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Need for a New Approach to Genetic Toxicity Assessment:

Lessons Learned and New Opportunities

James T. MacGregor
Toxicology Consulting Services
Arnold, MD 21012

Regulatory Genetic Toxicology Begins in the 1970's

- **EPA and FDA testing batteries adopted**
- **Need for *in vivo* risk assessment recognized**
- **DHEW Subcommitte on Environmental Mutagenesis (1974-77)**
  - **"It is not sufficient to identify substances which may pose a genetic hazard to the human population."**
  - **"…it is necessary to obtain quantitative data from relevant animal model systems from which extrapolation to humans can be made to predict virtually safe or tolerable levels of exposure."**

Thinking changed significantly by the end of the 1970's

McCann and Ames (PNAS, 72: 5135-9, 1975): Carcinogens are mutagens

- Perception that mutagens & carcinogens are rare & simple screening can identify them & eliminate exposure
- Cancer became the main health consequence of concern, especially at FDA
- Regulatory testing and decision-making based largely on qualitative test outcomes

Only recently has it been recognized that regulation based on qualitative test outcomes is inadequate

- Kirkland et al. Mutat. Res. 584: 1-256, 2005: I*n vitro* tests correlate poorly with *in vivo* cancer test outcome ("too many "false positives"")
- Thybaud et al., Mutation Research 633: 67-79, 2007: We need to move to a more quantitative risk assessment paradigm
- FDA, 2006: overall weight of evidence emphasized, but quantitative approach still not endorsed
- CHMP, 2006: Threshold of toxicological concern recognized for genotoxic pharmaceutical impurities
- ICH, S2(R1) proposed genetic toxicology testing revision: more weight on *in vivo* outcomes

Regulation based on qualitative outcomes of *in vivo* tests is also inadequate

- For example, limiting exposure of agents giving positive mutagenic effects *in vivo* would lead to the following case

