Current Strategies in Assessing Genotoxic Risk

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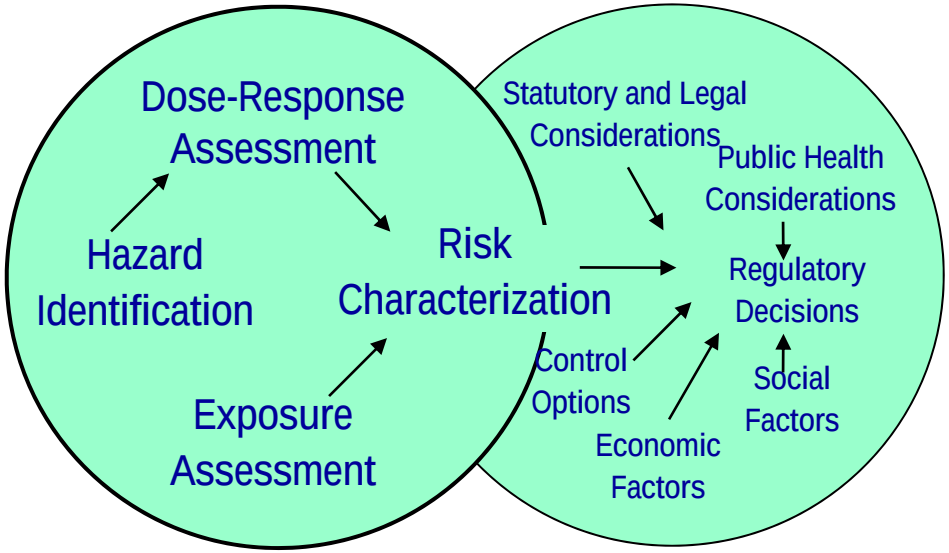
Presented at the HESI Annual Meeting
May 12, 2010

