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Attn: TSCA 8(e) Coordinator

RE: **Union Carbide Corporation's TSCA 8(e) CAP Submissions**
Concerning Vinylnorbornene (VNB; CASRN 3048-64-4) [8EHQ-
0392-2859; 8EHQ-0592-3538; 8EHQ-0592-4197]

Dear Sir or Madam:

As a follow-up to the above-noted submissions concerning the toxicology of vinylnorbornene (VNB; CASRN 3048-64-4), Union Carbide Corporation ("Union Carbide") herewith submits the following report:

"Vinylnorbornene: Mutagenic potential in the CHO/HGPRT Forward Mutation Assay", Bushy Run Research Center, BRRC Report 94U1372, October 18, 1994 (55 pp).

In the attached report the term "Confidential" may appear. This precautionary statement was for internal use at the time of issuance of the report, and is hereby waived for the purposes of the needs of the Agency in assessing health and safety information. The Agency is advised, however, that the publication rights to the contained information are the property of Union Carbide.

Very truly yours,

William C. Kuryla, Ph.D.
Associate Director
Product Safety



8EHQ-92-4197
SP003 11/09/94



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Attachment

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10/31/94



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STUDY TITLE

Vinylnorbornene: Mutagenic Potential in the CHO/HGPRT Forward Mutation Assay

TEST SUBSTANCE

Vinylnorbornene

DATA REQUIREMENT

Not Applicable

AUTHOR

J. S. Vergnes

STUDY COMPLETION DATE

October 18, 1994

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Vinylnorbornene: Mutagenic Potential in the CHO/HGPRT Forward Mutation Assay

COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

The portions of this study conducted by BRRC meet the requirements of the following Good Laboratory Practice Standards: Toxic Substances Control Act (TSCA), 40 CFR Part 792; Organisation for Economic Co-operation and Development (OECD), C(81)30(Final).

1. The test substance was not analyzed for stability.
2. The positive control substances were not analyzed for chemical purity, stability, or uniformity.
3. Analyses for stability and homogeneity of the positive control substances in the dosing solutions were not conducted.

These exceptions are not expected to compromise the integrity of the results and conclusion of the study.

Study Director:

Jane S. Vergnes
Jane S. Vergnes, Ph.D.

10/18/94
Date

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Vinylnorbornene: Mutagenic Potential in the CHO/HGPRT Forward
Mutation Assay

SUMMARY

Vinylnorbornene [VNB, CAS No. 3048-64-4; 16219-75-3 (ethylidene norbornene)] was evaluated for potential genotoxic activity using the HGPRT forward mutation assay in cultured Chinese Hamster Ovary cells (CHO-K1-BH4). In a preliminary test to determine cytotoxicity, VNB produced excessive cytotoxicity at concentrations of 0.10 mg/ml or greater both in the absence and in the presence of a rat liver S9 metabolic activation system (S9). Relative survival at the lowest concentration tested (0.01 mg/ml) was 98% in the absence of S9 and 80% in the presence of S9.

Based upon the results of the preliminary cytotoxicity test, duplicate cultures of CHO cells were treated with 5 concentrations of VNB ranging from 0.01 to 0.10 mg/ml in the absence of S9 and with 6 VNB concentrations ranging from 0.005 to 0.05 mg/ml in the presence of S9. Two additional concentrations, 0.03 and 0.04 mg/ml, were tested in the absence of S9. In a second complete test, CHO cells were treated with 5 VNB concentrations ranging from 0.01 to 0.05 mg/ml in the absence of S9 and from 0.005 to 0.10 mg/ml in the presence of S9. In a third complete test, CHO cells were treated with 5 VNB concentrations ranging from 0.01 to 0.06 mg/ml in the absence of S9 and from 0.01 to 0.08 mg/ml in the presence of S9. Three independent repetitions of the full test and a partial repetition of the first test in the absence of S9 were performed due to problems in obtaining a reproducible dose response. In spite of these problems, a full range of cytotoxic responses was represented in the 3 full and one partial repetitions of the test combined.

In the absence of S9, a statistically significant ($p < 0.05$) increase in mutation frequency was observed at 0.04 mg/ml VNB. However, this increase was not considered to be biologically significant since the mutation frequencies for each of the replicates (5.4×10^{-6} , 4.7×10^{-6}) were less than that of an untreated culture medium control (13.3×10^{-6}). No other significant or concentration-related increases in mutation frequency were observed in the absence of S9 activation. Mutation frequencies for the DMSO-treated vehicle control cultures ranged from 1.1×10^{-6} to 13.2×10^{-6} in the absence of S9. Mutation frequencies for the EMS-treated positive controls ranged from 79.6×10^{-6} to 247.6×10^{-6} at a concentration of 200 μ g/ml. No significant or concentration-related increases in mutation frequency were observed in the presence of S9 activation. Mutation frequencies for the DMSO-treated vehicle control cultures ranged from 0 to 5.3×10^{-6} in the presence of S9. Mutation frequencies for the DMN-treated positive controls ranged from 64.4×10^{-6} to 175.6×10^{-6} at a concentration of 200 μ g/ml. Since no significant, concentration-related increases in mutation frequencies were observed at any of the VNB concentrations tested, either in the absence or in the presence of S9, VNB was not considered to be mutagenic to cultured CHO cells under the conditions of this in vitro assay.

OBJECTIVE

The objective of this study was to assess the potential of VNB to increase the frequency of HGPRT deficient mutants in cultured Chinese Hamster Ovary (CHO) cells.

BACKGROUND INFORMATION

Previous genotoxicity studies on VNB have been conducted in vitro. No mutagenic activity was observed in an Ames test (BRRC Report 94U1371). No reproducible increases in the frequencies of sister chromatid exchanges were observed either in the absence or in the presence of a rat liver S9 metabolic activation system (BRRC Report 94U1373).

DOSE SELECTION

Concentrations were initially selected on the basis of a preliminary cytotoxicity test, which is described in this report. Additional concentrations for confirmatory testing were based upon data obtained during testing. The actual concentrations tested are given in the report tables.

MATERIALS AND METHODS

The protocol and one protocol amendment detailing the design and conduct of this study are presented in Appendix 2.

Test Substance

A 1-gallon metal can (3.262 kg gross weight) of vinylnorbornene (VNB), Lot No. 59FGC-27, CAS No. 3048-64-4; 16219-75-3 (ethylidene norbornene), was received on November 5, 1993, from Union Carbide Corporation, South Charleston, WV, and assigned BRRC Sample No. 56-403. The test substance was a colorless liquid. The test substance was stored at room temperature. The purity of the test substance was determined by the GLP Analytical Skill Center at the UCC South Charleston, WV, Technical Center to be 98.44% and the report is included in Appendix 1. Test substance stability was not confirmed by BRRC.

