FYI-0794-000993

1R-480

FYI-94-000993
INIT 07/14/94

UNION CARBIDE

UNION CARBIDE CORPORATION
INDUSTRIAL CHEMICALS DIVISION

39 OLD RIDGEBURY ROAD, DANBURY, CT 06817-0001

84940000093

October 22, 1986

A

Dynamac Corporation
The Dynamac Building
11140 Rockville Pike
Rockville, Maryland 20852

Contains No CBI

RECEIVED
OPPT CBIC
94 JUL 14 AM 9:21

Attn: Roberta Wedge

Dear Ms. Wedge:

Per your request, I am enclosing copies of the following TETA studies for your use with the TSCA Interagency Testing Committee.

1. Triethylenetetramine - Lifetime Dermal Carcinogenesis Study in C3H/HeJ Male Mice. BRRC Project Report 45-17, dated October 11, 1982.

2. Triethylenetetramine (TETA) - In Vitro Mutagenesis Studies: 3-Test Battery. BRRC Project Report 43-127, dated February 13, 1981.

3. Triethylene Tetramine (1974-75 Results) - Range Finding Toxicity and 7-Day Dietary Inclusion Studies. Carnegie-Mellon Institute of Research Special Report 39-54, dated April 12, 1976.

4. The Acute Toxicity of Triethylene Tetramine. Mellon Institute of Industrial Research Special Report 11-67, dated May 12, 1948.

5. Triethylenetetramine-Linear (TETA-Linear) - In Vitro Mutagenesis Studies: 3-Test Battery. BRRC Project Report 44-11, dated March 4, 1981.

Please let me know if we can be of further assistance.

Very truly yours,

G. P. Merrill

G. P. Merrill
Product Manager

/dmg
Enclosures

RECEIVED
10/18/94

UNION CARBIDE

BUSHY RUN RESEARCH CENTER

R. D. 4, Mellon Road, Export, Pennsylvania 15632 Telephone (412) 327-1020

UCC BUSINESS CONFIDENTIAL: Not to be released outside UCC without the written consent of the UCC-sponsoring Division HS&EA Manager.

Project Report 45-17
7 Pages
Tel: (412) 327-1020
October 11, 1982

Triethylenetetramine

Lifetime Dermal Carcinogenesis Study in C3H/HeJ Male Mice

Authors: P. J. Gazzie and L. R. DePass

Sponsor: Union Carbide Corporation
Ethylene Oxide Derivatives Division
South Charleston, WV

* * * * *

Summary

The dermal carcinogenic potential of triethylenetetramine (TETA) was assessed by applying 25 µl of a 5% (v/v) solution in deionized water to the backs of 50 male C3H/HeJ mice. A negative control group was dosed with deionized water. Both applications were performed three times a week until the death of the animals. No treatment-related skin tumors were observed in the groups treated with TETA or deionized water. A papilloma was observed on the upper lip of a TETA-treated mouse, but this was considered unrelated to treatment because of its location. One negative control mouse had a sebaceous adenoma of the skin of the thorax. No statistically significant difference in mortality rates was observed between the treated group and the water control group. TETA was not locally carcinogenic when applied to the skin of C3H/HeJ mice.

Objective

The objective of this study was to determine the dermal neoplasm producing potential of TETA.

Sample

A commercial sample of triethylenetetramine (TETA) was received on October 10, 1978. Analytical data for this sample was supplied by P. R. Umberger and W. H. Nelson, Union Carbide Corporation, South Charleston, WV. Preshipment analyses were performed using a direct chromatographic procedure to characterize the components of the sample. The parent sample was shown to be a mixture containing 79.15% TETA, 4.03% tris(aminoethyl)amine, 8.08% diaminoethylpiperazine, 8.34% piperazinoethylethylenediamine and less than 1% of seven other higher amines.

Chemical name: Triethylenetetramine
Quantity received: 1 gallon
Date received: October 10, 1978
Submitted by: Union Carbide Corp.
South Charleston, WV

BRRC Sample No.: 41-390
Description: yellow liquid,

Reference No.: TF43442; 1PRU-22
CAS No. 112-24-3

The reports of analyses performed on the parent sample at 6, 18 and 24 months of dosing have not yet been received. The stability analyses of diluted TETA using NMR spectroscopy and titration analysis showed that TETA in water was stable for at least one month. Monthly samples of the diluted TETA used for dosing were sent to UCC, South Charleston, WV, for titration and NMR analyses to determine that concentrations used for dosing were accurate. Reports received for these analyses indicated slight dilution differences in the samples over the dosing period. Titration analysis showed values ranging from 4.29 to 7.06 wt % with a mean value of 5.39 ± 0.45 wt %. The solvent for this study was water (CAS #7732-18-5) that was deionized in a Milli-Q reagent-grade water system (#Z02011574, Millipore Corp., Bedford, MA).

Methods

The mice used in this study were part of a large shipment to be used in various dermal carcinogenesis studies. C3H mice were used in this study because of their low incidence of spontaneous skin tumors. Deionized water was selected for use as the negative control substance because no skin tumors have ever been observed at BRRC in approximately 30 years of skin painting of C3H mice with water. A total of 1797 C3H/HeJ male mice were received (763 were received on January 31, 1979 and 1034 were received on February 27, 1979) from Jackson Laboratories, Bar Harbor, ME. The mice for this study were housed individually in stainless steel wire suspension cages. Zeigler block feed (Zeigler Bros., Inc., Gardners, PA) and water (Municipal Water Authority of Westmoreland County, Greensburg, PA) from an automatic watering system were provided for the animals ad libitum. All mice were identified by a toe-clipping method according to preassigned, unique identification numbers.

Mice selected for randomization were within two standard deviations from the mean with respect to their individual weights. The mice were randomized into test groups, of which two groups of 50 mice per group were assigned to this study. The randomization was statistically verified by the Bartlett's test for homogeneity of variances and the analysis of variance (Sokal and Rohlf, 1979). The two groups were assigned to receive either TETA or deionized water and appropriate cage cards were prepared. The mice assigned to the TETA group had body weights from 17.6 to 26.6 grams and the deionized water group weighed from 17.2 to 27.8 grams on the day of randomization. Ten mice from each group were randomly selected and predesignated for interim sacrifice after eighteen months of dosing. The remaining mice not randomized into these test groups were culled, used as quality control sentinels or assigned to other skin carcinogenesis studies.

In a preliminary study, groups of five C3H/HeJ mice were dosed daily for 10 days with 25 µl of solutions of 10% or 5% TETA in deionized water. The preliminary test indicated that TETA should be applied as a 5% concentration. This dilution of TETA was relatively non-irritating and non-toxic to the mice in the preliminary study and it was the concentration used in the chronic study.

Testing was initiated on March 12, 1979 when the mice were approximately 46 to 77 days of age. On Tuesday or Thursday of each week, the fur was clipped from the back of each mouse. Mice were treated three times weekly, following a Monday, Wednesday, and Friday treatment schedule. A 25 µl dose was applied using an Eppendorf automatic pipette, by spreading the aliquot up the back of each mouse with a clean disposable tip. Mice were observed daily for mortality and were carefully examined monthly for lesions of the skin. Necropsies were performed on all mice shortly after death or after sacrifice of culled and moribund animals. Necropsy included the careful examination of the skin and body cavities, and the recording of observations. All suspect tumors and the dorsal skin of all mice, with or without tumors, were fixed in 10% neutral buffered formalin (NBF). In addition, all livers, kidneys and lungs, unless autolyzed, were fixed in NBF for possible histopathologic examination. Sections were prepared from the dorsal skin of all mice and any suspect internal tumors. Histopathologic examinations were performed and reported (see Pathology Report, attached as Appendix I, for details).

Mortality incidences were assessed by the product-limit method (Kaplan and Meier, 1958). The Mantel-Cox and Breslow statistics were used for testing the equality of the survival curves (Mantel, 1966; Breslow, 1970).

Records and Raw Data

All records, specimens, raw data, and final report will be stored in the BRRC Archives upon completion of this report.

Deviations from the Protocol

The Study Director, L. G. Peterson, left this laboratory on August 13, 1981. L. R. DePass, Manager of Oral/Dermal Toxicology, assumed his duties as Study Director.

Results

The results of this study are summarized in Table I and a survival curve is presented in Figure I. In the group that received TETA as a 5% (v/v) dilution in deionized water the mean survival time was not statistically different from that of the deionized water control group (627 versus 626 days, respectively).

No papillomas or carcinomas were observed on the skin of any mice in either the TETA-treated group or the deionized water controls at the site of application. One papilloma was diagnosed on the upper lip of a TETA-treated mouse, but this finding was considered unrelated to the treatment because of its location. A sebaceous adenoma of the skin of the thorax was observed in a mouse in the deionized water control group.

The last surviving mouse in the TETA-treated group died on May 25, 1981. The last mouse in the deionized water control group died on May 23, 1981.

The ability of the C3H/HeJ mice to produce dermal carcinomas under similar experimental conditions was verified with mice treated with 3-methylcholanthrene in this laboratory.

Conclusion

The results of the present study indicated that TETA was not carcinogenic to the skin of C3H/HeJ male mice when applied as a 5% (v/v) solution in deionized water until the death of the animals.

References

Breslow, N. A generalized Kruskal-Wallis test for comparing k samples subject to unequal patterns of censorship. Biometrika, 57:579-594, 1970.

Kaplan, E. L. and P. Meier. Nonparametric estimation from incomplete observations. J. Amer. Statis. Assoc., 53:457-481, 1958.

Mantel, N. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemotherapy Reports, 50:163-170, 1966.

Sokal, R. R. and F. J. Rohlf. Biometry. W. H. Freeman and Company, San Francisco, CA, 1969.

Reviewed and Approved by:

Linval R. DePass, Ph.D.
Study Director
Manager, Oral/Dermal Toxicology

Fred R. Frank, Ph.D.
Director

Acknowledgements:

Skin Carcinogenesis:

R. A. Bradley, Technologist
M. J. Cardella, AALAS Cert. III
A. G. Chiaramonte, AALAS Cert. I
J. H. Coleman, A.A.
S. L. Melick, AALAS Cert. I
D. R. Meckley, B.A.
L. G. Peterson, B.S.
H. L. Zimmer-Weaver, Technologist

Histotechnicians:

K. F. D'Aurora, HT(ASCP)
C. D. DeMann, AALAS Cert. I
G. J. Haines, HT(ASCP)
M. A. McGee, HT(ASCP)
L. A. Meckley, HT(ASCP)
M. G. Orlando, HT(ASCP)
T. A. Savine, HT(ASCP)
H. M. Steel, AALAS Cert. I

Pathology: E. H. Fowler, DVM, Ph.D.

