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UNION CARBIDE CHEMICALS AND PLASTICS COMPANY INC.
HEALTH, SAFETY AND ENVIRONMENTAL AFFAIRS

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91 NOV -5 AM 8:38

Attn: Section 8(e) Coordinator (CAP Agreement)

Re: CAP Agreement Identification No. 8ECAP-0110

Dear Sir or Madam:

Union Carbide Corporation ("Union Carbide") herewith submits the following report pursuant to the terms of the TSCA §8(e) Compliance Audit Program and Union Carbide's CAP Agreement dated August 14, 1991 (8ECAP-0110). This report describes an *in vitro* SCE genotoxicity study with tetraethylenepentamine (CASRN 112-57-2).

"Tetraethylenepentamine - Sample A: In Vitro Genotoxicity Studies: sister Chromatid Exchange Assay", Bushy Run Research Center, Project Report 50-76, October 15, 1987.

A complete summary of this report is attached.

Previous TSCA Section 8(e) or "FYI" Submission(s) related to this substance are:

(none)

Previous PMN submissions related to this substance are: (none)

tetraeth

0 0 0 3

This information is submitted in light of EPA's current guidance. Union Carbide does not necessarily agree that this information reasonably supports the conclusion that the subject chemical presents a substantial risk of injury to health or the environment.

In the attached report the term "BUSINESS CONFIDENTIAL" is entered on the first page. This precautionary statement was for internal use at the time of issuance of the report. Confidentiality is hereby waived for 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.

Yours truly,



William C. Kuryla, Ph.D.
Assistant Director
Product Safety
(203/794-5230)

WCK/cr

Attachment (3 copies of cover letter, summary, and report)

SUMMARY

50-46

**Tetraethylenepentamine - Sample A
In Vitro Genotoxicity Studies:
Sister Chromatid Exchange Assay**

**Sponsor: Union Carbide Corporation
Industrial Chemicals Division**

* * * * *

SUMMARY

Tetraethylenepentamine - Sample A was evaluated for potential genotoxic activity using the Sister Chromatid Exchange (SCE) test in Chinese hamster ovary (CHO) cells in vitro. The results indicated that the test chemical produced a dose-related, statistically significant genotoxic effect in tests conducted both with and without addition of a rat-liver S9 metabolic activation system. The quantitative SCE increases were approximately the same in the tests with and without metabolic activation, indicating that the test material was active by direct mechanisms and did not require metabolic conversion.

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Project Report 50-76
14 Pages
October 15, 1987

Tetraethylenepentamine - Sample A
In Vitro Genotoxicity Studies:
Sister Chromatid Exchange Assay

Sponsor: Union Carbide Corporation
Industrial Chemicals Division

SUMMARY

Tetraethylenepentamine - Sample A was evaluated for potential genotoxic activity using the Sister Chromatid Exchange (SCE) test in Chinese hamster ovary (CHO) cells *in vitro*. The results indicated that the test chemical produced a dose-related, statistically significant genotoxic effect in tests conducted both with and without addition of a rat-liver S9 metabolic activation system. The quantitative SCE increases were approximately the same in the tests with and without metabolic activation, indicating that the test material was active by direct mechanisms and did not require metabolic conversion.

RESULTS AND INTERPRETATION

Selection of Test Concentrations - Preliminary experiments were performed with CHO cells to determine an appropriate range of test concentrations in which the highest concentration would produce moderate cytotoxicity but still allow sufficient numbers of cells in the second division (M2) for determination of SCEs. Test results with tetraethylenepentamine - sample A indicated that concentrations of 1.0 mg/ml or higher were excessively cytotoxic to CHO cells in the test without S9 activation and the treated cells were detached from the culture flask. For the SCE test without S9 activation, a maximum concentration of 0.8 mg/ml was tested. With S9 activation, a slightly lower degree of growth inhibition was noted and a maximum dose of 3.0 mg/ml was used for the definitive SCE test. In the SCE test with S9 activation, the 2.0 and 3.0 mg/ml doses produced excessive mitotic inhibition and too few cells were available for SCE scoring.

SCE Test- Tetraethylenepentamine - Sample A produced dose-related and statistically significant increases in SCEs in tests both with and without addition of a rat-liver S9 metabolic activation system. The highest increases in SCEs above control values were approximately 1.8 fold without S9 and 1.6 fold with S9 activation. The test chemical was considered to be a positive genotoxic agent in the SCE test system.

