Optimal Design for in Vivo Mutation Studies to Inform Cancer Mode-of-Action Assessment

Martha M. Moore, Ph.D.
Director, Division of Genetic and Reproductive Toxicology
National Center for Toxicological Research
Food and Drug Administration
Jefferson, Arkansas

Disclaimer: Not FDA Policy

Topics

- Mutagenic mode of action (How high a burden of proof??)
- Hazard identification vs. mode of action
- Implications for dose response?

Two Basic (and Different) Uses of Genetic Toxicology Data

- For hazard ID, approval and registration, pesticides, pharmaceuticals and medical devices and other safety assessments.

- To inform MOA cancer risk assessment--choice of extrapolation model

Three Steps (Questions) To Determine if Mutagenic MOA for Tumor Induction

- Potential mutagen? Hazard Identification (Evaluated using standard mutation assays)
- In vivo rodent/human mutagen? (Mutagenic in the target tissue?)
- Is mutation a “key” event in the development of the tumor?

Weigh the Evidence (MOA)

How High a Burden of Proof?

How to weigh the evidence as to whether a chemical causes specific tumors by a mutagenic mode of action (Mutation is THE key event)

(Listed in decreasing order of relevance/importance)

1. Cancer relevant oncogene/tumor suppressor gene mutations can be detected in the target tissue following chemical exposure
2. Surrogate gene mutations can be detected in the target tissue following chemical exposure
3. DNA adducts (known to be mutagenic adducts) can be detected in the target tissue following chemical exposure
4. Primary DNA damage can be detected in the target tissue following chemical exposure
5. Gene mutations and/or DNA adducts or other measures of primary DNA damage can be detected in vivo.
6. Evidence that the chemical can induce mutations, cytogenetic damage, DNA adducts and/or primary DNA damage in vitro.

MOA Evaluation Should involve

- Assessment of mutation in the target tissue
- Time to mutation (temporality)
- Dose response concordance (Mutation and tumor induction)

Multi-Stage Tumor Induction Requires the Accumulation of Events over Time

(Many / most rodent carcinogens require long chronic exposure)

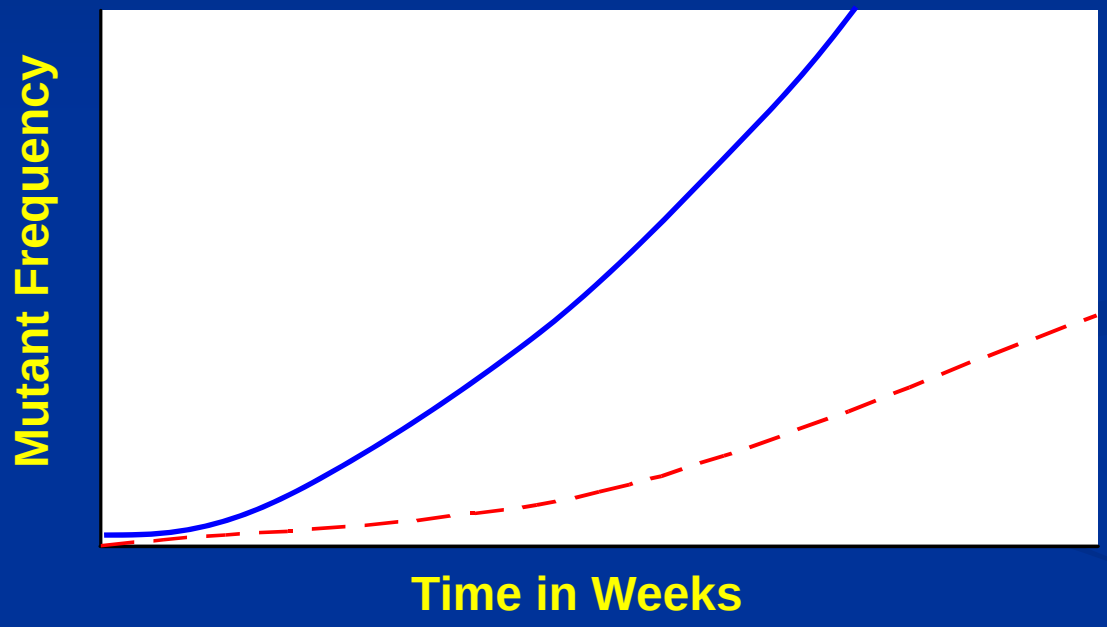
Mutagenic Carcinogen

Nonmutagenic Carcinogen

Temporality—time-to-mutation vs time-to-tumor

Predictions

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

Dose Response Concordance:

