

Heritable Germ Cell/Carcinogenicity

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■ Proteins

- Protamines – Heritable Germ
- Microtubules – no significant effects
- Kinesins
 - Aneuploidy
 - Micronuclei (kinetochore)
 - Dominant lethals

Heritable Germ Cell/Carcinogenicity

- DNA

- Clastogenic

- Chromosomal aberrations

- Deletions

- Micronuclei (parts chromosomes)

- Mutagenic

- Point mutations

- Frameshifts

Mutagenic Mode of Action

- AA convert to GA
- Mutations Hprt, cII
- DNA- GA adducts variety organs
- Mutagenic bacterial assays
- Postive mouse lymphoma
- Similar dose range dominant lethal and life time carcinogenicity

Mutagenic Mode of Action

- AA convert to GA - yes
- Mutations Hprt, cII – dose (50-125 mg/kg/d)
- DNA- GA adducts variety organs – yes, no correlation with target sites, dose? Except 1mg/kg/d
- Mutagenic bacterial assays – 1 mg/plate
- Postive mouse lymphoma – 300 mg/kg
- Similar dose range dominant lethal and life time carcinogenicity – 2.8-13 mg/kg/d (multiple)

Mutagenic Mode of Action

- Extend dosing into lower range consistent with one-hit, one target
- Concerns
 - Organ adducts no correlation with target
 - High doses except one study @ 1 mg/kg/d
 - Adducts formed are easily repaired
 - UDS @ 7.8 mg/kg
 - Dominant lethals limited to spermatids to spermatozoa
 - Spermatogonia @ 100-125 mg/kg
 - Threshold?

Doak et al 2007

- MMS – methylmethanesulfonate
- EMS – ethyl methanesulfonate
- MNU – methylnitrosourea
- ENU – ethylnitrosourea
- Micronuclei
- (CREST staining of kinetochore)
- HPRT mutation frequency
- TK mutation frequency

DNA Adducts

Table 1. DNA adduct profiles for MMS, MNU, EMS and ENU

Adduct	MMS	EMS	MNU	ENU
s value	>0.83	0.67	0.42	0.26
N⁷-G	81-83	58-65	65-70	11-11.5
N ³ -G	0.6	0.3-0.9	0.6-1.9	0.6-1.6
N ⁷ -A	1.8	1.1-1.9	0.8-2	0.3-0.6
N³-A	10.4-11.3	4.2-4.9	8-9	2.8-5.6
N ³ -T	0.1	Nd	0.1-0.3	0.8
N ³ -C	<1	0.4-0.6	0.06-0.6	0.2-0.6
O ⁶ -G	0.3	2	5.9-8.2	7.8-9.5
O ² -T	Nd	Nd	0.1-0.3	7.4-7.8
O ⁴ -T	Nd	Nd	0.1-0.7	1-2.5
O ² -C	Nd	0.3	0.1	2.7-2.8
Phosphotriesters	0.8	12-13	12-17	55-57

NOTE: Adapted from Beranek (13). Data are in percentages; all possible adducts were not included, so columns do not add up to 100%.

Abbreviation: Nd, not detected.

