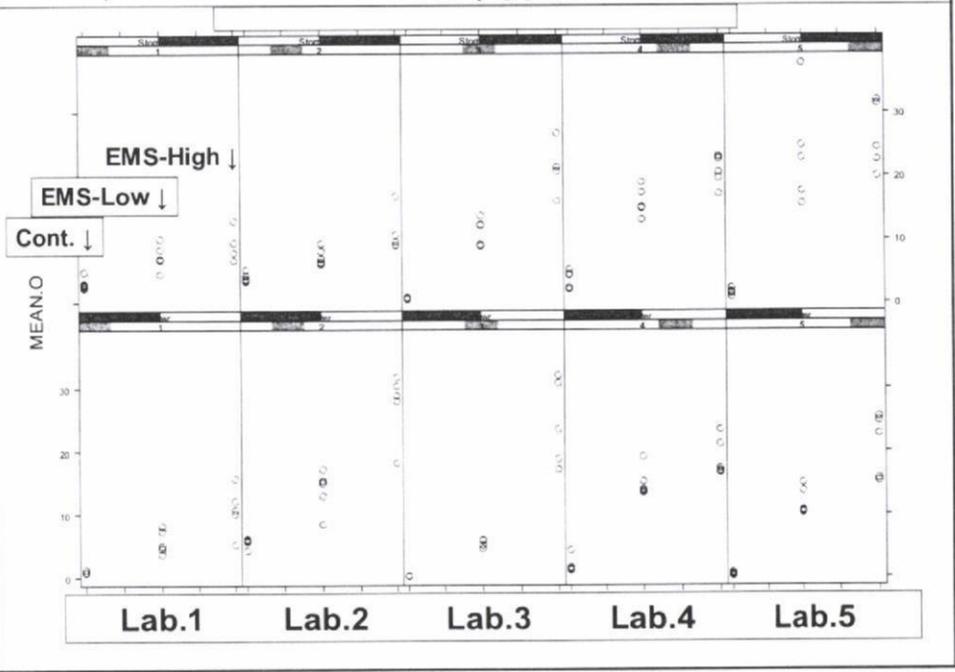
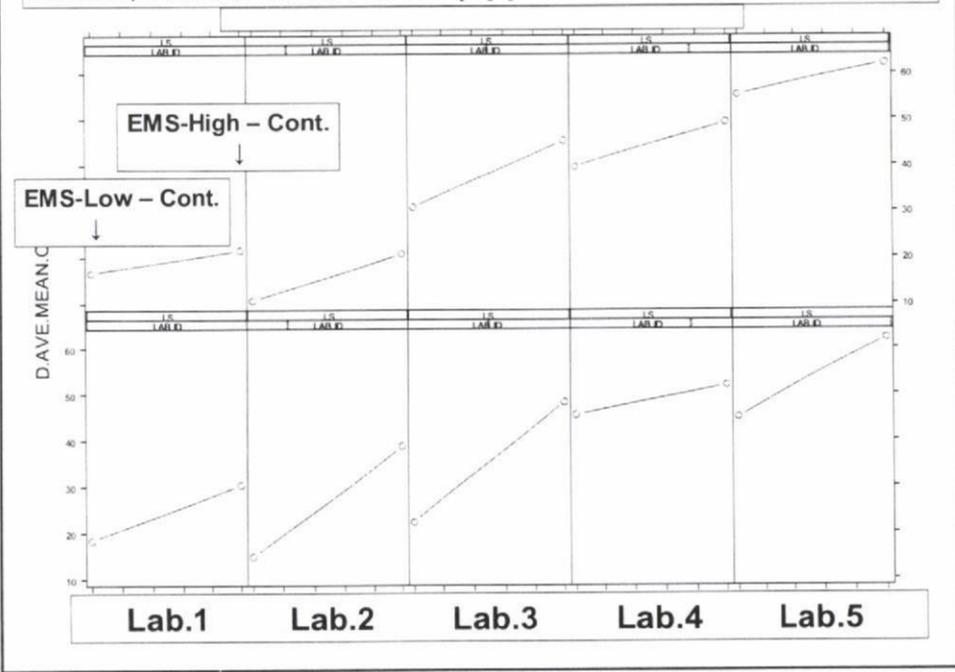