Predictions

If mutation is THE key event:

The mutation dose response will *lead* the tumor dose response

If the tumor dose response *leads* the mutation dose response or the mutation response is negative after long chronic exposures

Consistent with mutation is not the key event

How do you evaluate dose response concordance? (A work in progress)

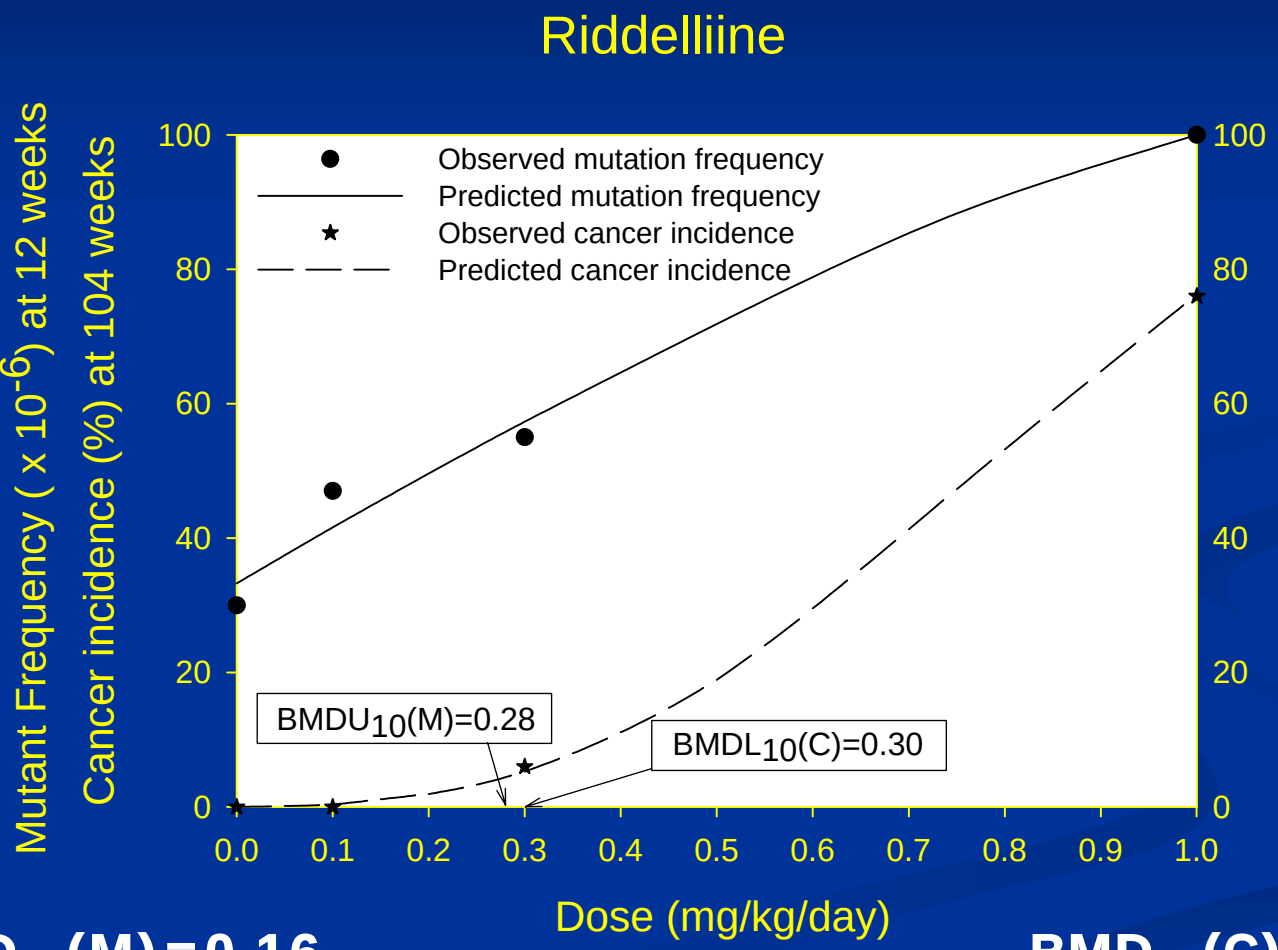
- Visual inspection of the curves
- Quantitative modeling to compare mutation and tumor response
  - Benchmark dose (BMD) (single dose assessment)
  - Compare the probability of an “adverse” MF response with the probability of tumor response (dose response curve assessment)