Non linear response MN – Crest staining negative = clastogenic

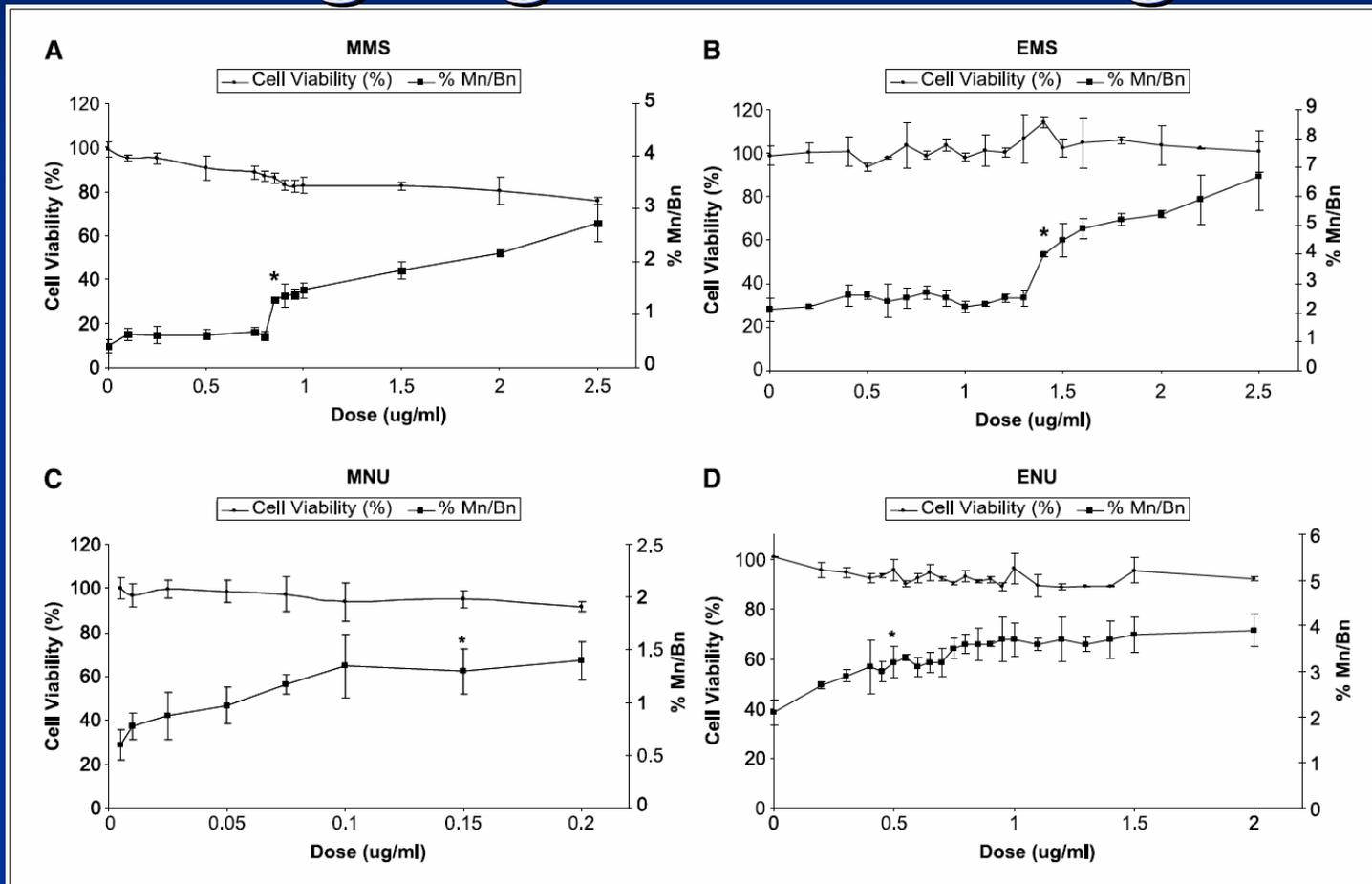


Figure 1. Influence of MMS (A), EMS (B), MNU (C), and ENU (D) dose upon micronucleus frequency in the AHH-1 cell line. Points, mean of treatments done in duplicate; bars, SD. *, the first statistically significant increases in chromosome damage at 0.85 $\mu\text{g}/\text{mL}$ MMS (A), 1.40 $\mu\text{g}/\text{mL}$ EMS (B), 0.15 $\mu\text{g}/\text{mL}$ MNU (C), and 0.50 $\mu\text{g}/\text{mL}$ ENU (D); %Mn/Bn, percentage of binucleated cells containing one or more micronuclei.