Positive Control Substances

A glass bottle containing 5 g of ethylmethanesulfonate (EMS), Lot No. 8EF-0531, CAS No. 62-50-0, was received on November 21, 1989, from Sigma Chemical Co. and assigned BRRC Sample No. 52-723. It was a liquid and was stored refrigerated. A glass bottle containing dimethylnitrosamine (DMN), Lot No. 29F-0679, CAS No. 62-75-9, was received on October 2, 1992, from Sigma Chemical Co. and assigned BRRC Sample No. 55-295. It was a liquid and was stored at 0 to 5°C.

Vehicle Control Substance

One 1-quart bottle of dimethylsulfoxide (DMSO), Lot No. BD469, CAS No. 67-68-5, was received on December 3, 1992, from Baxter Health Products, and assigned BRRC Sample No. 55-386. One 1-liter bottle of dimethylsulfoxide (DMSO), Lot No. BG326, CAS No. 67-68-5, was received on March 31, 1994, from Baxter Health Products, and assigned BRRC Sample No. 57-080. Both were liquids and were stored at room temperature.

Metabolic Activation

Rat liver S9 homogenate, prepared from Aroclor 1254-induced rats, was purchased from Microbiological Associates. The complete S9 metabolic activation system contained the following: 10 mM MgCl₂, 30 mM KCl, 5 mM glucose-6-phosphate, 4 mM NADP-oxidized form (nicotinamide adenine dinucleotide phosphate), 50 mM Na₂HPO₄, 10 mM CaCl₂ and S9 homogenate.

Study Organization

Cells were treated with the vehicle control substance, an appropriate positive control substance, and at least 5 dose levels of VNB both in the absence and in the presence of a rat liver S9 metabolic activation system. All treatments were performed on duplicate cultures except that single cultures were used for positive controls and untreated medium controls. Treated cultures were incubated at approximately 37°C in a humidified atmosphere containing approximately 5% CO₂ for approximately 4 hours. Three complete, independent tests were performed. The test substance was first administered to the test system on March 10, 1994, and testing was completed on June 22, 1994.

Cells and Culture Conditions

The CHO cells used in these studies, CHO-K1-BH4-(subclone D1), were obtained from Abraham Hsie at Oak Ridge National Laboratory. Cells were thawed from frozen stock cultures and maintained in active growth by subculturing 1-3 x 10⁵ cells 2 to 3 times/week in antibiotic-free, Ham's Modified F12 Medium supplemented to 10% (v/v) with heat-inactivated, fetal bovine serum (F12-10). For test substance exposures of cells, F12 medium without serum was employed. For determination of mutation frequencies, F12-D5 medium containing 2.0 µg/ml 6TG was used as a selective medium. Cells were counted with an electronic cell counter. Screening for Mycoplasma contamination was performed on all newly prepared frozen stock cultures by a commercial laboratory. All culture procedures and treatments with test substances were performed under aseptic conditions in vertical laminar-flow biohazard hoods.

Dosing Solution Preparation

Each dosing solution was prepared by diluting the appropriate amount of VNB with DMSO. Concentrations were not adjusted for percent active ingredient of the test substance. Dosing solutions were prepared on the day of treatment and all unused solutions were discarded by the end of the day. Aliquots of the dosing solutions were diluted on a weight/volume basis into the culture media already contained in cell culture flasks.

EMS and DMN positive control dosing solutions were prepared by diluting them with sterile, deionized water. Positive control dosing solutions were prepared on the day of treatment, and all unused solutions were discarded by the end of the day. EMS was used as the positive control in the absence of metabolic activation, and DMN was used as the positive control in the presence of metabolic activation. Aliquots of the dosing solutions were diluted on a weight/volume basis into the culture media already contained in cell culture flasks.

Preliminary Cytotoxicity Testing

In a preliminary cytotoxicity test, 7 VNB concentrations ranging from 0.01 to 5.0 mg/ml were tested on CHO cells, both in the absence and in the presence of S9, to determine the cytotoxicity of VNB. Approximately 24 hours before treatment, 5×10^5 cells were seeded in each 25 cm² tissue culture flask. The flasks were incubated at 37°C in a humidified atmosphere containing approximately 5% CO₂. Immediately prior to VNB treatment, the medium in each flask was replaced with 5 ml of F-12 medium without serum. S9 mixture was added to the appropriate cultures. After treatment with various concentrations of VNB for 4 hours, each flask was rinsed with phosphate-buffered saline (PBS), fresh F12-D5 medium was added, and the flasks were incubated for an additional 18 to 24 hours. Cytotoxicity was determined by comparing the number of cells in untreated control cultures to the number of cells in each of the VNB-treated cultures.

Mutagenicity Testing

Approximately 20 to 24 hours prior to the mutation test, CHO cells were plated at $5 \times 10^5/25$ cm² flask in F12-D5 medium and incubated at 37°C in a humidified 5-6% CO₂ atmosphere. On the day of testing, duplicate cultures were treated with 5 VNB concentrations ranging from 0.01 to 0.10 mg/ml in the absence of S9 and with 6 VNB concentrations ranging from 0.005 to 0.05 mg/ml in the presence of S9 for approximately 4 hours at 37°C. Two additional concentrations, 0.03 and 0.04 mg/ml, were tested in the absence of S9. In a second complete test, CHO cells were treated with 5 VNB concentrations ranging from 0.01 to 0.05 mg/ml in the absence of S9 and from 0.005 to 0.10 mg/ml in the presence of S9. In a third complete test, CHO cells were treated with 5 VNB concentrations ranging from 0.01 to 0.06 mg/ml in the absence of S9 and from 0.01 to 0.08 mg/ml in the presence of S9. Following treatment, the medium was removed by suction, cells were rinsed with PBS and fresh F12-D5 medium was added. The cells were allowed to recover for 18 to 24 hours before chemically-induced cytotoxicity was determined. Treatment of cells in the presence and absence of S9 was identical except that S9 activation mixture was added to each S9-treated culture.

The number of cells in the treated cultures was compared to the number of cells in the vehicle control cultures approximately 24 hours after treatment to measure treatment-induced cytotoxicity. The colony-forming potential of 100 cells in each of 4, 60 mm culture plates was also measured.