SAMPLE

Sample Name: Tetraethylenepentamine - Sample A

Quantity: 20 ml

BRRC Sample No.: 49-423

Submitted by: D. J. Schreck

Date Received: November 21, 1986

Division: Industrial Chemicals
South Charleston, WV

Identification: Ref. #36-ARD-31-7
viscous, light
yellow-brown liquid

CAS #: 112-57-2

Tetraethylenepentamine - Sample A
In Vitro Mutagenesis Studies

Sponsor: Union Carbide Corporation

OBJECTIVE

The purpose of this study was to determine whether tetraethylenepentamine - sample A was active in producing genetic damage (genotoxicity) in mammalian cells. The Sister Chromatid Exchange (SCE) test was used to evaluate the test chemical for potential genotoxic activity. A general description of the theoretical basis of this test is presented in Appendix 1 attached to the complete report.

SAMPLE CHARACTERISTICS

A sample of tetraethylenepentamine - sample A was received for testing on November 21, 1986 and the sample was assigned BRRC sample number 49-423. The physical and chemical data provided by the Sponsor for this chemical are attached to this report as Appendix 2.

Briefly, tetraethylenepentamine - sample A was described to have a density of 0.994, pH of 12 and a boiling point of 318°C. The test material was listed as completely soluble in water and stable for years (Appendix 2).

The test sample consisted of 89.735% pentamines with the following components:

38.522% AE-TAEA (aminoethyl-trisaminoethylamine)
33.701% L-TEPA (linear tetraethylenepentamine)
15.822% AE-DAEP (2-aminoethyl-di[2-amino-ethyl]piperazine
1.690% AE-PEEDA (aminoethyl-piperazinoethyl-ethylenediamine)

METHODS

A general description of the technical procedures used in the SCE test is presented in greater detail in Appendix 1. Testing was performed in compliance with Standard Operating Procedures used for these tests at the Bushy Run Research Center and deviations from procedures are noted in the individual test results.

A. SCE Test

(Detailed procedures in Appendix 1): SOP #7.2.12E was used for this study.

1. Selection of a suitable range of doses for testing was based upon cytotoxicity data obtained from preliminary experiments to determine relative cytotoxicity of the test chemical.

2. Production of SCEs following exposure to various concentrations of tetraethylenepentamine - sample A was studied with duplicate cultures of CHO cells tested both with and without the incorporation of a rat-liver S9 metabolic activation system. Various concentrations of tetraethylenepentamine - sample A for testing were attained by direct addition of various aliquots of the diluted or undiluted test agent into the culture medium. Cell-culture medium was used as the solvent. All dilutions were prepared immediately prior to testing.

For determination of direct mutagenic action, CHO cells were exposed to tetraethylenepentamine - sample A and appropriate controls for 5 hours without S9 activation. Indirect mutagenic action, requiring metabolic activation by liver S9 homogenate, was studied with a 2-hour exposure period. Bromodeoxyuridine (BrdU), required to differentiate between the individual "sister" chromatids by SCE staining, was present at a concentration of 3 µg/ml in the growth medium during treatment and during the culture period following exposure. A total of twenty-five cells/concentration was examined for SCE frequencies using duplicate cultures. At least 5 dose levels were tested both with and without metabolic activation. SCE production was determined for the highest 3 doses which did not produce excessive cytotoxic inhibition of cell division. The number of SCEs/cell, mean number of SCEs/chromosome and the level of statistical significance of the increases above the concurrent solvent control values are presented in tabular form. The percentages of cells at various mitotic stages of cell division were monitored and recorded for indicating comparative cytotoxic effects.

B. Control Agents

Positive, negative and solvent control materials were tested concurrently with the test sample to assure both the sensitivity of the test system and the concurrence of the results to historical test performance at BRRG. For the SCE assays, dimethylnitrosamine (DMN)-CAS #42-75-9 and ethylmethane-sulfonate (EMS)-CAS #62-50-0 were used as positive control agents to assure the sensitivity of the test system for detecting metabolic activation dependent and independent mutagens, respectively. Cell culture medium was used as the negative control and as the solvent for dilutions of the test chemical.

C. Metabolic Activation

S9 liver homogenate, prepared from Aroclor 1254-induced, Sprague-Dawley male rats, was purchased from Hazleton Biotechnologies (formerly Litton Bionetics), Kensington, MD. The S9 homogenate contained 40 mg/ml protein and had a benzo[a]pyrene-hydroxylase activity of 15 nmol hydroxybenzpyrene/20 min/mg protein (assayed by Litton); a concentration of 600 µg of S9 protein was added per 5 ml of culture medium.

