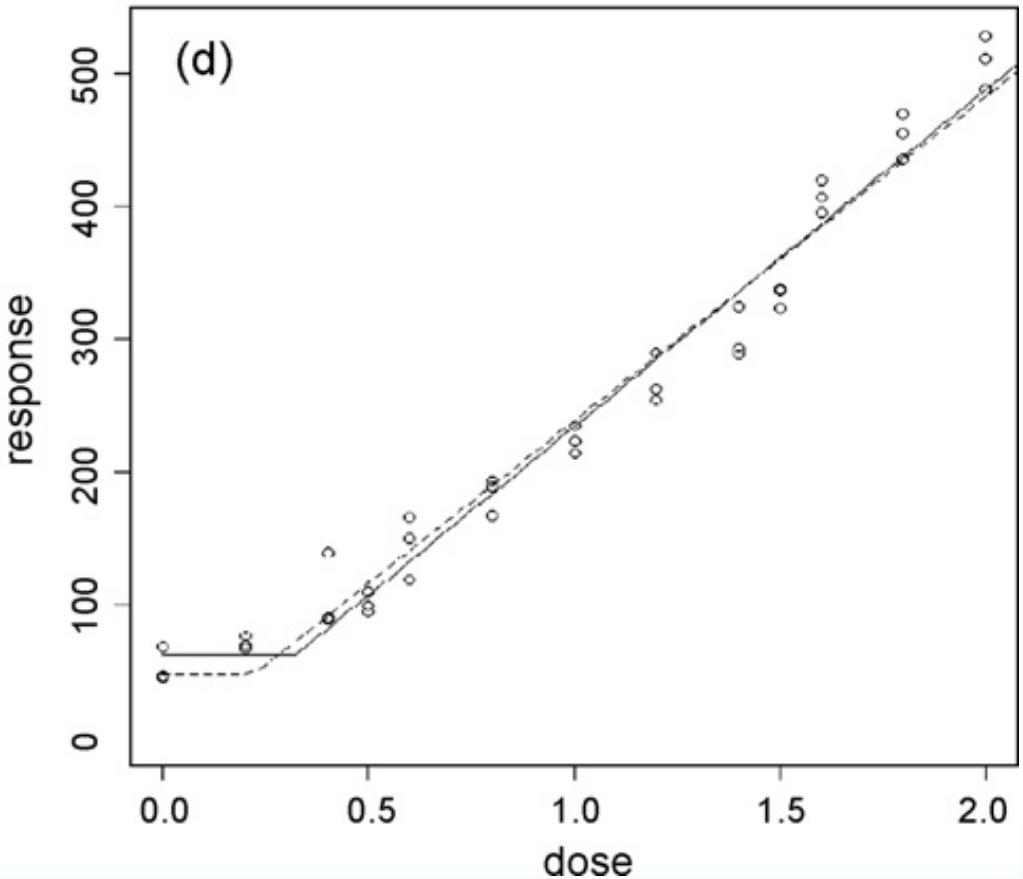
In vitro Mutation Dose-Response: ENU

Johnson *et al*., 2009



ENU Threshold Dose-response (Lutz & Lutz model)

Slide from Pottenger

Genetox – the Next Generation

- Emphasis on quantitation, rather than Yes / No
- Mutation as a toxicologically relevant endpoint
- Determining point of departure from *in vivo* genetox data for use in quantitative risk assessment
  - IWGT
  - HESI GTTC

Fanpop.com

POD Definitions

NOGEL – No-Observed-Genotoxic-Effect-Level. Highest study dose that yields a response that is not statistically different from the unexposed control. Analogous to NOAEL.

Td or BPD – Threshold or Breakpoint dose. Statistically identified dose below which the effect cannot be distinguished from the background observed in the absence of treatment. Piecewise, segmented or hockey-stick regression to identify "point of inflection".

