Beyond Positive or Negative: A Quantitative Approach for Interpreting Genotoxicity Data

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Presentation Outline

- A brief review of the current paradigm
- Dose-response in genotoxicity – the concept of NOGELs and Thresholds
- Application of Mode-of-Action and Key Event Framework to Mutagenicity
- Data Needs and Future Directions

Prevailing Paradigm in Genetic Toxicology

- Genotoxicity testing primarily for carcinogen identification.
- Data evaluation is binary – positive or negative.
- Default Assumption - dose-response linear without thresholds.
- Stigma associated with positive findings.
- Need for improvements in experimental design and data interpretation to inform risk assessment.

How can we use *in vitro* results to inform risk?

Vegetable	% Aqueous Juice (-S9/+S9)	% Aberrant Cells (-S9)	% Aberrant Cells (+S9)
Negative Control	-	3	1-3
Garlic	0.05	11	-
Peas	3	19	-
Broccoli	3	25	-
Carrots	10	3	3
Soybeans	10	11	21
Corn	3/10	8	9
Spinach	1/10	3	4
Bean sprouts	3/10	8	5
Asparagus	10/5	3	3
Positive Control	0.075 µg/mL MMC/6 µg/mL CP	34-46	52-56

Charles, Linscombe, Tornesi, Mattsson, Gollapudi: Food Chem. Toxicol., 40, 1391-1402, 2002

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The Concept of "Thresholds"

- **Definitions of Threshold:**
  - *Dose or exposure concentration of an agent below which a stated effect is not observed or expected to occur*
  - *transition point (observed or expected) between the highest dose that will not elicit a given biological effect, and the lowest dose that will.*
  - *the highest dose for which the response is not significantly (statistically or biologically) distinguishable from the control/background values,*
  - *etc. etc. ….*
- **Often accompanied by a descriptor such as "absolute", "theoretical", "operational", "practical", "apparent", "biological", etc.**

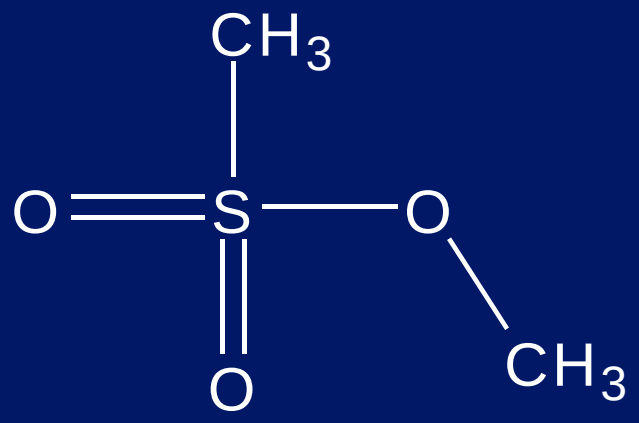
‘The “threshold” concept conflict is not likely to be resolved in the foreseeable future; proponents and opponents argue their case in a manner similar to those arguing religion.’

Gehring and Blau, 1978

IVGT Committee Recommendations - a bold first step in the right direction (Thybaud *et al*., 2007, Mutat. Res., 633, 67-79)

1. Examine suitability of applying thresholds of toxicological concern (TTC) concepts to genotoxicity,
   - BMDs and/or NOGELs from genotoxicity data.
2. Weight of evidence approach with robust qualitative and quantitative criteria for assessing genotoxic hazard,
   - utilize *in vivo* and *in vitro* dose–response data and human exposure information to characterize levels of concern.

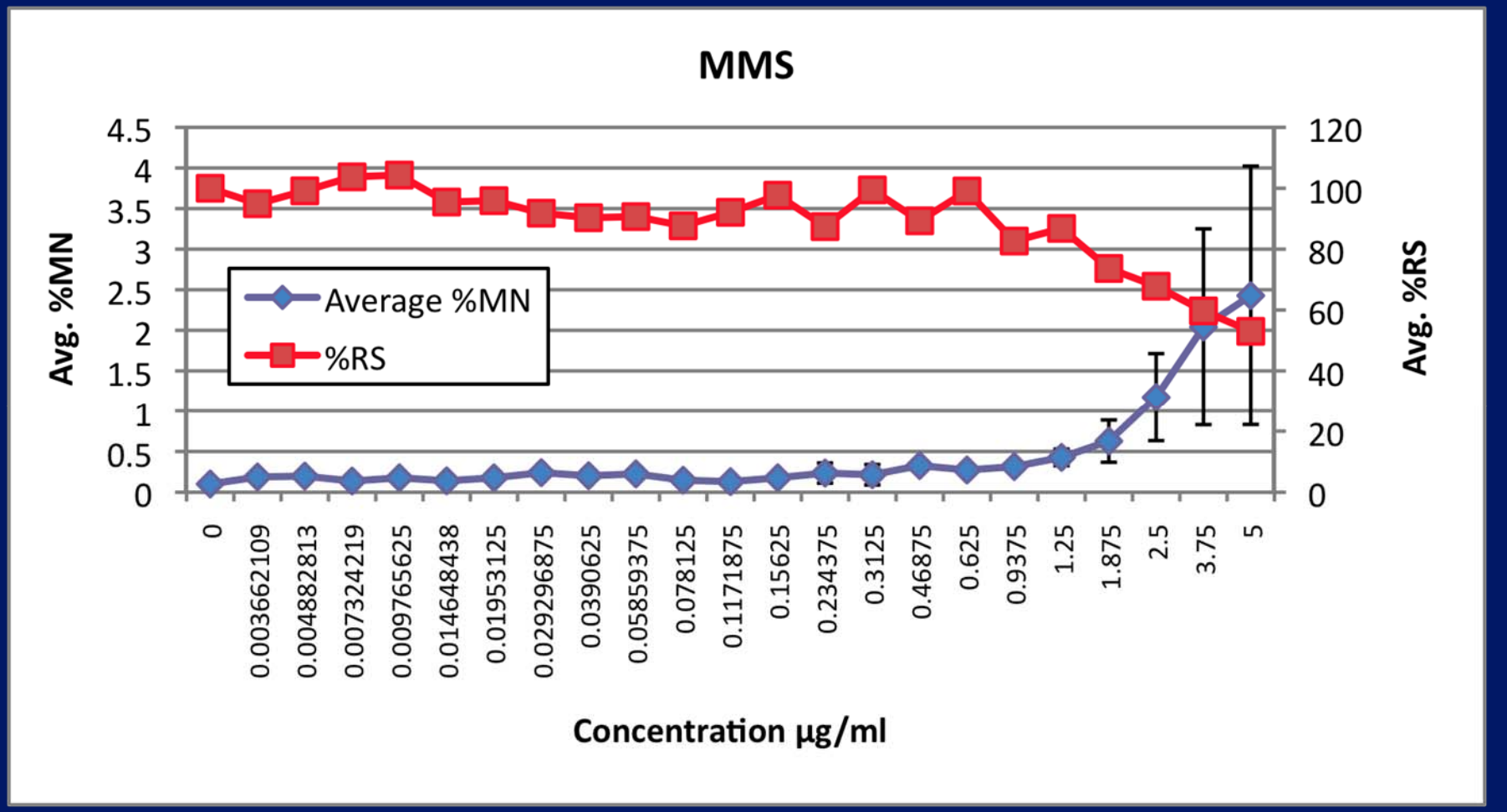
MMS : Dose-Response & NOGELs



Methyl methanesulfonate

- S_N2 alkylating agent
- 83% N7-MeG
  0.3% O^6-MeG

TK Cells Treated With MMS –Flow-based In Vitro MN (Courtesy: S. Dertinger, Littron Laboratories)