Pathology Report Preparation: P. A. Baker, R.N.

Project Report Preparation: P. J. Guzzie, B.S.

WPC/esk/0312A-3
05-17-82

BUSHY RUN RESEARCH CENTER
R. D. 4, Mellon Road, Export, Pennsylvania 15632 Telephone (412) 327-1020

CONFIDENTIAL: Not to be released outside UCC without the written consent of the UCC component sponsoring the work.

Project Report 43-127
18 Pages
Tel: (412) 327-1020
February 13, 1981

Triethylenetetramine (TETA)

In Vitro Mutagenesis Studies: 3-Test Battery

Authors: R. S. Slesinski, P. J. Guzzie, W. C. Hengler, K. J. Wagner

Sponsor: Union Carbide Corporation

* * * * *

SUMMARY

TETA was evaluated for potential mutagenic activity with a battery of three *in vitro* tests, which were: the Chinese Hamster Ovary (CHO) Mutation test, the Sister Chromatid Exchange (SCE) test and an assay for induction of Unscheduled DNA Synthesis (UDS) in rat liver cells. The results indicated that TETA produced a statistically significant mutagenic effect in all of the three *in vitro* tests performed. Increases in the mutation index were observed both with and without metabolic activation. These positive results are consistent with literature reports on positive effects of TETA in the Ames (Salmonella) mutation assay.

RESULTS AND INTERPRETATION

Selection of Test Concentrations - Preliminary experiments were performed to select an appropriate range of test concentrations in which the maximum concentration would allow survival of approximately 10% of the treated cells. A maximum concentration of 80 x 10^{-2}% (by volume) was chosen for the highest dose-level and a total of seven concentrations of TETA were tested for mutation induction because a steep dose response was suggested from prescreening data.

CHO Mutation Test - TETA produced a statistically significant increase in the frequency of mutations of CHO cells at several concentrations between 80 x 10^{-2}% to 2.5 x 10^{-2}% (by volume) in tests with and without the incorporation of a liver S9 metabolic activation system. The lack of a definite dose-related effect of treatment suggested that the alkaline effect of the test agent may have interfered with the tests. With S9 metabolic activation, the acidic S9 liver homogenate may have somewhat buffered the alkaline effect and a dose-related trend in the mutation index was observed for treatments between 10 x 10^{-2}% and 40 x 10^{-2}%.

SCE Test - TETA produced a highly statistically significant and dose-related increase in the frequency of SCE in CHO cells in tests without the incorporation of an S9 metabolic activation system. With metabolic activation, the effect on the frequency of SCE was decreased with respect to the results without S9, but the highest dose level produced a highly statistically significant effect. An overall range of concentrations between 40×10^{-2}% to 1.25×10^{-2}% (by volume) was used. The highly positive effects observed in the test without S9 metabolic activation indicated that TETA was an active mutagenic agent in the production of SCE in CHO cells.

UDS Test - TETA produced statistically significant increases in the amount of UDS activity in evaluations of concentrations between 100×10^{-2}% to 0.1×10^{-2}% (by volume). TETA was considered to be active in the present test with the hepatocyte test system and positive effects were observed in tests using both nuclei and DNA to detect increases in UDS.

Comparative Mutagenicity - The pattern of positive responses produced by TETA in the 3-test battery to determine potential mutagenicity indicated that TETA was active in comparison to known mutagenic agents, such as the positive controls tested concurrently with the test chemical. The consistent observation of a positive effect in each of the three tests employed indicates that TETA (or one of its many components) should be considered to be an active mutagenic agent. Additional studies in our laboratory on other samples of TETA will be presented in subsequent reports, but data with TETA-linear (99.42% purity) are consistent with the positive findings in this study. One other mutagenicity study on TETA by A. Hedenstedt [Mut. Res. 53, 198-199, 1978 (abstract)] also found a positive effect which was observed both with and without metabolic activation in the Ames (Salmonella) test.

SAMPLE

Quantity: 1 gallon

Submitted by: D. C. Best

Division: Specialty Chemicals and Plastics
Union Carbide Corporation
South Charleston, WV

CHF Sample No.: 41-390

Date Received: October 10, 1978

Identification: TF 43442;
yellow liquid

Ref.: PRU-22 (Analytical data)

CAS#: 112-24-3

Triethylenetetramine (TETA)

In Vitro Mutagenesis Studies: 3-Test Battery

Sponsor: Union Carbide Corporation

* * * * *

OBJECTIVE

The purpose of this study was to evaluate the potential of TETA to induce genetic damage in mammalian cells at the gene, chromosome and/or DNA (deoxyribonucleic acid) level of molecular organization. A battery of three *in vitro*, short-term tests which detect each of these genetic endpoints was employed to evaluate TETA for potential mutagenic activity. A general description of the theoretical basis of these three tests is presented in Appendices I, II and III attached to the complete report.

SAMPLE CHARACTERISTICS

A typical, commercial sample of TETA was received for testing on October 10, 1978 and was assigned sample #41-390. The physical and chemical information available from the Toxicology Data Bank or from "Material Safety Data Sheets" for this product are attached to this report as Appendix IV. Additional analytical data supplied by P. Umberger, Union Carbide Corporation, South Charleston, are attached to this report as Appendix V. TETA is a mixture containing linear, branched and cyclic molecules. The sample submitted for testing contained 79.15% TETA and ten other identified components; three other major components were trisaminoethylamine, 4.03%; diaminoethylpiperazine, 8.08%; and piperazinoethylethylenediamine, 8.34%.

METHODS

A description of the technical procedures used in the CHO test, the SCE test and the UDS assay are presented in greater detail in Appendices I, II and III, respectively (attached to the complete report). 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.

1. CHO Test (Detailed procedures in Appendix I): SOP #7.2.4A, 7.2.5, 7.2.11.

A. Dose Selection - Appropriate concentrations of TETA for testing were determined by measurements of cytotoxicity to CHO cells of six concentrations tested both in the presence and absence of a liver S9 metabolic activation system. Data from cytotoxicity tests on a sample of TETA-linear (CHF sample #42-447) were used to approximate the test concentrations for TETA. Selection of maximum concentration for testing depended upon an estimate of a dose level which would permit survival of at least 10% of the treated cells. Sterile water (H_2O) was used as the solvent and solvent control.

To simplify tables and to allow comparisons between different tests, concentrations of TETA in the following sections of the report are given in terms of volume percentages x 10^{-2} to eliminate zeros in the lower concentration values (eg. 0.025 % = 2.5 x 10^{-2}%).

B. Mutation - CHO cells were exposed for 5 hours to a minimum of five concentrations of TETA both with and without the addition of an S9 metabolic activation system. Dilutions of TETA for testing were prepared by either direct addition of various aliquots of the test agent into the cell culture media or by making sequential one-half dilutions of the stock solution for the maximum concentrations using sterile H_2O. The surviving fraction was determined at 20 to 24 hours after treatment and the mutant fraction was determined after a 7-day period to allow "expression" of the mutant phenotype. Only the data from the top five concentrations which allowed sufficient cell survival for assessment of survival and induction of mutants are usually presented in the tables. The percentage of cells surviving the treatment, the frequencies of mutant colonies and the number of mutants/ 10^6 viable cells are presented in tabular form.

2. SCE Test (Detailed procedures in Appendix II): 7.2.12A

Production of SCE's following exposure to various concentrations of TETA was studied in CHO cells both with and without the incorporation of an S9 metabolic activation system. Selection of a maximum dose level which would permit survival of at least 50% of the treated cells was based on the prescreening test for cytotoxicity performed as part of the CHO Mutation test. Dilutions of TETA for testing were prepared either by direct addition of various aliquots into the culture medium or by making sequential one-half dilutions of the stock solution for the maximum dose level using sterile H_2O. For determination of direct mutagenic action, CHO cells were exposed to TETA 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 ug/ml in the growth medium during treatment and during the culture period following exposure. A total of 20 cells/dose level and 5 dose levels, tested either with or without metabolic activation, were examined. The number of SCE/cell, mean number of SCE/chromosome and the level of statistical significance of the increases above the concurrent solvent control values are presented in tabular form.

3. UDS Test (Detailed procedures in Appendix III): 7.2.6, 7.2.7A, 7.2.8B, 7.2.9B

Induction of primary DNA damage in rat liver cells (hepatocytes), was studied at a minimum of six dose levels which spanned a 1000-fold range of concentrations. Cells were treated with TETA for 2 hours in culture medium

containing ^{3}H-thymidine, hydroxyurea and appropriate dilutions of TETA prepared in DMSO. Determination of UDS activity was performed by analyses of incorporation of ^{3}H-thymidine into isolated hepatocyte nuclei or in DNA (precipitated from aliquots of the isolated nuclei) using a Searle Analytic Model 81 or Packard Model 2650 scintillation spectrometer. Data are presented in tabular form with an indication of the level of statistical significance above the concurrent solvent control values.

4. Controls - Positive, negative and solvent controls were tested concurrently with the test sample to assure the sensitivity of the test system and the concurrence of the results to previous test performance. For the CHO and SCE assays, dimethylnitrosamine (DMN) and ethylmethanesulfonate (EMS) were used as positive control agents to assure the sensitivity of the test system for detecting indirect and direct-acting mutagens, respectively. Deionized water, sterilized by membrane filtration and glass-distilled dimethylsulfoxide (DMSO) were used as the solvent and negative controls, respectively.

   In the UDS assay, DMN and 4-nitroquinoline oxide (4-NQO) were used as positive controls representing indirect- or direct-acting mutagens, respectively. DMSO was used as the solvent and the solvent control.