At 2- to 3-day intervals after treatment, 5 to 10 x 10⁵ cells from each culture were subcultured into 100 mm tissue culture dishes containing F12-D5 medium and incubated at 37°C in a humidified 5-6% CO₂ atmosphere. Eight days after treatment, cells were dissociated with trypsin, counted and plated at a concentration of 2×10^5 cells/dish in 5, 100 mm culture dishes (1×10^6 total cells) containing 10 ml of F12-D5 (6TG) selective medium. To quantify the colony forming ability of the cells at the time of mutant selection, cells from the same cultures were diluted and 100 cells/dish were plated in each of 4, 60 mm culture plates containing F12-D5 medium (without 6TG). All cultures were then incubated for an additional 6-8 days to allow growth of colonies. Colonies were then fixed, stained, and counted (Hsie *et al.*, 1981). The number of colonies was counted either manually or using an Artek Model 880 Colony Counter.

Data Analyses

Mutation data were analyzed by the method of Irr and Snee (1979) after transformation according to the method of Box and Cox (1964). Data for positive control substances were not compared statistically whenever they were at least 5 times the concurrent culture medium control value.

The criteria for interpretation of the test results depend upon both the level of statistical significance with respect to the concurrent vehicle control and the evidence of a dose-response effect. Increases in the frequencies of mutations with respect to the vehicle controls which did not meet at least one of the following criteria were not considered indications of mutagenicity in this test system: statistically significant, concentration-related increases in mutation frequencies at two or more consecutive concentration levels; or a statistically significant, reproducible increase at one or more concentration levels. Furthermore, an increase in the mutation frequency of less than two-fold with respect to the vehicle control was not considered a positive result. A valid test also met the following criteria. The spontaneous mutation frequencies for the culture medium controls were within the literature range of 0 to 20 mutants/10⁶ viable (clonable) cells. The mutation frequencies induced by the positive control agents were at least twice the frequencies for the culture medium controls. The viable (clonable) fraction was at least 80% for the culture medium controls.

All statistical analyses were performed using BMDP Statistical Software. For all statistical tests, the probability value of < 0.05 (one-tailed) was used as the critical level of significance.

Various models of calculators, computers, and computer programs may have been used to analyze data for this study. Since various models round or truncate numbers differently, values in some tables may differ slightly from those in other tables or from independently calculated data. The integrity of the study and interpretation of the data were unaffected by these differences.

RETENTION OF RECORDS

All raw data, documentation, the protocol and any amendments, and a copy of the final report generated as a result of this study will be retained in the BRRC Archives for at least 5 years.

RESULTS AND DISCUSSION

All references to differences in group mean values in the following text refer to comparisons of statistically significant differences between the treatment group and the vehicle control group, unless otherwise noted. Repeated reference to the control and the statistical significance will not be made in order to simplify the text.

Mutagenicity Test

The results of the preliminary cytotoxicity study are shown in Table 1. VNB produced excessive cytotoxicity at concentrations of 0.10 mg/ml or greater, both in the absence and in the presence of S9. Relative survival at the

lowest concentration tested (0.01 mg/ml) was 98% in the absence of S9 and 80% in the presence of S9.

Cytotoxicity data obtained approximately 24 hours after VNB treatment, both in the absence and in the presence of S9, are presented in Tables 2, 4, 8, and 12 (without S9) and in Tables 6, 10, and 14 (with S9). Three independent repetitions of the full test and a partial repetition of the first test in the absence of S9 (Table 4) were performed due to problems in obtaining a reproducible dose response. Finding appropriate concentrations in the range between 0.03 mg/ml and 0.10 mg/ml was particularly problematic. Little to no toxicity was consistently observed at concentrations of 0.01 mg/ml or less, both in the absence and in the presence of S9 activation. In spite of the problems in obtaining cytotoxic responses that were reproducible from one experiment to the next, a full range of cytotoxic responses was represented in the 3 full repetitions of the test and the partial repeat of the first test in the absence of S9.

Mutation data are presented in Tables 3, 5, 9, and 13 (without S9) and in Tables 7, 11, and 15 (with S9). In the absence of S9, a statistically significant ($p < 0.05$) increase in mutation frequency was observed at 0.04 mg/ml VNB (Table 13). However, this increase was not considered to be biologically significant since the mutation frequencies for each of the replicates (5.4×10^{-6} , 4.7×10^{-6}) were less than that of an untreated culture medium control (13.3×10^{-6}). No other significant or concentration-related increases in mutation frequency were observed in the absence of S9 activation. Mutation frequencies for the DMSO-treated vehicle control cultures ranged from 1.1×10^{-6} to 13.2×10^{-6} in the absence of S9. Mutation frequencies for the EMS-treated positive controls ranged from 79.6×10^{-6} to 247.6×10^{-6} at a concentration of 200 μ g/ml. No significant or concentration-related increases in mutation frequency were observed in the presence of S9 activation. Mutation frequencies for the DMSO-treated vehicle control cultures ranged from 0 to 5.3×10^{-6} in the presence of S9. Mutation frequencies for the DMN-treated positive controls ranged from 64.4×10^{-6} to 175.6×10^{-6} at a concentration of 200 μ g/ml. Since no statistically significant, concentration-related increases in mutation frequencies were observed at any of the VNB concentrations tested, either in the absence or in the presence of S9, VNB was not considered to be mutagenic to cultured CHO cells.

CONCLUSION

VNB did not produce statistically significant, concentration-related increases in the frequencies of 6TG resistant CHO cells either in the absence or in the presence of a rat liver S9 metabolic activation system. Therefore, VNB was not considered mutagenic to CHO cells under the conditions of this in vitro assay.

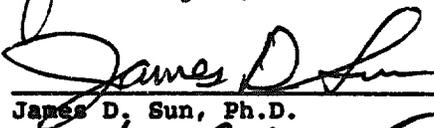
REVIEW AND APPROVAL

Study Director:


 Jane S. Vergnes, Ph.D.