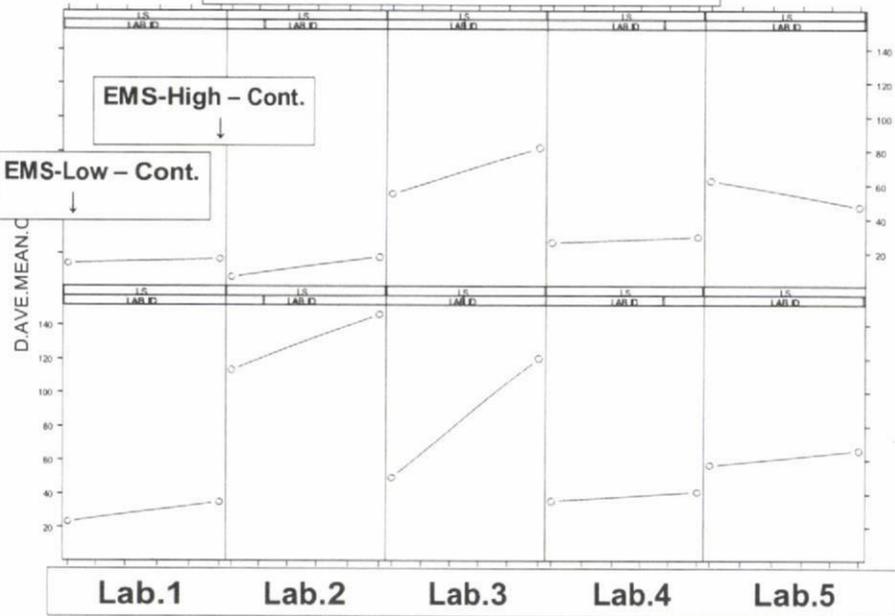
Result, Estimate: Tail moment (upper: Stomach, lower: Liver)



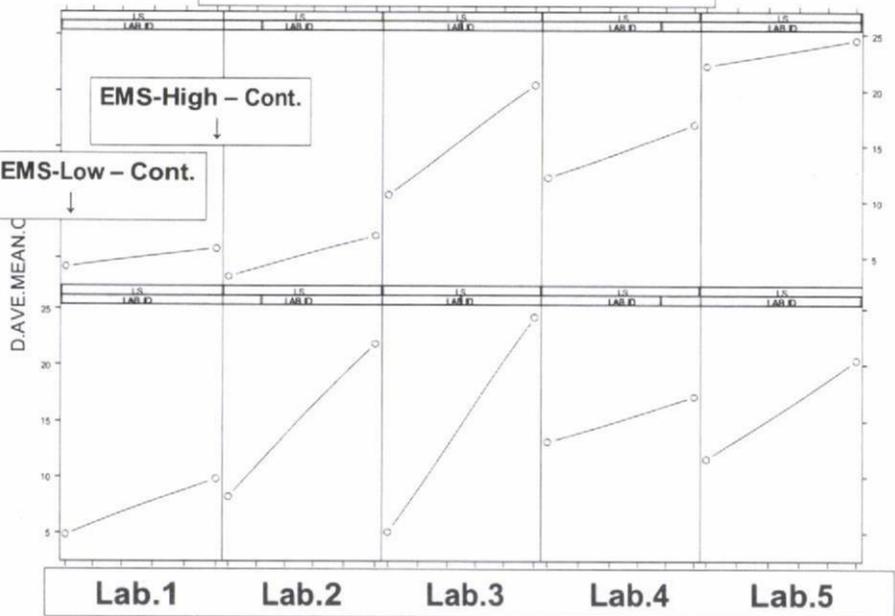
Result, Effect: %DNA in tail (upper: Stomach, lower: Liver)



Result, Effect: Tail length (upper: Stomach, lower: Liver)



Result, Effect: Tail moment (upper: Stomach, lower: Liver)



Note: Dunnett's one side test result

				Comparison					
				L - V			H - V		
Estimate	Parameter	Region	Lab.	Lower	Diff.	Sig.	Lower	Diff.	Sig.
Mean(Obs)	% tail DNA	Liver	1	10.5	18.4	Sig.	22.5	30.3	Sig.
			2	8.1	14.9	Sig.	32.1	38.8	Sig.
			3	17.5	22.3	Sig.	43.5	48.3	Sig.
			4	38.6	45.5	Sig.	45.1	52	Sig.
			5	37.5	45	Sig.	54.6	62.1	Sig.
		Stomach	1	8.1	16.6	Sig.	13	21.5	Sig.
			2	3.2	10.6	Sig.	13.4	20.8	Sig.
			3	27.8	30.9	Sig.	42.2	45.3	Sig.
			4	32.7	39.7	Sig.	42.4	49.4	Sig.
			5	45.6	55.3	Sig.	52.4	62	Sig.

"L-V" means "the difference between low dose and vehicle control", and "H-V" means "the difference between high dose and vehicle control", i.e. these means "Effect". "Lower" means a value of the lower limit for the confidence interval obtained from Dunnett's one side test, and the value is considered significant when it is zero or more. "Diff." means a value of "Effect", and comparison of these values would be a preferable yardstick (criterion) to understand the variation of parameters among testing facilities. "Sig." means the statistical significance between the vehicle control group and the treatment group.

Summary of the 1st Phase Validation Study on *in vivo* Comet Assay

- ◆ Well validated data were obtained.
- ◆ Standard protocol is optimized overall, however further optimization seems necessary
 - ✓ Add or not EDTA into low-melting agarose gel
 - ✓ Electrophoresis solution temp.: below 10°C vs. room temp. 20 °C
 - ✓ %DNA in tail in negative control: lesser is better vs. 1-15%
 - ✓ Effect of cytotoxicity: histopathology vs. neutral diffusion assay
 - ✓ Statistical analysis: acceptable criteria of variation etc.
 - ✓ Further reduction of animal use

Please access Comet Assay Interest Group homepage
<http://cometassay.com/>
If you are interested in this validation effort.