Lutz "Hockey Stick" Model: MMS Flow MN Data

$a
[1] 0.18845

$CIa
[1] 0.1118479 0.2648027

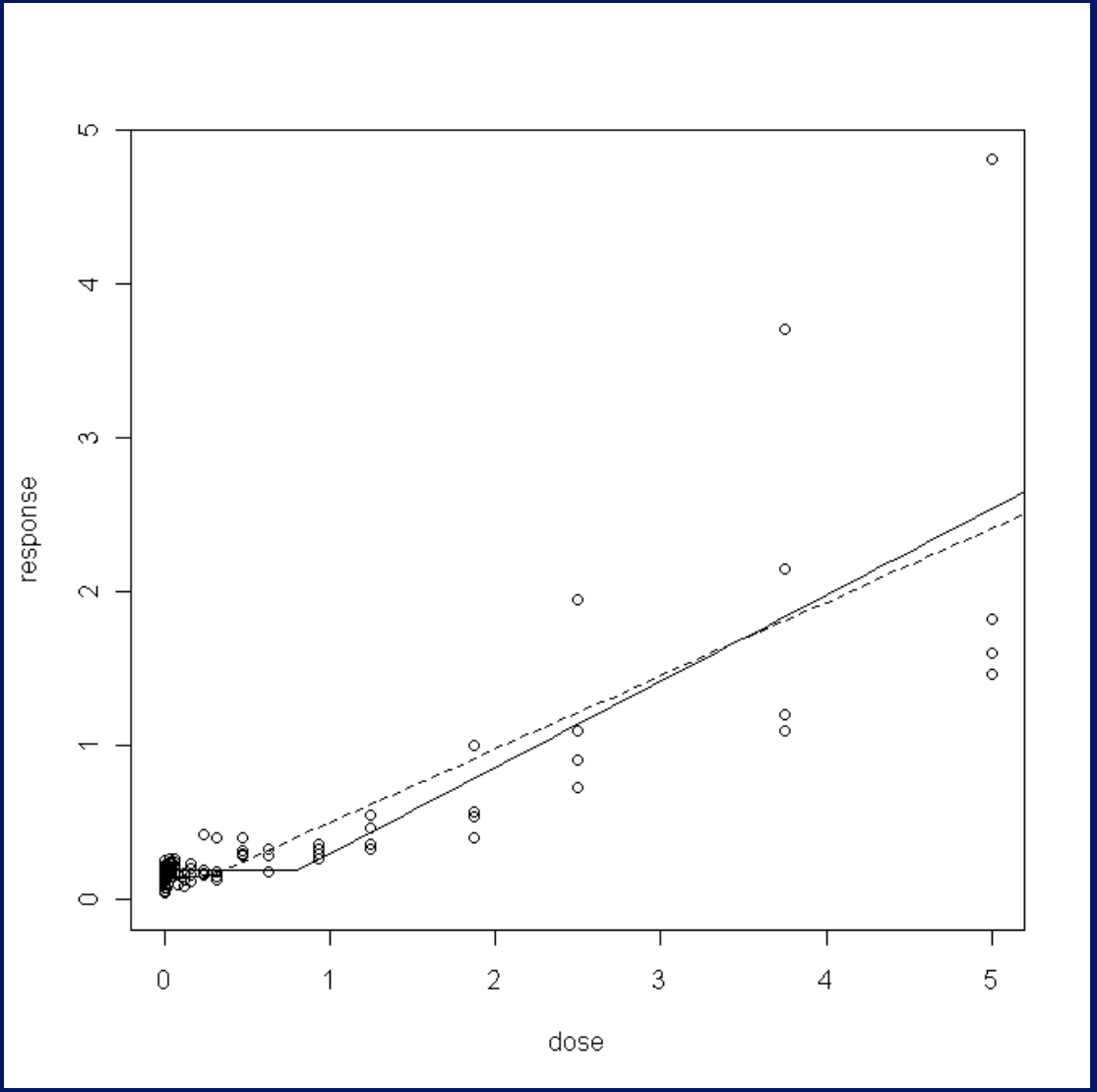
$td
[1] 0.80445

$CItd
[1] 0.2661150 1.2797277

$b
[1] 0.5607

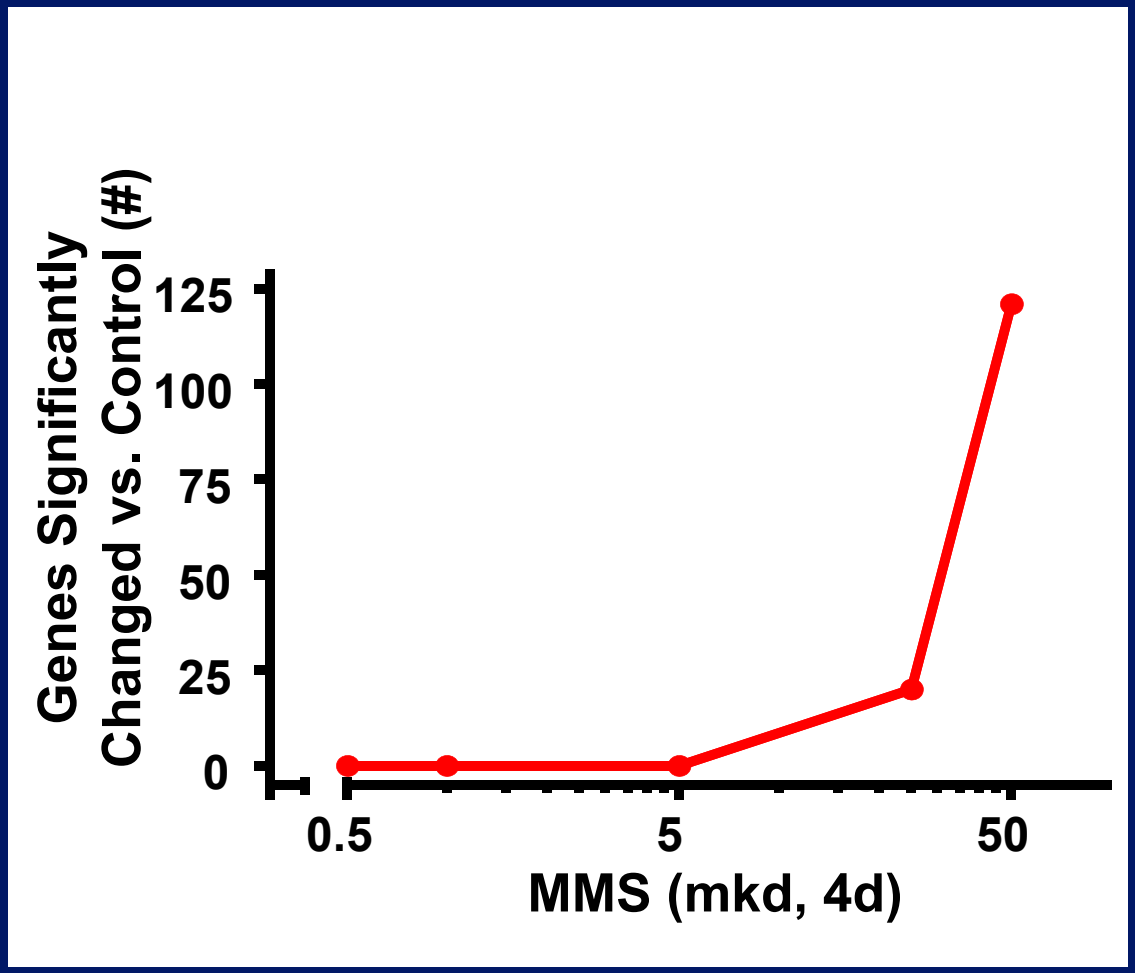
$CIb
[1] 0.4616451 0.6726306

$pvalue
[1] 0.02151534



Gene Expression Changes in MMS-treated rat livers

Agilent complete rat genome array (41,121 features)