HPRT mutation frequencies

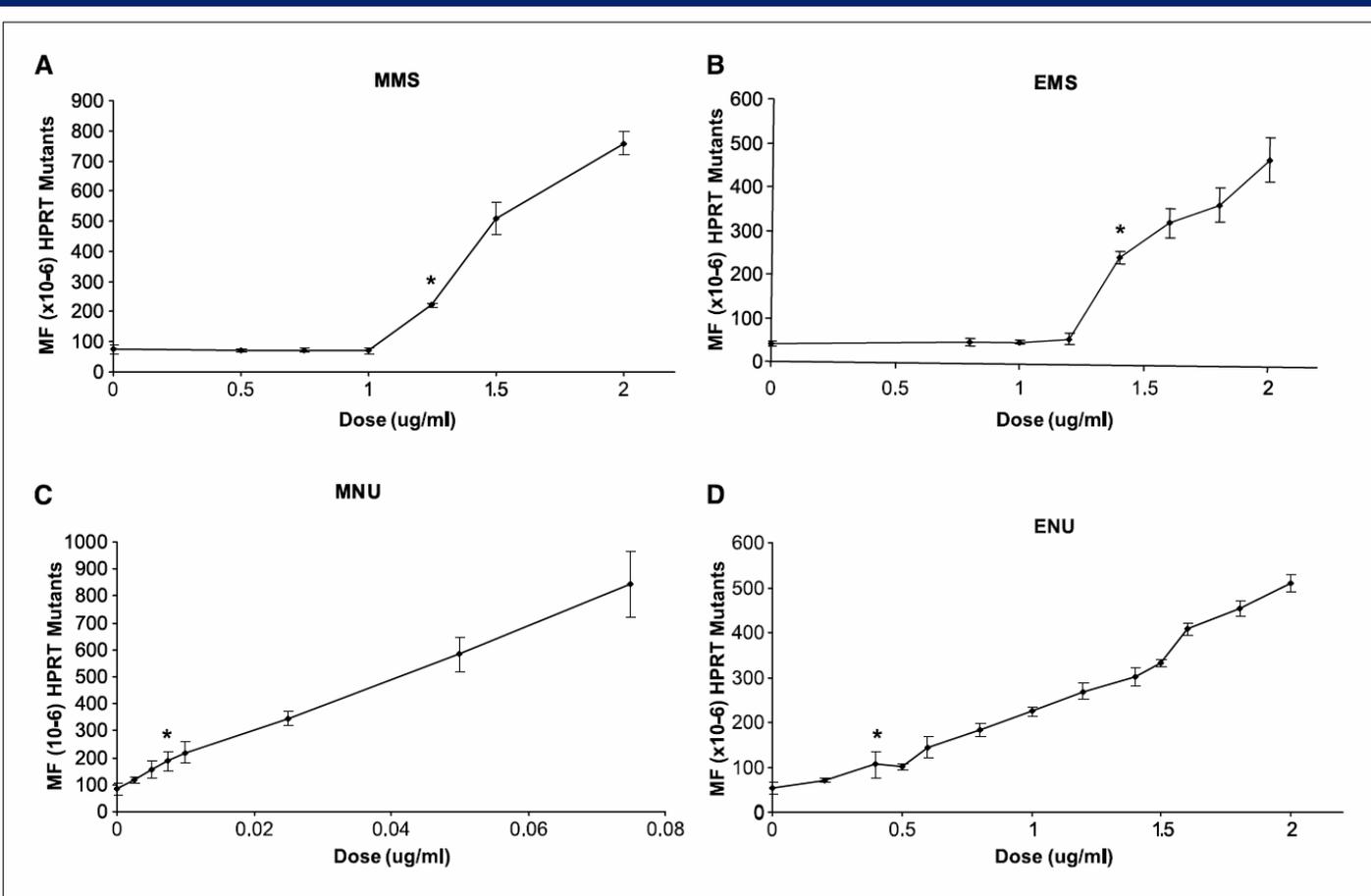


Figure 2. Dose-response relationships of MMS (A), EMS (B), MNU (C), and ENU (D) with respect to *HPRT* gene mutation frequency (MF). *, the first statistically significant increases in mutation frequency at 1.25 $\mu\text{g}/\text{mL}$ MMS (A), 1.40 $\mu\text{g}/\text{mL}$ EMS (B), 0.0075 $\mu\text{g}/\text{mL}$ MNU (C), and 0.40 $\mu\text{g}/\text{mL}$ ENU (D); MF, number of 6-thioguanine resistant clones/ 10^6 clone-forming cells. Points, average mutation frequency calculated from 100×96 -well plates, each dose done in triplicate; bars, SD.

TK mutation assay

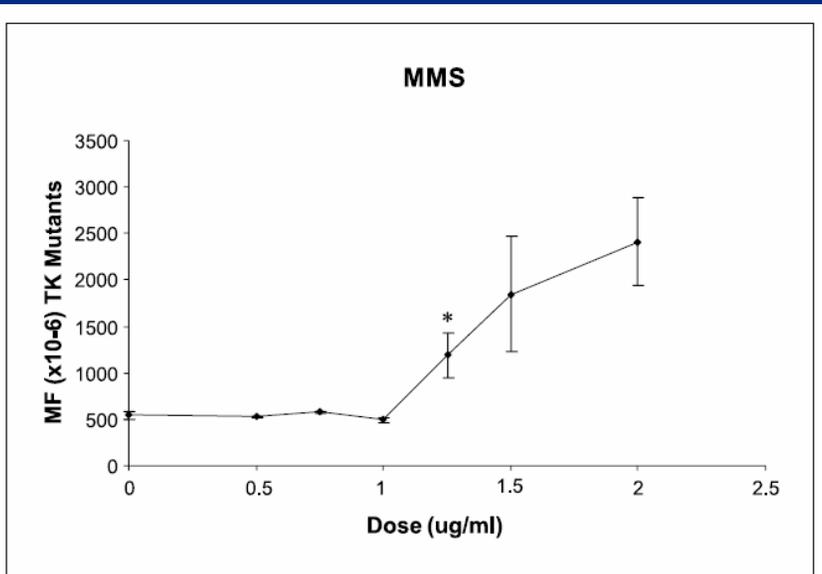


Figure 3. MMS mutation frequency dose-response relationship according to the TK forward mutation assay. *MF*, the number of trifluorothymidine resistant clones/ 10^6 clone-forming cells. *Points*, average mutation frequency calculated from 100×96 -well plates, with each dose done in triplicate. *, the first statistically significant increase in mutation frequency at 1.25 $\mu\text{g}/\text{mL}$ MMS.

- N7G, N3A repaired by base excision repair enzymes (robust)
- O6 repaired by methylguanine DNA methyltransferase (easily saturated)
- Conclusion: Linearity tests for AA