Thank you very much for your attention!!

In Vivo Alkaline Comet Assay:
Room Temperature Compared to 4° C Unwinding and
Electrophoresis;
2 mM EDTA Compared to 10 mM EDTA in Gel
(2,6-Diaminotoluene, Acrylamide, 2,4-Diaminotoluene)

Andy Kraynak

Merck Research Labs, Merck & Co., Inc., West Point, Pa USA

Procedure

JaCVAM Protocol Version 12

- 5 male Sprague-Dawley rats/group, 6 - 8 weeks of age.
- Treated 2X (24 hours and 3 hours before tissue harvest).
- Gamma-irradiated positive control: Sample of cell suspension from two vehicle control rats - irradiated just prior to making slides.
- 2 slides/rat/tissue for each unwinding and electrophoresis condition:
 - Room temperature (20-23° C) and refrigerated (4° C).
 - Constant 0.7 volts/cm, ~300 mA, 20 minutes
- Perceptive Comet Assay IV image analysis.
- 50 comets scored/slide, hedgehogs counted.

Notes for Data Tables

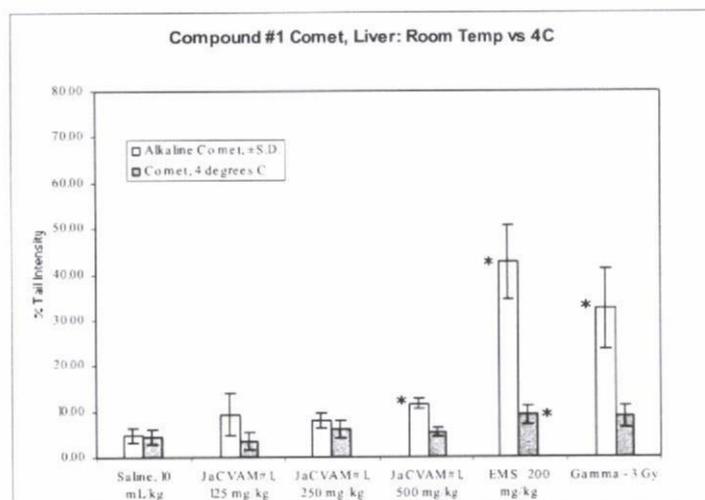
Comet data for treatment group: Values are a mean \pm standard deviation of animal means in each treatment group.

Statistical significance: Indicated by *, $p \leq 0.03$, analysis of non-transformed data at animal level, ANOVA with multiplicity adjustment.

"Hedgehogs": Number of hedgehogs observed (but not scored) in the process of scoring 50 comets/slide. Expressed as a percent of total cells observed for each dose group.

2,6-Diaminotoluene

Compound #1 (2,6-Diaminotoluene)
Liver



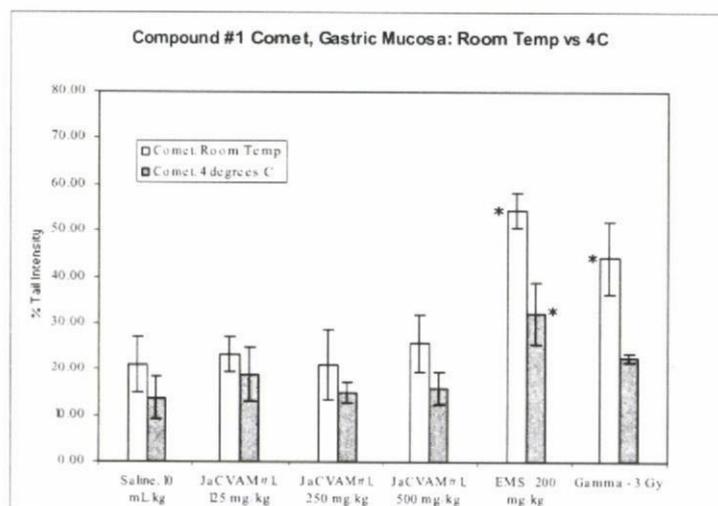
* $p \leq 0.03$, analysis of non-transformed data at animal level, ANOVA with multiplicity adjustment.

Compound #1 (2,6-Diaminotoluene) – Liver

Treatment	Group Mean % Tail Intensity ¹		% Hedgehogs	
	Room Temp	4° C	Room Temp	4° C
<u>Vehicle Control</u>				
Corn oil 10 mL/kg	4.8 ± 1.7	4.5 ± 1.6	4	5
<u>Positive Control:</u>				
Gamma Radiation 3 Gy	32.4 ± 8.7*	8.8 ± 2.4	3	4
EMS 200 mg/Kg	42.6 ± 8.1*	9.1 ± 2.1*	5	4
<u>2,6-DAT</u>				
125 mg/Kg	9.4 ± 4.5	3.5 ± 2	4	3
250 mg/Kg	8.0 ± 1.6	6.1 ± 2	6	4
500 mg/Kg	11.6 ± 1.2*	5.5 ± 1	6	5

1. Mean % tail intensity calculated from average % tail intensity value for each of 5 rats (2 rats for gamma-irradiated control cells).