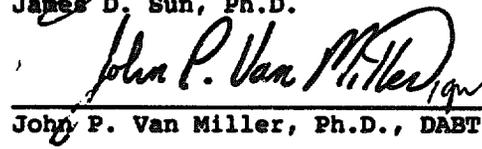
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REFERENCES

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TABLE 1
 VINYLORBORNENE: MUTAGENIC POTENTIAL IN THE CHO/HGPRT FORWARD
 MUTATION ASSAY

PRELIMINARY CYTOTOXICITY TEST

VRE Concentration (mg/ml)	Initial Cell Density ^a (Cells/flask x 10 ⁵)	Final Cell Density ^b (Cells/flask x 10 ⁵)		Relative Survival (% of Control)	
		<u>Without S9</u>	<u>With S9</u>	<u>Without S9</u>	<u>With S9</u>
Vehicle - DMSO (20 µl/ml)	5.0	23.2	32.3	100	100
0.01	5.0	22.8	25.8	98	80
0.03	5.0	18.4	24.0	79	74
0.10	5.0	C	C		
0.30	5.0	C	C		
1.0	5.0	C	C		
3.0	5.0	C	C		
5.0	5.0	C	C		

C - cytotoxic

a - Cells were inoculated into culture flasks approximately 24 hr prior to treatment.

b - Determined approximately 18 to 24 hr after treatment.

0 0 1 5

TABLE 2
 VINYLCHLORIDE: MUTAGENIC POTENTIAL IN THE CHO/HGPRT FORWARD MUTATION ASSAY
 DETERMINATION OF CYTOTOXICITY 24 HOURS AFTER TREATMENT
 TEST #1 - WITHOUT S9

Test Substance Concentration (mg/ml)	Cells/Plate ($\times 10^5$) ^a	Cells/Plate ($\times 10^5$) Mean	Plating Efficiency (% of Control)	Colonies/Plate ^b	Colonies/Plate Mean (S.D.)	Cloning Efficiency (% of Control)
0.01A	43.8	43.2	93	77, 110, 105, 82	91.4 (12.5)	95
0.01B	42.6			76, 91, 95, 95		
0.025A	33.8	34.2	73	77, 86, 84, 89	90.9 (20.5)	94
0.025B	34.6			76, 71, 130, 114		
0.05A ^c						
0.05B ^c						
0.075A ^c						
0.075B ^c						
0.10A ^c						
0.10B ^c						
Controls:						
Vehicle:						
DMSO A	45.5	46.6	100	84, 132, 105, 77	96.4 (17.6)	100
DMSO B	47.6			87, 91, 107, 88		
Negative: Culture Medium	45.1		97	80, 93, 97, 108	94.5 (11.6)	98
Positive: EMS (200 μ g/ml)	44.3		95	48, 55, 53, 101	64.3 (24.7)	67

a - 5 $\times 10^5$ cells/culture were plated in flasks approximately 24 hours prior to treatment.

b - 100 cells plated.

c - All cells lysed and floating 24 hours after treatment.

Abbreviations: EMS - ethylmethanesulfonate; DMSO - dimethylsulfoxide; S.D. - standard deviation.

TABLE 3

VITILIGENOMES: MUTAGENIC POTENTIAL IN THE CHO/HGPRT FORWARD MUTATION ASSAY
 PLATING EFFICIENCIES AND MUTATION FREQUENCIES DETERMINED AFTER EXPRESSION PERIOD
 TEST #1 - WITHOUT S9

Test Substance Concentration (µg/ml)	Colonies/Plate ^a	Plating Efficiency		% of Combined Vehicle Controls	Mutant Determination		Corrected Mutation Frequency ($\times 10^{-6}$) ^c
		Colonies/Plate Mean (S.D.)	Colony/Plate ^b		Mutant Colonies	Total Colonies	
0.01A	83, 84, 88, 79	83.5 (3.7)	4, 0, 1, 2, 0	94	7	8.4	
0.01B	93, 74, 129, 87	95.8 (23.5)	0, 0, 0, 0, 0	108	0	0.0	
0.025A	65, 93, 87, 88	83.3 (12.4)	0, 0, 0, 0, 0	94	0	0.0	
0.025B	81, 67, 79, 82	77.3 (6.9)	0, 0, 0, 0, 0	87	0	0.0	
0.05A	Cytotoxic						
0.05B	Cytotoxic						
0.075A	Cytotoxic						
0.075B	Cytotoxic						
0.10A	Cytotoxic						
0.10B	Cytotoxic						
Controls:							
Vehicle:							
DMSO A	102, 87, 96, 77	90.5 (10.9)	0, 1, 0, 1, 0	102	2	2.2	
DMSO B	84, 88, 90, 88	87.5 (2.5)	0, 0, 0, 0, 1	98	1	1.1	
Negative: Culture Medium	95, 113, 106, 90	101.0 (10.4)	1, 1, 0, 0, 0	113	2	2.0	
Positive: EMS (200 µg/ml)	93, 95, 117, 97	100.5 (11.1)	17, 11, 18, 14, 20	113	80	79.6	

a - 100 cells inoculated into each plate.
 b - 2×10^5 cells inoculated in each of 5 plates (1×10^6 total cells).
 c - Mutants/ 10^6 clonable cells: total number of mutant colonies divided by viable fraction.
 Abbreviation: EMS - ethylmethanesulfonate; DMSO - dimethylsulfoxide; S.D. - standard deviation.

74
80
86
92
98
04
10

TABLE 4
 VINYLCHLORIDE: MUTAGENIC POTENTIAL IN THE CHO/HGRT FORWARD MUTATION ASSAY
 DETERMINATION OF CYTOTOXICITY 24 HOURS AFTER TREATMENT
 TEST #1 - WITHOUT S9 - ADDITIONAL DOSES

Test Substance Concentration (mg/ml)	Cells/Plate (x 10 ⁵) ^a	Cells/Plate (x 10 ⁵) Mean	Plating Efficiency (% of Control)	Colonies/Plate ^b	Colonies/Plate Mean (S.D.)	Cloning Efficiency (% of Control)
0.03A	18.6	15.0	56	75, 87, 81, 76	71.5 (9.8)	76
0.03B	11.4			62, 65, 59, 67		
0.04A	7.3	12.6	47	30, 38, 45, 39	47.3 (11.6)	50
0.04B	17.9			51, 50, 53, 68		
Controls:						
Vehicle:						
DMSO A	25.8	27.0	100	98, 116, 82, 104	94.6 (11.9)	100
DMSO B	28.1			85, 92, 86, 92		

a - 5 x 10⁵ cells/culture were plated in flasks approximately 24 hours prior to treatment.
 b - 100 cells plated.

Abbreviations: DMSO - dimethylsulfoxide; S.D. - standard deviation.