5. Metabolic Activation - S9 liver homogenate, prepared from Arochlor 1254-induced, Sprague-Dawley male rats, was purchased from Litton Bionetics. The S9 preparation used for the CHO test contained 40 mg/ml protein and had a benzo(a)pyrene hydroxylase (BPH) activity of 13.93 nmol of hydroxybenzpyrene/20 min/mg protein, (assayed by Litton). A concentration of 3600 ug of S9 protein was added to 5 ml of culture media.

   For the SCE test, another lot of S9 homogenate, containing 40 mg/ml protein and a BPH of 15 nmol hydroxybenzpyrene/20 min/mg protein (assayed by Litton); a concentration of 600 ug of S9 protein was added per 5 ml culture media.

6. Statistical Analyses - Data from the SCE and UDS tests were analyzed by appropriate parametric tests following Standard Operating Procedures for statistical analyses at the Bushy Run Research Center. Data from the CHO test do not follow a normal distribution according to experience with historical controls. Thus, the Student's t-test was used after transformation of the mutation frequencies (MF) according to the method of Irr and Snee $(MF + 1)^{0.15}$ (Irr, J. D. and R. Snee, Proceedings of the Cold Spring Harbor-Banbury Conference, II (1979), 263-274).

   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. The degree of statistical significance is denoted by: a: $0.05 > p > 0.01$, b: $0.01 > p > 0.001$, or c: $p < 0.001$. No superscript (or NS) indicates $p > 0.05$.

7. Raw Data Storage - Copies of the final report, statistical analyses, analytical data and data used to prepare the final report are stored in the BRRC Archives. Slides are stored in the Genetic Toxicology slide storage area.

RESULTS

SECTION I - CHO MUTATION TEST - Triethylenetetramine (TETA)

A. Test Dates - Initiated: April 17, 1980
Completed: July 10, 1980

B. Selection of Test Concentration (Data not shown in tables)

Test concentrations were selected from prior test results on another sample of TETA submitted for mutagenicity tests (TETA-linear; CHF Sample Number 42-447). CHO cells were exposed for five hours to concentrations of TETA-linear which spanned a concentration range from 100 x 10^{-2}% to 0.3 x 10^{-2}% by volume. The percentage of cells which survived the exposure, both in the presence and absence of an S9 metabolic activation system, was determined by counting the number of colonies produced by the survivors after a 5- to 7-day incubation period. A concentration of 80 x 10^{-2}% was selected as the maximum concentration for testing with and without S9 activation. A total of seven concentrations were tested in the later mutation tests because the prescreening results suggested the existence of a probable steep dose-response effect.

C. Determination of Mutation Induction

1. Survival (Cytotoxicity)

Table 1 presents the cytotoxicity data for CHO cells treated with TETA in the presence and absence of a liver S9 metabolic activation system. A steep dose-response effect with the test agent was observed from the > 99% cell killing obtained with the top concentration of TETA, in tests without S9 activation, in comparison to the markedly lower cytotoxicity obtained at only one-half the top dose-level. The results on cytotoxicity in the presence of an S9 activation system were unusual because the 40 x 10^{-2}% dose level produced a significant decrease in the percentage of survival which was not obtained at the next higher dose level. These data suggest the possibility of an experimental error in pipetting or dilution and the same effect was seen in the cell counts at the time the cells were plated for determination of the surviving fraction.

2. Mutation

Table 2 presents the data for induction of mutants by TETA and control agents. In tests with and without addition of an S9 metabolic activation system, TETA produced statistically significant increases in the frequency of mutants at all but one dose level in both tests. The effect was not definitively dose-related and the absolute number of mutants obtained in the

test without metabolic activation was within the historical experience for the spontaneous occurrence of mutants with the solvent and negative controls in other tests. The presence of a marginal indication of a dose-related response in the test with S9 activation for dose levels between 10 x 10^{-2}% and 40 x 10^{-2}% and the production of highly significant mutation frequencies outside the historical control variation for the test indicated that TETA was an active agent in the induction of gene mutations in CHO cells.

Mutation frequencies for the solvent controls for tests both with and without S9 activation were in an acceptable and low range based upon experience with historical control values. Statistically significant increases in the mutation frequencies were obtained for the DMN and EMS positive controls for both experiments and these values were within the expected range of values observed in historical control data. The statistically significant effect obtained with DMSO, the negative control in the test with metabolic activation, was not considered to be biologically significant because DMSO has been observed in other tests to increase the inherent mutagenic action of the liver homogenate.

D. Deviations from Standard Procedures - F_{12} cell culture media was purchased from Kansas City Biological instead of from Grand Island Biological as stated in the Standard Operating Procedure. Also, 0.075% trypsin, rather than 0.05%, was used to improve cell dissociation from the culture dish. We attempted to minimize the highly alkaline effect of TETA in the cell culture medium by equilibrating the culture flasks with 10% CO_2 in air.

E. Conclusions

TETA was consistently active as a mutagenic agent for CHO cells when tested with and without the incorporation of an S9 metabolic activation system over a 32-fold range of concentrations. Several statistically significant increases above the concurrent solvent control were produced at a majority of the dose levels tested and TETA was considered to be mutagenic in the CHO test, especially in the test which included an S9 metabolic activation system.

SECTION II - SCE TEST - Triethylenetetramine (TETA)

A. Test Dates - Initiated: April 21, 1980
Completed: November 11, 1980

B. Selection of Test Concentrations

A maximum concentration of 40 x 10^{-2}% of TETA was chosen as the top dose level for testing with and without S9 activation respectively, based on cytotoxicity data from the CHO mutation test. Higher concentrations were expected to produce delays in the mitotic cycle and to decrease the number of cells with SCE staining. A 32-fold range of TETA concentrations from 40 x 10^{-2}% to 1.25 x 10^{-2}% was examined in SCE experiment #1 without S9 activation. A 16-fold range of concentrations of TETA from 40 x 10^{-2}% to 2.5 x 10^{-2}% (by volume) was examined in SCE experiment #2 with S9

activation. A lower concentration range was examined in experiment #2 because the highest dose level tested (80 x 10^{-2}%) was cytotoxic and inhibited cell division necessary to visualize SCE.

C. Determinations of SCE Induction

1. The data for SCE induction in CHO cells treated with various dose levels of TETA or with positive, negative or solvent control agents without an S9 metabolic activation system are summarized in Table 3. A highly statistically significant increase in the SCE frequency was produced by three out of five dose levels of TETA tested for direct action in the absence of a metabolic activation system. All five of the five concentrations of TETA tested produced an increased frequency of SCE in CHO cells and the effect was directly related to the treatment dose. A positive dose-related effect is considered a strong indication for the existence of a biologically significant mutational effect. The test without S9 activation was considered a positive indication for potential direct mutagenic action of the test agent.

The number of SCE produced by the concurrent EMS positive control was highly statistically significant from the concurrent solvent control and these data indicated an appropriate sensitivity of the test system comparable to our historical positive control data. The numbers of SCE obtained with the solvent and 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 TETA in the presence of an S9 metabolic activation system are presented in Table 4. A statistically significant increase in the SCE values was observed at two of the five tested concentrations of TETA. However, there was no evidence for dose-response effect of TETA treatments, in contrast to the findings in the test without addition of S9. The production of a highly significant effect at the highest dose level suggested that additional higher concentrations should be tested to determine the existence of a reproducible, biologically significant response. However, these tests were not considered necessary to adequately evaluate the mutagenic potential of TETA which was clearly a positive mutagen in the other tests of the mutation test battery; including the positive effect upon SCE in the test without 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 SCE were produced by the DMN positive control which indicated that the metabolic activation system was suitably active.

D. Deviations from Standard Procedures - Numerous minor deviations were employed to improve the reproducibility of this test and these changes will be incorporated into our revised SOP for this procedure.

1. F_{12} culture medium was purchased from Kansas City Biological Co.
2. Trypsin was used at a concentration of 0.075% rather than 0.05% to improve cell dissociation.

3. Flasks were equilibrated with 10% CO_2 in air to attempt to minimize the effect of the test agent upon the pH of the medium.
4. Cell cultures for the experiment were started at 4×10^5 cells/flask 72 hours prior to use.
5. Colchicine was employed at a concentration of 0.5 ug/ml at 1 hour prior to harvest of cells for chromosome preparation.
6. An extended interval of 33 hours post-treatment was employed to improve the mitotic index.
7. Seven concentrations were used for treatment and only the highest five which permitted adequate cell survival were analyzed.

E. Conclusions

TETA produced highly statistically significant effects on the frequency of SCE over the 32-fold range of concentrations tested without addition of an active S9 metabolic activation system. A dose-related effect of TETA exposure on the SCE frequency was evident in the test without S9 metabolic activation and the test agent was considered to be active in the present *in vitro* assay.

SECTION III - UDS TEST - Triethyenetetramine (TETA)

A. Test Dates - Initiated: May 7, 1980
Completed: July 2, 1980

B. Selection of Test Concentrations

Standard procedures were followed and TETA was tested over a 3-log range of concentrations from 100×10^{-2}% to 0.1×10^{-2}% by volume. The maximum dose-level was selected with consideration of the cytotoxicity data obtained in the CHO Mutation test which indicated that higher values would result in excessive cell killing. These dose levels were considered to be appropriate for testing because uptake and incorporation of ^{3}H-thymidine into hepatocytes are generally even more sensitive to chemical effects than survival measurements with CHO cells (in which cytotoxicity is measured after a 24-hour recovery period following treatment).

C. Determination of UDS Induction

1. Nuclear-Bound Radioactive Label (Data in Table 5)

Induction of "unscheduled" incorporation of ^{3}H-thymidine into nuclei of hepatocytes exposed to TETA or to appropriate positive and negative controls is presented in Table 5. In hepatocytes treated with TETA, three concentrations tested for potential activity induced a statistically significant increase in the amount of ^{3}H-thymidine incorporation and four of six concentrations induced a numerical elevation in the amount of UDS. The level of the increases were substantially outside the confidence interval of

the historical data with the solvent controls for this assay but the effects were not clearly dose-related. The results in this test were interpreted as a probable positive effect on primary DNA damage in rat hepatocytes.

Both of the positive control agents, NQO and DMN, induced numerically elevated and statistically significant increases in UDS over values obtained with the solvent control. With both positive controls, all dose levels produced a numerical elevation in UDS, but the highest two dose levels of DMN appeared to be cytotoxic and the responses were not significant with respect to the concurrent control.