Compound #1 (2,6-Diaminotoluene)
Stomach



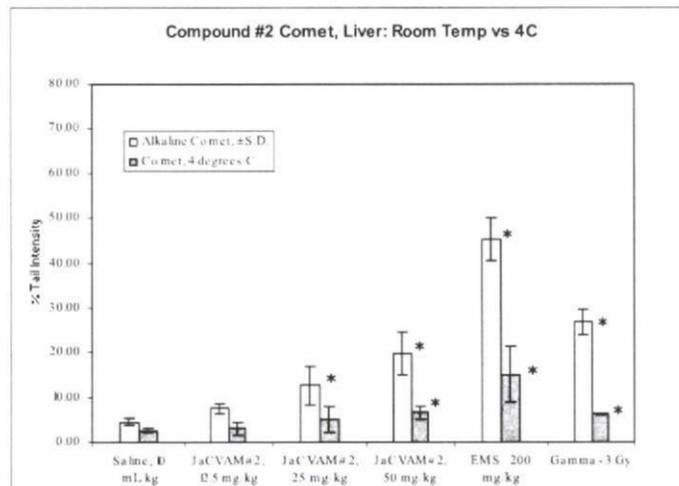
* $p \leq 0.03$, analysis of non-transformed data at animal level, ANOVA with multiplicity adjustment.

Compound #1 (2,6-Diaminotoluene) - Stomach

Treatment	Group Mean % Tail Intensity		Group Mean % Hedgehogs	
	Room Temp	4° C	Room Temp	4° C
<u>Vehicle Control</u>				
Com oil 10 mL/kg	21.1 ± 6.1	13.8 ± 4.7	26	19
<u>Positive Control:</u>				
Gamma Radiation 3 Gy	44.2 ± 7.8*	22.6 ± 1.0	23	20
EMS 200 mg/Kg	54.5 ± 3.9*	32.2 ± 6.8*	23	18
<u>2,6-DAT</u>				
125 mg/Kg	23.3 ± 3.8	18.9 ± 5.9	22	22
250 mg/Kg	21.1 ± 7.7	15.0 ± 2.2	25	22
500 mg/Kg	25.7 ± 6.3	15.9 ± 3.4	27	18

Compound #2 (Acrylamide)

Compound #2 (Acrylamide), Liver

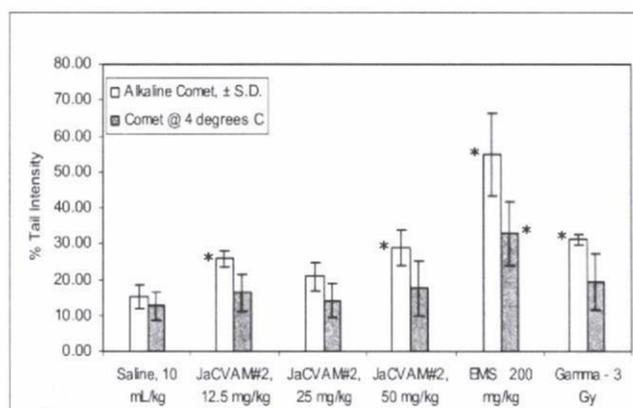


* $p \leq 0.03$, analysis of non-transformed data at animal level, ANOVA with multiplicity adjustment.

Compound #2 (Acrylamide) - Liver

Treatment	Group Mean % Tail Intensity		% Hedgehogs	
	Room Temp	4° C	Room Temp	4° C
<u>Vehicle Control</u>				
0.9% Saline 10 mL/kg	4.52 ± 0.8	2.7 ± 0.6	3.3	1
<u>Positive Control:</u>				
Gamma Radiation 3 Gy	26.7 ± 2.8*	6.3 ± 0.1*	1.3	0.5
EMS 200 mg/Kg	45.2 ± 4.9*	15.0 ± 6.2*	1.6	2.3
<u>Acrylamide</u>				
12.5 mg/Kg	7.6 ± 1.1	3.1 ± 1.5	2.3	1.1
25 mg/Kg	12.6 ± 4.2*	5.1 ± 2.9	4.4	4
50 mg/Kg	19.9 ± 4.8*	6.5 ± 1.4*	3.1	3.5

Compound #2 (Acrylamide), Stomach



* p<0.03, analysis of non-transformed data at animal level, ANOVA with multiplicity adjustment.