Number of Genes Significantly Changed		
	25 mkd	50 mkd
1.5 fold	8	112
2 fold	3	79
3 fold	2	38
4 fold	2	31
5 fold	0	20

MMS - Clear NO(G)ELs demonstrated for a number of other end point points

1. *In Vitro* Mouse Lymphoma Cell Cultures
   - DNA Adducts
   - Mutations
2. *In Vivo* Rat Peripheral Blood
   - DNA Adducts
   - MN-reticulocytes by flow
3. *In Vivo* Rat Liver
   - DNA Adducts
   - Gene expression changes

Comparison of NOGELs Across Assays

Assay	NOGEL (µg/mL)
In Vitro MLA - Mutation	1
In Vitro TK – MN[1]	1
In Vitro AHH –MN & hprt[2]	1
In Vivo – Rat MN[3]	5

[1]Dertiner et al., 2010; [2]Doak et al., 2009; [3]Estimate

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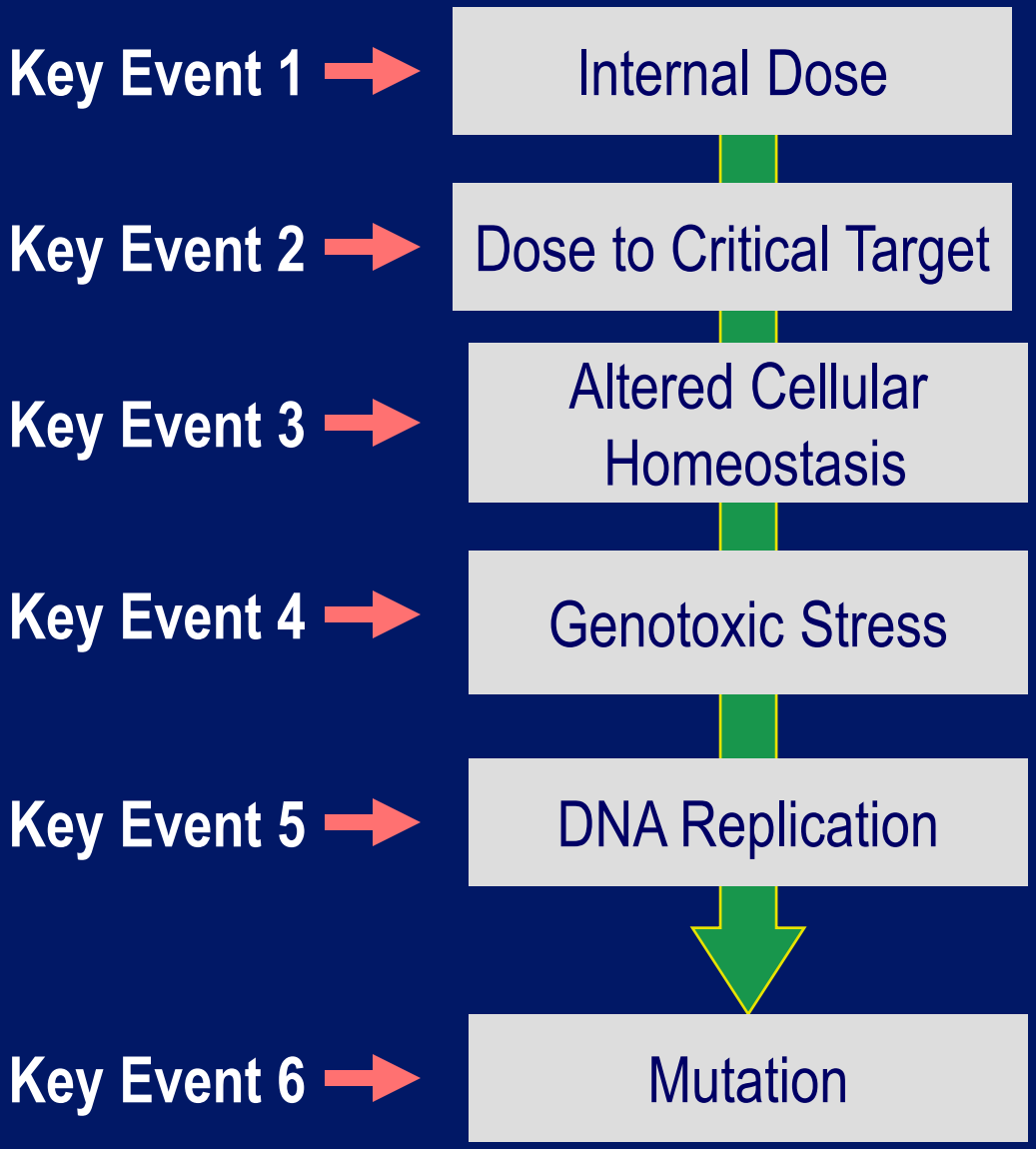
Key Event Dose-Response Framework (KEDRF)