NRC Risk Assessment Paradigm

Risk Assessment

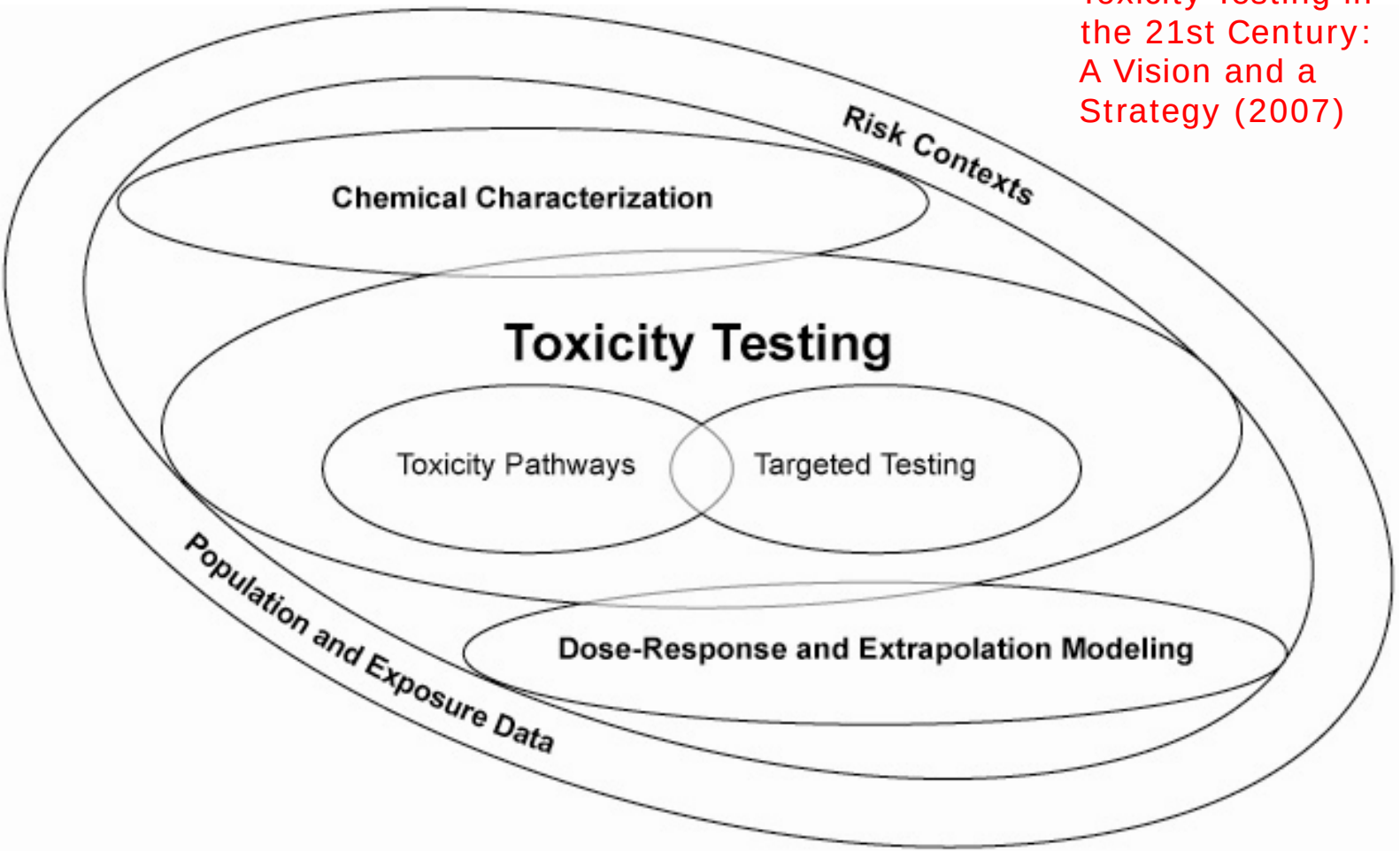


Risk Management

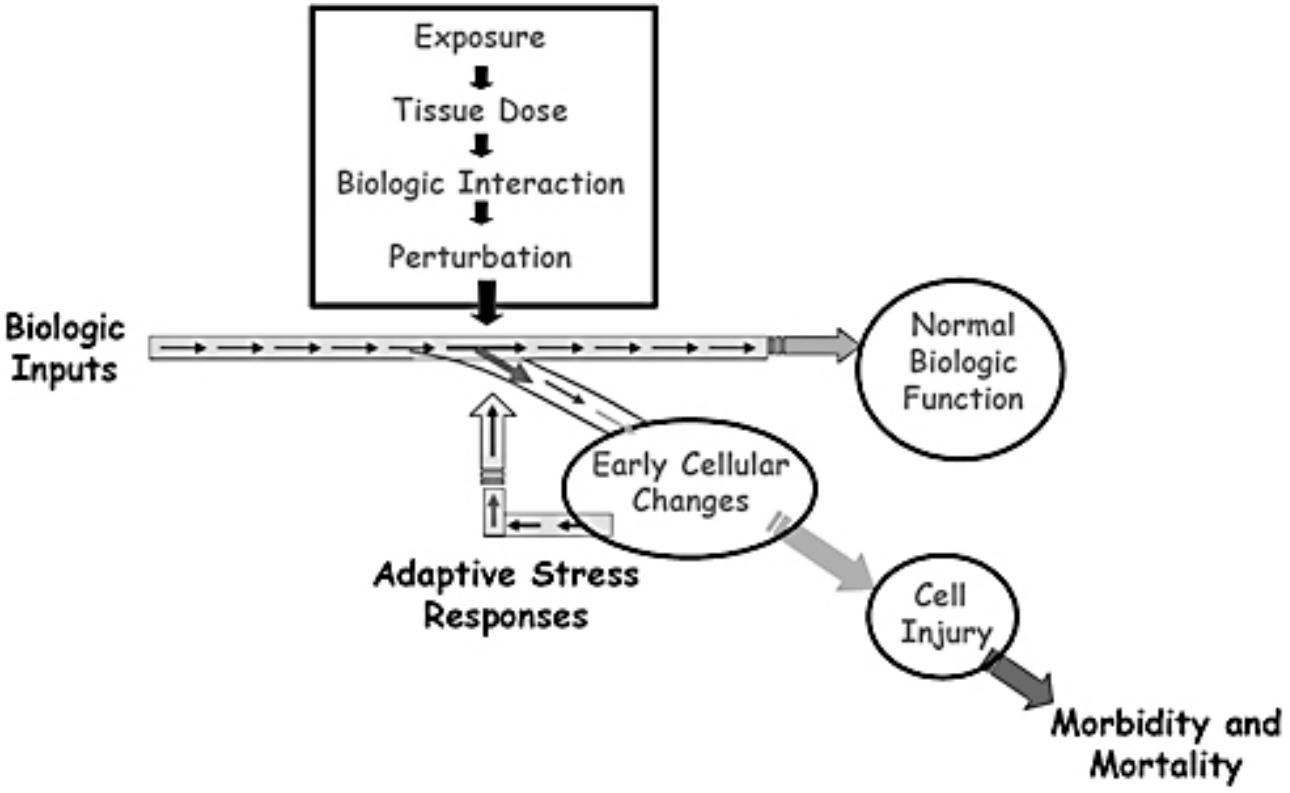
National Research Council, 1983

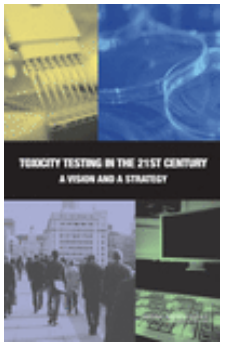
National Academies Current RA Vision

Toxicity Testing in the 21st Century: A Vision and a Strategy (2007)



Toxicity Testing in the 21st Century





Toxicity Testing in the 21st Century: A Vision and a Strategy (2007)

FIGURE 2-2 Biologic responses viewed as results of an intersection of exposure and biologic function. The intersection leads to perturbation of biologic pathways. When perturbations are sufficiently large or when the host is unable to adapt because of underlying nutritional, genetic, disease, or life-stage status, biologic function is compromised, and this leads to toxicity and disease.
Source: Adapted from Andersen et al. 2005. Reprinted with permission; copyright 2005, *Trends in Biotechnology*.