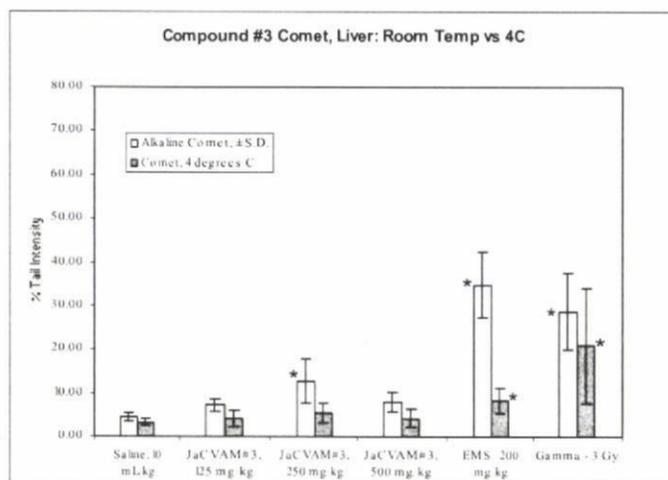
Compound #2 (Acrylamide) - Stomach

Treatment	Group Mean % Tail Intensity		% Hedgehogs	
	Room Temp	4° C	Room Temp	4° C
<u>Vehicle Control</u>				
0.9% Saline 10 mL/kg	15.2 ± 3.2	12.6 ± 4	10.4	8
<u>Positive Control:</u>				
Gamma Radiation 3 Gy	31.2 ± 1.4*	19.4 ± 7.8	19.4	8
EMS 200 mg/Kg	54.8 ± 11.6*	32.8 ± 8.7*	20.2	11.6
<u>Acrylamide</u>				
12.5 mg/Kg	25.8 ± 2.4*	16.3 ± 5.3	14	9.8
25 mg/Kg	20.9 ± 4	14.2 ± 4.7	11	10.5
50 mg/Kg	28.9 ± 5*	17.7 ± 7.6	16.4	11

1. Many of these cells were atypical: branched streams or strands of DNA emanating from nucleus rather than even diffusion.
 2. N.D. = Not Done.

Compound #3 (2,4-Diaminotoluene)

Compound #3 (2,4-Diaminotoluene), Liver



* p<0.03, analysis of non-transformed data at animal level, ANOVA with multiplicity adjustment.

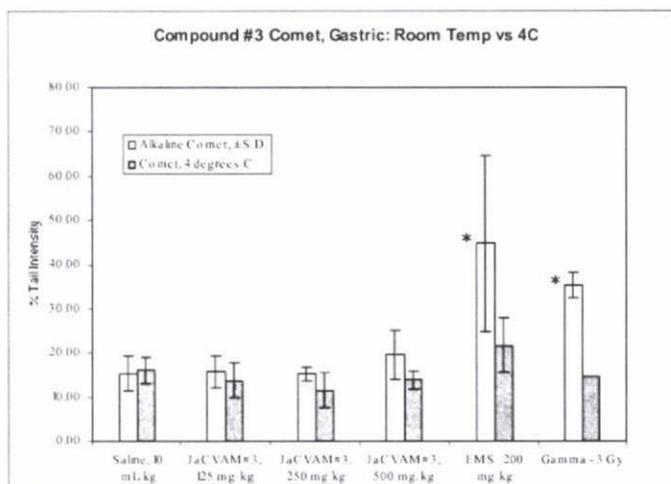
Compound #3 (2,4-Diaminotoluene) - Liver

Treatment	Group Mean % Tail Intensity		% Hedgehogs	
	Room Temp	4° C	Room Temp	4° C
Vehicle Control 2% Tween 80 10 mL/kg	4.4 ± 1.0 ¹	3.3 ± 0.8	4	4
<u>Positive Control:</u> Gamma Radiation 3 Gy	28.8 ± 8.8*	20.9 ± 13.2*	2	7
EMS 200 mg/Kg	34.8 ± 7.5*	8.3 ± 3.0*	2	5
<u>2,4-DAT</u> 125 mg/Kg	7.2 ± 1.4	4.1 ± 1.9	2	4
250 mg/Kg	12.9 ± 5.1* ²	5.5 ± 2.2	3	3
500 mg/Kg	8.0 ± 2.2	4.3 ± 2.0	5	2

¹ Only 450 comets scored due to technical problem.

² Only 350 comets scored (from 5 rats) due to technical problem.

Compound #3 (2,4-Diaminotoluene), Stomach



* p<0.03, analysis of non-transformed data at animal level, ANOVA with multiplicity adjustment.

Compound #3 (2,4-Diaminotoluene) - Stomach, Rm Temp

Treatment	Group Mean % Tail Intensity		% Hedgehogs	
	Room Temp	4°C	Room Temp	4°C
<u>Vehicle Control</u>				
2% Tween 80 10 mL/kg	15.4 ± 3.9	16.1 ± 2.9	23	4
<u>Positive Control</u>				
Gamma Radiation 3 Gy	35.4 ± 3.0*	14.8 ¹	11	18
EMS 200 mg/Kg	44.8 ± 20.0* ³	21.7 ± 6.2 ²	21	22
<u>2,4-DAI</u>				
125 mg/Kg	15.8 ± 3.6	13.8 ± 3.9	17	17
250 mg/Kg	15.3 ± 1.6	11.6 ± 4.0	17	15
500 mg/Kg	19.7 ± 5.6	13.9 ± 2.1	24	19

¹ Data from only one rat due to technical error.

² Data from four rats due to technical error.