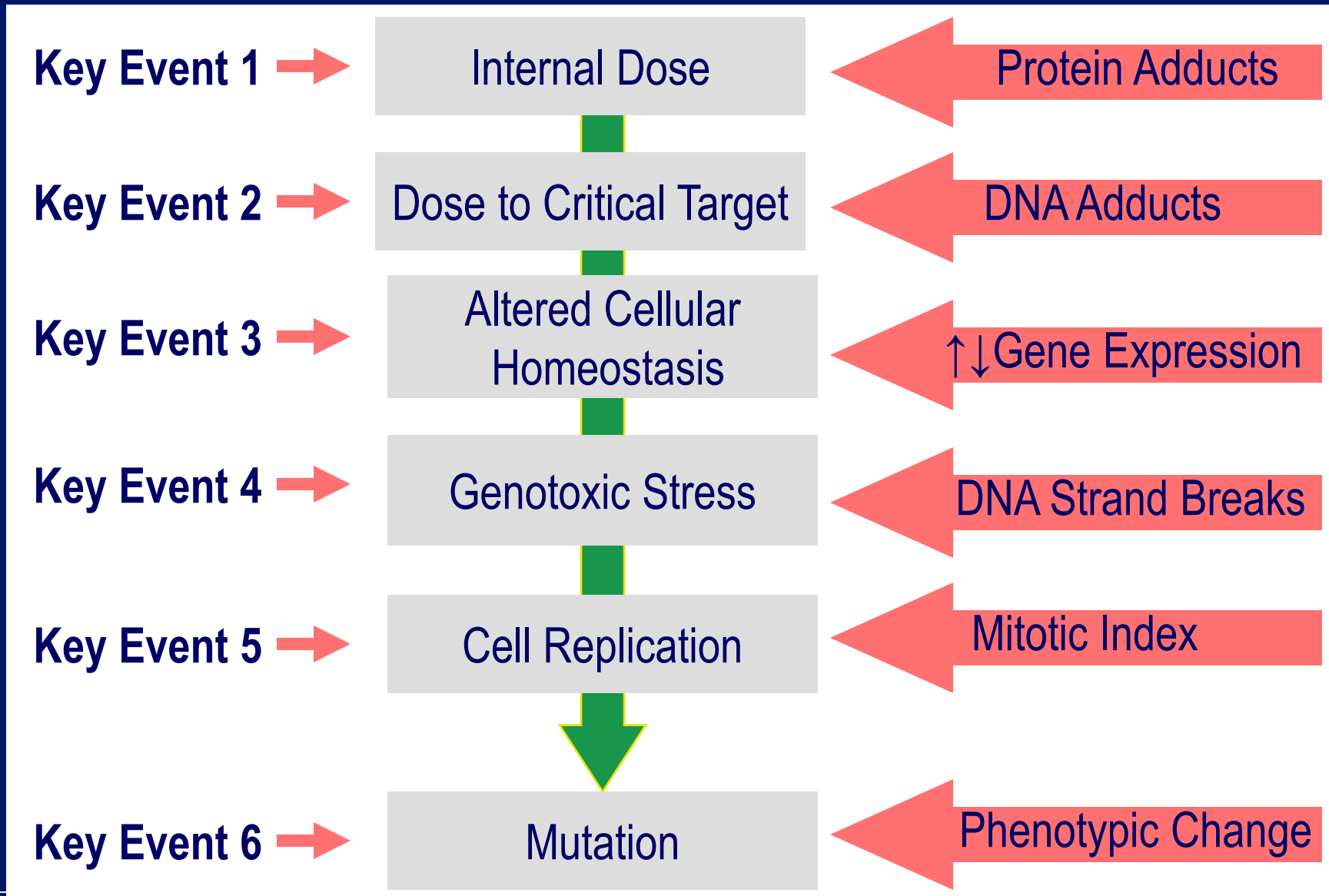
- A series of key events lead to the effect,
  - Key event: a necessary, but not sufficient, step
- Dose-response relationship for each individual event in the pathway is described to determine
  - how individual dose-response relationships combine to generate the overall dose-response curve, and
- KEDRF helps to critically evaluate existing data for knowledge gaps.

- *Ref: Julien et al. (2009): The key event dose-response framework: A cross-disciplinary mode-of-action based approach to examining dose-response and thresholds, Critical Reviews in Food Science Nutrition, 49, 682-689.*

Direct Acting Alkylating Agent – Postulated MOA and Key Events



Biomarkers of Postulated Key Events - An Example



Biomarkers of KE - Dose-Response and Temporality

Temporal

Dose

Dose (mg/kg or µM)	*KE 1:* Protein Adducts	*KE 2:* DNA Adducts	*KE 3:* Gene Expression Changes	*KE 4* DNA Strand Breaks	*KE 5:* Cell Repliction	KE 6: Mutation
1	+	-	-	-	-	-
2	++	-	-	-	-	-
3	+++	++	+	-	-	-
4	++++	+++	+	+	+	-
5	++++	+++	++	++	+	+

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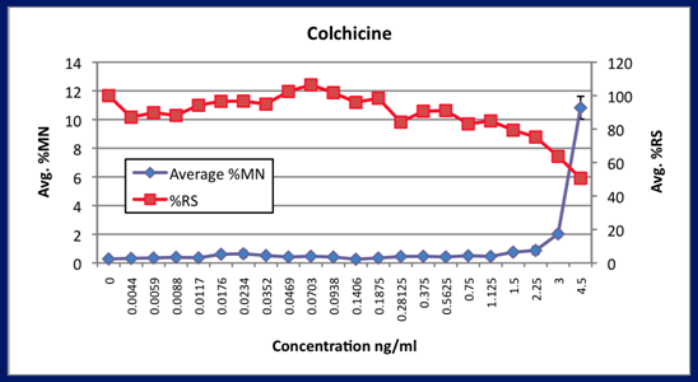
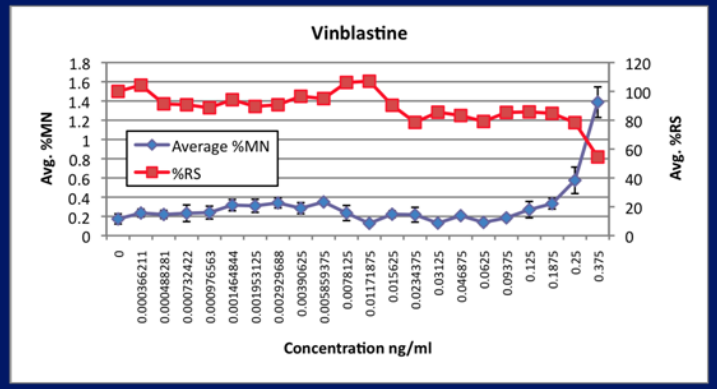
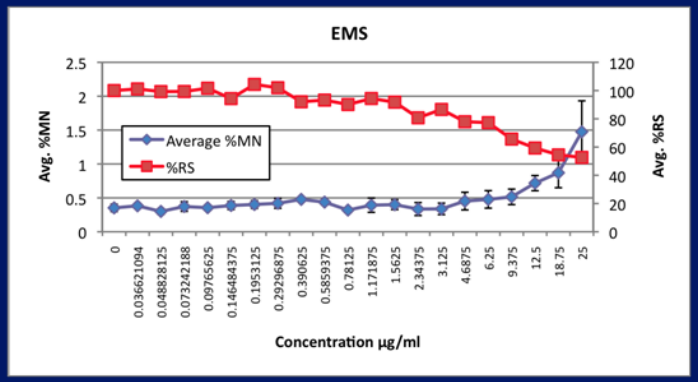
Data Needs for Establishing Thresholds for DNA-reactive Agents (Boobis et al., 2009, Crit. Rev. Food Sci. Nutri., 49, 690-707)

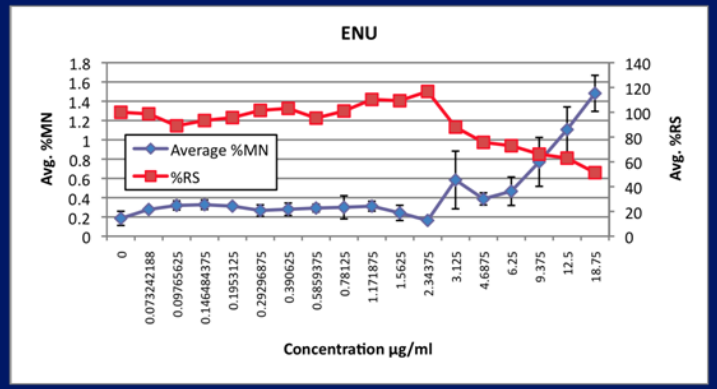
- Background levels of DNA damage.
- Background levels of genetic alterations.
- Dose-response curves for DNA adducts and genetic alterations over a low dose range.
- DNA repair characteristics at low doses.
- Rate of induced cell proliferation at low doses.
- Reliable biomarkers of response based on key events for characterizing low dose effects.