Cofactor concentrations in the complete S9 metabolic activation mixture are described in detail in Appendix 1. Typically 1.0 ml of the complete metabolic activation system (S9 homogenate plus cofactors) was added per each 4.0 ml of culture medium.

D. Statistical Analyses

The specific test used to obtain the level of statistical significance is indicated with a footnote on the table. Data from the SCE test do not follow a normal distribution according to experience with historical controls. Thus, the data were analyzed after transformation of the SCE values according to the conversion method of Box and Cox (1964). For SCE data, statistical analyses of historical data at BRRC indicate that an exponent of 0.15 is the appropriate value for transformation of SCE values (BRRC Intramural Report 46-64).

Rounding of data to either two decimal places or to the appropriate number of significant figures was performed for presentation on tables. Although statistically significant decreases in mutation indices can occur because of cytotoxic responses, only statistically significant increases in responses above control values are indicated on tables for simplicity. All statistical tests were performed to determine whether the treatment with the test agent produced a response statistically different from the value(s) obtained with the concurrent solvent control. The degree of statistical significance at BRRC is denoted by the letters: a: $0.05 > p > 0.01$, b: $0.01 > p > 0.001$, or c: $p < 0.001$. No superscript (or NS) indicates $p > 0.05$.

E. Data Storage

Copies of the final report, statistical analyses, analytical data, slides, and data used to prepare the final report will be stored in the BRRC Archives. A reserve sample of the test chemical will be saved for at least one year after completion of testing unless limited by sample stability or when directed otherwise by the sponsor.

F. Historical Control Data Summary

Evaluation of results from *in vitro* mutagenicity tests must take into consideration a comparison of both concurrent and historical control data for interpretation of the biological significance of the results. Each test system has been found to vary both within and between laboratories and evaluations only against concurrent controls may be misleading. The range of variability of negative control data for the tests at BRRC, is provided for comparative purposes. Because the data from many systems do not follow a normal distribution, the mean and median are both given as well as the 95 percentile range for typical test variability.

1. SCE test

Negative controls: n = 30
mean = 0.509 SCEs/chromosome
S.D. = 0.087
median = 0.498 SCEs/chromosome
95 percentile range = 0.227 to 0.666
SCEs/chromosome

G. Cell Culture History - CHO Cells

CHO-K1-B24 (Subclone D1) cells were obtained from Dr. Abraham Hsieh, Oak Ridge National Laboratory. Cells were passed once after receipt and stored in liquid nitrogen until used to prepare larger batches of stock cultures which were also stored in liquid nitrogen. Stock cultures were prepared from cells thawed at approximately 1- to 2-month intervals for use in studies. Cells used for the SCE test were passage number 3 for tests with and without S9 activation.

RESULTS

SECTION I - Tetraethylenepentamine - Sample A

A. Test Dates: Cell-culture intervals

	<u>Cytotoxicity</u>	<u>SCE Test</u>
Initiated:	January 19, 1987	February 3, 1987
Completed:	January 21, 1987	February 6, 1987

