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CAS 100-00-5 1 OF 1

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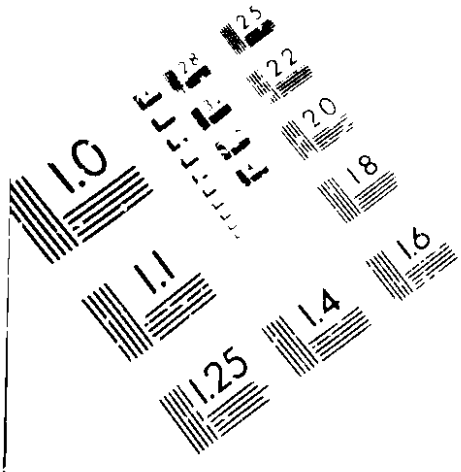

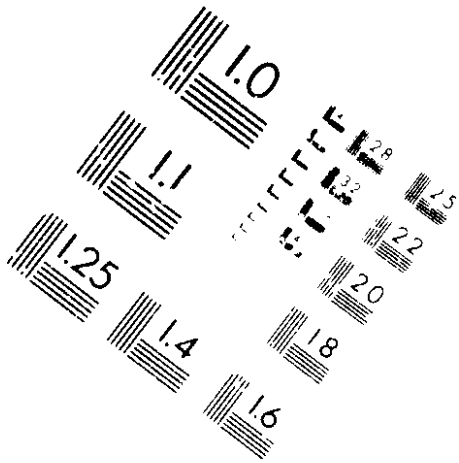

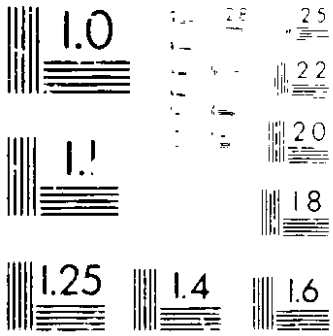

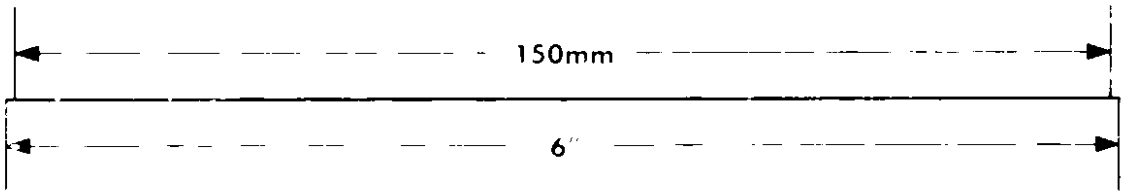

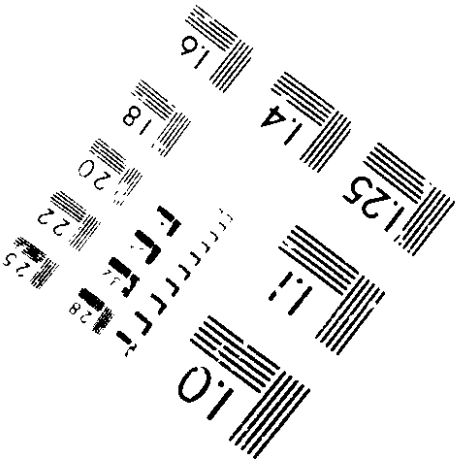

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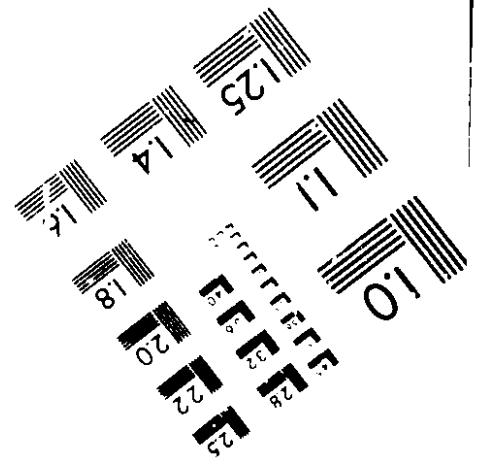
FAX (716) 288-5989