TABLE 5
 VINYLCHLORIDE: MUTAGENIC POTENTIAL IN THE CHO/HGPRT FORWARD MUTATION ASSAY
 PLATING EFFICIENCIES AND MUTATION FREQUENCIES DETERMINED AFTER EXPRESSION PERIOD
 TEST #1 - WITHOUT S9 - ADDITIONAL DOSES

Test Substance Concentration (mc/ml)	Plating Efficiency		% of Combined Vehicle Controls	Mutant Determination		Corrected Mutation Frequency ($\times 10^{-6}$) ^c
	Colonies/Plate ^a	Colonies/Plate Mean (S.D.)		Colonies/Plate ^b	Total Colonies	
0.03A	89, 82, 67, 78	79.0 (9.2)	86	0, 0, 0, 0, 0	0	0.0
0.03B	79, 82, 82, 96	84.8 (7.6)	93	0, 2, 0, 0, 1	3	3.5
0.04A	72, 115, 78, 81	86.5 (19.4)	95	0, 0, 0, 1, 0	1	1.2
0.04B	76, 72, 101, 81	83.0 (12.6)	91	0, 0, 1, 0, 1	2	2.4
Controls:						
Vehicle:						
DMSO A	84, 133, 79, 108	101.0 (24.8)	110	0, 0, 0, 2, 1	3	3.0
DMSO B	70, 74, 90, 94	82.0 (11.8)	90	0, 0, 0, 1, 0	1	1.2

a - 100 cells inoculated into each plate.
 b - 2 x 10⁵ cells inoculated in each of 5 plates (1 x 10⁶ total cells).
 c - Mutants/10⁶ clonable cells: total number of mutant colonies divided by viable fraction.
 Abbreviation: DMSO - dimethylsulfoxide; S.D. - standard deviation.

TABLE 6
 VINYLCHLORIDE: MUTAGENIC POTENTIAL IN THE CHO/HGPRT FORWARD MUTATION ASSAY
 DETERMINATION OF CYTOTOXICITY 24 HOURS AFTER TREATMENT
 TEST #1 - WITH S9

Test Substance Concentration (mg/ml)	Cells/plate ($\times 10^5$) ^a	Cells/plate ($\times 10^5$) Mean	Plating Efficiency (% of Control)	Colonies/Plate ^b	Colonies/Plate Mean (S.D.)	Cloning Efficiency (% of Control)
0.005A	36.0	33.7	119	144, 84, 107, 105	103.3 (21.4)	112
0.005B	30.5			80, 95, 122, 89		
0.01A	34.5	32.5	115	87, 95, 91, 67	88.5 (15.1)	96
0.01B	30.4			67, 99, 91, 111		
0.02A	30.4	31.8	112	97, 100, 96, 84	95.1 (13.7)	104
0.02B	33.2			83, 91, 125, 85		
0.03A	28.2	29.5	104	82, 101, 76, 81	84.3 (10.6)	92
0.03B	30.7			80, 82, 100, 72		
0.04A	26.4	26.2	92	103, 101, 89, 84	74.0 (23.9)	81
0.04B	25.9			45, 65, 64, 41		
0.05A	22.9	22.0	78	79, 93, 71, 65	80.1 (13.2)	87
0.05B	21.0			64, 95, 96, 78		
Controls:						
Vehicle:						
DMSO A	26.8	28.3	100	88, 97, 87, 91	91.9 (10.7)	100
DMSO B	29.8			112, 86, 76, 98		
Negative: Culture Medium	36.2		128	73, 88, 74, 92	81.8 (9.7)	89
Positive: DMN (200 µg/ml)	32.4		114	36, 35, 37, 39	36.8 (1.7)	40

a - 5×10^5 cells/culture were plated in flasks approximately 24 hr prior to treatment.

b - 100 cells plated.

Abbreviation: DMN - dimethylnitrosamine; DMSO - dimethylsulfoxide; S.D. - standard deviation.

TABLE 7
 VINYLBORNE: MUTAGENIC POTENTIAL IN THE CHO/HGPRT FORWARD MUTATION ASSAY
 PLATING EFFICIENCIES AND MUTATION FREQUENCIES DETERMINED AFTER EXPRESSION PERIOD
 TEST #1 - WITH 89

Test Substance Concentration (µg/ml)	Plating Efficiency		% of Combined Vehicle Controls	Mutant Determination		Corrected Mutation Frequency ($\times 10^{-6}$) ^c
	Colonies/Plate ^a	Colonies/Plate Mean (S.D.)		Colonies/Plate ^b	Total Colonies	
0.005A	67, 67, 79, 76	72.3 (6.2)	74	0, 0, 0, 0, 2	2	2.8
0.005B	93, 74, 81, 87	83.3 (8.1)	86	0, 0, 0, 0, 0	0	0.0
0.01A	93, 67, 85, 88	83.3 (11.3)	85	2, 3, 2, 2, 1	10	12.0
0.01B	73, 92, 102, 71	84.5 (15.0)	86	0, 0, 0, 0, 0	0	0.0
0.02A	95, 142, 95, 114	111.5 (22.2)	114	1, 1, 0, 0, 0	2	1.8
0.02B	108, 98, 136, 109	112.8 (16.3)	115	0, 0, 0, 0, 1	1	0.9
0.03A	88, 74, 81, 82	81.3 (5.7)	83	2, 1, 2, 0, 0	5	6.2
0.03B	89, 87, 100, 102	94.5 (7.6)	97	2, 0, 0, 0, 1	3	3.2
0.04A	83, 94, 89, 98	88.5 (6.9)	91	2, 0, 0, 0, 0	2	2.3
0.04B	100, 81, 109, 104	98.5 (12.2)	101	1, 1, 0, 0, 1	3	3.0
0.05A	88, 78, 79, 54	74.8 (14.5)	76	0, 0, 0, 0, 1	1	1.3
0.05B	87, 88, 109, 88	93.0 (10.7)	95	2, 2, 6, 1, 0	11	11.8
Controls:						
Vehicle:						
DMSO A	101, 87, 75, 112	93.8 (16.2)	96	2, 1, 0, 1, 1	5	5.3
DMSO B	80, 97, 128, 102	101.8 (19.9)	104	0, 0, 0, 0, 0	0	0.0
Negative:						
Culture Medium	99, 93, 102, 82	94.0 (8.8)	96	0, 0, 0, 0, 0	0	0.0
Positive:						
DMS (200 µg/ml)	52, 68, 64, 106	72.5 (23.3)	74	9, 14, 18, 15, 18	74	102.1

a - 100 cells inoculated into each plate.
 b - 2 x 10⁵ cells inoculated in each of 5 plates, (1 x 10⁶ total cells).
 c - Mutants/10⁶ clonable cells: total number of mutant colonies divided by viable fraction.
 Abbreviation: DMS - dimethylnitrosamine; DMSO - dimethylsulfoxide; S.D. - standard deviation.