Unfortunately most of the available in vivo mutation studies were conducted for hazard identification—not optimally designed for MOA evaluation

We present 2 case studies that can be used to demonstrate the general approach

- Riddelliine (consistent with mutagenic MOA)
- Dichloroacetic Acid (DCA) (consistent with nonmutagenic MOA)

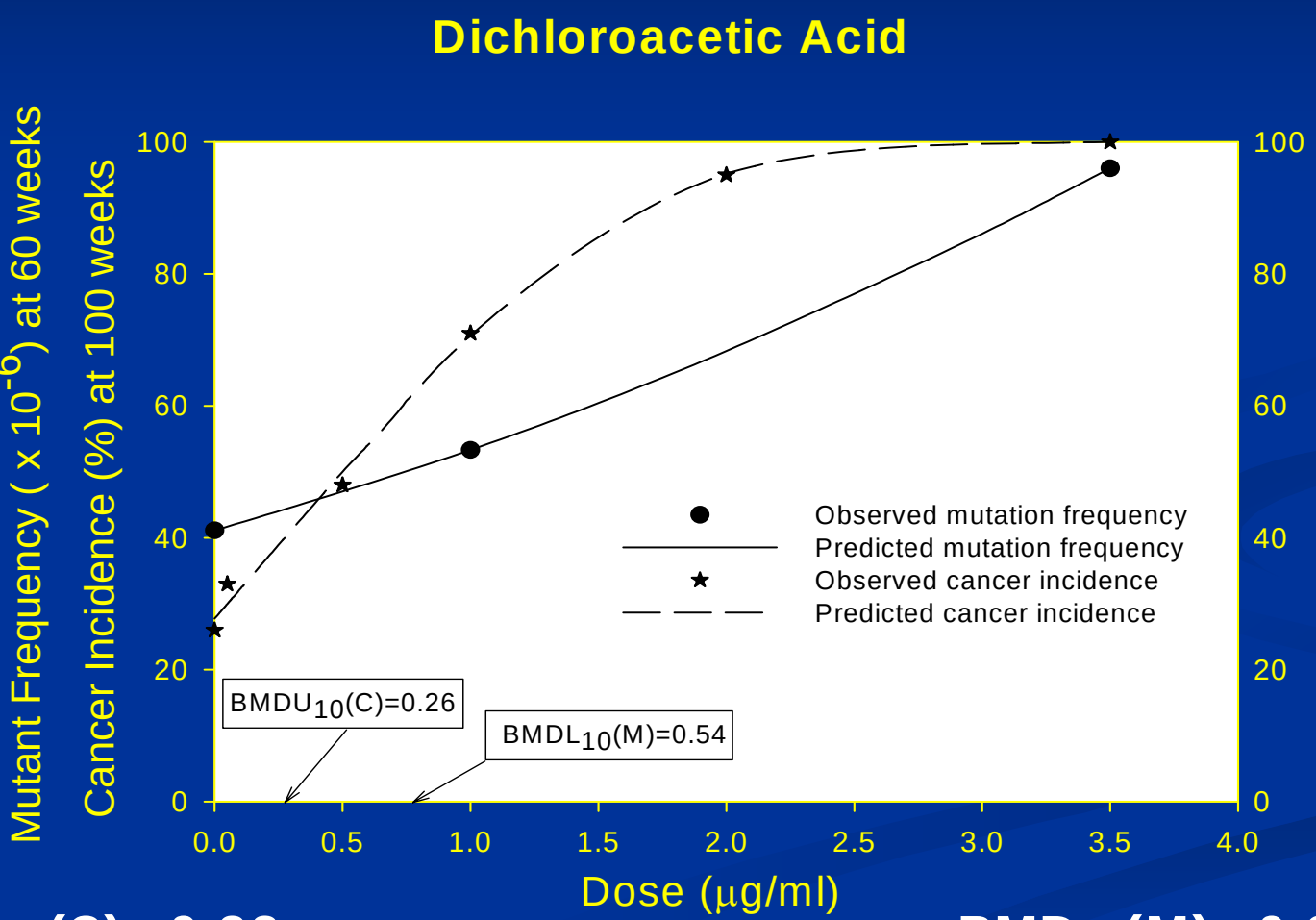
Case study 1: Visual inspection and BMD analysis for riddelline: (Consistent with mutagenic MOA)