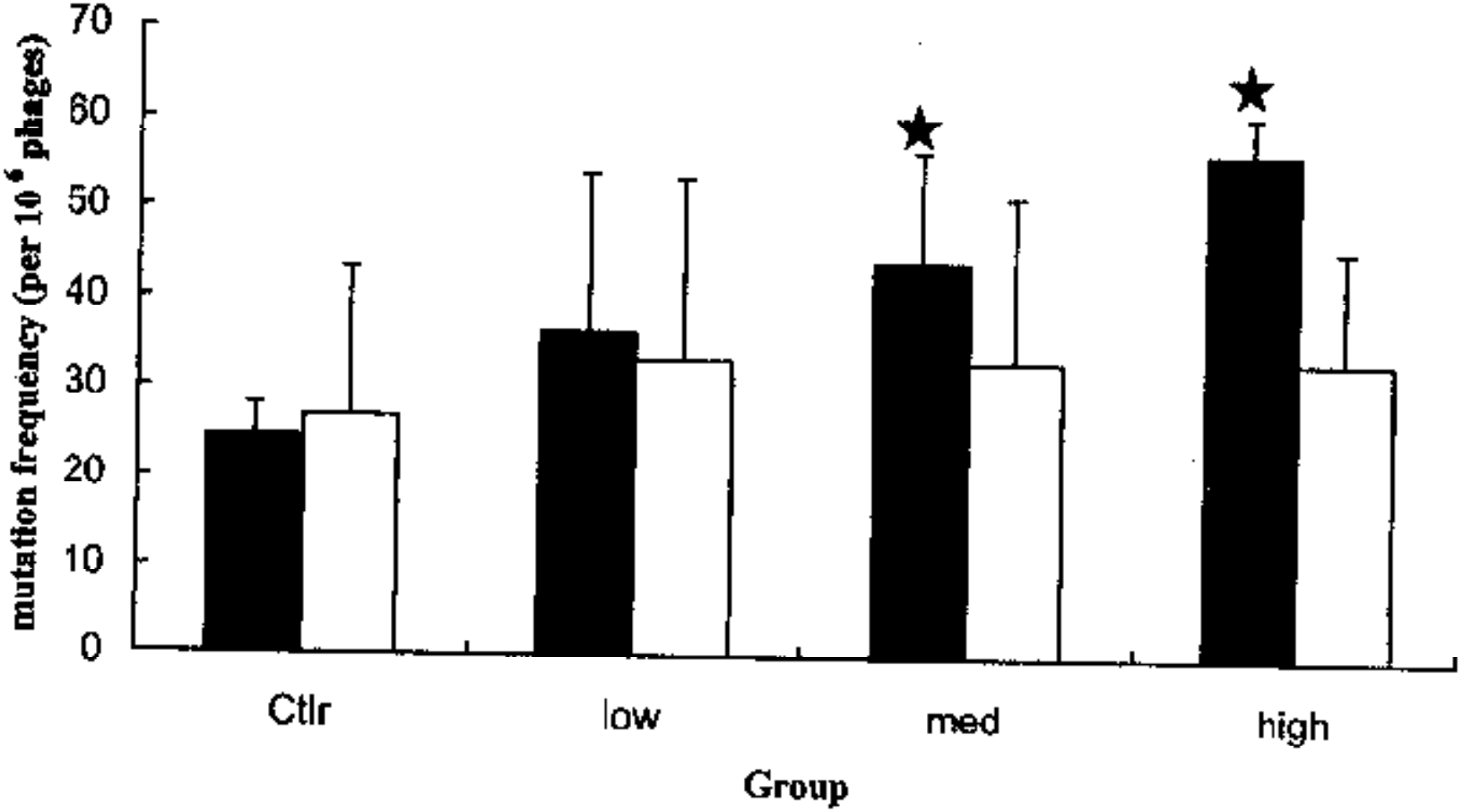


Fig. 1. Mutation frequency of *cII* in liver (□) and colon (▨) in the four groups. *Bars* represent means, and *lines* denote 1 SD. Groups that are significantly different ($P < 0.05$) from the control group (group 1) are denoted with a *star*.

Note that, in the preceding example:

- The route is relevant (dietary administration at a non-toxic level)
- The result clearly shows genotoxicity under “relevant” test conditions
- The test substance is sucrose[1]

[1]A Sucrose-rich Diet Induces Mutations in the Rat Colon, L.O. Dragsted et al, Cancer Res. 62: 4339-45, 2002.

The HESI IVGT Initiative

- Recognition that *in vitro* hazard screening is insufficient led to HESI IVGT initiative in 2005
- Early consensus (June 2006): Quantitative dose-response & exposure information could contribute to better risk categorization. Useful to determine:
  - If *in vitro* potency in mammalian cell assays and projected human exposure can be used to categorize risk into broad levels
    - For direct-acting agents only, or can metabolism can be taken into account? Only for agents with expected thresholds? etc.
  - If analysis of dose-response parameters and/or benchmark doses *in vivo* can be used to identify acceptable margins of exposure
    - Does this depend on mechanism of action (*e.g.*, DNA-reactive agents *vs.* "indirect" mechanisms) ?

HESI IVGT Quantitative Sub-Group

- June 2007: work group formed to develop a decision tree based on the existing 2007 IWGT framework
  - "Quantitative Subgroup" charged to develop quantitative approaches to support the decision tree
- Need for support to develop a database for the needed quantitative analyses led to application to Health Canada to support a collaboration to:
  - Develop the database, conduct analyses, and support additional laboratory work
  - Grant was approved and funded in August 2008

Health Canada Grant

- 3 yr grant to develop improved understanding of the relationship between *in vitro* and *in vivo* genetic toxicology assays
  - HESI/IVGT database in year 1
    Database manager: Beth Julien
- Co-PIs: Paul White, George Douglas
- Project Team: B. Meek, A. Williams, J. Kim., M. Holsapple, D. Phillips (Health Canada, HESI, Inst. Cancer Res., UK)
- Steering Committee: B. Gollapudi, P. Kasper, D. J.-Kram, J. MacGregor, V. Thybaud