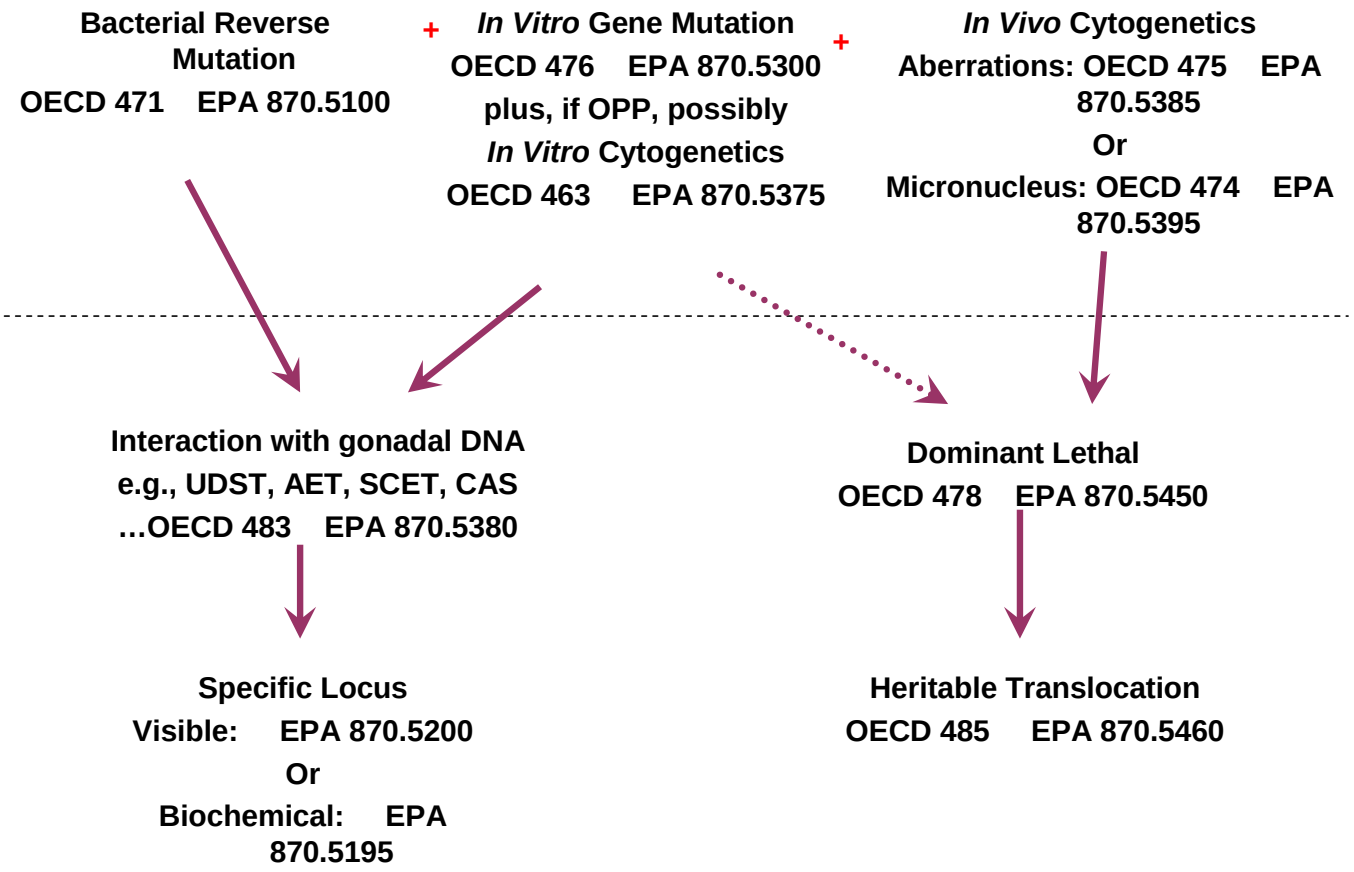
Testing Batteries

- Currently no single test can detect the entire spectrum of induced mutations.
- Assays and test batteries have developed to assess effects on the three major endpoints of genetic damage associated with human disease:
  - mutations in single genes (point mutations) or in blocks of genes;
  - clastogenicity (structural chromosome aberrations);
  - aneuploidy (numerical chromosome aberrations).

EPA Testing Scheme

- Current EPA genotoxicity testing battery is required for pesticides and toxic substances that are regulated under:
  - Federal Insecticide, Fungicide and Rodenticide Act (FIFRA)
  - Toxic Substances Control Act (TSCA)
- Three-tiered system of various genotoxicity tests
- Discussed in Dearfield *et al*. 1991. *Mutat. Res*. 258:259-283

OPPTS Mutagenicity Testing Scheme for Existing Chemicals and Pesticides



ICH Testing Scheme

- International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH Guidelines)
- The standard test battery:
  - A test for gene mutation in bacteria
  - An *in vitro* test with cytogenetic evaluation of chromosomal damage with mammalian cells or an *in vitro* mouse lymphoma tk assay
  - An *in vivo* test for chromosomal damage using rodent hematopoietic cells
- Discussed in Muller *et al*. 1999. *Mutat. Res*. 436(3):195-225

MOA Framework

- 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

MOA Simple Scheme

(from Dearfield & Moore, EMM 46: 236-245, 2005)