TABLE 8
 VINYLCHLORIDE: MUTAGENIC POTENTIAL IN THE CHO/HGPRT FORWARD MUTATION ASSAY
 DETERMINATION OF CYTOTOXICITY 24 HOURS AFTER TREATMENT
 TEST #2 - WITHOUT S9

Test Substance Concentration (mg/ml)	Cells/plate (x 10 ⁵) ^a	Cells/plate (x 10 ⁵) Mean	Plating Efficiency (% of Control)	Colonies/Plate ^b	Colonies/Plate Mean (S.D.)	Cloning Efficiency (% of Control)
0.01A	29.1	33.0	85	114, 92, 220, 156	117.1 (48.3)	80
0.01B	36.6			101, 91, 75, 88		
0.02A	36.8	35.3	91	94, 98, 105, 210	117.8 (38.8)	81
0.02B	33.8			108, 99, 129, 99		
0.03A	36.3	36.6	94	149, 89, 95, 78	102.6 (22.5)	70
0.03B	36.8			106, 86, 100, 118		
0.04A	32.3	28.9	74	84, 108, 86, 80	93.8 (12.7)	64
0.04B	25.5			89, 116, 88, 99		
0.05A	23.0	15.1	39	78, 84, 96, 87	63.9 (24.8)	44
0.05B	7.2			42, 35, 50, 39		
Controls:						
Vehicle:						
DMSO A	40.0	38.9	100	129, 144, 170, 244	145.9 (45.5)	100
DMSO B	37.7			132, 141, 113, 94		
Negative: Culture Medium	43.0		111	98, 96, 134, 86	103.5 (21.0)	71
Positive: EMS (200 µg/ml)	42.6		110	88, 89, 99, 159	108.8 (33.9)	75

a - 5 x 10⁵ cells/culture were plated in flasks approximately 24 hours prior to treatment.

b - 100 cells plated.

Abbreviation: EMS - ethylmethanesulfonate; DMSO - dimethylsulfoxide; S.D. - standard deviation.

79
80
81
82
83
84
85

TABLE 9
 VITIMORBONUMS: MUTAGENIC POTENTIAL IN THE CHO/HGRT FORWARD MUTATION ASSAY
 PLATING EFFICIENCIES AND MUTATION FREQUENCIES DETERMINED AFTER EXPRESSION PERIOD
 TEST #2 - WITHOUT 89

Test Substance Concentration (mg/ml)	Plating Efficiency			Mutant Determination		
	Colonies/Plate ^a	Colonies/Plate Mean (S.D.)	% of Combined Vehicle Controls	Mutant Colonies		Corrected Mutation Frequency (x 10 ⁻⁶) ^c
				Colonies/Plated	Total Colonies	
0.01A	89, 87, 97, 95	92.0 (4.8)	94	2, 2, 3, 3, 2	12	13.0
0.01B	70, 116, 167, 93	111.5 (41.5)	114	3, 7, 3, 3, 4	20	17.9
0.02A	106, 120, 106, 77	102.3 (18.1)	105	2, 3, 3, 3, 3	14	13.7
0.02B	92, 92, 89, 123	99.0 (16.1)	102	2, 0, 1, 1, 0	4	4.0
0.03A	122, 148, 126, 119	128.8 (13.1)	132	3, 1, 2, 1, 0	7	5.4
0.03B	109, 85, 93, 97	96.0 (10.0)	98	0, 1, 1, 1, 1	4	4.2
0.04A	d			2, 1, 4, 1, 0	8	4.8
0.04B	102, 91, 104, 120	104.3 (12.0)	107	0, 0, 1, 1, 3	5	4.8
0.05A	131, 86, 88, 104	102.3 (20.8)	105	0, 3, 4, 0, 2	9	8.8
0.05B	79, 140, 105, 104	107.0 (25.1)	110	1, 0, 0, 1, 1	3	2.8
Controls:						
Vehicle:						
DMSO A	98, 70, 92, 95	88.8 (12.7)	91	2, 2, 2, 0, 1	7	7.9
DMSO B	121, 119, 95, 90	106.3 (16.0)	109	4, 3, 1, 2, 4	14	13.2
Negative:						
Culture Medium	87, 83, 93, 78	85.3 (6.3)	87	1, 2, 2, 3, 4	12	14.1
Positive:						
EMS (200 µg/ml)	83, 92, 74, 99	87.0 (10.9)	89	11, 32, 33, 30, 33	139	159.8

a - 100 cells inoculated into each plate.
 b - 2 x 10⁵ cells inoculated in each of 5 plates (1 x 10⁶ total cells).
 c - Mutants/10⁶ cloneable cells: total number of mutant colonies divided by viable fraction.
 d - Cloning efficiency plate missing, corrected mutation frequency can not be calculated.
 Abbreviation: EMS - ethylmethanesulfonate; DMSO - dimethylsulfoxide; S.D. - standard deviation.

TABLE 10
 VINYLCHLORIDE: MUTAGENIC POTENTIAL IN THE CHO/HGPRT FORWARD MUTATION ASSAY
 DETERMINATION OF CYTOTOXICITY 24 HOURS AFTER TREATMENT
 TEST #2 - WITH S9

Test Substance Concentration (mg/ml)	Cells/Plate (x 10 ⁵) ^a	Cells/Plate (x 10 ⁵) Mean	Plating Efficiency (% of Control)	Colonies/Plate ^b	Colonies/Plate Mean (S.D.)	Cloning Efficiency (% of Control)
0.005A	47.6	48.8	105	96, 99, 90, 97	90.6 (6.0)	94
0.005B	50.0			89, 83, 85, 86		
0.01A	39.8	41.4	89	80, 93, 97, 56	86.4 (14.0)	90
0.01B	43.0			87, 85, 92, 101		
0.03A	40.6	40.6	87	109, 111, 85, 94	94.5 (14.4)	98
0.03B	40.6			82, 103, 70, 102		
0.05A	18.0	20.9	45	114, 72, 60, 67	78.3 (16.0)	81
0.05B	23.8			80, 78, 79, 76		
0.10A ^c						
0.10B ^c						
Controls:						
Vehicle:						
DMSO A	47.6	46.6	100	84, 99, 84, 91	96.4 (10.0)	100
DMSO B	45.5			93, 105, 111, 104		
Negative: Culture Medium	45.1		97	99, 110, 88, 108	101.3 (10.0)	105
Positive: DMN (200 µg/ml)	43.0		92	39, 41, 29, 36	36.3 (5.3)	38

a - 5 x 10⁵ cells/culture were plated in flasks approximately 24 hr prior to treatment.

b - 100 cells plated.

c - All cells lysed and floating 24 hours after treatment.