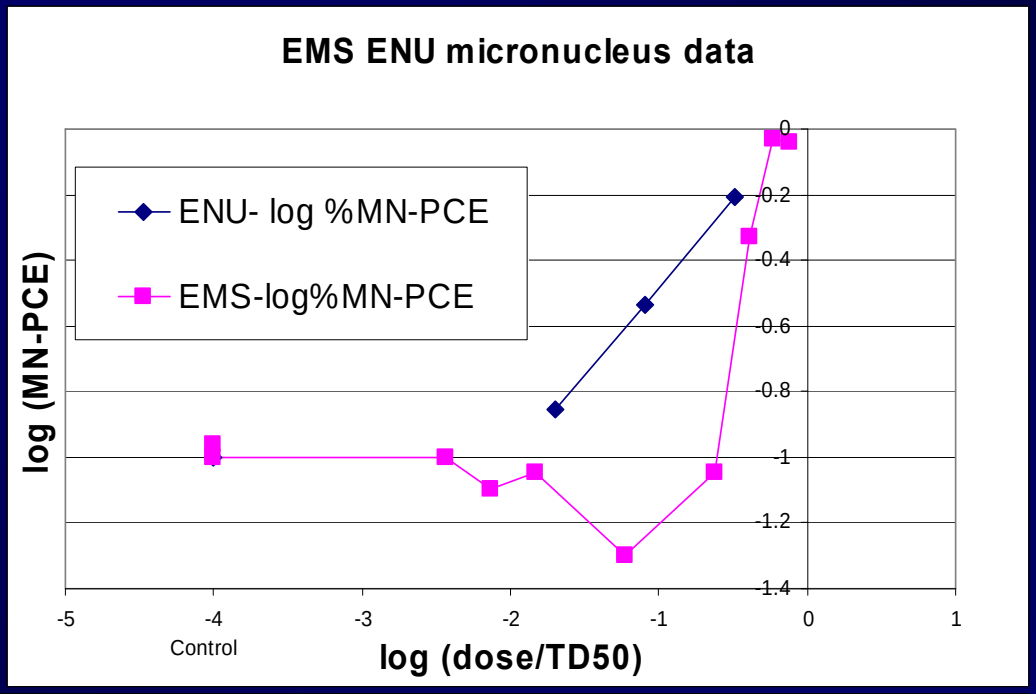
One important question: Are there practical thresholds for genotoxicants?

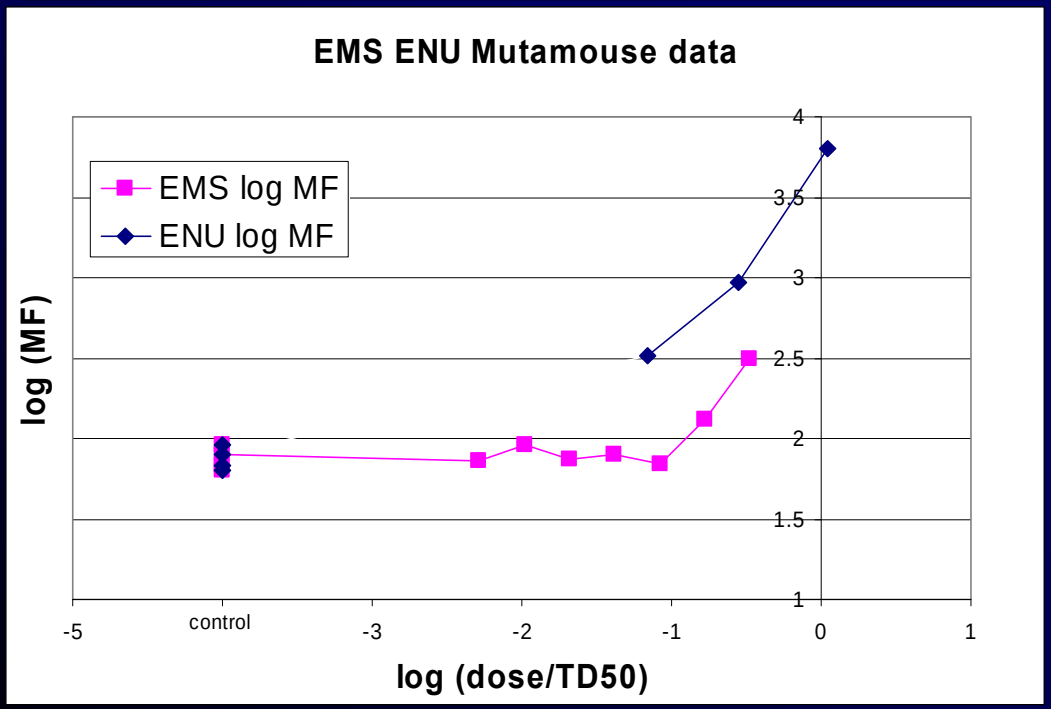
- Initial consensus was yes, for some classes of **non-DNA- reactive** genetic toxicants (*e.g.*, many aneugens, disturbance of nucleotide pool balance, glutathione depletion, DNA synthesis inhibitors)
- Need a systematic compilation and analysis of data, including DNA-reactive mutagens, that examines the dose response and modes of action, including the presumption of low dose linearity
- Need consensus on appropriate methods (and parameters) to describe the dose-response curve

Other Questions:

Can “negligible risk” be defined as an exposure that does not increase the already-present spontaneous rate more than a defined increment?