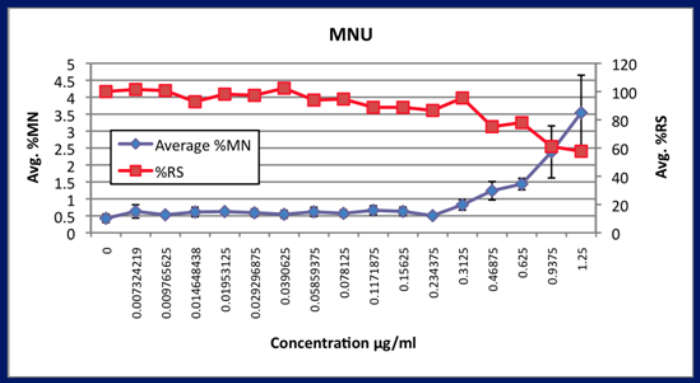
Flow-based In Vitro MN Dose-Response Studies (Courtesy: S. Dertinger, Littron Laboratories)

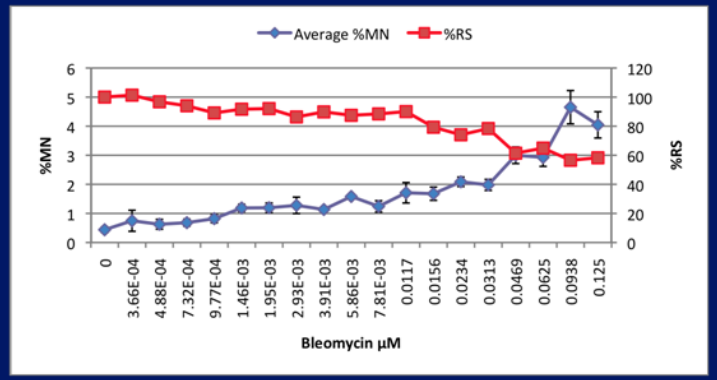




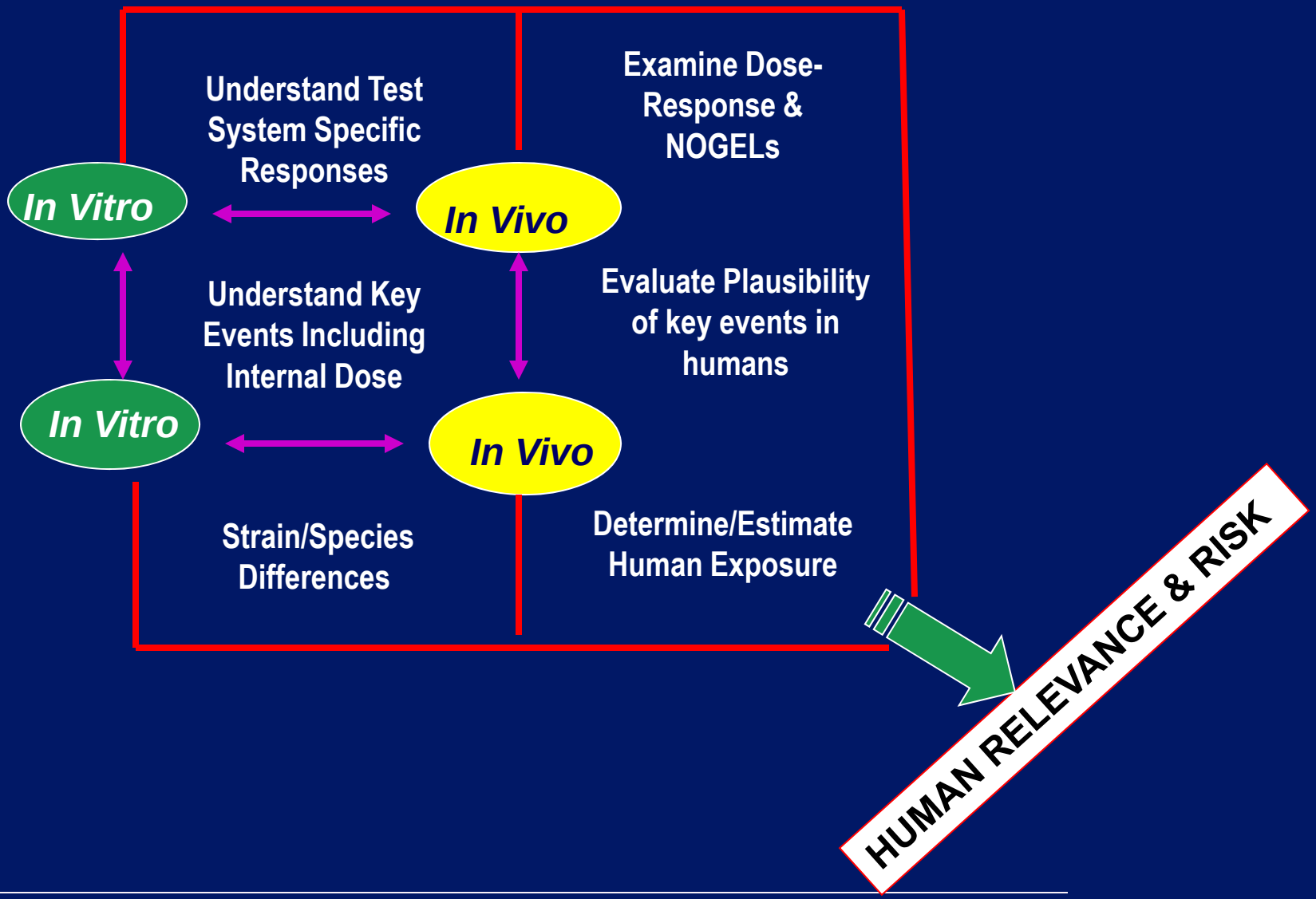








Use Available Data to Determine Human Relevance



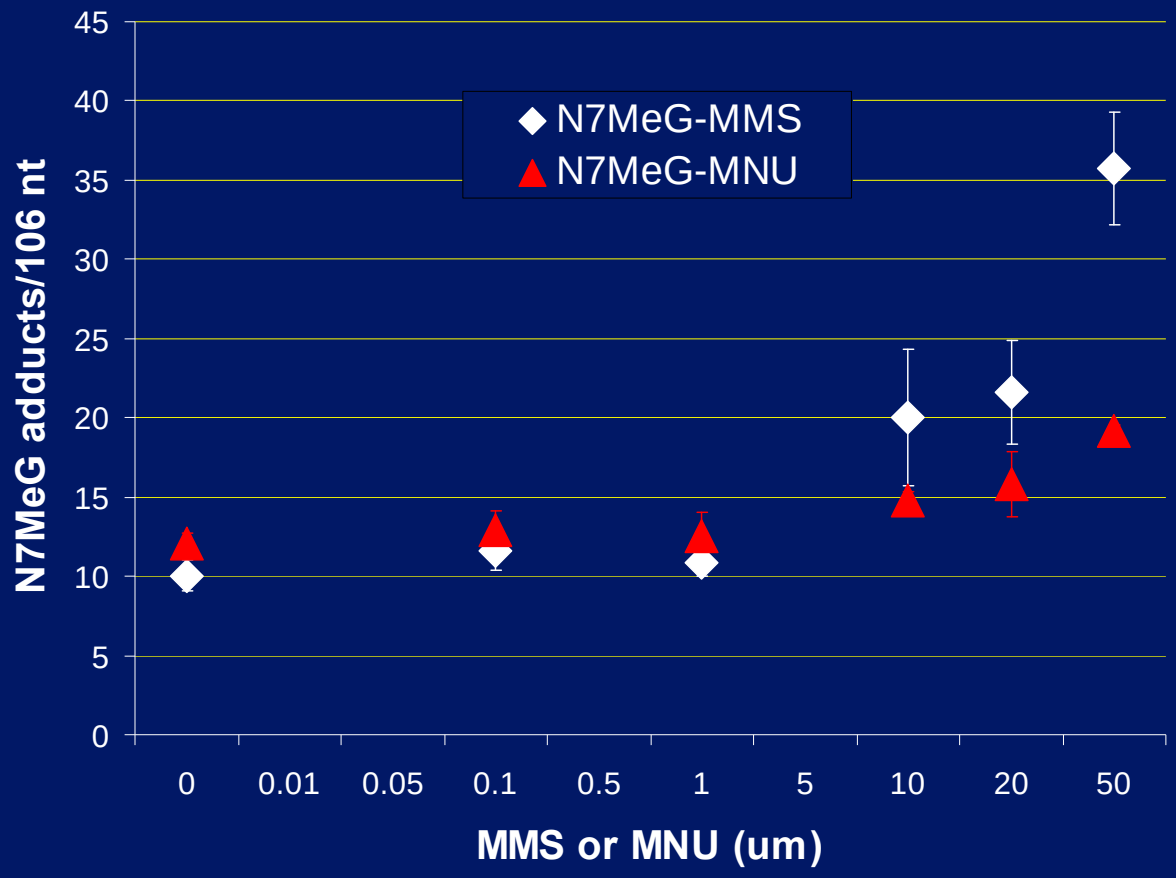
A Genetic Toxicologist Adopts/adapts Paracelsus