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Submitting Organization		
E I DUPONT DE NEMOURS & CO		
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HASKELL LABORATORY		
Document Title		
MUTAGENIC ACTIVITY IN THE CHINESE HAMSTER OVARY ASSAY OF 1-CHLORO-4-NITRO- WITH COVER LETTER OUTLINING CURRENT STUDY OF TETRAHYDROFURAN TOXICITY DATED 05/10/94 (SANITIZED)		
Chemical Category		
BENZENE, 1-CHLORO-4-NITRO- (100-00-5)		

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Microfiche No. (7) *	1	No. of Pages	2
Doc I.D.	3	Old Doc I.D. 8694 0000 7175	4
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Pub/Journal Name	9
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Organ. Name	11
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Office of Pollution Prevention and Toxics
Environmental Protection Agency
401 M Street SW
Washington, DC 20460
Attention: 8(d) Reporting

Dear Sir/Madam:

OPPTS-82042; FRL-4745-5

In response to the subject final rule published in the Federal Register on February 9, 1994 (59FR 5956, and effective on March 11, 1994, DuPont Specialty Chemicals submits the enclosed toxicity reports under TSCA section 8(d) Health and Safety Data Reporting on the following chemicals: Dimethyl sulfate, o-Chloronitrobenzene, m-Toluidine, 1,3-Dinitrobenzene, Diphenylamine, 2,4-Dinitrotoluene, o-Anisidine, Chlorobenzene, Tetrahydrofuran, o-Nitrotoluene, Xylidine, p-Nitroaniline and Phenylhydrazine.

Additionally, we are aware of the following ongoing studies on Tetrahydrofuran (THF).

Under a TSCA section 4 (a) test rule THF is being evaluated for neurotoxicity potential. The testing is being conducted by DuPont on behalf of an industry consortium, the THF Task Force umbrellaed under the Synthetic Organic Chemical Manufacturers Association (SOCMA). To date the in-life phase of an acute neurotoxicity study has been completed. Subchronic neurotoxicity testing including neuropathology and evaluations for Motor Activity and Functional Observation Battery will be conducted in the near future.

A two-generation reproduction toxicity study is underway at BASF's toxicology laboratories in Germany to determine the reproductive toxicity potential of THF through the oral route of exposure (compound administered in drinking water). The in-life phase of the one generation rangefinder (for the main study) has been completed. The main study is expected to begin later this year. The studies are being conducted by BASF on behalf of an industry consortium of which DuPont is a member

Final reports from both studies will be submitted to EPA when they issue, the final report from the reproductive toxicity rangefinder has been submitted to EPA under the 8(d) rule by BASF.

You may contact me on 302/774-6467 if there are any questions regarding this submission.