- Appropriate metrics? NOGEL, benchmark dose, MOE
- Can response curves be normalized across test systems?
  - Can exposure parameters (*e.g.*, Cmax, AUC) or adducts (DNA, protein) be used to normalize across systems? When?
  - Can it be assumed that the ratio of toxicity to mutagenicity is similar in vitro and in vivo, and in different tissues?
  - Can a virtually safe dose be defined in terms of a fraction of the toxic dose?

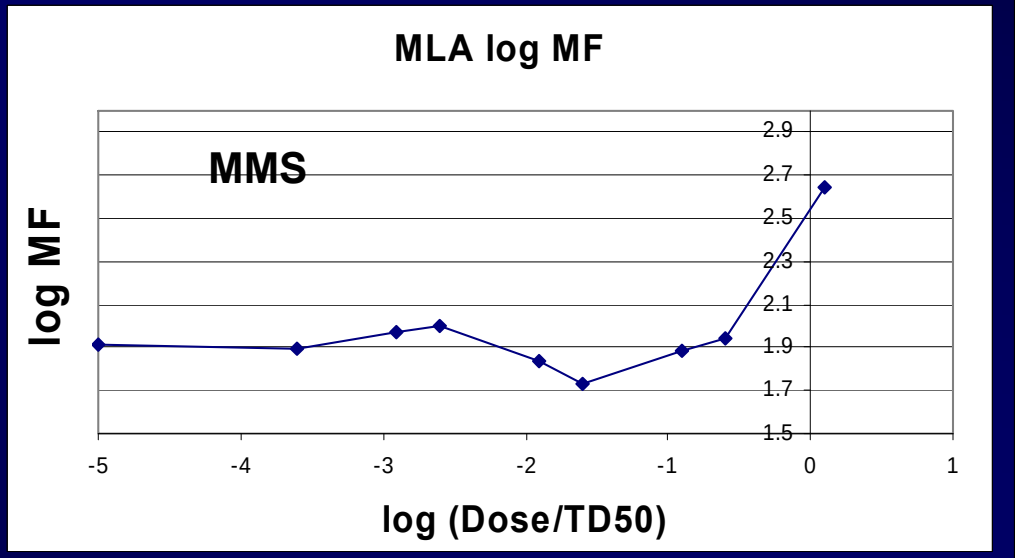