- *"What is there that is not a genotoxin? All things are genotoxins and nothing [is] without genotoxicity. Solely the dose and/or the test system determines that a thing is not a genotoxin."*

Acknowledgements

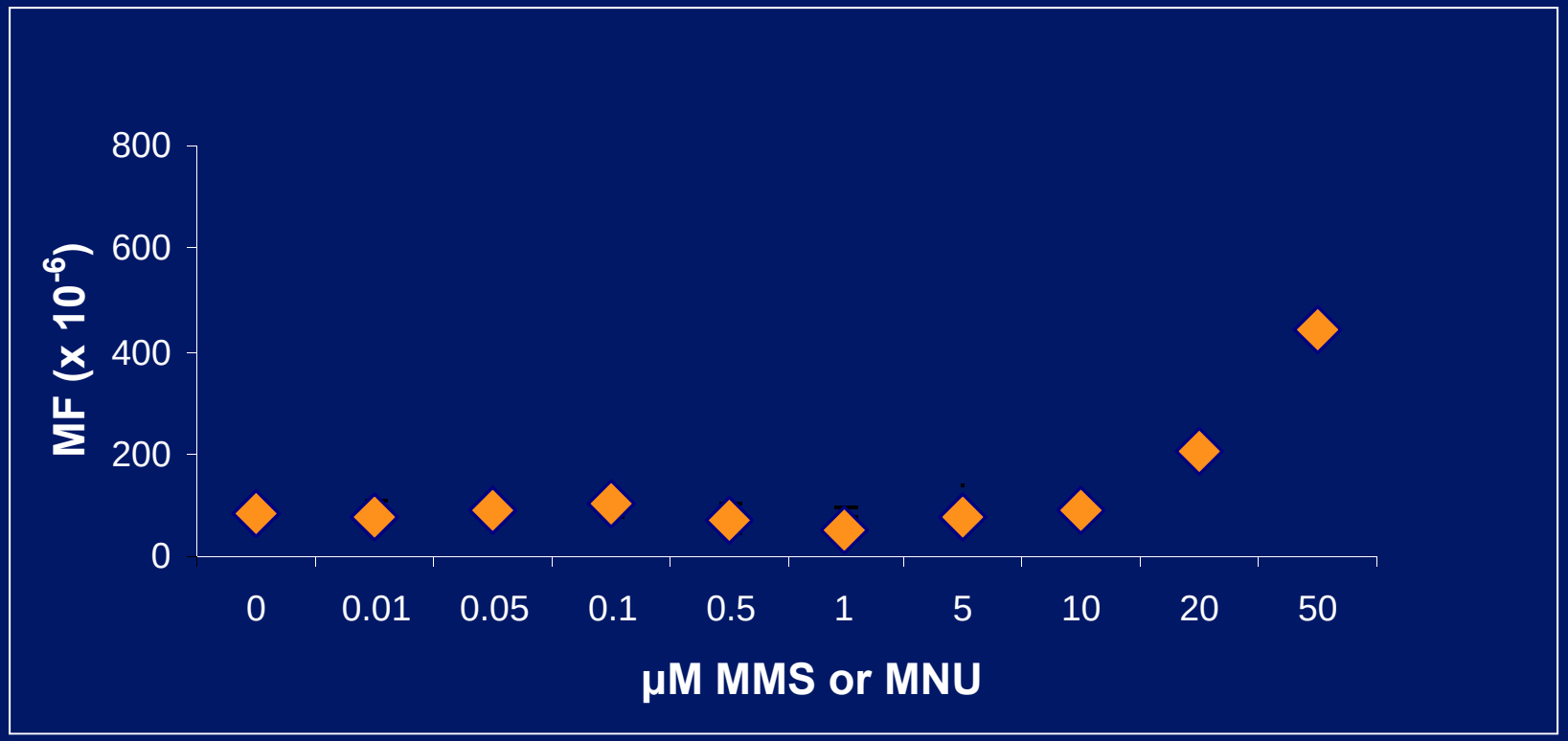
- Steve Dertinger and Littron Laboratories for kindly providing the dose-response data on MN induction in TK Cells.
- Dow Colleagues: Lynn Pottenger, Matt LeBaron, Melissa Schisler, Fagen Zhang.