Yours truly,

K D Dastur

K. D. Dastur
Manager, Product Toxicology
and Chemical Regulations

/pmt
Enclosure

LIST OF SUBMITTED STUDIES

Title	Report #
Dimethyl Sulfate (CAS #77-78-1)	
1. Industrial Hygiene Survey of DMS Manufacture and Use (CAI) Plant	HLR# 203-77
2. Industrial Hygiene Survey of DMS Manufacturing Facilities - (CAI) Plant	HLR# 202-77
3. Acute Inhalation Toxicity and Antidote Study	HLR# 318-71
4. Preliminary Tests on the Toxicity of Diethyl Sulfate and Diethyl Peroxide (DMS data included)	NA
5. Primary Skin Irritation and Sensitization Tests on Guinea Pigs	HLR# 22-72
6. Mutagenicity Evaluation in Salmonella Typhimurium	HLR# 348-81
7. Dimethyl Sulfate Permeation Testing	HLR# 261-81
8. Dimethyl Sulfate Permeation Testing	HLR# 538-81
9. Teratogenicity Study of Dimethyl Sulfate in Rats	HLR# 535-91
10. Carcinogenicity and Chronic Toxicity of Inhaled Dimethyl Sulfate	NA
1-Chloro-4-nitrobenzene (p-Nitrochlorobenzene) (CAS#100-00-5)	
1. Inhalation Median Lethal Concentration (LC50)	HLR# 751-81
2. Subchronic Inhalation Toxicity Study of p-Chloronitrobenzene (PCNB) in Rats	HLR# 429-84
3. 96-Hour LC50 to Fathead Minnows	HLR#62-79
4 Toxicity of Compounds Used in (CAI) Building	HLR# 13-49
5. Eye Irritation Test in Rabbits	HLR# 57-82
6. In Vitro Microbial Mutagenicity Studies of p-Nitrochlorobenzene	HLR# 474-75
7. Mutagenic Activity of Mixed Nitrochlorobenzene [o-, p-] in the Salmonella Microsome Assay	HLR# 539-77
8. Mutagenic Activity of para-Chloronitrobenzene In Salmonella/Microsome Assay	HLR# 530-77
9. Mutagenic Activity of Benzene, para-Nitrochloro-46.5% in Salmonella/Microsome Assay	HLR# 965-77
10. Mutagenic Activity of Mixed Nitrochlorobenzene in Salmonella/Microsome Assay	HLR# 964-77
11. Mutagenic Activity in the Salmonella/Microsome Assay	HLR# 392-78
12. Mutagenic Activity in the Salmonella/Microsome Assay	HLR# 535-78
13. Mutagenic Activity in the Salmonella/Microsome Assay	HLR# 536-78
14. Mutagenic Activity in the Salmonella/Microsome Assay	HLR# 705-78
15. Mutagenic Activity in the Salmonella/Microsome Assay	HLR# 275-79
16. Mutagenic Activity in the Chinese Hamster Ovary Assay	HLR# 24-80

List of Submitted Studies (continued)

Title	Report #
1,3-Dinitrobenzene (CAS#99-65-0)	
1. Nitrobenzene Derivatives	NA
Diphenylamine (CAS#122-39-4)	
1. Para Chlor Aniline Quinaldine and (CBI)	NA
2. Results of Inorganic Lead and Diphenylamine Air Samples Collected at the (CBI) Plant	HLR# 484-76
3. Department of Transportation Skin Corrosion Test on Rabbit Skin	HLR# 421-75
2,4, - Dinitrotoluene (CAS#121-14-2)	
1. Industrial Hygiene Survey of the (CBI) Involving Dry Chemical Handling Procedures	HLR# 71-77
2. Evaluation of Exposure to 2,4-Dinitrotoluene While Handloading Shotshell - (CBI)	HLR# 654-79
3. Class B Poison Labelling Test	HLR# 26-66
4. 96-Hour LC50 to Fathead Minnows	HLR# 61-79
5. Eye Irritation Test in Rabbits	HLR# 713-81
6. Department of Transportation Skin Corrosion Test on Rabbit Skin	HLR# 556-73
7. Oral Bioassay Study (Dogs)	HLR# 490-76
8. The Toxicity of TNT and DNT	NA
9. Teratological and Postnatal Evaluation of Dinitrotoluene in Fischer 344 Rats	RTI Report RTI/1938/0003F
10. A thirty day toxicology study in Fischer 344 rats given dinitrotoluene-technical grade. (Study performed at Hazleton Laboratories America, Inc. for Chemical Industry Institute of Toxicology, Research Triangle Park, NC-	

List of Submitted Studies (Continued)