Summary of Room Temp vs Refrigerated Lysis/Electrophoresis

Tissue	Treatment	% DNA in Tail - 20-23° C	% DNA in Tail - 4° C	Fold Increase Over Control - 20-23° C	Fold Increase Over Control - 4° C
Liver 2,6-DAT	Vehicle	4.8 ± 1.7	4.5 ± 1.6	-	-
	Gamma-irradiated ¹	32.4 ± 8.7*, p<.010	8.8 ± 2.4 (p=.038)	6.8	2.0
	EMS	42.6 ± 8.1*, p<.001	9.1 ± 2.1*, p=.005	8.9	2.0
	Low	9.4 ± 4.5 (p=.074)	3.5 ± 2.0	2.0	(0.8)
	Mid	8.0 ± 1.6	6.1 ± 2.0	1.7	1.4
	High	11.6 ± 1.7*, p<.009	5.5 ± 1.0	2.4	1.2
Stomach 2,6-DAT	Vehicle	21.1 ± 6.1	13.8 ± 4.7	-	-
	Gamma-irradiated	44.2 ± 7.8*, p<.008	22.6 ± 1.0 (p=.056)	2.1	1.6
	EMS	54.5 ± 3.9*, p<.001	32.2 ± 6.8*	2.6	2.3
	Low	23.3 ± 3.8	18.9 ± 5.9	1.1	1.4
	Mid	21.1 ± 7.7	15.0 ± 2.2	1.0	1.1
	High	25.7 ± 6.3	15.9 ± 3.4	1.2	1.2
Liver (Acrylamide)	Vehicle	4.52 ± 0.8	2.7 ± 0.6	-	-
	Gamma-irradiated	26.7 ± 2.8*, p<.001	6.3 ± 0.1*, p=.001	5.9	2.3
	EMS	45.2 ± 4.9*, p<.001	15.0 ± 6.2*, p=.002	10.0	5.6
	Low	7.6 ± 1.1	3.1 ± 1.5	1.7	1.1
	Mid	12.6 ± 4.2*, p=.003	5.1 ± 2.9	2.8	1.9
	High	19.9 ± 4.8*, p<.001	6.54 ± 1.4*, p=.011	4.4	2.4
Stomach (Acrylamide)	Vehicle	15.2 ± 3.2	12.6 ± 4.0	-	-
	Gamma-irradiated	31.2 ± 1.4*, p<.001	19.4 ± 7.8 (p=.169)	2.1	1.5
	EMS	54.8 ± 11.6*, p<.001	32.8 ± 8.7*, p=.002	3.6	2.6
	Low	25.8 ± 2.4*, p=.003	16.3 ± 5.3	1.7	1.3
	Mid	20.9 ± 4.0, (p=.085)	14.2 ± 4.7	1.4	1.1
	High	28.9 ± 5.0*, p<.001	17.7 ± 7.6	1.9	1.4
Liver 2,4-DAT	Vehicle	4.4 ± 1.0	3.3 ± 0.8	-	-
	Gamma-irradiated	28.8 ± 8.8*, p<.001	20.9 ± 13.2*, p=.017	6.5	6.3
	EMS	34.8 ± 7.5*, p<.001	8.3 ± 3.0*, p=.007	7.9	2.5
	Low	7.2 ± 1.4	4.1 ± 1.9	1.6	1.2
	Mid	12.9 ± 5.1*, p=.001	5.5 ± 2.2	2.9	1.7
	High	8.0 ± 2.2	4.3 ± 2.0	1.8	1.3
Stomach 2,4-DAT	Vehicle	15.4 ± 3.9	16.1 ± 2.9	-	-
	Gamma-irradiated	35.4 ± 3.0*, p<.001	14.8	2.3	(0.9)
	EMS	44.8 ± 20.0*, p=.014	21.7 ± 6.2	2.9	1.34
	Low	15.8 ± 3.6	13.8 ± 3.9	1.0	(0.9)
	Mid	15.3 ± 1.6	11.6 ± 4.0	1.0	(0.7)
	High	19.7 ± 5.6	13.9 ± 2.1	1.3	(0.9)

Additional Experiments: Temperature Effects and Increasing EDTA in Gel

- Treated one rat with saline, one rat with EMS (50 mg/kg).
- Liver and stomach harvested 3 hours after treatment.
- Portion of saline control cell sample irradiated 3 Gy gamma radiation.
- Cell suspensions embedded in standard low-melt agarose (2 mM EDTA) or low-melt agarose containing 10 mM EDTA.
 - 3 slides from each treatment and each EDTA level; unwinding and electrophoresis either at 4° C or ambient (approximately 23° C).
 - Slides blinded for reading, all slides read by two biologists at different times.