Abbreviation: DMN - dimethylnitrosamine; DMSO - dimethylsulfoxide; S.D. - standard deviation.

TABLE 11
 VINYLCHLORIDE: MUTAGENIC POTENTIAL IN THE CHO/HGPRT FORWARD MUTATION ASSAY
 PLATING EFFICIENCIES AND MUTATION FREQUENCIES DETERMINED AFTER EXPRESSION PERIOD
 TEST #2 - WITH S9

Test Substance Concentration (mg/ml)	Plating Efficiency			Mutant Determination			Corrected Mutation Frequency (x 10 ⁻⁶) ^c
	Colonies/Plate ^a	Colonies/Plate Mean (S.D.)	% of Combined Vehicle Controls	Colonies/Plate ^b	Total Colonies		
0.005A	72, 85, 80, 84	80.3 (5.9)	75	2, 2, 2, 1, 1	8		10.0
0.005B	113, 104, 188, 138	135.8 (37.7)	127	0, 1, 0, 0, 0	1		0.7
0.01A	82, 130, 92, 79	95.8 (23.5)	89	0, 0, 0, 0, 0	0		0.0
0.01B	97, 75, 121, 90	95.8 (19.2)	89	0, 2, 0, 0, 0	2		3.1
0.03A	107, 117, 121, 107	113.0 (7.1)	106	0, 0, 0, 1, 0	1		0.9
0.03B	94, 95, 138, 93	105.0 (22.0)	98	1, 2, 0, 3, 2	8		7.5
0.05A	78, 97, 94, 88	89.3 (8.4)	83	3, 0, 0, 1, 2	7		7.8
0.05B	116, 109, 113, 109	111.8 (3.4)	104	0, 0, 0, 0, 0	0		0.0
0.10A	Cytotoxic						
0.10B	Cytotoxic						
Controls: Vehicle:							
DMSO A	78, 88, 112, 89	91.8 (14.4)	86	0, 0, 0, 0, 0	0		0.0
DMSO B	115, 156, 121, 97	122.3 (24.7)	114	0, 1, 1, 0, 0	2		1.6
Negative: Culture Medium ^d	131, 127, 197, 131	146.5 (33.7)	137	1, 1, 1, 1, 0	4		2.7
Positive: DMEM (200 µg/ml)	50, 66, 50, 55	55.3 (7.5)	52	23, 17, 19, 14, 24	97		175.6

a - 100 cells inoculated into each plate.
 b - 2 x 10⁵ cells inoculated in each of 5 plates, (1 x 10⁶ total cells).
 c - Mutants/10⁶ clonable cells: total number of mutant colonies divided by viable fraction.
 d - 150 cells were inoculated into each plate for plating efficiency and 1.58 x 10⁶ total cells were inoculated for mutant determination.
 Abbreviation: DMEM - dimethylmitrosamine; DMSO - dimethylsulfoxide; S.D. - standard deviation.

TABLE 12
 VINYLCHLORIDE: MUTAGENIC POTENTIAL IN THE CHO/HGPRT FORWARD MUTATION ASSAY
 DETERMINATION OF CITOTOXICITY 24 HOURS AFTER TREATMENT
 TEST #3 - WITHOUT S9

Test Substance Concentration (mg/ml)	Cells/plate (x 10 ⁵) ^a	Cells/plate (x 10 ⁵) Mean	Plating Efficiency (% of Control)	Colonies/Plate ^b	Colonies/Plate Mean (S.D.)	Cloning Efficiency (% of Control)
0.01A	31.6	34.6	91	107, 111, 126, 104	102.9 (12.4)	126
0.01B	37.6			96, 89, 102, 88		
0.03A	37.6	38.5	101	102, 128, 74, 94	97.5 (16.7)	119
0.03B	39.4			108, 80, 94, 100		
0.04A	37.4	33.2	87	111, 114, 166, 120	133.3 (19.7)	163
0.04B	28.9			134, 143, 124, 154		
0.05A	4.3	5.0	13	42, 39, 49, 41	42.6 (4.1)	52
0.05B	5.6			41, 39, 49, 41		
0.05AC						
0.05BC						
Controls:						
Vehicle:						
DMSO A	37.9	38.1	100	82, 79, 83, 71	81.6 (10.1)	100
DMSO B	38.2			69, 91, 78, 100		
Negative: Culture Medium	22.2		58	83, 93, 82, 82	85.0 (5.4)	104
Positive: EMS (200 µg/ml)	33.8		89	69, 73, 82, 53	69.3 (12.1)	85

a - 5 x 10⁵ cells/culture were plated in flasks approximately 24 hours prior to treatment.
 b - 100 cells plated.
 c - All cells lysed and floating 4 hours after treatment.
 Abbreviation: EMS - ethylmethanesulfonate; DMSO - dimethylsulfoxide; S.D. - standard deviation.

TABLE 13
 VINYLCHLORIDE: MUTAGENIC POTENTIAL IN THE CHO/HGPRT FORWARD MUTATION ASSAY
 PLATING EFFICIENCIES AND MUTATION FREQUENCIES DETERMINED AFTER EXPRESSION PERIOD
 TEST #3 - WITHOUT S9

Test Substance Concentration (ug/ml)	Plating Efficiency			Mutant Determination		
	Colonies/Plate ^a	Colonies/Plate Mean (S.D.)	% of Vehicle Controls	Colonies/Plate ^b	Mutant Colonies Total	Corrected Mutation Frequency (x 10 ⁻⁶) ^c
0.01A	74, 86, 82, 81	80.8 (5.0)	101	0, 1, 1, 7, 1	10	12.4
0.01B	84, 78, 84, 99	86.3 (9.0)	108	1, 0, 0, 0, 0	1	1.2
0.03A	83, 108, 100, 87	97.0 (10.4)	121	1, 1, 0, 1, 0	3	3.1
0.03B	88, 107, 105, 94	98.5 (9.0)	123	1, 2, 2, 2, 2	9	9.1
0.04A	75, 73, 66, 82	74.0 (6.6)	93	0, 1, 0, 2, 1	4	5.4 *
0.04B	74, 78, 103, 87	85.5 (12.9)	107	3, 1, 0, 0, 0	4	4.7 *
0.05A	104, 99, 105, 133	110.3 (15.4)	138	0, 3, 2, 3, 2	10	9.1
0.05B	89, 100, 81, 99	92.3 (9.0)	115	0, 0, 0, 0, 0	0	0.0
0.06A	Cytotoxic					
0.06B	Cytotoxic					
Controls:						
Vehicle:						
DMSO A	70, 82, 78, 67	74.3 (6.9)	93	0, 0, 0, 1, 1	2	2.7
DMSO B	83, 96, 79, 85	85.8 (7.3)	107	2, 0, 0, 1, 0	3	3.5
Negative: Culture Medium	70, 63, 98, 70	75.3 (15.5)	94	2, 1, 3, 1, 3	10	13.3
Positive: EMS (200 ug/ml)	80, 87, 101, 102	92.5 (10.8)	116	52, 51, 49, 35, 42	229	247.6