	Title	Report #
	o-Anisidine (CAS#90-04-0)	
1	Toxicity of Compounds Used in (Hydrogen Reduction) Building	NA
2.	Department of Transportation Skin Corrosion Test on Rabbit Skin	HLR# 532-73
3.	In Vitro Microbial Mutagenicity Studies of ortho-Anisidine	HLR# 247-75
4.	Toxic Hazards Evaluation of Five Atmospheric Pollutants from Army Ammunition Plants	NA
	Chlorobenzene (CAS#108-90-7)	
1	Mutagenic Acitivty of Monochlorobenzene in the Salmonella/Microsome Assay	HLR# 537-77
	Tetrahydrofuran (CAS#109-99-9)	
1.	Acute Toxicity Evaluation By Aspiration and Insufflation	HLR# 374-71
2.	Toxicity of Tetrahydrofuran	NA
3.	Acute Inhalation Toxicity in Rats	HLR# 848-79
4.	48-Hour LC50 to Daphnia Magna	HLR# 744-80
5.	96-Hour LC50 to Fathead Minnows	HLR# 745-80
6.	Federal Hazardous Substances Act Test - Rabbit Eye Irritation	HLR# 290-71
7.	96-Hour LC50 to Fathead Minnows	HLR# 135-82
8.	Tetrahydrofuran (THF) Inhalation Effect on the Rat Conceptus	HLR# 750-82
	o-Nitrotoluene (CAS#88-72-2)	
1.	Inhalation Class B Poison	HLR# 98-72
2.	Class B Poison Tests on Rabbit Skin	HLR# 84-72
3.	Acute Oral Test	HLR# 56-72
4.	Department of Transportation Skin Irritation Test on Rabbit Skin	HLR#626-73
	Phenylhydrazine (CAS#100-63-0)	
1	Acute Skin Absorption Toxicity	HLR# 106-63

List of Submitted Studies (Continued)

Title	Report #
Xylidine (CAS#1300-73-8)	
1. Class B Poison Test	HLR# 228-69
2. Class B Poison Test	HLR# 172-69
3. Department of Transportation Skin Corrosion Test on Rabbit Skin	HLR# 538-73
4-Nitroaniline (p-Nitroaniline) (CAS#100-01-6)	
1 Subchronic Inhalation Toxicity Study of p-Nitroaniline (PNA) in Rats	HLR# 372-84
2. Toxicity of Compounds Used in Hydrogen Reduction Building	NA
3. Inhalation Median Lethal Concentration (LC50)	HLR# 856-81
4 Micronucleus assay with p-Nitroaniline	MSL-9283
m-Toluidine (CAS#108-44-1)	
1. Oral Class B Poison Test	HLR# 543-74
2. Oral LD50 Test in Rats	HLR# 955-80
3. Eye Irritation Test in Rabbits	HLR# 945-80
4 Skin Irritation Test in Rabbits	HLR# 948-80
5. DOT Skin Corrosion Test	HLR# 527-73

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Copies to: [CBI]

E. I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine
Elkton Road, Newark, DE 19711

HASKELL LABORATORY REPORT NO. 24-80 MR NO. [3095-005]

Material Tested: Benzene, 1-chloro-4-nitro- (see page 3 for composition)

Haskell No. 13,044
Other Code: [CBI]

Material Submitted by: [CBI]

Date of Initiation 6-28-79
Date of Completion 12-19-79

MUTAGENIC ACTIVITY IN THE CHINESE HAMSTER OVARY ASSAY

I. Introduction

The Chinese hamster ovary (CHO) assay measures the capability of a chemical to induce forward mutations in cultured Chinese hamster ovary cells. Cells possessing mutations at the hypoxanthine-guanine phosphoribosyltransferase (HGPRT) locus form colonies in the presence of the purine analogue, 6-thioguanine (6-TG). The assay was developed by A. W. Hsie at Oak Ridge National Laboratory.

II. Methods

A. General Procedure

A preliminary cytotoxicity evaluation of the test material is performed to select concentrations for mutagenesis testing. In general, concentrations which kill from 0% to 90% of the cells are chosen.

The cytotoxic and mutagenic activities of the test material are evaluated in the mutagenesis assay. All assays are performed in the presence and absence of a metabolic activation system composed of rat-liver homogenate and cofactors.

In the mutagenesis assay, 1×10^6 cells are treated with each concentration of test material. The cells are exposed to the test compound for 5 hours in the activated assay and 18 hours in the nonactivated assay. For every concentration of test material, cell survival is determined 24 hours after beginning exposure by plating some of the treated

cells at a low density. The number of colonies that arise after seven days incubation is an indication of how well the cells survived treatment. Other cells from the same treatment level are plated at a high density and maintained in an exponential growth phase for seven days to permit expression of the mutant phenotype. At the end of this period, 1 x 10^6 cells are placed in medium containing 6-thioguanine to select for 6-TG resistant mutants. Resistant cells form colonies which are visible when stained one week later. Also at the time of selection, some cells are plated at a low density in 6-TG free medium to determine the percentage of cells that are viable at this time. This indicates the actual number of cells from which the mutants were selected.