2. DNA-Bound Radioactive Label (Data in Table 6)

Analyses of DNA, from aliquots of hepatocyte nuclei used for the UDS studies presented on Table 5, were performed as a second assessment of "unscheduled" incorporation of radioactive thymidine. Values for radioactivity incorporated into the DNA of these hepatocyte nuclei are presented in Table 6.

For hepatocytes treated with TETA, three of the test concentrations induced levels of UDS which were statistically significant from the solvent control. However, we did not observe a definite dose-related effect upon ^{3}H-thymidine incorporation following treatments with TETA. Positive results on ^{3}H-thymidine incorporation into DNA were consistent with the data obtained in the assessment of UDS measured using nuclei from cells treated with the same range of concentrations. TETA was considered to be active in the induction of DNA damage discernible by UDS activity in the present study.

The pattern of UDS activity produced by varying concentrations of the positive control agents NQO and DMN were similar and consistent with the data obtained in the assessment of ^{3}H-thymidine incorporated into nuclei presented in Table 5. All doses of the positive control agents produced a numerical elevation in the amount of UDS but the variability was lower (than with nuclear measurements) and with both NQO and DMN all dose-levels produced statistically significant increases from the solvent control.

D. Deviations from Standard Procedures - Nuclei were stored in the lysing solution for one week to attempt to reduce flocculence in the tubes.

E. Conclusion

TETA stimulated a statistically significant increase in the incorporation of radioactive thymidine in cells treated over a 1000-fold range of test concentrations. TETA was considered to be active in producing primary DNA damage in the present test with the hepatocyte test system.

Reviewed and Approved by:

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

Elton R. Homan, Ph.D.
Associate Director, Toxicology

Fred R. Frank, Ph.D.
Director

Contributors:

Chinese Hamster Ovary test	Peggy J. Guzzie, B.S. Master Technologist
Sister Chromatid Exchange test	Michelle W. Gaunt, B.S. Master Technologist
	Kathy J. Wagner, B.S. Master Technologist
	Peggy J. Guzzie, B.S. Master Technologist
Unscheduled DNA Synthesis Assay	W. Christopher Hengler, M.S. Assistant Scientist

WPC/1123-2

REFERENCES

A search of the major computer data files produced only one reference concerning previous testing of TETA for mutagenicity/carcinogenicity:

Hedenstedt, A. Mutagenicity screening of industrial chemicals: seven aliphatic amines and one amide tested in the Salmonella/microsomal assay. Mutat. Res. 53(2), 298-199, 1978.

Table 1
Chinese Hamster Ovary (CHO) Mutation Assay:
Determination of Toxic Effects of Chemical Treatment During 5 Hr Mutation Induction Period

Test Chemicals	Total # Colonies	Total # Cells Plated	% Survival	% of Solvent Control
Without S9 Activation				
[Triethylenetetramine] (%, v/v)				
80.0×10^{-2}	-	TOXIC -	-	-
40.0×10^{-2}	394	400	98.5	83.3
20.0×10^{-2}	440	400	110.0	93.0
10.0×10^{-2}	400	400	100.0	84.6
5.0×10^{-2}	424	400	106.0	89.6
2.5×10^{-2}	426	400	106.5	90.1
Controls				
H_2O (20 ul/ml) - Solvent	473	400	118.2	-
DMSO (20 ul/ml)	475	400	118.8	100.4
EMS (200 ug/ml)	393	400	98.2	83.1
With S9 Activation				
[Triethylenetetramine] (%, v/v)				
80.0×10^{-2}	479	400	119.8	111.9
40.0×10^{-2}	100	800	12.5	11.7
20.0×10^{-2}	397	400	99.2	92.8
10.0×10^{-2}	409	400	102.2	95.6
5.0×10^{-2}	360	400	90.0	84.1
2.5×10^{-2}	430	400	107.5	100.5
Controls				
H_2O (20 ul/ml) - Solvent	428	400	107.0	-
DMSO (20 ul/ml)	399	400	99.8	93.2
DMN (3700 ug/ml)	71	400	17.8	16.6

Abbreviations: H_2O - water; S9 - liver homogenate; DMSO - dimethylsulfoxide
EMS - ethylmethanesulfonate; DMN - dimethylnitrosamine

WPC/1123-1

Table 2
Chinese Hamster Ovary (CHO) Mutation Assay:
Results on Evaluation of Mutant Induction by Triethylenetetramine

Test Chemicals	Plating Efficiency			Mutation Induction		
	Total # Colonies	Total # Cells Plated	Viable Fraction	Total # Mutant Colonies	Total # Cells Plated	Mutants[1] / 10^6 Viable Cells
Without S9 Activation						
[Triethylenetetramine] (%, v/v)						
80.0×10^{-2}	-	-	TOXIC	-	-	-
40.0×10^{-2}	403	400	1.008	22	1×10^6	21.8[a]
20.0×10^{-2}	338	400	0.845	16	1×10^6	18.9[b]
10.0×10^{-2}	261	400	0.652	14	1×10^6	21.5[a]
5.0×10^{-2}	404	400	1.010	18	1×10^6	17.8
2.5×10^{-2}	395	400	0.988	14	1×10^6	14.2[b]
Controls:						
H_2O (20 ul/ml) - Solvent	459	400	1.148	4	1×10^6	3.5
DMSO (20 ul/ml)	368	400	0.920	6	1×10^6	6.5
EMS (200 ug/ml)	390	400	0.975	110	1×10^6	112.8[c]
With S9 Activation						
[Triethylenetetramine] (%, v/v)						
80.0×10^{-2}	372	400	0.930	25	1×10^6	26.9[a]
40.0×10^{-2}	393	400	0.982	74	1×10^6	75.3[c]
20.0×10^{-2}	361	400	0.902	31	1×10^6	34.3[b]
10.0×10^{-2}	383	400	0.958	11	1×10^6	11.5
5.0×10^{-2}	407	400	1.018	15	1×10^6	14.7[b]
2.5×10^{-2}	369	400	0.922	26	1×10^6	28.2[b]
Controls:						
H_2O (20 ul/ml) - Solvent	399	400	0.998	7	1×10^6	7.0
DMSO (20 ul/ml)	341	400	0.852	21	1×10^6	24.6[b]
DMN (3700 ug/ml)	194	400	0.485	58	1×10^6	119.6[c]

[1]Total # mutant colonies per 10^6 cells plated divided by viable fraction.
Statistical significance above solvent control: a: $0.05 > p > 0.01$; b: $0.01 > p > 0.001$; c: $p < 0.001$
No superscript indicates $p > 0.05$. Data analyzed by Student's t-test.

Abbreviations: H_2O - water; S-9 - liver homogenate; DMSO - dimethylsulfoxide; EMS - ethylmethanesulfonate; [illegible]thylnitrosamine.

Table 3
Sister Chromatid Exchange (SCE) Assay:
Induction of SCE's by Triethylenetetramine Without S9 Metabolic Activation
5 Hour Treatment

Test Chemicals	Total # of Chromosomes	Total # of SCE		SCE/Cell[1]	Mean Number SCE/Chromosome[2] ± S.D.	Significance Above Solvent Control[3]
[Triethylenetetramine] (%, v/v)						
40.0×10^{-2}	-	-	TOXIC	-	-	-
20.0×10^{-2}	403	349		17.45	0.866 ± 0.139	c
10.0×10^{-2}	410	268		13.40	0.654 ± 0.184	c
5.0×10^{-2}	406	246		12.30	0.606 ± 0.112	c
2.5×10^{-2}	405	181		9.05	0.448 ± 0.112	NS
1.25×10^{-2}	410	194		9.70	0.472 ± 0.129	NS
Controls						
H_2O (5 ul/ml) - Solvent	399	153		7.65	0.385 ± 0.150	-
DMSO (5 ul/ml)	398	133		6.65	0.334 ± 0.100	NS
EMS (100 ug/ml)	398	486		24.30	1.217 ± 0.262	c

[1]Twenty cells examined per dose level.

[2]Mean value of SCE/chromosome determined from the values of the individual cells examined.

[3]Statistical significance above solvent control: c: $p < 0.001$; NS: $p > 0.05$. Data analyzed by Student's t-test.

Abbreviations: H_2O - water; S9 - liver homogenate; DMSO - dimethylsulfoxide; EMS - ethylmethanesulfonate; S.D. - standard deviation

WPC/1123-1

Table 4
Sister Chromatid Exchange (SCE) Assay:
Induction of SCE's by Triethylenetetramine With S9 Metabolic Activation
2 Hour Treatment

Test Chemicals	Total # of Chromosomes	Total # of SCE	SCE/Cell[1]	Mean Number SCE/Chromosome[2] ± S.D.	Significance Above Solvent Control[3]
[Triethylenetetramine] (%, v/v)					
40.0×10^{-2}	402	238	11.90	0.592 ± 0.203	b
20.0×10^{-2}	386	165	8.25	0.430 ± 0.190	NS
10.0×10^{-2}	397	161	8.05	0.409 ± 0.151	NS
5.0×10^{-2}	400	163	8.15	0.414 ± 0.209	NS
2.5×10^{-2}	389	199	9.95	0.515 ± 0.144	a
Controls					
H_2O (5 ul/ml) - Solvent	396	164	8.20	0.414 ± 0.141	-
DMSO (5 ul/ml)	391	103	5.15	0.263 ± 0.146	NS
DMN (500 ug/ml)	398	639	31.95	1.600 ± 0.795	c

[1]Twenty cells examined per dose level.

[2]Mean value of SCE/chromosome determined from the values of the individual cells examined.

[3]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.

Abbreviations: H_2O - water; S9 - liver homogenate; DMSO - dimethylsulfoxide; DMN - Dimethylnitrosamine; S.D. - standard deviation

WPC/1123-1

Table 5
Unscheduled DNA Synthesis in Hepatocytes from Rat Liver

Nuclear-bound label: all DPM values are calculated from nuclei per 10^6 viable hepatocytes. Each average is calculated from duplicate samples, except for DMSO which was done in quadruplicate.