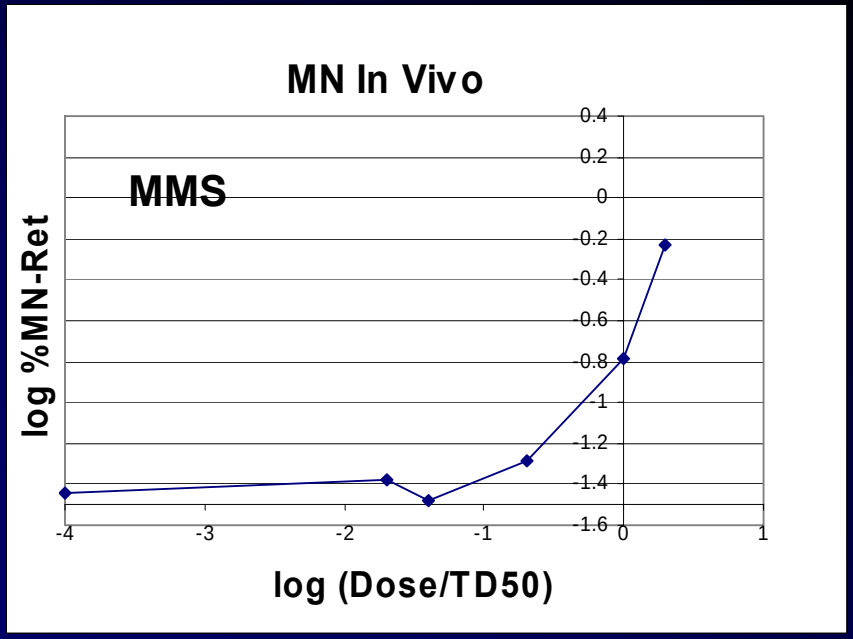


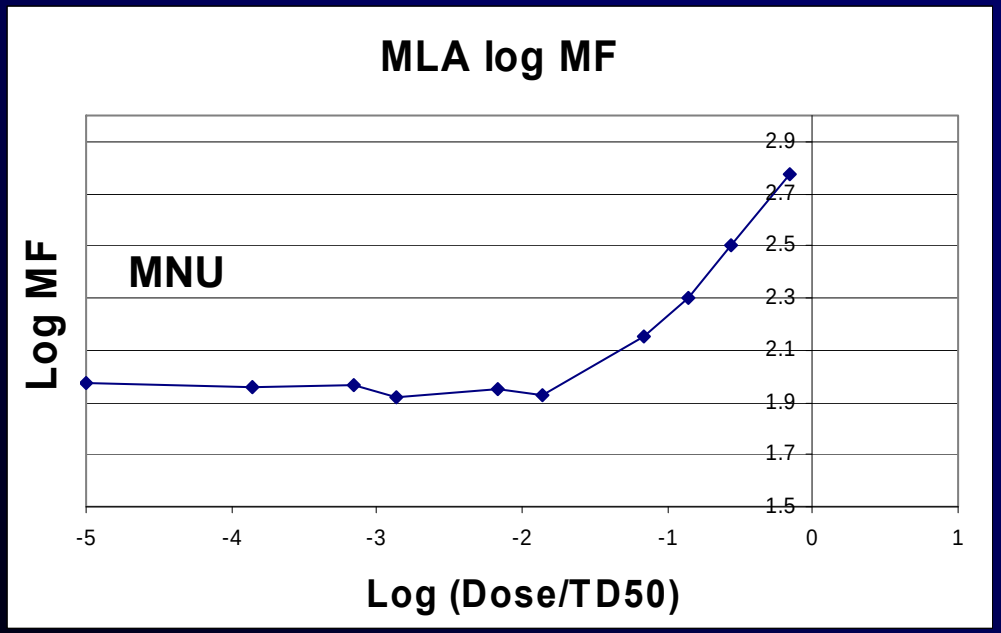
When dose is expressed as fraction of TD50:

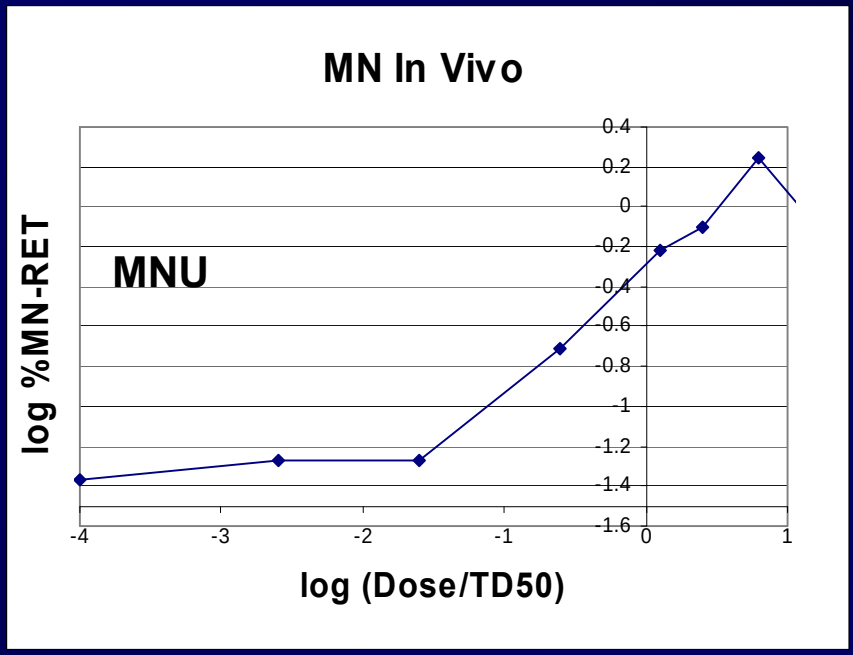
- MN and lacZ mutation freqs behave similarly
- EMS & ENU similar at toxic dose but EMS is at baseline at 1/10 toxic dose & ENU more mutagenic
- EMS clearly non-linear; ENU data insufficient to determine
  - If linear, will rapidly become insignificant relative to spontaneous
- Do in vitro results show similar dose-responses?