Effects of 10 mM EDTA in Gel

4 °C Lysis/Electrophoresis

Treatment-Gel-Electrophoresis	Rat Stomach (Glandular)				Rat Liver			
	% Tail Intens.		Average	Average Fold-Increase	% Tail Intens.		Average	Average Fold-Increase
	Read 1	Read 2			Read 1	Read 2		
Saline	12.76	9.41	11.09		4.13	1.53	2.83	
Saline, 10 mM EDTA	11.20	9.73	10.47		2.23	2.51	2.37	
EMS, 50 mg/kg	17.42	13.88	15.65	1.42	2.80	2.64	2.72	1.20
EMS, 50 mg/kg, 10 mM EDTA	18.30	16.09	17.20	1.64	1.33	2.11	1.72	0.72
Gamma, 3 Gy	25.59	20.79	23.19	2.11	11.37	4.17	7.77	2.74
Gamma, 3 Gy, 10 mM EDTA	20.34	17.05	18.70	1.78	5.80	5.04	5.42	2.30

23 °C Lysis/Electrophoresis

Treatment-Gel-Electrophoresis	Rat Stomach (Glandular)				Rat Liver			
	% Tail Intens.		Average	Average Fold-Increase	% Tail Intens.		Average	Average Fold-Increase
	Read 1	Read 2			Read 1	Read 2		
Saline	18.08	10.31	14.20		3.49	3.35	3.42	
Saline, 10 mM EDTA	17.51	13.17	15.34		4.78	2.78	3.78	
EMS, 50 mg/kg	29.70	30.6	30.15	2.31	9.30	6.72	8.01	2.34
EMS, 50 mg/kg, 10 mM EDTA	38.32	29.21	33.77	2.20	7.25	4.84	6.04	1.63
Gamma, 3 Gy	30.77	27	28.89	2.16	23.94	9.31	16.62	4.82
Gamma, 3 Gy, 10 mM EDTA	25.84	21.83	23.83	1.57	29.46	19.32	24.39	6.55

Conclusions

- Comet assay conducted at room temperature correctly predicted genotoxicity of 2,4-DAT, 2,6-DAT and Acrylamide in liver and for acrylamide in stomach.
- Ambient temperatures (20-23° C) for lysing and electrophoresis result in a more sensitive assay compared to 4 ° C.
 - If refrigeration is necessary, the minimum temperature should be closer to 10° C.
 - Studies should include a weaker positive control to confirm sensitivity
 - This control could be from frozen stocks of cells from one animal to reduce animal use.
- Additional EDTA in low-melt gel did not have a significant effect on background for stomach or liver tissue.
 - Overall, our data highlights the need for very consistent and careful control of all conditions.

(Additional Slides)

Effects of Lysing/Electrophoresis Temperature

Treatment - Electrophoresis	Rat Stomach (Glandular)				Rat Liver			
	% Tail Intens.		Average	Average Fold-Increase	% Tail Intens.		Average	Average Fold-Increase
	Read 1	Read 2			Read 1	Read 2		
Saline - 4C	12.76	9.41	11.09		4.13	1.53	2.83	
Saline - ambient	18.08	10.31	14.20		3.49	3.35	3.42	
EMS, 50 mg/kg - 4C	17.42	13.88	15.65	1.42	2.80	2.64	2.72	1.20
EMS, 50 mg/kg - ambient	29.70	30.6	30.15	2.31	9.30	6.72	8.01	2.34
Gamma, 3 Gy - 4C	25.59	20.79	23.19	2.11	11.37	4.17	7.77	2.74
Gamma, 3 Gy - ambient	30.77	27	28.89	2.16	23.94	9.31	16.62	4.82

Effect of 10 mM EDTA in Gel and Comparison of Lysis/Electrophoresis Temperature

Treatment-Gel-Electrophoresis	Rat Stomach (Glandular)			Rat Liver		
	Mean % Tail Intens. Read 1	Read 2	Fold-Increase Average	Mean % Tail Intens. Read 1	Read 2	Fold-Increase Average
Saline-No EDTA-4C	12.76	9.41		4.13	1.53	
Saline-No EDTA-ambient	18.08	10.31		3.49	3.35	
Saline-EDTA-4C	11.20	9.73		2.23	2.51	
Saline-EDTA-ambient	17.51	13.17		4.78	2.78	
EMS-No EDTA-4C	17.42	13.88	1.42	2.80	2.64	1.20
EMS-No EDTA-ambient	29.70	30.6	2.31	9.30	6.72	2.34
EMS-EDTA-4C	18.30	16.09	1.64	1.33	2.11	0.72
EMS-EDTA-ambient	38.32	29.21	2.20	7.25	4.84	1.63
Gamma-No EDTA-4C	25.59	20.79	2.11	11.37	4.17	2.74
Gamma-No EDTA-ambient	30.77	27	2.16	23.94	9.31	4.82
Gamma-EDTA-4C	20.34	17.05	1.78	5.80	5.04	2.30
Gamma-EDTA-ambient	25.84	21.83	1.57	29.46	19.32	6.55

Merck experiment AE07-001. One rat treated with single oral dose of saline, one with 50 mg/kg ethylmethane sulfonate. Portion of saline control cell samples irradiated with 3 Gy gamma radiation for the gamma positive control. Cell suspensions embedded in standard low-melt agarose, or low-melt agarose containing 10 mM EDTA. Sets of 3 slides from each condition subjected to unwinding and electrophoresis either at 4 degrees C or at ambient (approximately 23 degrees C). Slides blinded for reading, all slides read by two biologists on separate occasions.

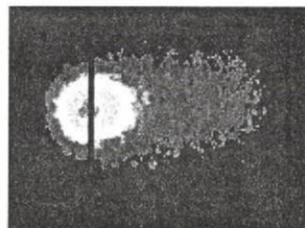
Fewer Animals, but More Slides?