Test Chemical	Concentration	Radioactivity in Nuclei Avg. DPM ± S.D.	% of Solvent Control ± S.D.	Significance Above Solvent Control[1]
Solvent - DMSO	2%	2902 ± 524	100.0% ± 18.0%	-
Positive Controls: 4 - NQO	3.0 ug/ml	15528 ± 590	535.1% ± 20.3%	c
	0.9 ug/ml	5879 ± 1945	202.6% ± 67.0%	b
	0.3 ug/ml	6615 ± 1053	228.0% ± 36.3%	b
DMN	1000 ug/ml	4135 ± 717	142.5% ± 24.7%	NS
	300 ug/ml	4764 ± 472	164.2% ± 16.3%	NS
	100 ug/ml	5116 ± 142	176.3% ± 4.9%	a
Test Chemical: [Triethylenetetramine] (%, v/v)	100×10^{-2}%	357 ± 197	12.3% ± 6.8%	NS
	30×10^{-2}%	2210 ± 2250	76.2% ± 77.5%	NS
	10×10^{-2}%	4745 ± 2215	163.5% ± 76.3%	NS
	3×10^{-2}%	5154 ± 356	177.6% ± 12.3%	a
	1×10^{-2}%	6482 ± 1580	223.4% ± 54.5%	b
	0.1×10^{-2}%	5752 ± 186	198.2% ± 6.4%	b

[1]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 Duncan's Multiple Range Analysis.

Abbreviations: DMSO - dimethylsulfoxide; 4-NQO - 4-nitroquinoline oxide; DMN - dimethylnitrosamine; DPM - disintegrations per minute; S.D. - standard deviation

WPC/1123-3

2199

Table 6
Unscheduled DNA Synthesis in Hepatocytes from Rat Liver

DNA-bound label: all DPM values are calculated from DNA precipitated per 10^6 viable hepatocytes. Each average is calculated from duplicate samples, except for DMSO which was done in quadruplicate.

Test Chemical	Concentration	Radioactivity in DNA Avg. DPM ± S.D.	% of Solvent Control ± S.D.	Significance Above Solvent Control[1]
Solvent - DMSO	2%	1893 ± 71	100.0% ± 3.8%	-
Positive Controls:				
4 - NQO	3.0 ug/ml	13459 ± 1004	711.0% ± 53.1%	c
	0.9 ug/ml	4722 ± 1949	249.4% ± 102.9%	a
	0.3 ug/ml	4798 ± 1296	253.5% ± 68.4%	b
DMN	1000 ug/ml	3360 ± 576	177.5% ± 30.4%	b
	300 ug/ml	3488 ± 128	184.3% ± 6.8%	c
	100 ug/ml	3829 ± 121	202.3% ± 6.4%	c
Test Chemical: [Triethylenetetramine] (%, v/v)	100×10^{-2}%	428 ± 69	22.6% ± 3.6%	NS
	30×10^{-2}%	1731 ± 1473	91.4% ± 77.8%	NS
	10×10^{-2}%	3591 ± 1844	189.7% ± 97.4%	NS
	3×10^{-2}%	3986 ± 434	210.6% ± 22.9%	c
	1×10^{-2}%	4069 ± 1287	214.9% ± 68.0%	a
	0.1×10^{-2}%	3897 ± 160	205.8% ± 8.4%	c

[1]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.

Abbreviations: DMSO - dimethylsulfoxide; 4-NQO - 4-nitroquinoline oxide; DMN - dimethylnitrosamine; DPM - disintegrations per minute; S.D. - standard deviation

Special Report 39-54
8 Pages
Confidential

April 12, 1976
Tel: (412) 327-1020

CHEMICAL HYGIENE FELLOWSHIP
Carnegie-Mellon Institute of Research
Carnegie-Mellon University
4400 Fifth Avenue
Pittsburgh, Pa. 15213

Triethylene Tetramine

(1974-75 Results)

Range Finding Toxicity and 7-Day Dietary Inclusion Studies

Sponsor: *Union Carbide Corporation*, Chemicals and Plastics Operations Division

* * * * * * *

Summary

	1974-75 Results	1948 Results[1]
Stomach Intubation, rat LD50	2.83 ml/kg; undiluted	4.34 gm/kg; 1 ml = 0.10 gm in water
Skin Penetration, rabbit LD50	0.561 ml/kg; undiluted	0.820 ml/kg; undiluted
		2.00 gm/kg killed 4 of 10; 1 ml = 0.10 gm in water
Inhalation, rat Substantially saturated vapor	static conditions at 21°C; 8 hours killed 0 of 6	dynamic conditions (room temperature); 8 hours killed 1 of 6
Mist from saturation at 170°C, LT50	-	3.17 hours
Uncovered Skin Irritation, rabbit	moderate, Grade 6	moderate, Grade 6
Eye Injury, rabbit	moderate, Grade 7	moderate, Grade 5
Seven-Day Dietary Inclusion, rat	minimum effect at 1.30 gm/kg/day; maximum no ill effect at 0.49 gm/kg/day	-

1 Smyth, Henry F., Jr. *et al.*, "Range Finding Toxicity Data List III". Journal of Industrial Hygiene and Toxicology. Vol. 31, No. 1, 60-62 (January 1949) and Chemical Hygiene Fellowship (CHF) Report 11-67; 1948.

Interpretation

Triethylene tetramine was moderately toxic following acute peroral intubation and covered dermal application. Administration of the undiluted material to uncovered rabbit skin resulted in necrosis in the current and previously-reported tests. Therefore, it is rated by the Chemical Hygiene Fellowship as a Department of Transportation (D.O.T.) "corrosive" although the actual D.O.T. covered skin irritation test was not performed. Application of a 10% dilution in distilled water resulted in no irritation to rabbit skin and moderate corneal injury in rabbit eyes resulted from 15 to 40% in distilled water. No hazard is anticipated from the infrequent inhalation of substantially saturated vapor evolved at room temperature under normal handling conditions. Rats that received triethylene tetramine in their diets for 7 days were affected by 1.30 gm/kg/day; this is the minimum effect level (MiE). No significant effect, in the criteria examined, resulted at 0.49 gm/kg/day. The ratio of single peroral LD50/MiE was 2.18, probably indicating a low degree of chronicity.

The results of these latest range finding tests were similar to those done previously and reported in 1948 (Report 11-67) with the possible exception of the rabbit eye irritation test. In this, the new sample was somewhat more irritating than the previous one (Grade 7 compared to Grade 5).

Included in the literature on triethylene tetramine are reports of severe dermatoses among workers that contacted "Hardener 951", which is actually triethylene tetramine (Grandjean, E., "Danger of Dermatoses due to Cold-Setting Ethoxyline Resins (Epoxide Resins)." Brit. J. Ind. Med. 14, 1-4 (1957) and Markicevic, A. *et al.* "Noxious Effect of Epoxide Resins". Arhiv. Hig. Rada Toksilcol. 15 (1), 9-14, 1964). The skin reaction noted in these papers was characterized as erythematous itching and severe allergic eczema. Another paper includes the results of some tests on animals using triethylene tetramine (Kowalski, Z., *et al.*,"Toxic Effects of Triethylenetetramine." Med. Pracy 13, 265-72, 1962). In this study, the rat LD50 for intraperitoneal dosing was 78.4 mg/kg. Application of 20 mg of the material resulted in necrotic foci and extravasations on rabbit skin; the same dose to rabbit eyes resulted in inflamation of the conjunctivae and lymphatic exudation. There were no sensitizing effects noted as a result of patch tests on guinea pigs.

Sample

Quantity: 2 quarts Date Received: 7-26-74 CHF Sample No.: 37-418

Submitted By: R. V. Berthold Division: Chemicals and Plastics
South Charleston, WV

Charge No: 01067

Peroral, Single Dose to Rats

LD50 - 2.83 (2.05 to 3.89) ml/kg; undiluted.

Conditions - standard.

Dosage; ml/kg	Dead / Dosed	Days to Death	Weight Change	Signs and/or Symptoms
8.0	5/5	0,0,0,1,1	-	Sluggish 20 min, death 2.25 to 3 hr.
4.0	5/5	1,1,2,3,4	-	Sluggish 30 min.
2.0	0/5	-	77 to 111	Sluggish 40 min.

Gross Pathology - in victims, petechial hemorrhages in the lungs; livers and spleens mottled; stomachs distended, liquid-filled, pylori hemorrhaged; intestines distended, liquid-filled, hemorrhaged and yellow; kidneys mottled or speckled and slightly congested. Nothing remarkable in survivors.

Conclusions - moderately toxic following acute peroral intubation.

Skin Penetration, Single Dose to Rabbits

LD50 - 0.561(0.264 to 1.19) ml/kg; undiluted.

Conditions - standard. Dosed under polyethylene sheeting.

Dosage; ml/kg	Dead / Dosed	Days to Death	Weight Change	Skin Irritation	Signs and/or Symptoms
1.0	3/4	1,3,4	-144	necrosis	-
0.5	2/4	4,9	-403,-28	necorsis	-
0.25	0/4	-	-111,170, 227,275	necrosis	-

Gross Pathology - in victims, lungs congested; livers congested or mottled; kidneys pale. Nothing remarkable in survivors.

Conclusions - moderately toxic following acute covered dermal application.

Inhalation, Single, by Rats

Conditions - static exposure at 21°C. Procedure B of standard test procedures.

Procedure	Time	Concentration	Dead / Dosed	Death	Weight Change	Signs and/or Symptoms
B	8 hr	Substantially saturated vapor	0/6	-	58 to 83	-

Gross Pathology - Nothing remarkable.

Conclusions - no hazard is anticipated from the infrequent inhalation of substantially saturated vapor evolved at room temperature under normal handling conditions.

Skin Irritation, Rabbit, Uncovered

Conditions - standard.
Applied undiluted or in distilled water.

Conclusions - necrosis on 2 of 2 rabbits from the undiluted material; no irritation on 5 rabbits from 10% in distilled water. Grade 6.

Eye Irritation, Rabbit

Conditions - standard.
Instilled undiluted or in distilled water.

Conclusions - moderate corneal injury from 0.005 ml undiluted per eye; severe corneal injury, with iritis, from 0.5 ml per eye of 40% in distilled water; trace corneal injury from 15%. Grade 7.