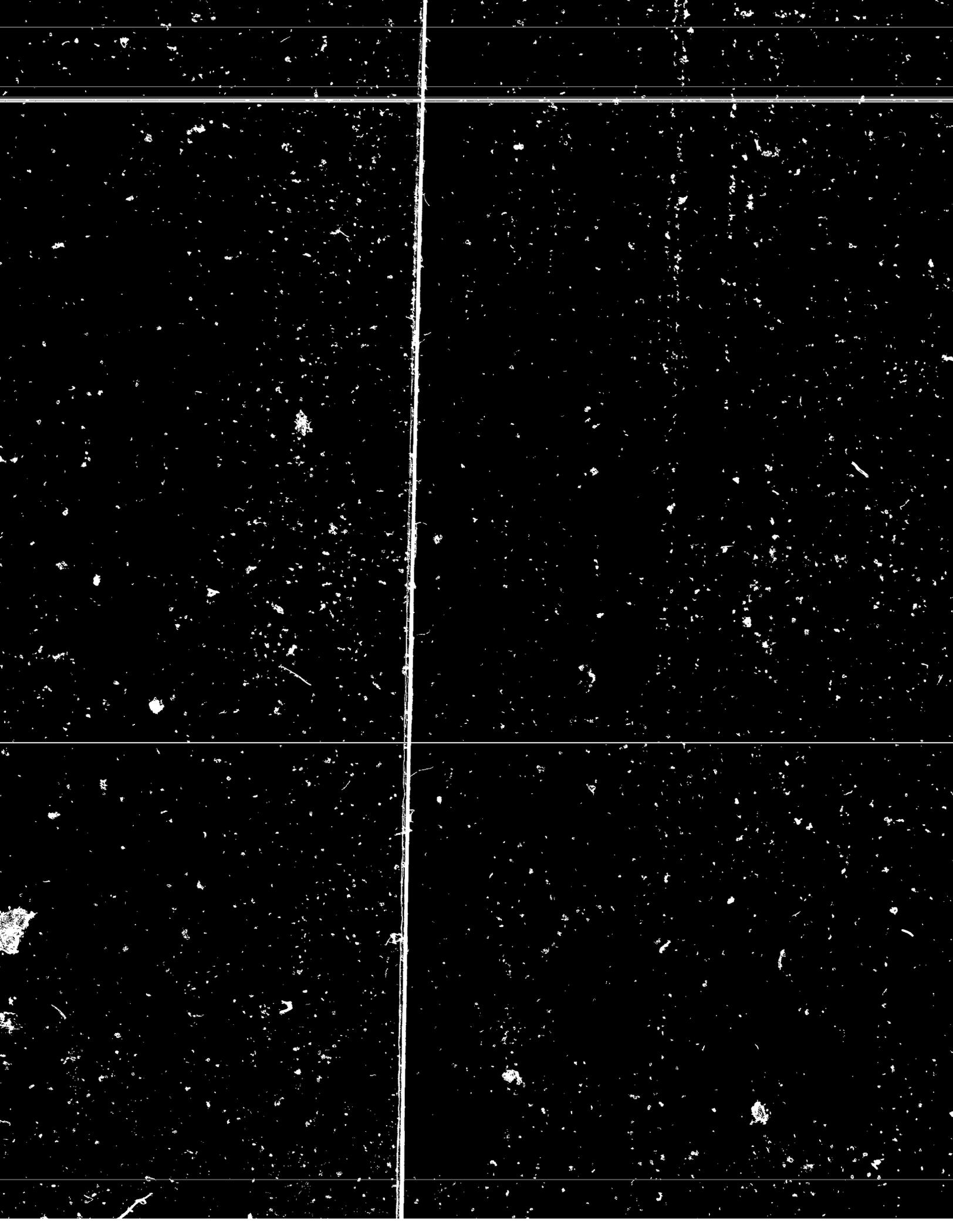
B. Selection of Test Concentrations

Concentrations of 3.0 and 0.8 mg/ml were tested as the highest doses with and without S9 activation, respectively, based on cytotoxicity data from preliminary experiments. Higher concentrations were expected to produce delays in the mitotic cycle and to decrease the number of cells with SCE staining. In preliminary cytotoxicity tests (Table 1), a concentration of 3.0 mg/ml produced approximately 65% inhibition of growth when tested with an S9 metabolic activation system. The same dose tested without metabolic activation produced death of the cell culture evident by detachment of the cell monolayer. A concentration of 1.0 mg/ml produced a similar high degree of cytotoxicity and a maximum dose of 0.8 mg/ml was used for the definitive test without S9 activation.

C. Determinations of Effects upon SCEs

1. The data for SCE production in CHO cells treated with various dose levels of tetraethylenepentamine - sample A or with positive, negative or solvent control agents without an S9 metabolic activation system are summarized in Table 2. Statistically significant increases in the numbers of SCEs were observed with all of the doses of the test agent evaluated for SCE induction. The increases followed a dose-related trend and the 0.8 mg/ml high dose produced approximately a 1.8-fold increase above control values. The data showed that tetraethylenepentamine - sample A was a direct-acting positive genotoxic agent in this *in vitro* test system.

The number of SCEs produced by the concurrent EMS positive control was highly statistically different from the values for the concurrent solvent controls. These data indicated an appropriate sensitivity of the test system comparable to our historical positive control data. The number of SCEs obtained with the solvent and medium controls were also in an acceptable range of values included in the variability encountered in our historical control values for this test.



2. SCE values obtained following treatments of CHO cells with tetraethylenepentamine - sample A in the presence of an S9 metabolic activation system are presented in Table 3. Statistically significant increases in SCEs were produced by all of the doses of the test agent evaluated. The quantitative increases in SCEs showed a similar dose-related trend evident in the test without S9 activation, and SCE values attained approximately a 1.6-fold increase over concurrent controls. Tetraethylenepentamine - Sample A was considered a positive genotoxic agent in the test with S9 activation.