Treatment	Room Temperature				Refrigerated				Alkaline Elution	
	Slide 1	Slide 2	Animal Mean ± Std Dev	Group Mean	Slide 1	Slide 2	Animal Mean ± Std Dev	Group Mean	Animal Mean ± Std Dev	Group Mean
Vehicle	5.5	5.9	5.7 ± 0.3	4.4 ± 1.0	4.1	2.1	3.1 ± 1.4	3.3 ± 0.8	0.031 ± 0.01	.031 ± .002
	8.2	2.2	5.2 ± 4.3		6.5	2.8	4.7 ± 2.6		0.030 ± 0.03	
	3.5	3.5	3.5 ± 0.0		2.1	3.1	2.6 ± 0.7		0.034 ± 0.06	
	4.1	x	4.1		2.3	4.4	3.4 ± 1.5		0.030 ± 0.02	
	2.3	4.7	3.5 ± 1.7		2.7	2.8	2.7 ± 0.1		0.031 ± 0.03	
EMS	55.1	32.6	43.9 ± 15.9	34.8 ± 7.5	6.9	5.6	6.2 ± 0.9	8.3 ± 3.0	0.224 ± 0.09	.211 ± .044
	34.7	46.2	40.4 ± 8.1		9.6	14.5	12.0 ± 3.5		0.233 ± 0.06	
	32.7	37.2	34.9 ± 3.2		5.7	13.3	9.5 ± 5.4		0.246 ± 0.18	
	23.5	31.4	27.4 ± 5.6		9.3	9.2	9.2 ± 0.1		0.219 ± 0.13	
	28.5	26.3	27.4 ± 1.6		4.3	4.5	4.4 ± 0.2		0.135 ± 0.13	
Low Dose	9.3	5.6	7.5 ± 2.6	7.2 ± 1.4	6.3	2.2	4.2 ± 2.9	4.1 ± 1.9	0.038 ± 0.03	.037 ± .002
	1.1	10.9	6.5 ± 7.0		1.4	1.4	1.4 ± 0.0		0.038 ± 0.02	
	8.6	10.4	9.5 ± 1.2		5.4	2.3	3.9 ± 2.1		X	
	6.8	5.7	6.2 ± 0.7		6.8	x	6.8		0.034 ± 0.02	
	7.1	6.2	6.7 ± 0.7		7.1	1.3	4.2 ± 4.1		0.036 ± 0.01	
Mid Dose	x	11.3	11.3	12.9 ± 5.1	5.5	3.3	4.4 ± 1.5	5.5 ± 2.2	0.039 ± 0.03	.039 ± .005
	5.4	11.9	8.6 ± 4.6		8.6	9.6	9.1 ± 0.7		0.037 ± 0.02	
	9.2	10.9	10.1 ± 1.2		4	8	6.0 ± 2.9		0.048 ± 0.15	
	21.6	x	21.6		2.7	5.7	4.2 ± 2.1		0.038 ± 0.06	
	6.1	17.4	12.8 ± 6.5		3.7	4	3.9 ± 0.2		0.035 ± 0.01	
High Dose	6.5	12.1	9.3 ± 4.0	8.0 ± 2.1	2.7	2.5	2.6 ± 0.2	4.3 ± 2.2	0.033 ± 0.02	.036 ± .002
	8.3	6.9	7.6 ± 1.0		8.4	7.3	7.9 ± 0.8		0.038 ± 0.03	
	7.8	2.5	5.1 ± 3.7		3	2.3	5.1 ± 3.1		0.035 ± 0.03	
	6.7	7.9	7.3 ± 0.9		2.3	3.6	2.9 ± 0.9		0.037 ± 0.02	
	6.5	14.8	10.7 ± 5.8		3.8	2.5	3.1 ± 0.9		0.038 ± 0.02	

Red font = >2-fold difference between duplicate slides.
Blue field = lowest animal mean for the group.
Yellow field = highest animal mean for the group.

Validation of *in Vitro* Comet Assay

-Pre-validation to make a robust protocol and consensus for evaluation and interpretation-



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Genotoxicity Tests (Prediction of Carcinogenicity)

In vitro Tests

- 1, Rec Assay
- 2, Phage Induction Test
- 3, Umu Test
- 4, Bacterial Reverse Mutation Test (Ames assay)
- 5, Bacterial Forward Mutation Test
- 6, Mutation Assay Using *S. Cerevisiae*
- 7, Mammalian Gene Mutation Assay
- 8, UDS Test
- 9, SCE Test
- 10, Chromosome Aberration (CA) Test
- 11, Micronuclei (MN) Test
- 12, Comet Assay
- 13, Cell Transformation Test
- etc.

In vivo Tests

- 1, Micronuclei Test
- 2, SCE Test
- 3, Chromosome aberration Test
- 4, UDS Test
- 5, Endogenous Gene Mutation Assay (Hprt, GPA, HLA, etc.)
- 6, Transgenic Gene Mutation Assay (MutaMouse, BigBlue, etc.)
- 7, Spot Test
- 8, Comet Assay
- etc.