Seven-Day Dietary Inclusion, Rats

Procedure

Triethylene tetramine was added to ground PURINA Chow and fed in the diet for 7 days. Groups of 5 male and 5 female Harlan-Wistar albino rats, 30 days of age at the start of the study, were randomly assigned to each dosage level and to a control level.

Results

The results are summarized in Table 39-1.

Inclusion of triethylene tetramine in the diet of rats for 7 days resulted in severe body weight depression (or significant weight loss in some cases) among rats at the highest dosage levels, 2.98 gm/kg/day for males and 2.63 gm/kg/day for females. At these levels, there was also depression of liver weight and liver weight as percentage of body weight. Kidney weight as percentage of body weight was significantly higher for both males and females as compared to control values. The kidney weight *per se* was somewhat depressed for the females as expected because the rats at this level did not gain a normal amount of weight.

At the middle dosage levels, 1.23 gm/kg/day for males and 1.38 gm/kg/day for females, kidney weight as percentage of body weight was increased. There was also an increase in kidney weight *per se* among the females, as well as a slight body weight depression observed only after the first day of the study. No significant body weight or organ weight effects were noted at the lowest dosage levels, 0.51 gm/kg/day for males and 0.47 gm/kg/day for females.

Conclusions

The maximum no significant ill-effect level was an average of 0.49 gm/kg/day based on body weight, liver weight and kidney weight. The ratio between the single peroral LD50 and the minimum effect level (MiE) for the 7-day feeding study was 2.83 gm/kg/1.30 gm/kg (average) or 2.18, probably indicating a low degree of chronicity. The median predicted minimum effect level for 90-day rat feeding is 0.43 gm/kg; that for 2 years is 0.24 gm/kg (Weil *et al.*, "Toxicology and Applied Pharmacology" 14, 426-431, 1969).

290

Roy C. Myers

Roy C. Myers, B.S.
Research Associate

Carrol S. Weil

Carrol S. Weil, M.A.
Senior Fellow

Approved:

Eugene F. Cox

Eugene F. Cox, Ph.D.
Director

Acknowledgments:

Skin Penetration, Irritation Tests	Naomi I. Condra, B.S. Fellow
Inhalation Studies	Daniel L. Geary, Jr., M.S. Junior Fellow
Single Peroral Test	Deborah S. Burton, B.A. Research Associate
Diet Preparation and Feeding	Murray D. Woodside, B.S. Fellow

Typed: April 14, 1976
acc

Table 39-1

Summary of Results of 7 Days of Inclusion of Triethylene Tetramine in the Diet of Rats

	Male Rats			
Dosage goal, gm/kg	3.35	1.35	0.55	0.00
Concentration in diet, %	2.81	1.16	0.47	0.00
Dosage attained, gm/kg/day	2.98	1.23	0.51	0.00
Diet consumed, gm/rat/day	12.0	16.0	16.7	16.1
Body weight change, gm				
1 day of doses	-2.8[c]	6.0	5.8	6.0
5 days of doses	4.0[c]	25.4	25.2	26.0
7 days of doses	6.8[c]	42.2	47.4	46.8
Liver weight, gm	4.77[c]	7.05	7.32	7.30
Liver wt as % of body wt	4.20[b]	4.67	4.72	4.83
Kidney weight, gm	1.42	1.70	1.55	1.53
Kidney wt as % of body wt	1.25[c]	1.13[c]	1.00	1.01
Mortality	0	0	0	0

	Female Rats			
Dosage goal, gm/kg	3.35	1.35	0.55	0.00
Concentration in diet, %	3.02	1.31	0.51	0.00
Dosage attained, gm/kg/day	2.63	1.38	0.47	0.00
Diet consumed, gm/rat/day	8.3	15.1	12.6	14.4
Body weight change, gm				
1 day of doses	-2.6[c]	2.2[a]	3.6	7.0
5 days of doses	1.0[c]	18.8	17.0	23.2
7 days of doses	-6.4[c]	35.6	33.4	39.2
Liver weight, gm	3.63[c]	7.29	6.77	6.82
Liver wt as % of body wt	3.82[c]	5.10	4.91	4.88
Kidney weight, gm	1.13[a]	1.61[a]	1.38	1.34
Kidney wt as % of body wt	1.19[c]	1.13[b]	1.01	1.00
Mortality	0	0	0	0

[a] $0.05 > P > 0.01$

[b] $0.01 > P > 0.001$

[c] $P < 0.001$

Standard Test Procedures

In all tests, the nonfasted animals are maintained on appropriate Wayne diets and water *ad lib* except during period of manipulation or confinement. Dosage levels differ by a factor of 2 in a geometric series. LD50s or LC50s are calculated by the moving average method based on a 14-day observation period.

Toxicity Terminology for Peroral and 24-Hr Dermal LD50s (A)/Inhalation 4-Hr LC50 (B)

	A, gm/kg	B, ppm		A, gm/kg	B, ppm
Extremely low order	> 15	> 100M	Highly	0.05-0.5	100-1M
Slightly	5-15	10M-100M	Seriously	0.01-0.05	10-100
Moderately	0.5-5	1M-10M	Dangerously	< 0.001	< 10

Peroral. Compounds administered by stomach intubation to Wistar derived male rats, 90-120 grams in weight and 3 to 4 weeks of age, reared in our own colony.

Skin Penetration. Male albino rabbits, 3 to 5 months of age, are immobilized during the 24-hour contact period with the compound retained under impervious sheeting on the clipped intact skin of the trunk. Thereafter, excess fluid is removed to prevent ingestion. Maximum dosage that can be retained is 16 to 20 ml/kg.

Inhalation. Procedure A. Concentrated vapor is generated in a gas washing bottle by passing dried air at 2.5 liters/min through a fritted glass disc immersed to a depth of at least 1-1/2 inches in the chemical which is delivered to rats in a 9-liter glass exposure chamber. Mean vapor concentration is calculated from the loss in weight of the liquid or estimated from the vapor pressure at the actual temperature of the chemical during aeration.

Procedure B. Substantially saturated vapor is prepared by spreading 50 grams of chemical over 200 cm^2 area on shallow tray placed near the top of a 120-liter glass chamber which is then sealed for at least 16 hours while an intermittently operated fan agitates the internal chamber atmosphere. Rats are then introduced in a gasketed drawer-type cage designed and operated to minimize vapor loss.

Procedure C. Mist, vapor and any oxidation or decomposition products of the chemical held at 170°C are generated and delivered as in A.

Procedure D. Vapor at metered concentration, not checked analytically, is generated by feeding the liquid at a constant rate down the inside of a spirally corrugated surface of a minimally heated one-inch Pyrex tube, through which metered air is passed. Resultant vapor is delivered as in A.

Procedure E. Spray - Solutions or suspensions are atomized in a glass VAPONEFRIN nebulizer using dried compressed air at 9 liters/min (corrected) and 22 psi. The resultant aerosol of droplets averaging 2 microns in diameter is conducted directly into a 60-liter cubic glass chamber containing rats. Mean aerosol concentration is calculated from the amount of material atomized.

Procedure F. Dust - Dust clouds are generated by a baffled Wright Dust Feed through which air is passed at 14 liters/min (uncorrected) at 5 psi. The dust is delivered directly to a 120-liter plexiglas chamber containing rats. Airborne dust concentrations are measured gravimetrically every half hour.

Skin Irritation. Chemical is applied in 0.01 ml amounts to clipped, uncovered intact skin of 5 rabbit bellies either undiluted or in progressive dilutions of 10, 1, 0.1, and 0.01% in solvent. Ten grades are recognized based on appearance of moderate or marked capillary injection, erythema, edema or necrosis within 24 hours. No injury from undiluted = Grade 1.

Eye Irritation. Eyes not staining with 5% fluorescein in 20 seconds contact are accepted. Single instillation of 0.005, 0.02, 0.10 or 0.5 ml undiluted or of 0.5 ml of 40, 15, 5 and 1% dilutions are made into conjunctival sac of 5 rabbits. Read immediately unstained and after fluorescein at 24 hours, with ten grades recognized. Trace or no injury from 0.5 ml undiluted = Grade 1.

Confidential

Report 11-67

R: 5-12-48

MELLON INSTITUTE OF INDUSTRIAL RESEARCH

UNIVERSITY OF PITTSBURGH

Number copies made 30

SPECIAL REPORT

Number copies mailed 15 by MRC

Date of mailing 5-21-48

on

Addressed to 4-F.W. Hale, 1-H.B. McGhee, 1-W.E. Reich, 1-B.D. Crane, 1-C.V. Dornehl, 1-R.B. Wellman, 1-F.R. Rayland, 1-E.W. Rugeley, 1-A.S. Kanaan, 1-L.D. Stanley, 1-C.H. Arnold, 1-D.C. Lewis

The Acute Toxicity of Triethylene Tetramine

Carbide and Carbon Chemicals Corporation Industrial Fellowship No. 274-11

Tables of Protocols Attached

Summary

Based upon the results of its acute oral toxicity for male albino rats, triethylene tetramine is a moderstely toxic compound with an LD_{50} of 4.34 (3.81 to 4.94) gm./kg. There is little difference between it and tetraethylene pentamine by the oral route; it is about 1/3 to 1/2 as toxic as diethylene triamine or ethylene diamine fed in a comparable concentration in water.

By skin penetration he LD_{50} for rabbits, using undiluted compound, is 0.82 (0.72 to 0.94) ml./kg. while 2.0 gm./kg. of a 10% aqueous dilution killed 4 of 10 rabbits dosed. The wide variation between the oral and skin penetration LD_{50}'s is due to (I) the use of diluted compound in the former test and undiluted in the latter, (2) to species difference, and (3) the fact that the compound once it has penetrated the skin can be absorbed directly into the blood stream without the intervention of the protective action of the liver.

Saturated vapors produced at room temperature were lethal to only 1 of 6 rats in an 8-hour exposure, and a mist produced by heating the compound to 170°C. while it was aerated, killed all of 6 rats in 8 hours, 5 of 6 in 4 hours, and none of 6 in a 2-hour exposure to the cooled effluent mist.

Its irritative action on the skin of the rabbit is equivalent to that of n-butyl amine. In contact with the cornea of the rabbit eye, damage about as severe as that produced by acetone results.

Sample

The sample was received 1-5-48 from Texas City bearing identification 550-A-2952.