All mutagenesis assays include solvent controls and positive controls. All dose levels are done in duplicate and all experiments are performed at least twice.

B. Activation System

The activation system (S-9) is derived from the 9,000 x g supernatant of homogenized rat liver. The liver is taken from Sprague-Dawley rats previously treated with the polychlorinated biphenyl mixture, Aroclor 1254. The S-9 is diluted in 0.15 M KCl so that the treatment medium contains 1 mg/protein/ml. In addition, the treatment medium contains: $MgCl_2 \cdot 6H_2O$, 5 mM; NADP, 1.5 mM, glucose-6-phosphate, 5 mM and NADH, 1 mM.

A detailed description of the protocol is available upon request.

Results Acetone was the solvent in this study because it was more effective than other solvents tested. The lower concentrations tested with and without activation were soluble in acetone and did not precipitate when added to treatment medium. The higher concentrations tested with and without activation were soluble in acetone and precipitated when added to the treatment medium.

Toxicity was observed at higher concentrations.

The results of the toxicity and mutagenicity studies with this compound are presented in Tables 1, 2, 3, 4, and 5.

The statistical analysis included only replicate dose points from all experiments. The results of the analysis are presented in Tables 6 and 7. A detailed description of the statistical methods used is available upon request.

Conclusion: Benzene, 1-chloro-4-nitro- is not mutagenic to cultured Chinese hamster ovary cells. The statistical analyses showed no significant increase in the mutation frequency over the control.

Composition: [CBI]

Report by: _____
Carolyn H. Smith
Molecular Biologist

Approved by: David F. Krahn
David F. Krahn
Chief, Molecular Biology Section

Notebook No. [CBI]

CHS:DFK:ms
Report No. 24-80
Date Issued: January 21, 1980

TABLE 1 (TRIAL 1)

TOXICITY AND MUTAGENICITY OF H-13,044 IN CHINESE HAMSTER OVARY CELLS WITH ACTIVATION

Compound	Concentration (μM)	TOXICITY: Cell Survival (% Plated)	TOXICITY: Cell Survival (% of Control)	MUTAGENICITY: Cell Survival (% Plated)	MUTAGENICITY: Mutants Total	MUTAGENICITY: Mutants Frequency*
Acetone	0	68.6	100.0§	70.6	7	9.9
		70.8		77.9	15	19.2
13,044	1.59	61.3	87.9	79.8	7	8.8
		56.2	80.6	71.8	12	16.7
"	1.90	26.7	38.3	46.4	2	4.3
		51.8	74.3	59.2	19	32.1
"	2.22	18.7	26.8	53.2	0	0
		21.9	[illegible]	49.3	2	4.0
"	2.38	15.6	22.4	45.8	5	10.9
		15.0	21.5	41.8	8	19.1
DMN	8.10	56.0	80.3	66.9	64	95.7
		36.4	52.2	73.2	56	76.5

*Mutants per 10^6 surviving cells

H-13,044 = Benzene, 1-chloro-4-nitro-

Acetone = Solvent control

DMN = Dimethylnitrosamine

§ = The average of the duplicate plating efficiencies is set equal to 100%

TABLE 2 (TRIAL 2)

TOXICITY AND MUTAGENICITY OF H-13,044 IN CHINESE HAMSTER OVARY CELLS WITH ACTIVATION

Compound	Concentration (mM)	TOXICITY Cell Survival (% Plated)	TOXICITY Cell Survival (% of Control)	MUTAGENICITY Cell Survival (% Plated)	MUTAGENICITY Mutants Total	MUTAGENICITY Mutants Frequency*
Acetone	0	59.8	100.0§	81.2	0	0
		68.1		77.7	2	2.6
13,044	1.59	37.2	58.1	66.4	4	6.2
		61.9	96.7	82.7	2	2.4
"	1.90	46.7	73.0	65.3	0	0
		27.8	43.4	74.1	3	4.0
"	2.22	17.8	27.8	65.8	0	0
		23.7	37.0	62.5	1	1.6
"	2.38	14.4	22.5	61.2	0	0
		6.6	10.3	47.8	0	0
DMN	8.10	57.2	89.4	70.8	70	98.9
		49.1	76.7	79.8	57	71.4