The SCE values for the negative and solvent controls in the test with S9 activation were in an acceptable range of variability as encountered in previous experiments with this test system. Highly statistically significant numbers of SCEs were produced by the DMN positive control which indicated that the metabolic activation system was suitably active.

3. Table 4 presents a summary of the proportion of cells at various mitotic stages of cell division following exposures to tetraethylenepentamine - sample A. These data are often helpful to determine the extent of biological effects of the test agent or to identify doses which cause severe mitotic inhibition. Observation of increases in the numbers of first division cells would indicate cytotoxic inhibition of the cell progression of the cell population.

In the data from the test with S9, the proportion of 1st division cells increased commensurate with increasing test concentration. The 1.0 mg/ml dose produced approximately a 14% average decrease in the number of cells which completed two rounds of cell division in comparison to the control culture. In the test without S9 activation, no remarkable increases in 1st division cells were evident. The data from these two tests demonstrate that the doses evaluated for SCEs were in a biologically effective range of concentrations, but did not produce excessive cytotoxicity.

D. Deviations from Standard Operating Procedures (SOP): None.

E. Conclusions

Tetraethylenepentamine - Sample A produced dose-related and statistically significant increases in the incidence of SCEs in the CHO cells exposed both in the presence and absence of an S9 metabolic activation system. Tetraethylenepentamine - Sample A was considered to produce a positive genotoxic effect in this in vitro screening test.

Reviewed and Approved by:

Ronald S. Slesinski 5/17/88

Ronald S. Slesinski, Ph.D., DABT Date
Study Director
Manager, Genetic Toxicology

F. R. Frank 12/15/87

Fred R. Frank, Ph.D. Date
Director

Contributors:

Sister Chromatic Exchange Test

Mileen R. Morabit, P S
Master Technologist

Statistical Analyses of Data

Pege J. Guzzie, M.S.
Scientist

WPC/kam/0826K-1
05/04/87

Table 1

**Preliminary Cytotoxicity Test for Determination of
Appropriate Dose Range for Subsequent Genotoxicity Tests**

Test Chemical	Initial Cell Concentration* (Cells/flask x 10 ⁵)	Final Cell Concentration** (Cells/flask x 10 ⁵)		Percentage Relative to Solvent Control	
		Without S9	With S9	Without S9	With S9
Tetraethylene- diamine - Sample A] (ug/ml)					
0 (Solvent)	5.0	34.9	34.6	100.0	100.0
.1	5.0	38.7	36.8	110.8	106.4
.3	5.0	37.2	34.4	106.4	99.3
.6	5.0	29.6	31.4	84.7	90.8
.0	5.0	Cytotoxic	28.0	Cytotoxic	81.0
.0	5.0	Cytotoxic	12.0	Cytotoxic	34.8
.0	5.0	----- Cytotoxic -----			

* CHO cells were inoculated uniformly into all culture flasks approximately 24 hrs prior to chemical treatments. Solvent used for the test chemical was cell-culture medium.

** Determined approximately 20 to 24 hrs after removal of the test agents. Cytotoxic effects indicated at the upper concentration ranges were noted by detachment and death of the cell culture.

Abbreviations: S9 - rat-liver homogenate metabolic activation system

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Slater Chromatid Exchange (SCE) Assay
Production of SCEs by Tetraethylammonium - Sample A Treated With S9 Metabolic Activation
2-Hour Treatment

Test Chemicals	Total # of Chromosomes	Total # of SCEs	SCEs/Cell*	Mean Number SCEs/Chromosome ± S.D.	Significance Above Solvent Control†
[Tetraethylene-pentamine - Sample A]					
0.6A	502	233	13.2	0.66 (0.20)	ns(a)
0.6B	500	263	10.5	0.53 (0.14)	ns
0.6C	499	342	13.7	0.69 (0.20)	c(c)
0.6D	503	417	16.7	0.84 (0.20)	c
1.0A	500	354	14.2	0.71 (0.23)	c(c)
1.0B	502	389	15.6	0.78 (0.21)	c
2.0 & 3.0	- - - - - Cytotoxic - too few mitotic cells to evaluate - - - - -				
CONTROLS:					
Solvent:					
Cell-culture Medium	498	250	10.0	0.50 (0.17)	-
Cell-culture Medium	498	249	10.0	0.50 (0.17)	-
Positive:					
DMS (300 µg/ml)	503	1468	58.7	2.92 (0.78)	c

* Twenty-five cells examined per dose level.
 ** Mean value of SCE/chromosome determined from the values of the individual cells examined.
 † Statistical significance above solvent control: a: 0.05 > p > 0.01; b: 0.01 > p > 0.001; c: p < 0.001; NS: p > 0.05
 Data analyzed by Student's t-test by comparing individual test groups with the combined solvent control groups. Significances noted in parentheses are the combined test groups with the combined solvent controls.