Weight of evidence (WOE) analysis to determine if mutagenic or not

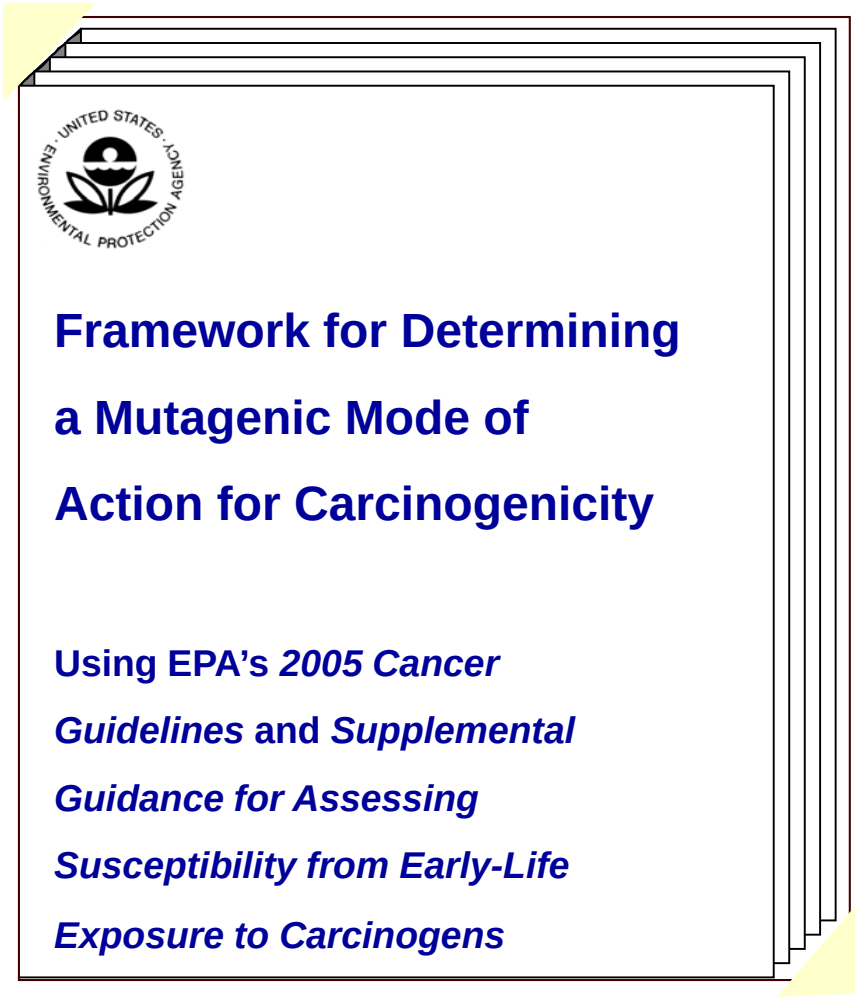
↓

WOE analysis to determine if mutagenic activity is mode of action (MOA) for the adverse effect of concern

↓

Inform low-dose extrapolation (i.e., is MOA likely to be linear, non-linear, or unknown at low exposure levels)