MMS and MNU-induced N7MeG Adducts in the mouse lymphoma cells in culture

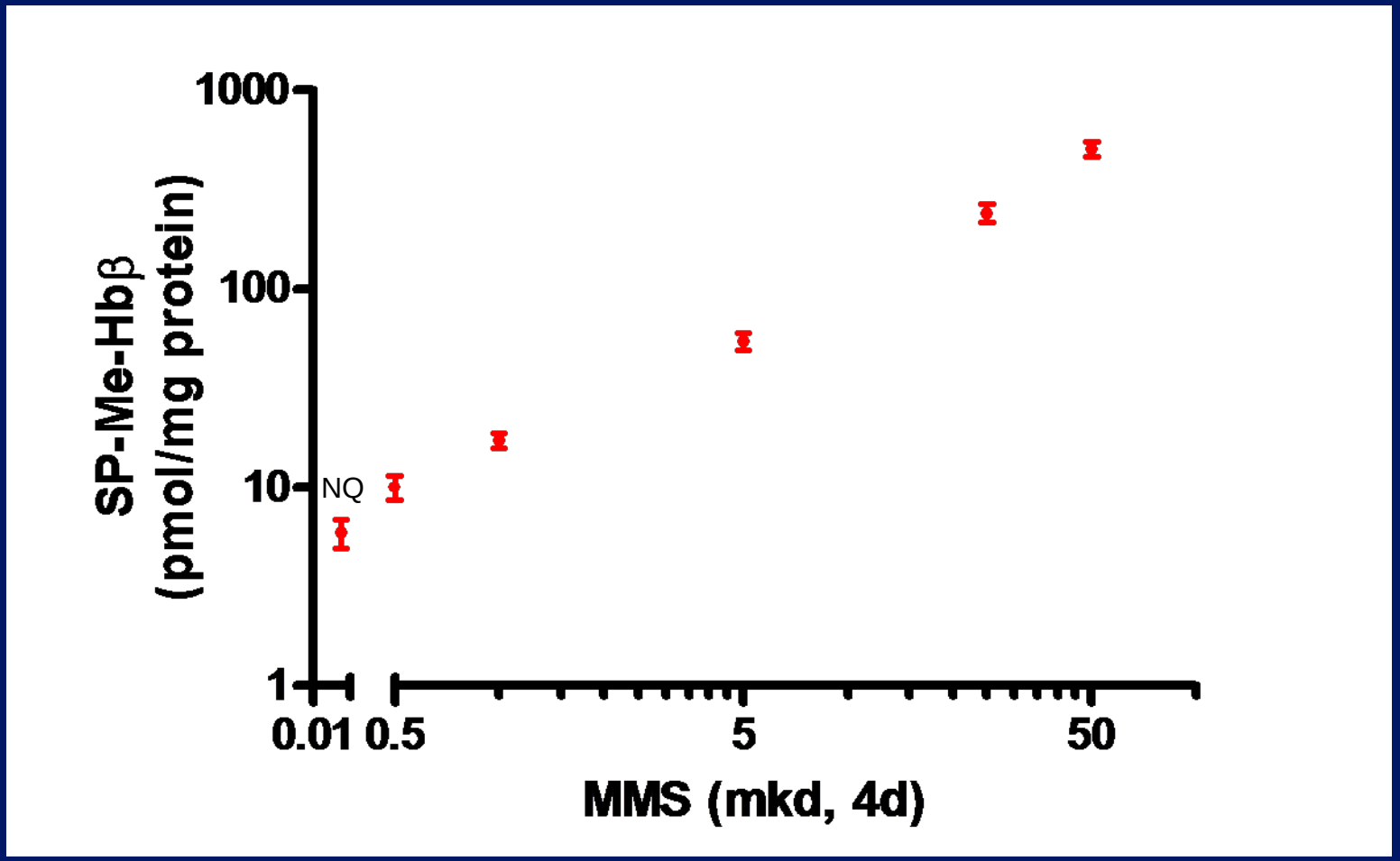


In Vitro Cell Cultures

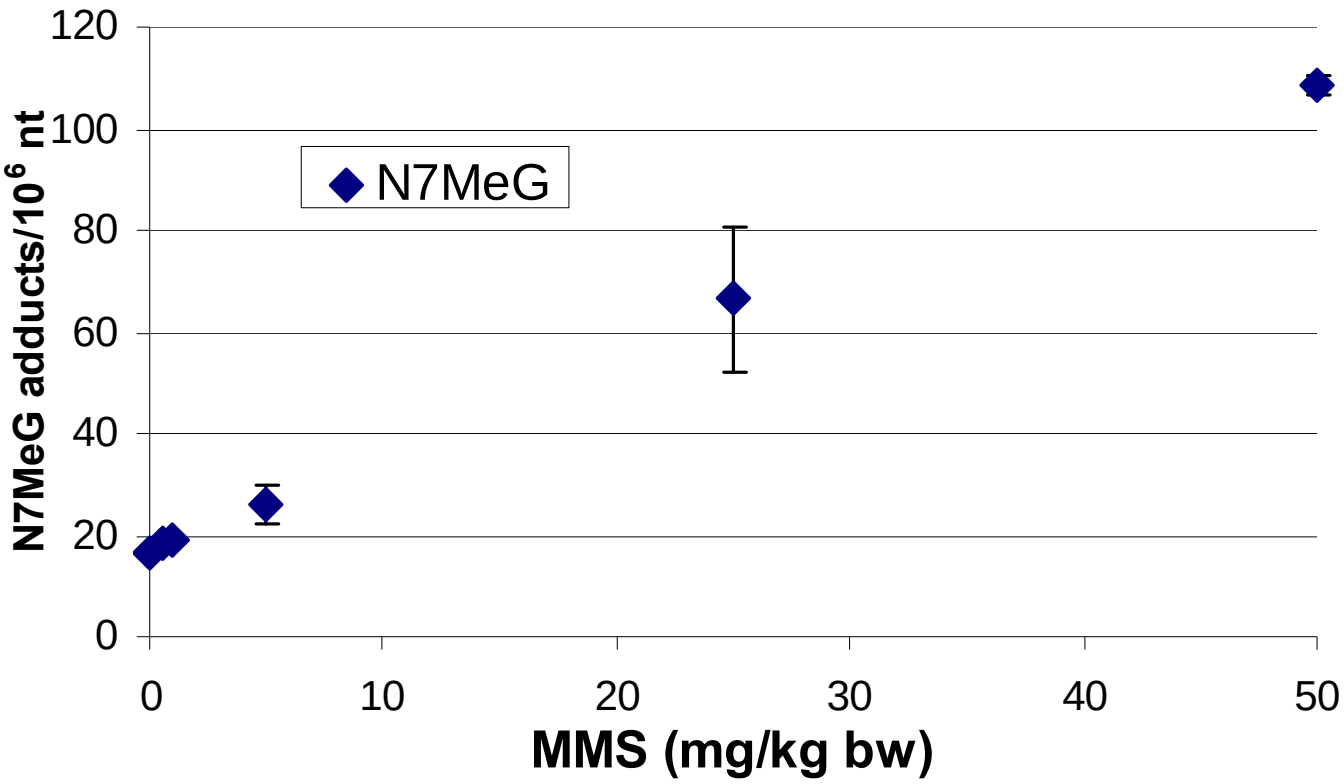
In Vitro dose-response for the induction of *tk* mutations in the mouse lymphoma cell cultures