*Mutants per 10^6 surviving cells

H-13,044 = Benzene, 1-chloro-4-nitro-

Acetone = Solvent control

DMN = Dimethylnitrosamine

§ = The average of the duplicate plating efficiencies is set equal to 100%

TABLE 3 (TRIAL 1)

TOXICITY AND MUTAGENICITY OF H-13,044 IN CHINESE HAMSTER OVARY CELLS WITHOUT ACTIVATION

Compound	Concentration (mM)	TOXICITY: Cell Survival (% Plated)	TOXICITY: Cell Survival (% of Control)	MUTAGENICITY: Cell Survival (% Plated)	MUTAGENICITY: Mutants Total	MUTAGENICITY: Mutants Frequency*
Acetone	0	54.9	100.0§	83.3	0	0
		63.2		77.8	3	3.8
H-13,044	1.59†	66.5	112.7	91.8	15	16.3
"	1.90	41.6	70.5	69.4	8	11.5
		58.7	99.5	59.7	16	26.8
"	2.22	35.7	60.5	80.9	7	8.6
		25.5	43.2	77.9	5	6.4
"	2.38	34.4	58.3	77.0	14	18.2
		30.7	52.0	98.4	31	31.5
EMS	0.80	46.9	79.5	73.8	179	242.5
		40.0	67.8	66.1	227	343.1

*Mutants per 10^6 surviving cells

H-13,044 = Benzene, 1-chloro-4-nitro-

Acetone = Solvent control

EMS = Ethylmethane sulfonate (positive control)

† = Duplicate point loss due to toxicity

§ = The average of the duplicate plating efficiencies is set equal to 100%

TABLE 4 (TRIAL 2)

TOXICITY AND MUTAGENICITY OF H-13,044 IN CHINESE HAMSTER OVARY CELLS WITHOUT ACTIVATION

Compound	Concentration (mM)	TOXICITY: Cell Survival (% Plated)	TOXICITY: Cell Survival (% of Control)	MUTAGENICITY: Cell Survival (% Plated)	MUTAGENICITY: Mutants Total	MUTAGENICITY: Mutants Frequency*
Acetone	0	44.9	100.0§	71.8	12	16.7
		65.5		61.9	14	22.6
13,044	1.59	70.6	127.9	75.1	12	16.0
		60.5	109.6	78.3	4	5.1
"	1.90	41.8	75.7	86.2	7	8.1
		62.8	113.8	63.3	0	0
"	2.22	63.2	114.5	52.4	0	0
		63.6	115.2	68.2	11	16.1
"	2.38	8.2	14.8	73.2	0	0
		62.7	113.6	67.7	4	5.9
"	2.54	64.6	117.0	70.6	0	0
		40.1	72.6	58.7	0	0
EMS	0.80	60.1	108.9	63.7	170	266.9
		51.4	93.1	100.8	270	267.9

*Mutants per 10^6 surviving cells

H-13,044 = Benzene, 1-chloro-4-nitro-

Acetone = Solvent control

EMS = Ethylmethane sulfonate (positive control)

§ = The average of the duplicate plating efficiencies is set equal to 100%

TABLE 5 (TRIAL 3)