$BMD_{10}(M) = 0.16$

$BMD_{10}(C) = 0.39$

Case study 2: Visual inspection and BMD analysis for DCA (60-week exposure for MF) (Consistent with nonmutagenic MOA)



$BMD_{10}(C) = 0.22$

$BMD_{10}(M) = 0.97$

Available online at www.sciencedirect.com
ScienceDirect
Regulatory Toxicology and Pharmacology 51 (2008) 151–161
Regulatory Toxicology and Pharmacology
www.elsevier.com/locate/yrtph

Analysis of in vivo mutation data can inform cancer risk assessment

Martha M. Moore [a,*], Robert H. Heflich [a], Lynne T. Haber [b], Bruce C. Allen [c], Annette M. Shipp [d], Ralph L. Kodell [a,1]

[a] *National Center for Toxicological Research, Food and Drug Administration, Division of Genetic and Reproductive Toxicology, HFT-120, 3900 NCTR Road, Jefferson, AR 72079, USA*
[b] *Toxicology Excellence for Risk Assessment, Cincinnati, OH, USA*
[c] *Bruce Allen Consulting, Chapel Hill, NC, USA*
[d] *ENVIRON Health Sciences Institute, Ruston, LA, USA*

General Features of the Hazard Identification Design

- Acute exposure (single or small # of doses)
- IWGT recommended design is 28 days treatment and 3 days for mutant manifestation
- Generally a negative control and 2 doses
- Generally the high dose is the MTD and the low dose is approximately ½ the top dose

MOA Basic Design

- Same species/strain as cancer study
- Same exposure route as cancer study
- Multiple doses (6 or 7 or more) based on tumor data--Enough doses to adequately “define” the dose response curve
- Chronic treatment (up to 12 months) modeled on the tumor bioassay
- Interim analysis of MF (to define time-to-mutation)
- Detection of mutation in the target tissue(s)
- Evaluate the dose response concordance

Bottom-line Questions for MOA Assessment

- What happens?
- When does it happen?
- At what dose does it happen?

- This information can then be used to develop a timeline for the various events and also to understand the dose response curves for the various events

Implications for Dose Response

- "Mutagenic" carcinogens are "expected" to have a linear dose response
- Point mutations are "expected" to have linear kinetics
- Chromosomal mutations are "expected" to have nonlinear kinetics

Nonlinear or Thresholds for Point Mutagens??

- There is some evidence that the answer is yes—for some, but probably not all point mutagens
- Ethyl methanesulfonate (EMS) vs. ethyl nitrosourea (ENU)

EMS and ENU

- Both are rodent carcinogens
- Both are used as positive controls for genetox assays
- Direct-acting ethylating agents

EMS vs ENU mutagenicity in the rat *Hprt* lymphocyte assay (in vivo treatment)