Single Oral Doses

An LD_{50} of 4.34 (3.81 to 4.94) gm./kg. resulted from the oral administration of a 10% aqueous dilution of triethylene tetramine to male albino rats. Symptoms developing after the doses included sluggishness, prostration, and occasionally narcosis. Lung hemorrhage, and congestion of visceral organs were noted at autopsy of those that succumbed. Triethylene tetramine is the least toxic of the series for rats. Ethylene diamine (76%) has an LD_{50} of 2.43 gm./kg. or 1.85 gm./kg. on the basis of contained diamine,

diethylene triamine 2.33 and tetraethylene pentamine 3.99 gm./kg. for 10% aqueous dilutions of the compounds.

Skin Penetration

The application of undiluted tetramine to the clipped trunk of rabbits for 24 hours under "Vinylite" sheeting resulted in an LD_{50} of 0.82 (0.72 to 0.94) ml./kg. Severe skin damage resulted and visceral organs showed gross damage. There often was found an increase in peritoneal fluid, and the animals had watery feces. A dosage of 2.0 gm./kg. of a 10% dilution in water, the maximum dosage possible, killed 4 of 10 rabbits. The diluted material produced severe erythema of the skin but necrosis did not result as it did with the undiluted compound. Internal organs were also grossly damaged.

Inhalation

Substantially saturated vapors produced at room temperature killed one of six rats exposed for 8 hours. Death was due to a lung hemorrhage. The remainder gained weight normally in the 14-day observation period.

Inhalation of a cooled mist produced by heating the aerated compound in a bath at 170°C. for 8 hours killed all of 6 rats; 4 hours 5 of 6; and 2 hours none of six. Lung damage accounted for most of the deaths.

Irritation

The application of the undiluted compound to the clipped skin of the rabbit belly in the vesicant test produced necrosis. A 10% dilution in acetone produced erythema, capillary injection or no reaction. This places it in the same grade 6 as the other members of this series, comparable in activity to n-butyl amine.

Applied to the rabbit eye in 0.02 ml. amounts, this material caused severe corneal necrosis. Doses of 0.005 ml. produced severe damage in only one of 5 eyes. The compound is relegated to Grade 5 of our 10-grade rating system, also represented by acetone. This reaction is much less severe than that produced by ethylene diamine 76%, and diethylene triamine.

Charles P. Carpenter
SENIOR INDUSTRIAL FELLOW

Charles P. Carpenter

Typed: May 12, 1948 - met

Table 11-112

Triethylene Tetramine

Single Doses to Male Albino Rats by Mouth

Fed by Stomach Tube as a Dilution in Water, 1 ml. = 0.10 gm.

Rat Number	1948 Date Dosed	Grams Wt.	Weight Change in 14 Days	Dosage; Grams per Kilo	Dose in Grams	Dose in ml. of 10% Dilution	Days to Death
69134	2-17	90	-	6.30	0.568	5.7	1
69133	"	92	-	6.30	0.580	5.8	1
68069	"	102	-	6.30	0.645	6.5	1
68107	"	96	-	6.30	0.605	6.1	1
68109	"	98	-	6.30	0.617	6.2	1
68100	"	96	-	6.30	0.605	6.1	4
69154	"	96	-	6.30	0.605	6.1	0
69151	"	94	-	6.30	0.592	5.9	1
69565	2-24	92	-	6.30	0.580	5.8	8
69551	"	110	-	6.30	0.693	6.9	7
69554	2-24	106	-	5.00	0.530	5.3	0
69555	"	110	-	5.00	0.550	5.5	2
69556	"	104	-	5.00	0.520	5.2	0
69548	"	114	-	5.00	0.570	5.7	0
69549	"	98	-	5.00	0.490	4.9	3
69547	"	112	-	5.00	0.560	5.6	1
69557	"	100	-	5.00	0.500	5.0	0
69558	"	94	+ 28	5.00	0.470	4.7	-
69553	"	106	+ 44	5.00	0.530	5.3	-
69545	"	108	+ 60	5.00	0.540	5.4	-
67668	2-10	93	-	3.98	0.370	3.7	2
67583	"	98	-	3.98	0.390	3.9	1
69035	"	100	-	3.98	0.398	4.0	1
68123	"	90	+ 36	3.98	0.358	3.6	-
68171	"	108	+ 57	3.98	0.430	4.3	-
68126	"	90	+ 53	3.98	0.358	3.6	-
67598	"	91	+ 59	3.98	0.362	3.6	-
67599	"	101	+ 63	3.98	0.402	4.0	-
67664	"	103	+ 72	3.98	0.410	4.1	-
67585	"	93	+ 71	3.98	0.370	3.7	-
69723	2-26	90	-	3.16	0.284	2.8	1
69708	"	90	-	3.16	0.284	2.8	1
69561	2-24	100	+ 50	3.16	0.316	3.2	-
69712	2-26	90	+ 32	3.16	0.284	2.8	-
69714	"	93	+ 39	3.16	0.294	2.9	-
69715	"	90	+ 40	3.16	0.284	2.8	-
69717	"	94	+ 41	3.16	0.297	3.0	-
69728	"	90	+ 25	3.16	0.284	2.8	-
69704	"	90	+ 48	3.16	0.284	2.8	-
69710	"	91	+ 41	3.16	0.288	2.9	-

Table 11-113

Triethylene Tetramine

Single Doses to Male Albino Rabbits by Skin Absorption

Administered undiluted under "Vinylite" dam for 24 hours

Rabbit Number	1948 Date Clipped	1948 Date Applied	Grams Wt.	Weight Change in 14 Days	Dosage; Ml. per Kilo	Dose in ml.	Days to Death
70513	3-2	3-2	2570	-	1.00	2.6	2
70514	"	"	2650	-	1.00	2.6	2
70516	"	"	2412	-	1.00	2.4	4
70520	"	"	2376	-	1.00	2.4	2
70521	"	"	2671	-	1.00	2.7	3
70725	4-2	4-2	2798	-	1.00	2.8	3
70780	"	"	2276	-	1.00	2.3	3
70784	"	"	2516	-	1.00	2.5	3
70712	"	"	2380	+ 222	1.00	2.4	-
70755	"	"	2316	- 190	1.00	2.3	-
70745	3-30	3-30	2886	-	0.89	2.6	3
70811	"	"	2540	-	0.89	2.3	3
70814	"	"	2136	-	0.89	1.9	2
70818	"	"	2306	-	0.89	2.1	6
70820	"	"	2330	-	0.89	2.1	6
70825	"	"	2668	-	0.89	2.4	3
70826	"	"	2370	-	0.89	2.1	2
70813	"	"	2356	- 558	0.89	2.1	-
70817	"	"	2388	- 338	0.89	2.1	-
70822	"	"	2270	- 412	0.89	2.0	-
70705	3-9	3-9	2280	-	0.795	1.8	3
70737	3-22	3-23	2638	-	0.795	2.1	2
70747	3-23	"	2516	-	0.795	2.0	2
70698	3-8	3-9	2216	- 60	0.795	1.8	-
70701	"	"	2792	- 628	0.795	2.2	-
70703	3-9	3-9	2284	- 518	0.795	1.8	-
70704	"	"	2136	?	0.795	1.7	-
70736	3-22	3-23	2184	+ 38	0.795	1.7	-
70739	3-23	"	1952	+ 82	0.795	1.6	-
70746	"	"	2172	- 538	0.795	1.7	-
70871	4-7	4-7	2290	-	0.710	1.6	4
70870	"	"	2340	-	0.710	1.7	2
70891	"	"	2312	-	0.710	1.6	4
70892	"	"	2448	-	0.710	1.7	7
70895	"	"	2298	- 440	0.710	1.6	-
70886	"	"	2224	- 416	0.710	1.6	-
70887	"	"	2104	- 304	0.710	1.5	-
70888	"	"	2270	- 40	0.710	1.6	-
70889	"	"	2424	- 854	0.710	1.7	-
70893	"	"	2196	- 706	0.710	1.6	-

BUSHY RUN RESEARCH CENTER
R. D. 4, Mellon Road, Export, Pennsylvania 15632 Telephone (412) 327-1020

CONFIDENTIAL: Not to be released outside UCC without the written consent of the UCC-sponsoring Division HS&EA Manager.

Project Report 44-11
19 Pages
Tel: (412) 327-1020
March 4, 1981

Triethylenetetramine-Linear (TETA-Linear)

In Vitro Mutagenesis Studies: 3-Test Battery

Authors: R. S. Slesinski, P. J. Guzzie, W. C. Hengler, K. J. Wagner

Sponsor: Union Carbide Corporation

* * * * *

SUMMARY

TETA-linear was evaluated for potential mutagenic activity with a battery of three *in vitro* tests, which were: the Chinese Hamster Ovary (CHO) Mutation test, the Sister Chromatid Exchange (SCE) test and an assay for induction of Unscheduled DNA Synthesis (UDS) in rat liver cells. The results indicated that TETA-linear produced a statistically significant mutagenic effect in both the SCE test (without metabolic activation) and in the hepatocyte UDS test. The steep slope of the cytotoxicity response to TETA-linear resulted in an inconclusive result with the CHO mutation test. However, the pattern of positive responses in the other two tests (UDS and SCE) suggested that TETA-linear was a likely mutagenic agent for mammalian cells in the battery of tests performed.

RESULTS AND INTERPRETATION

Selection of Test Concentrations - Preliminary experiments were performed to select an appropriate range of test concentrations in which the maximum concentration would allow survival of approximately 10% of the treated cells. A maximum concentration of 80×10^{-2}% (by volume) was chosen for the highest dose-level and a total of six concentrations of TETA-linear were tested in subsequent mutation determinations because a steep dose response effect was suggested by the prescreening data.

CHO Mutation Test - TETA-linear did not produce a statistically significant increase in the frequency of mutations of CHO cells at any concentration between 80×10^{-2}% to 2.5×10^{-2}% (by volume) in tests with and without the incorporation of a liver S9 metabolic activation system. The lack of a dose-related effect of treatment on the induction of mutations indicated that TETA-linear was probably not active in producing gene mutations in CHO cells. However, the steep slope of the cytotoxic effect of the test agent upon cell survival prevented an unequivocal negative classification of TETA-linear because the possibility of a narrow effective dose range could not be excluded with the available data. The results were considered inconclusive and no definitive classification of TETA-linear as a mutagen or non-mutagen was possible for this test.