TOXICITY AND MUTAGENICITY OF H-13,044 IN CHINESE HAMSTER OVARY CELLS WITHOUT ACTIVATION

Compound	Concentration (mM)	TOXICITY Cell Survival (% Plated)	TOXICITY Cell Survival (% of Control)	MUTAGENICITY Cell Survival (% Plated)	Mutants Total	Mutants Frequency*
Acetone	0	75.3	100.0§	70.6	3	4.2
		93.2		50.8	11	21.6
13,044	1.59	71.1	84.4	66.8	0	0
		63.4	75.3	80.2	2	2.5
"	1.90	67.0	79.6	27.5	0	0
		57.2	67.9	76.4	1	1.3
"	2.22	45.0	53.4	55.8	1	1.8
		34.1	40.5	56.6	1	1.8
"	2.54	34.9	41.4	66.5	8	12.0
		30.7	36.5	36.1	0	0
"	2.86	38.2	45.4	43.0	0	0
		30.6	36.3	33.2	18	54.2
EMS	0.80	69.0	81.9	51.1	96	187.9
		69.6	82.7	75.4	194	257.3

*Mutants per 10^6 surviving cells

H-13,044 = Benzene, 1-chloro-4-nitro-

Acetone = Solvent control

EMS = Ethylmethane sulfonate (positive control)

§ = The average of the duplicate plating efficiencies is set equal to 100%

TABLE 6

PNCB-ACTIVATED MUTAGENICITY
STATISTICAL ANALYSIS OF MUTAGENICITY

DOSE	AVERAGE	T	PROB	MUTATION FREQUENCY
0.00	1.3031			4.8
1.59	1.3733	0.63	0.5400	7.3
1.90	1.3119	0.08	0.9383	5.1
2.22	1.1068	-1.76	0.1008	1.0
2.38	1.2546	-0.43	0.6708	3.5

T IS STUDENT'S T-STATISTIC FOR THE COMPARISON OF EACH DOSE LEVEL TO THE NEGATIVE CONTROL (DOSE = 0).

DOSE-RESPONSE ANALYSIS OF VARIANCE

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F RATIO	PROB
TOTAL	19	0.9699			
TRIAL	1	0.4586			
DOSE	4	0.1616			
LINEAR	1	0.0263	.0263	1.05	.3226
QUADRATIC	1	0.0547	.0547	2.19	.1610
HIGHER ORDER	2	0.0806	.0403	1.61	.2341
RESIDUAL	14	0.3497	.0250		

DOSE X TRIAL INTERACTION

F RATIO	DEGREES OF FREEDOM	PROBABILITY
1.36	4 AND 10	.3161

A SIGNIFICANT DOSE-TRIAL INTERACTION INDICATES THAT THE DOSE-RESPONSE RELATIONSHIP IS DIFFERENT IN THE DIFFERENT TRIALS . THE DOSE-RESPONSE ANOVA ABOVE ASSUMES THAT THIS INTERACTION IS NOT STATISTICALLY SIGNIFICANT.

TABLE 7

PNCB NONACTIVATED MUTAGENICITY
STATISTICAL ANALYSIS OF MUTAGENICITY

DOSE	AVERAGE	T	PROB	MUTATION FREQUENCY
0.00	1.3813			7.6
1.59	1.3301	0.37	0.7152	5.7
1.90	1.2722	0.92	0.4159	4.0
2.22	1.2698	-0.85	0.4061	3.9

T IS STUDENT'S T-STATISTIC FOR THE COMPARISON OF EACH DOSE LEVEL TO THE NEGATIVE CONTROL (DOSE = 0).

DOSE-RESPONSE ANALYSIS OF VARIANCE

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F RATIO	PROB
TOTAL	22	1.0889			
TRIAL	2	0.1671			
DOSE	3	0.0506			
LINEAR	1	0.0456	.0456	0.89	.3592
QUADRATIC	1	0.0014	.0014	0.03	.8694
HIGHER ORDER	1	0.0036	.0036	0.07	.7953
RESIDUAL	17	0.8729	.0513		

DOSE X TRIAL INTERACTION

F RATIO	DEGREES OF FREEDOM	PROBABILITY
2.40	6 AND 11	.0990

A SIGNIFICANT DOSE-TRIAL INTERACTION INDICATES THAT THE DOSE-RESPONSE RELATIONSHIP IS DIFFERENT IN THE DIFFERENT TRIALS . THE DOSE-RESPONSE ANOVA ABOVE ASSUMES THAT THIS INTERACTION IS NOT STATISTICALLY SIGNIFICANT.

AMTEK CORP.

CERTIFICATE OF AUTHENTICITY

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