Data courtesy of E. Gocke Roche Pharmaceuticals

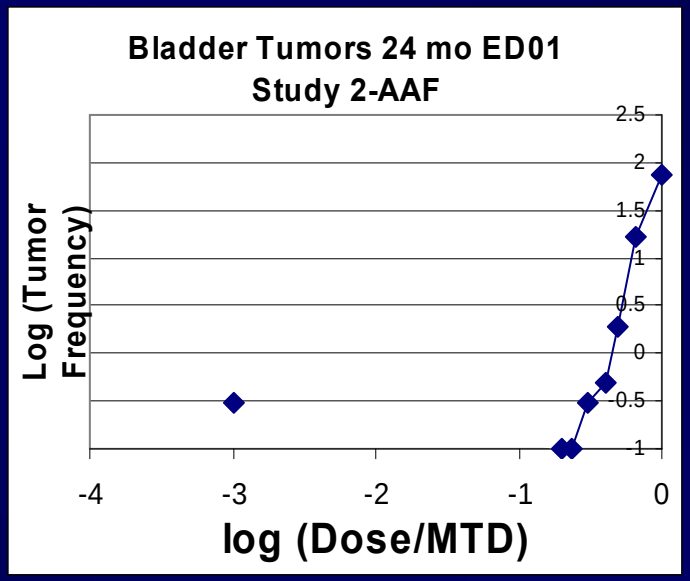


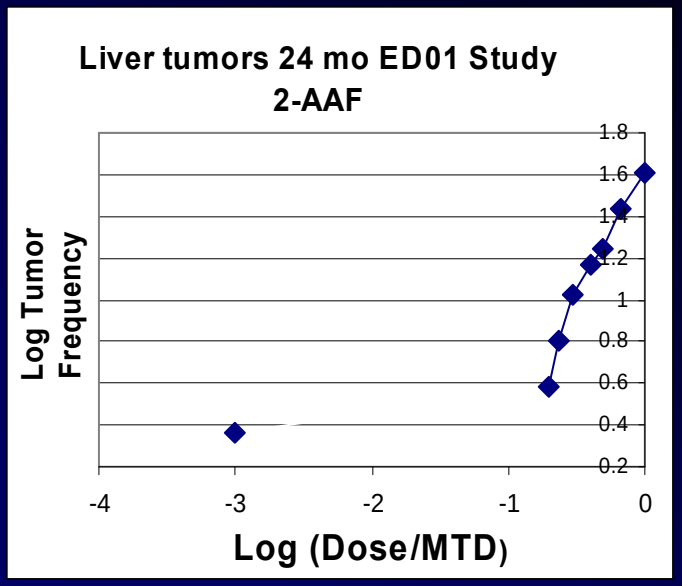


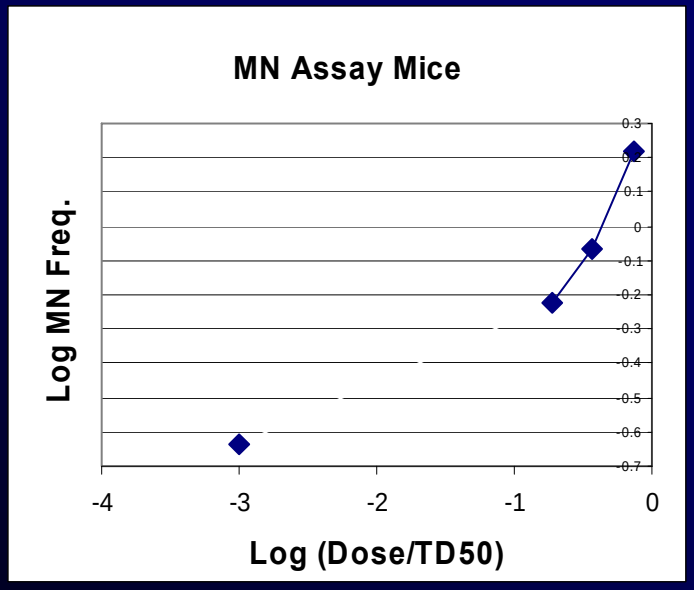


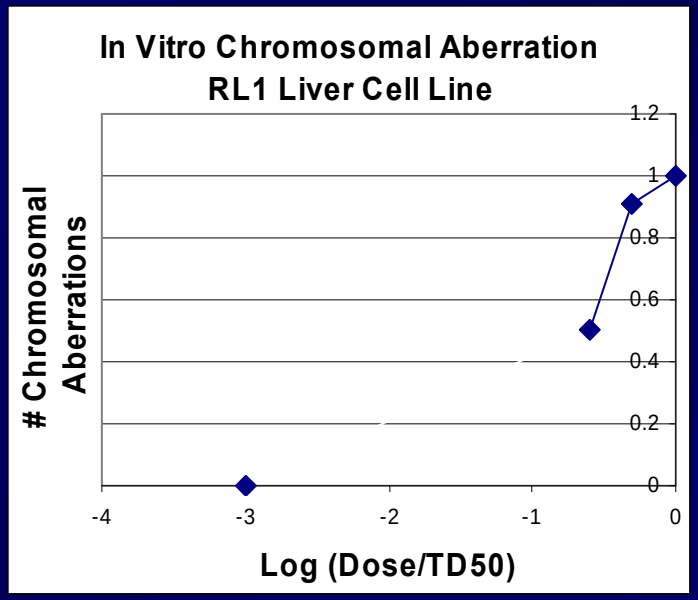


Data courtesy of B.Gollapudi, Dow Chemical









Data from ED01 study and IPCS short-term test studies (Tates,1988; Dean,1981)

Summary of IVGT Status

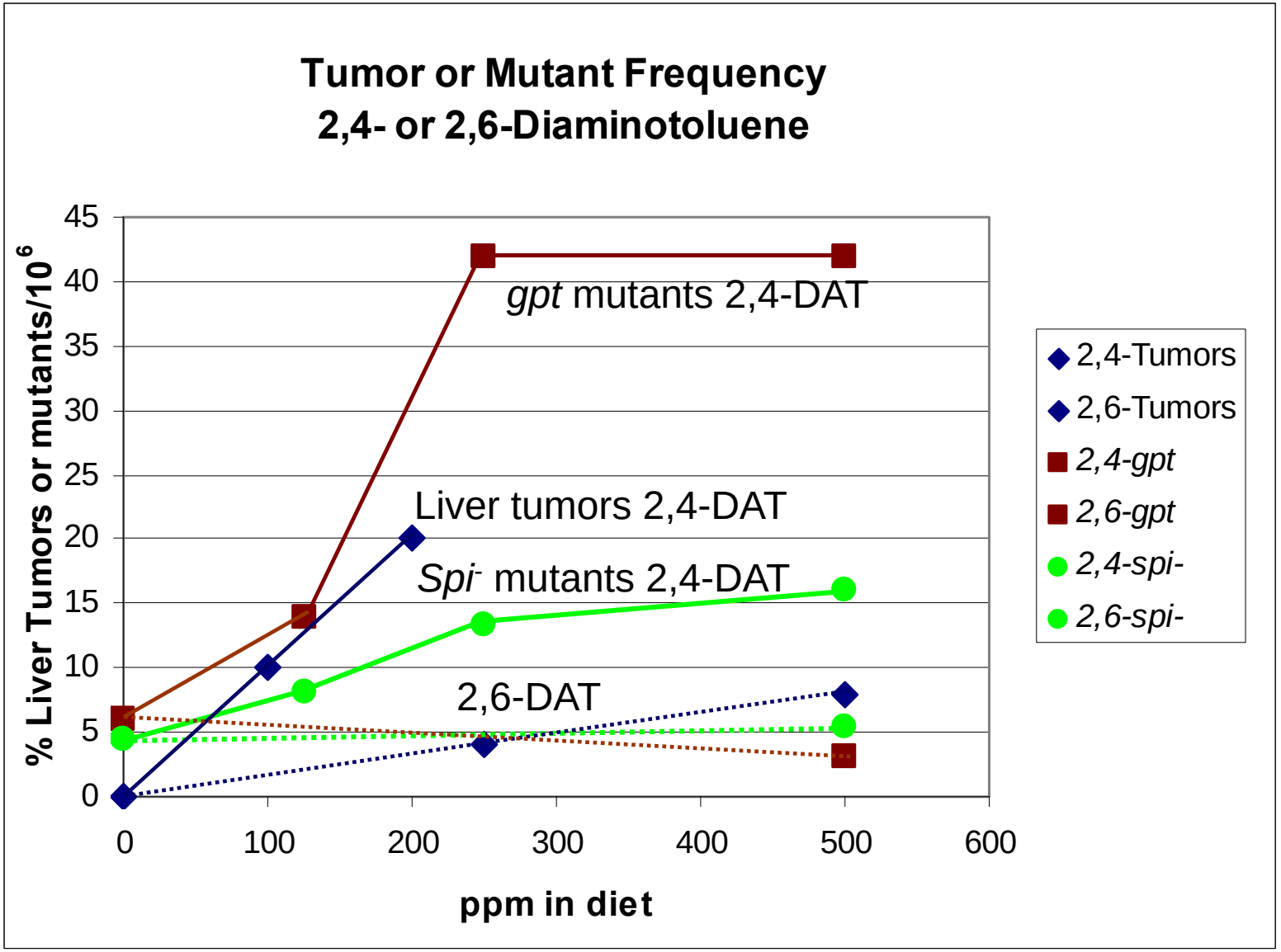
- HESI has established project committee to implement improved approaches to genetic toxicology assessment
- Health Canada grant and HESI contract are in place
- Database is under construction
- Meeting to discuss initial analyses will be held in mid-2010
  - It is likely that experimental work will be needed to generate additional dose-response data
- Recommendations to supplement decision tree will be made as data analysis permits consensus on approaches