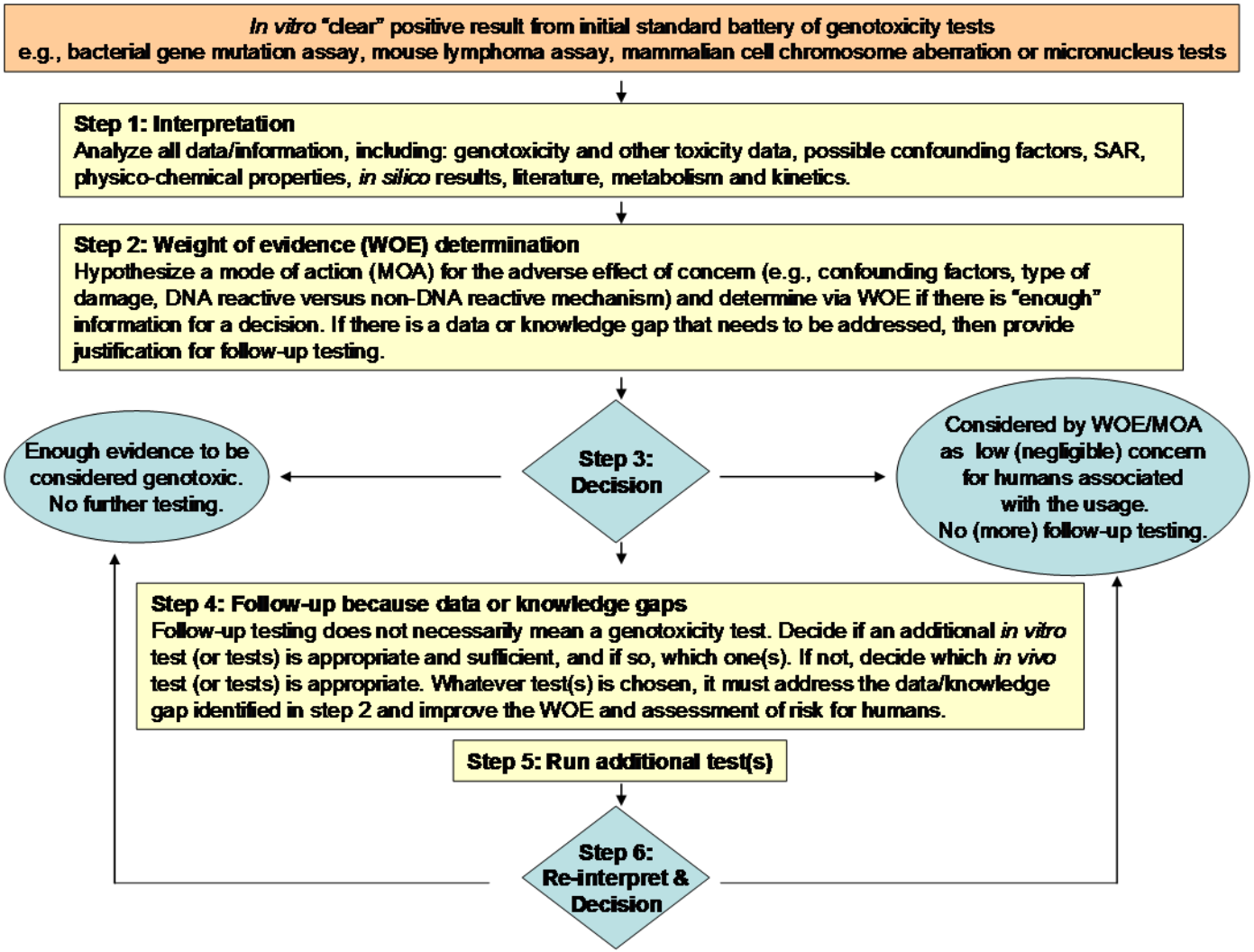
EPA's Mutagenic MOA Framework



External peer review completed 05/08

www. epa.gov/osa/mmoaframework/pdfs/MMOA-ERD-FINAL-83007.pdf

IVGT: Follow-up testing and decision making in case of in vitro positive results



Ranking of existing genotoxicity assays

Type of assay	Category	OECD Guideline(s)	Endpoint(s)	Strengths	Limitations	Opportunities	References
Gene mutations							
Gene mutation assays in transgenic models	2	No guideline	Gene mutations (point mutations including base pair substitutions and frameshift mutations) in mammals *in vivo.* Reporter genes (e.g., *lacZ*, *lacI*, *gpt*) in shuttle vectors (e.g., lambda phage). Some models (e.g., *spi*, plasmid) also have the ability to detect deletions.	Can be applied to any tissue. Relevant end-point: gene specific. No selective pressure on mutations, therefore accumulation of damage over time. Uses a small number of animals.	Labor intensive and expensive. Requires multiple dosing. Requires transgenic animals. Need to optimize protocols for different tissues, or to apply the recommended design (28 treatment days, sampling after 3 and/or 28-day recovery period). Mutamouse, Big Blue and *gpt* delta models do not detect large deletions. Relatively high mutant frequency background shown to impact the sensitivity.	Mutant sequencing for mechanistic information (mutational spectrum) and confirmation of mutation (increase in mutant frequency versus clonal effect). Quantitation of dose response possible.	Heddle et al. (2000), Thybaud et al. (2003), Lambert et al. (2005), OECD (2009)

Example

The End

Thank you very much

Any questions?

Except where noted, the views presented in this presentation are solely those of the presenter.

IVGT: Potential follow-up assays in case of in vitro positive findings

Assays that can be chosen		DNA adduct assay	UDS assay	Comet assay	Gene mutation assays			Micronucleus assay		Chromosome Aberration assay	Assays for non DNA reactive mechanisms
					In vitro assays (e.g. hprt)	Transgenic models (a)		Micronuclei without centromere	Micronuclei with centromere		
End-points detected by the above assays		DNA Primary damage									
		Adducts		Breaks	Point mutations	Point mutations	Deletions	Structural chromosome damage	Numerical chromosome damage	Structural chromosome damage	
Follow-up in case of positive findings in the *in vitro* gene mutation assays: for mechanistic purpose and/or confirmation of the *in vitro* findings	*In vitro* assays	To evaluate DNA reactivity			To confirm the gene mutation end-point *in vitro*						To evaluate the evidence supporting non DNA mechanisms
	In vivo assays	To further evaluate DNA reactivity *in vivo*				To further evaluate the gene mutation end-point *in vivo*					
Follow-up in case of positive findings in the *in vitro* chromosome damage tests: for mechanistic purpose and/or confirmation of the *in vitro* findings	*In vitro* assays	To evaluate DNA reactivity					To confirm the induction the chromosome damage end-point *in vitro* and to differentiate clastogen from aneugen mechanism				
	In vivo assays	To further evaluate DNA reactivity *in vivo*					To further evaluate the chromosome damage end-point *in vivo*	To further evaluate chromosome damage end-point *in vivo*, and in case of *in vivo* findings to differentiate clastogen from aneugen mechanism			

(a): for more details on transgenic mutation assays and their ability to detect point mutations and deletions see Heddle et al. (2000) and Thybaud et al. (2003)