Abbreviations: S9 - rat-liver; homogenate metabolic activation system; DMF-Dimethylnitrosamine; S.D. - standard deviation
 VPC/kam/0826K-1
 05-04-87

Table 4

Determination of Cytotoxic and Mitotic Inhibitory Effects of Chemical Exposure: Proportion of Cells at Various Stages of Cell Division after Chemical Exposure

Dose (Z)	Test Without S9 Activation			Test With S9 Activation		
	1st Division	2nd Division	3rd Division	1st Division	2nd Division	3rd Division
[Tetraethylene-pentamine - Sample A]						
0.4 A	13	87	0			
0.4 B	6	94	0			
Mean	9.5	90.5	0			
0.6 A	2	98	0	1	99	0
0.6 B	C	100	0	3	97	C
Mean	1.0	99.0	0	2.0	98.0	0
0.7 A	0	100	0			
0.7 B	0	100	0			
Mean	0	100	0			
0.8 A	Cytotoxic - Too few mitotic cells					
0.8 B	1	99	0	3	97	0
Mean	1	99	0	2	98	0
				2.5	97.5	0
1.0 A	Not tested					
1.0 B	Not tested					
Mean	Not tested					
CONTROLS:						
Solvent:						
Cell-culture Medium	6	94	0	2	98	0
Cell-culture Medium	0	100	0	0	100	0
Mean	3.0	97.0	0	1.0	99.0	0
Positive:						
EMS (100 µg/ml)	1	99	0			
DMN (300 µg/ml)						
				0	100	0

Abbreviations: S9 - rat-liver homogenate metabolic activation system; DMN: Dimethylnitrosamine; S.D. - standard deviation; EMS - ethylmethanesulfonate.

MSD/9001/10/10/06/01

**BUSHY RUN RESEARCH CENTER**

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Telephone (412) 733-5200

Good Laboratory Practices Compliance

This study was conducted in accordance with the Environmental Protection Agency's Good Laboratory Practices (GLP) Standards (effective December 29, 1983) with the following exceptions:

1. The sponsor indicated that tetraethylenepentamine - sample A was completely soluble in water and no additional analyses of stability in the dosing solution were performed.
2. The positive and solvent control substances were the highest purity available and were analyzed for concurrent biological activity and not for chemical purity, stability, or uniformity. The test results confirmed the appropriate responses for these control agents in the respective test systems.
3. The content of the test control mixtures was documented by gravimetric analysis and no additional chemical analyses were performed.

These deviations from the GLP standards, in my opinion, do not affect the validity or conclusions of this study.

Prepared by:

Ronald S. Slesinski 5/19/87
Ronald S. Slesinski, Ph.D., DABT Date

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05-04-87

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Quality Assurance Unit Study Inspection Summary

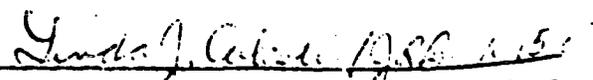
Test Substance: Tetraethylenepentamine - Sample A

Study: In Vitro Genotoxicity Studies: Sister Chromatid Exchange Assay

Study Director: R. S. Slesinski, Ph.D., D.A.B.T.

The Quality Assurance Unit of BRRC conducted the inspections listed below and reported the results to the study director and to management on the dates indicated. It is the practice of this Quality Assurance Unit to report the results of each inspection to both the study director and management.

<u>Date</u>	<u>Inspection Type</u>	<u>Date QAU Report Issued</u>	
		<u>To Study Director</u>	<u>To Management</u>
11-17-86	Standard Protocol	11-18-86	11-21-86
2-3-87	Standard Protocol Amendment	2-3-87	2-6-87
2-5-87	Event-Rinsing	2-5-87	3-31-87
6-18-87 to 10-12-87	Raw Data, Report	8-14-87	10-14-87
10-13-87	Archives	10-14-87	10-14-87


Linda J. Calisti, Group Leader Date
Good Laboratory Practices/Quality Assurance

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