Where Might the Field Go From Here?

Is it practical to re-structure genetic toxicology testing to include risk assessments based on exposure-response information using existing methodologies?

Key Questions

- Are assays available and sufficiently sensitive to identify relevant risks?
- Is it economically feasible to measure them during toxicity testing?
- How many tissues/endpoints need to be monitored?
  - *E.g.*, are there enough "genotoxic carcinogens" specific for tissues other than liver, lung, kidney, bone marrow, intestine, urinary bladder to be of concern?
  - If there are, how can we expect one or two *in vitro* systems to identify them?
- If an objective is to identify "genotoxic carcinogens", can insignificant risk be defined as a specified increment of the spontaneous background mutagenicity?



In vivo mutation correlates with *in vivo* tumorigenesis: Data from Nohmi et al., Tox. Sci. 114: 71-78, 2010 and NTP Technical Reports 162 and 200

What is the cost of current *vs.* alternative screening practices?

- ICH 3-test battery is at least $55,000 to $65,000 (if cleanly negative)
- What would be the cost of integrating relevant *in vivo* endpoints into toxicology studies?
  - an integrated design would allow measurement during a GLP repeat-dose toxicology protocol
  - cost would then be dependent on only genetic endpoint measurement & be independent of other study costs

One Hypothetical Battery

- **Bacterial mutagenicity to flag potential hazard ($6000)**
- **Micronuclei in reticulocytes and pig-a mutations in RBC (and/or lymphocytes?) ($10,000 - $15,000 ??)**
- **Screening for base change and deletion mutations in 5-6 tissues—e.g., gpt-Δ rat using liver, lung, intestine, kidney, urinary bladder using a selectable marker**

  (Cost uncertain but 4-5 weeks of technical time at $60,000/yr salary x10% 4x OH multiplier = ~$24,000)

Sum of above =~ $40,000 to $45,000

Strategic considerations

- **A battery such as described would be far more comprehensive than current practice, and no more expensive (after initial validation and adoption into practice)**
- **Site specific analysis could be added or substituted for local exposures (e.g., comet, MN, or transgene analysis at exposure site for dermal, inhalation, etc.)**
- **Specific gene analysis could be used when MOA is known or hypothesized (*e.g., ras* or other oncogenes)**

Conclusions

Technical tools for a better approach are available

- **An integrated scheme might be very cost effective, if the required "up-front" assessments are made and agencies commit to a new approach**
  - **Analysis of tumor site specificity needed**
  - **Commitment to the use of animals with appropriate genetic markers in routine toxicology needed**
    - **NTP could develop path/clin chem. bkgrd. if animal model could be agreed upon (*e.g.*, *gpt*Δ Spi$^-$ selection?)**
    - **Mass breeding would dramatically ↓ cost of transgenic animals**
- **We should make these commitments and move toward a better testing paradigm**