a - 100 cells inoculated into each plate.
 b - 2 x 10⁵ cells inoculated in each of 5 plates (1 x 10⁶ total cells).
 c - Mutants/10⁵ cloneable cells; total number of mutant colonies divided by viable fraction.
 * significantly different from control group (p < 0.05).
 Abbreviation: EMS - ethylmethanesulfonate; DMSO - dimethylsulfoxide; S.D. - standard deviation.

TABLE 14
 VINYLCHLORIDE: MUTAGENIC POTENTIAL IN THE CHO/HGERT FORWARD MUTATION ASSAY
 DETERMINATION OF CYTOTOXICITY 24 HOURS AFTER TREATMENT
 TEST #3 - WITH S9

Test Substance Concentration (mg/ml)	Cells/plate ($\times 10^5$) ^a	Cells/Plate ($\times 10^5$) Mean	Plating Efficiency (% of Control)	Colonies/Plate ^b	Colonies/Plate Mean (S.D.)	Cloning Efficiency (% of Control)
0.01A	31.0	31.9	96	74, 94, 88, 95	90.1 (7.8)	93
0.01B	32.8			91, 98, 85, 96		
0.05A	5.2	10.0	30	25, 33, 39, 27	53.4 (24.3)	55
0.05B	14.9			73, 78, 78, 74		
0.06A	1.5	1.8	5	36, 56, 31, 43	50.0 (20.8)	52
0.06B	2.0			39, 54, 44, 97		
0.07A	0.5	0.8	2	20, 21, 26, 29	32.5 (9.6)	34
0.07B	1.0			44, 40, 40, 40		
0.08A ^c						
0.08B ^c						
Controls:						
Vehicle:						
DMSO A	31.8	33.2	100	91, 85, 93, 97	96.6 (10.8)	100
DMSO B	34.5			91, 93, 120, 103		
Negative: Culture Medium	32.9		99	69, 86, 99, 77	82.8 (12.9)	86
Positive: DMN (100 µg/ml)	34.6		104	23, 29, 34, 35	30.3 (3.5)	31

a - 5 x 10⁵ cells/culture were plated in flasks approximately 24 hr prior to treatment.

b - 100 cells plated.

c - All cells lysed and floating 24 hours after treatment.

Abbreviation: DMN - dimethylnitrosamine; DMSO - dimethylsulfoxide; S.D. - standard deviation.

TABLE 15
 VINYLCHLORIDE: MUTAGENIC POTENTIAL IN THE CHO/HGPRT FORWARD MUTATION ASSAY
 PLATING EFFICIENCIES AND MUTATION FREQUENCIES DETERMINED AFTER EXPRESSION PERIOD
 TEST #3 - WITH 89

Test Substance Concentration (µg/ml)	Plating Efficiency		% of Combined Vehicle Controls	Mutant Determination		Corrected Mutation Frequency (x 10 ⁻⁶) ^c
	Colonies/Plate ^a	Colonies/Plate Mean (S.D.)		Colonies/Plate ^b	Total Colonies	
0.01A	102, 109, 81, 90	95.5 (12.4)	98	1, 1, 1, 0, 0	3	3.1
0.01B	135, 110, 199, 92	134.0 (46.8)	138	0, 0, 0, 0, 0	0	0.0
0.05A	84, 96, 94, 72	86.5 (11.0)	89	0, 0, 0, 0, 1	1	1.2
0.05B	69, 72, 66, 72	69.8 (2.9)	72	0, 0, 0, 0, 0	0	0.0
0.06A	106, 103, 82, 86	94.3 (12.0)	97	0, 0, 0, 0, 0	0	0.0
0.06B	85, 76, 92, 113	91.5 (15.8)	94	0, 0, 0, 0, 0	0	0.0
0.07A	85, 89, 109, 101	96.0 (11.0)	99	0, 0, 0, 0, 0	0	0.0
0.07B	76, 100, 105, 79	90.0 (14.6)	93	0, 0, 0, 0, 0	0	0.0
0.08A	Cytotoxic					
0.08B	Cytotoxic					
Controls: Vehicle:						
DMSO A	96, 120, 84, 103	102.8 (18.6)	106	0, 0, 0, 0, 0	0	0.0
DMSO B	92, 83, 120, 64	91.8 (26.9)	94	1, 2, 0, 0, 0	3	3.3
Negative: Culture Medium	89, 104, 157, 58	102.0 (41.4)	105	0, 0, 0, 0, 0	0	0.0
Positive: DMN (200 µg/ml)	95, 76, 78, 74	80.8 (9.6)	83	6, 10, 18, 9, 10	52	64.4

a - 100 cells inoculated into each plate.
 b - 2 x 10⁵ cells inoculated in each of 5 plates, (1 x 10⁶ total cells).
 c - Mutants/10⁶ clonable cells: total number of mutant colonies divided by viable fraction.
 Abbreviation: DMN - dimethylnitrosamine; DMSO - dimethylsulfoxide; S.D. - standard deviation.

Vinylornbornene: Mutagenic Potential in the CHO/HGPRT Forward Mutation Assay

QUALITY ASSURANCE UNIT INSPECTION SUMMARY

<u>Inspection Date(s)</u>	<u>Inspection Type</u>	<u>Date QAU Report Issued To</u>	
		<u>Study Director</u>	<u>Management</u>
03-07-94 to 03-08-94	PROTOCOL	03-09-94	03-15-94
04-06-94	EVENT-SUBCULTURE	04-06-94	04-06-94
06-30-94	PROTOCOL AMENDMENT #1	06-30-94	06-30-94
07-26-94 to 08-03-94	RAW DATA, REPORT	08-04-94	10-17-94
10-14-94	ARCHIVES	10-14-94	10-17-94

James H. Coleman
 James H. Coleman
 Representative, Quality Assurance Unit

10-17-94
 Date