In Vivo Rat PB MNT - Internal Dose



DNA Adducts Peripheral Blood WBC - Rat



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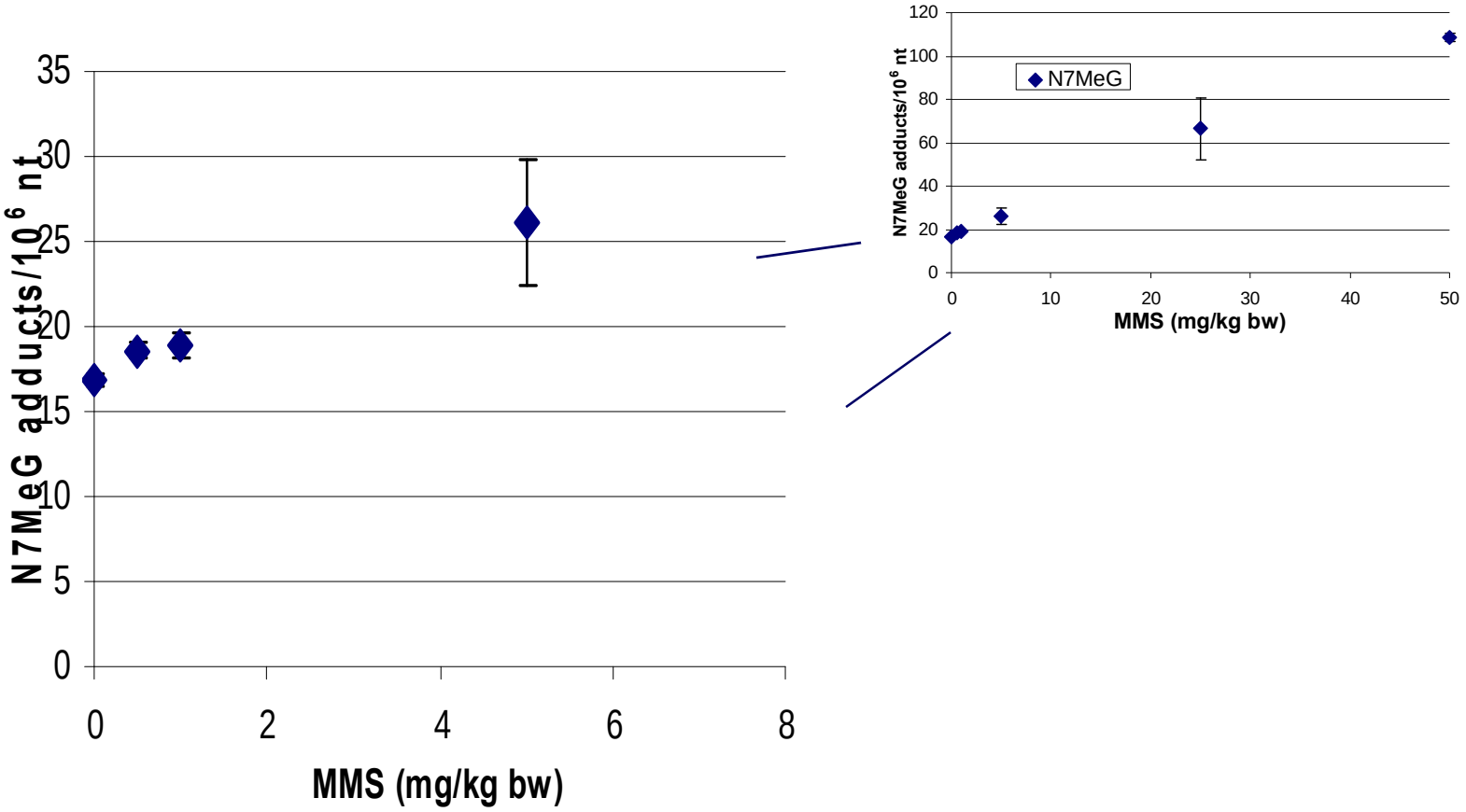
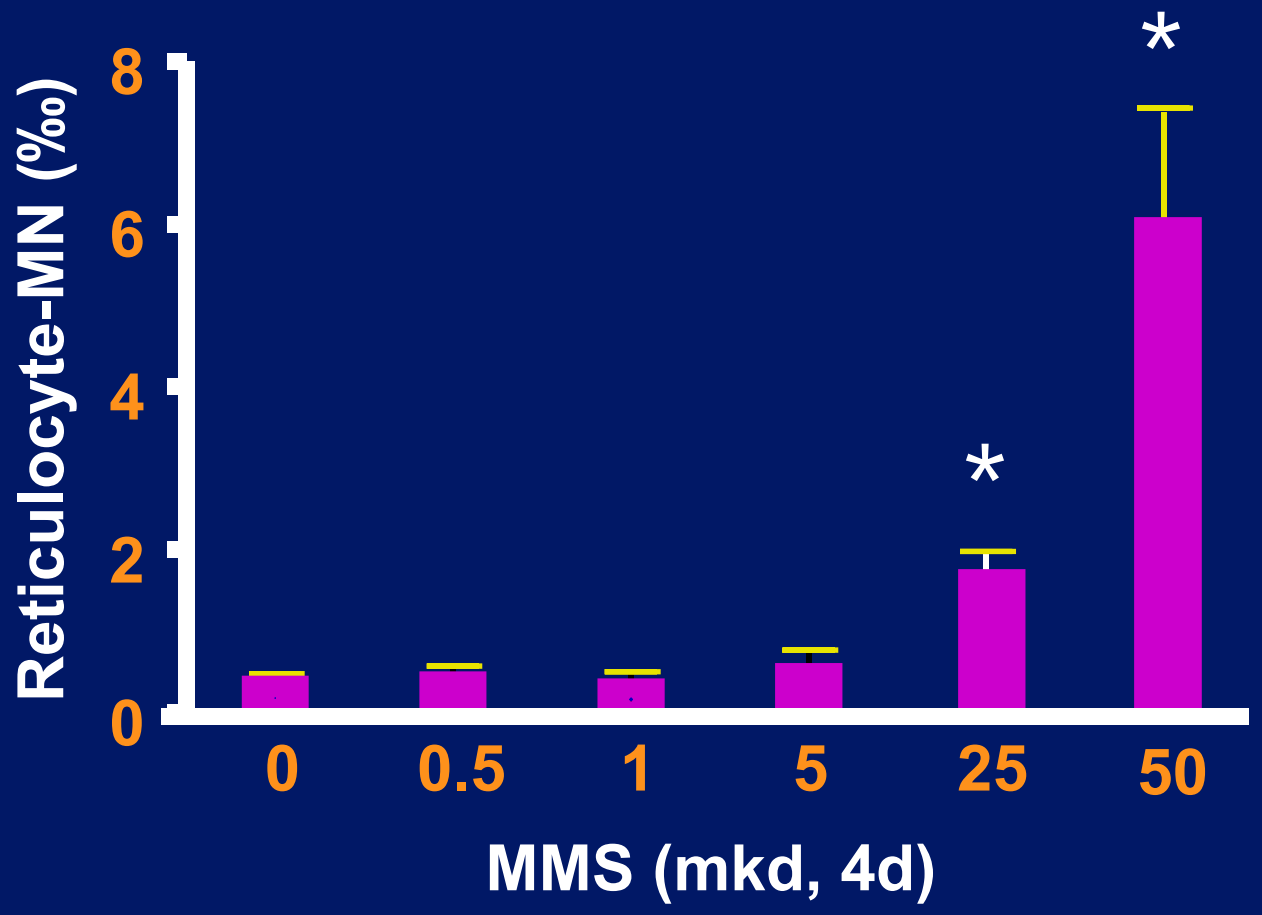


Dose Response: *In Vivo* Rat PB MNT



DNA adducts in liver of MMS-treated rats