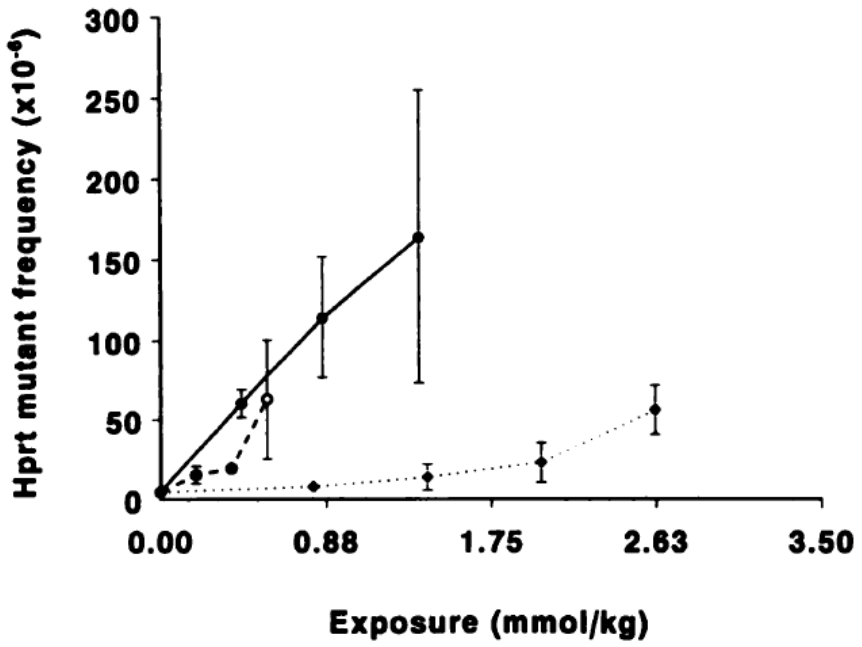


Fig. 1. Dose effect of EMS (◆), HOENU (○) or ENU (●) on the *hprt* mutant frequency in rat T-lymphocytes *in vivo*. *Bars*, SD. Young male Lewis rats were treated i.p. with single exposures of alkylating agent. After 28–32 days, cells were isolated from the spleen and T-lymphocytes were stimulated by Con A *in vitro* for 44 h. Then, the cells were incubated in either medium containing 6-thioguanine for selection of *hprt* mutants or nonselective medium for determining the clone-forming ability for 6–9 days at 37°C under 5% CO_2. The *hprt* mutant frequency is expressed as the number of 6-thioguanine-resistant clones/10^6 clone-forming cells.

Single exposure

0.88 mM = 109 mg

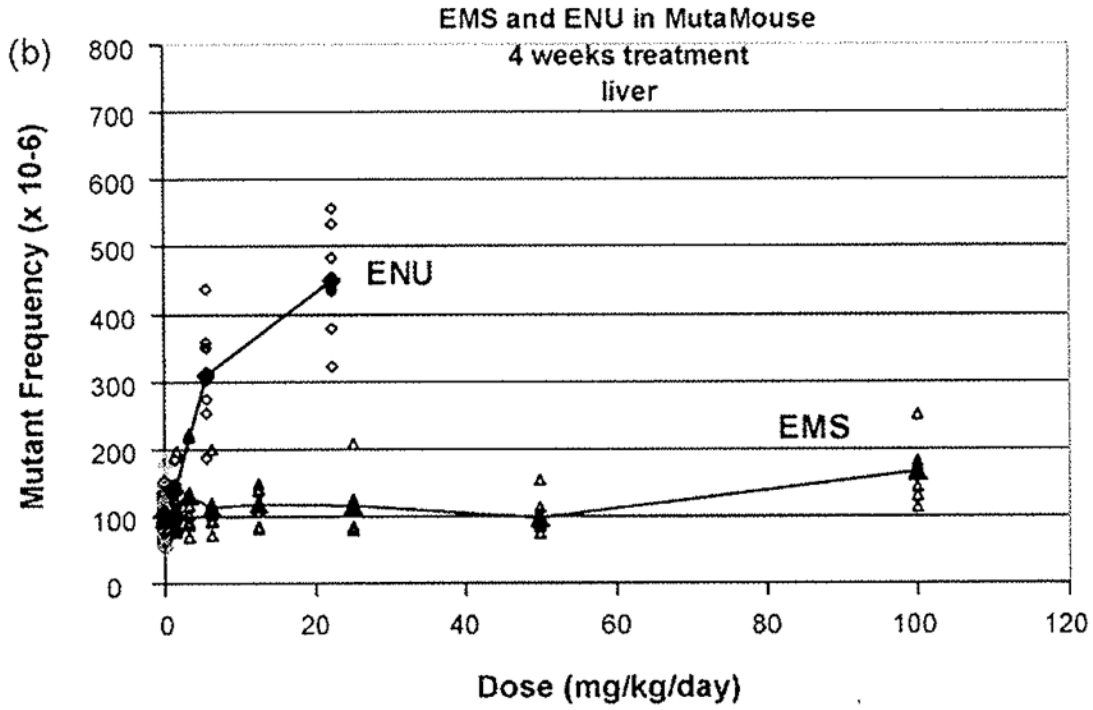
2.63 mM = 326 mg

Jansen JG et al., 1995

Cancer Research

55:1875-1882

EMS has a threshold?: data from 28-day repeat-dose treatment



Gocke et al. (2009)

Toxicology Letters

190: 286 to 297

Conclusions

- Future research to assess mutation as a key event should use a MOA design rather than hazard ID design
- Research is needed to understand the shapes of the dose response curves for mutation (in vivo)

Acknowledgements

- Robert Heflich, NCTR
- Mugamine Manjanatha, NCTR
- Lynne Haber, *TERA*