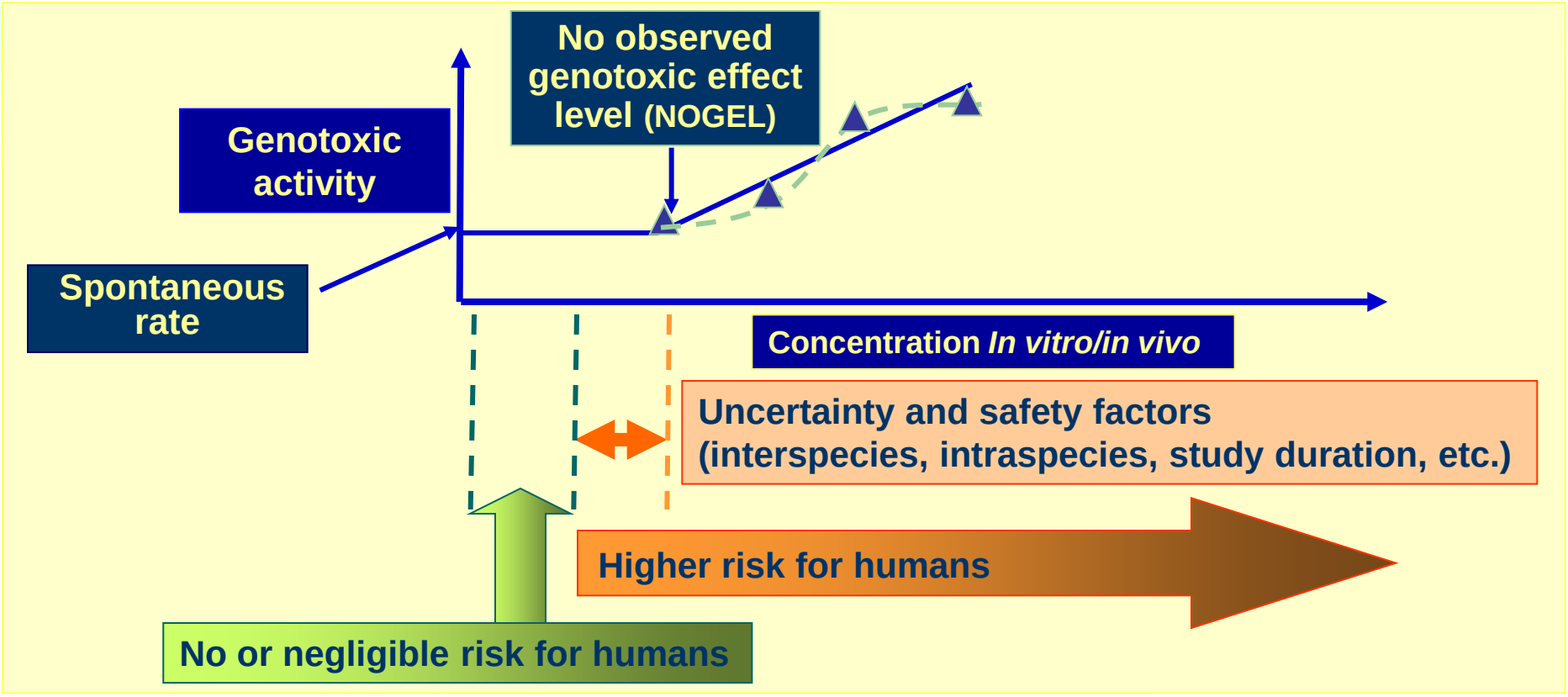
STD – Slope Transition Dose. Lowest dose in a non-linear dose-response relationship where the first derivative >0 (i.e., slope transition to positive).

BMD – Benchmark Dose. Dose of a substance that yields a predetermined level of response (i.e., the BMR or Benchmark Response). BMR often specified as 10% increase above control (i.e., BMD_{10}). Can also be 1 standard deviation above control (i.e., BMD_{1SD})

PoD	Full Name	Defined Using	Advantages	Disadvantages
NOGEL	No Observed Genotoxic Effect Level	Dunnett's, Dunn's (*drsmooth**)	Easy to determine, analogous to NO(A)EL	Dependent of study design, sparse data tends to provide larger PoDs.
BPD	Breakpoint Dose	L&L Method, *Segmented* in R (*drsmooth*)	Simple bi-linear functional form, appropriate for some MOAs.	Single functional form, ability to define BPD dependant on study design, data censoring often required.
STD	Slope Transition Dose	*mgcv* in R (*drsmooth*)	Readily accommodates non-linear models, uses all data, flexible non-linear algorithms.	Algorithms undergoing validation, ability to define STD highly dependant on study design.
BMD_{10}	Benchmark Dose 10%	PROAST or BMDS	Flexible methodology and functions, uses all available data, comparable to analyses for other endpoints, requires fewer doses.	Requires consensus on appropriate BMR for each endpoint.
BMD_{1SD}	Benchmark Dose 1 Standard Deviation	BMDS	Flexible methodology and functions, uses all available data, comparable to analyses for other endpoints, requires fewer doses.	Historical PoD comparisons influenced by precision of response measurement (i.e., variance of control).

*drsmooth (Dose-Response Modeling with Smoothing Splines) available from CRAN (Comprehensive R Archive Network)

Conceptual Framework



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

Slide from V. Thybaud, Sanofi

References

- Gollapudi, B, *et al.* 2013. Quantitative Approaches for Assessing Dose-Response Relationships in Genetic Toxicology Studies. *Environ. Molec. Mutagen.* 54:8-18.

- Johnson, G, *et al.* 2014. Derivation of Point of Departure (POD) Estimates in Genetic Toxicology Studies and Their Potential Applications in Risk Assessment. *Environ. Molec. Mutagen.* 55:609-623.

- MacGregor, JT, *et al.* 2015. IWGT Report on Quantitative Approaches to Genotoxicity Risk Assessment I. Methods and metrics for defining exposure-response relationships and points of departure (PoDs). *Mutat. Res.* 783:55–65.

- MacGregor JT, *et al.* 2015. IWGT Report on Quantitative Approaches to Genotoxicity Risk Assessment II. Use of Point-of-Departure (PoD) metrics in defining acceptable exposure limits and assessing human risk. *Mutat Res.* 783: 66-78.

Acknowledgements

- Mutagenic Mode of Action AOP: Lynn Pottenger, Martha Moore, J Klapacz, Marcy Banton

- Genetic Toxicology at the Crossroad: Dave Eastmond, Bob Heflich, Dan Levy, Paul White, John Wills