SCE Test - TETA-linear produced a statistically significant and dose-related effect on the frequency of SCE in CHO cells in tests without the incorporation of an S9 metabolic activation system and a highly significant effect at only the highest dose tested with metabolic activation. The increases of the SCE frequency were below the 2-fold level generally accepted as a positive effect and the cytotoxicity of TETA-linear may have contributed to the moderate increases in the dose range examined. Additional testing would be necessary to determine the reproducibility of the positive effects observed in tests with and without S9 activation, but the pattern of dose-related effects obtained without S9 indicated that TETA-linear should be considered to be an active agent in the induction of SCE in CHO cells.

UDS Test - TETA-linear produced statistically significant increases in the amount of UDS activity at three of six concentrations between $100 \times 10^{-2}\%$ to $0.1 \times 10^{-2}\%$ (by volume). Although the cytotoxicity of TETA-linear affected the test of the highest two dose levels, TETA-linear was considered to be active in producing primary DNA damage in the present test with the hepatocyte test system.

Comparative Mutagenicity - The pattern of responses produced by TETA-linear in the 3-test battery to determine potential mutagenicity indicated that TETA-linear was an active mutagenic agent in the *in vitro* tests employed. The cytotoxicity of the test sample was a complicating factor in demonstrating definitive dose-related responses characteristic of known mutagenic agents. Thus, additional testing would be required to test very narrow concentration ranges which may more clearly demonstrate dose-related effects. The pattern of positive responses in this study, tests on other samples of TETA in our laboratory and literature reports on positive effects of TETA (see reference section) are consistent with the conclusion that TETA-linear was an active mutagenic agent in the present study. However, testing in our laboratory of a sample of TETA which had been treated with sodium borohydride to attempt to reduce and minimize contamination by nitrosamines (BRRC Report 44-12) suggested that the level of mutagenic activity was decreased by this treatment. The possibility that nitrosamines in this sample of TETA may be responsible for the positive effects obtained cannot be excluded (or proven) with the available data.

SAMPLE

Quantity: 4 ounces

Submitted by: D. C. Best

Division: Specialty Chemicals and Plastics
Union Carbide Corporation
South Charleston, WV 25303

CHF Sample No.: 42-447

Date Received: November 12, 1979

Identification: 99CAG 84-2B
yellowish liquid

CAS#: 112-24-3

Triethylenetetramine-Linear (TETA-Linear)

In Vitro Mutagenesis Studies: 3-Test Battery

Sponsor: Union Carbide Corporation

* * * * *

OBJECTIVE

The purpose of this study was to evaluate the potential of TETA-linear to induce genetic damage in mammalian cells at the gene, chromosome and/or DNA (deoxyribonucleic acid) level of molecular organization. A battery of three *in vitro*, short-term tests which detect each of these genetic endpoints was employed to evaluate TETA-linear for potential mutagenic activity. A general description of the theoretical basis of these three tests is presented in Appendices I, II and III attached to the complete report.

SAMPLE CHARACTERISTICS

A typical, commercial sample of TETA-linear was received for testing on November 12, 1979. The physical and chemical information available from the Toxicology Data Bank or from "Material Safety Data Sheets" for this product are attached to this report as Appendix IV. Additional analytical data on the composition and purity of this sample were received from P. Umberger, Union Carbide Corporation and the pertinent data for TETA-linear are attached as Appendix V. Briefly, the sample contained 99.42% TETA and 0.58% of diaminoethylpiperazine.

METHODS

A description of the technical procedures used in the CHO test, the SCE test and the UDS assay are presented in greater detail in Appendices I, II and III, respectively (attached to the complete report). 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.

1. CHO Test (Detailed procedures in Appendix I): SOP #7.2.4A; 7.2.5, 7.2.11.

A. Dose Selection - Appropriate concentrations of TETA-linear for testing were determined by measurements of cytotoxicity to CHO cells of six concentrations tested both in the presence and absence of a liver S9 metabolic activation system. Selection of a maximum concentration for testing depended upon an estimate of a dose level which would permit survival of at least 10% of the treated cells. Sterile water (H_2O) was used as the solvent and solvent control.

To simplify tables and to allow comparisons between different tests, concentrations of TETA-linear in the following sections of the report are given in terms of volume percentages x 10^{-2} to eliminate zeros in the lower concentration values (eg. 0.001 % = 0.1 x 10^{-2}%).

B. Mutation - CHO cells were exposed for 5 hours to a minimum of five concentrations of TETA-linear both with and without the addition of an S9 metabolic activation system. The highest concentration of TETA-linear tested without S9 metabolic activation produced excessive cell killing and cells treated at this dose level could not be assessed for survival or mutation induction. Dilutions of TETA-linear for testing were prepared by either direct addition of various aliquots of the test agent into the cell culture media or by making sequential one-half dilutions of the stock solution for the maximum concentrations using sterile H_2O. The surviving fraction was determined at 20 to 24 hours after treatment and the mutant fraction was determined after a 7-day period to allow "expression" of the mutant phenotype. Only the data from the top five concentrations which allowed sufficient cell survival for assessment of survival and induction of mutants are usually presented in the tables. The percentage of cells surviving the treatment, the frequencies of mutant colonies and the number of mutants/10^6 viable cells are presented in tabular form.

2. SCE Test (Detailed procedures in Appendix II): 7.2.12A

Production of SCE's following exposure to various concentrations of TETA-linear was studied in CHO cells both with and without the incorporation of an S9 metabolic activation system. Selection of a maximum dose level which would permit survival of at least 50% of the treated cells was based on the prescreening test for cytotoxicity performed as part of the CHO Mutation test. Dilutions of TETA-linear for testing were prepared either by direct addition of various aliquots into the culture medium or by making sequential one-half dilutions of the stock solution for the maximum dose level using sterile H_2O. For determination of direct mutagenic action, CHO cells were exposed to TETA-linear 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 ug/ml in the growth medium during treatment and during the culture period following exposure. A total of 20 cells/dose level and 5 dose levels, tested either with or without metabolic activation, were examined. The number of SCE/cell, mean number of SCE/chromosome and the level of statistical significance of the increases above the concurrent solvent control values are presented in tabular form.

3. UDS Test (Detailed procedures in Appendix III): 7.2.6, 7.2.7, 7.2.8A, 7.2.9A

Production of primary DNA damage in rat liver cells (hepatocytes), was studied at a minimum of six dose levels which spanned a 1000-fold range of concentrations. Cells were treated with TETA-linear for 2 hours in

culture medium containing ^{3}H-thymidine, hydroxyurea and appropriate dilutions of TETA-linear prepared in DMSO. Determination of UDS activity was performed by analyses of incorporation of ^{3}H-thymidine into isolated hepatocyte nuclei or in DNA (precipitated from aliquots of the isolated nuclei) using a Searle Analytic Model 81 or Packard Model 2650 scintillation spectrometer. Data are presented in tabular form with an indication of the level of statistical significance above the concurrent solvent control values.

4. Controls - Positive, negative and solvent controls were tested concurrently with the test sample to assure the sensitivity of the test system and the concurrence of the results to previous test performance. For the CHO and SCE assays, dimethylnitrosamine (DMN) and ethylmethanesulfonate (EMS) were used as positive control agents to assure the sensitivity of the test system for detecting indirect and direct-acting mutagens, respectively. Deionized water, sterilized by membrane filtration and glass-distilled dimethylsulfoxide (DMSO) were used as the solvent and negative controls, respectively.

   In the UDS assay, DMN and 4-nitroquinoline oxide (4-NQO) were used as positive controls representing indirect- or direct-acting mutagens, respectively. DMSO was used as the solvent and the solvent control.

5. Metabolic Activation - S9 liver homogenate, prepared from Arochlor 1254-induced, Sprague-Dawley male rats, was purchased from Litton Bionetics. The S9 preparation used for the CHO test contained 40 mg/ml protein and had a benzo(a)pyrene hydroxylase (BPH) activity of 13.93 nmol of hydroxybenzpyrene/20 min/mg protein, (assayed by Litton). A concentration of 600 ug/ml of S9 protein was used.

   For the SCE test, another lot of S9 homogenate, containing 40 mg/ml protein and a BPH of 15 nmol hydroxybenzpyrene/20 min/mg protein (assayed by Litton); a final concentration of 120 ug/ml of S9 protein was used.

6. Statistical Analyses - Data from the SCE and UDS tests were analyzed by appropriate parametric tests following Standard Operating Procedures for statistical analyses at the Bushy Run Research Center. Data from the CHO test do not follow a normal distribution according to experience with historical controls. Thus, the Student's t-test was used after transformation of the mutation frequencies (MF) according to the method of Irr and Snee $(MF + 1)^{0.15}$ (Irr, J. D. and R. Snee, Proceedings of the Cold Spring Harbor-Banbury Conference, II (1979), 263-274).

   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. The degree of statistical significance is denoted by: a: $0.05 > p > 0.01$, b: $0.01 > p > 0.001$, or c: $p < 0.001$. No superscript (or NS) indicates $p > 0.05$.

7. Raw Data Storage - Copies of the final report, statistical analyses, analytical data and data used to prepare the final report are stored in the BRRC Archives. Slides are stored in the Genetic Toxicology slide storage area.

RESULTS

SECTION I - CHO MUTATION TEST - Triethylenetetramine-Linear (TETA-Linear)

A. Test Dates - Initiated: January 24, 1980
Completed: July 10, 1980

B. Selection of Test Concentration (Data not shown in tables)

CHO cells were exposed for five hours to concentrations of TETA-linear which spanned a concentration range from 100 x 10^{-2}% to 0.3 x 10^{-2}% by volume. The percentage of cells which survived the exposure, both in the presence and absence of an S9 metabolic activation system, was determined by counting the number of colonies produced by the survivors after a 5 to 7 day incubation period. A second method for determination of cytotoxicity was also used in which the inhibition of growth of the cells was measured following treatments over the same range of concentrations as tested in the colony determination method above. A concentration of 80 x 10^{-2}% was selected as the maximum concentration for testing with and without S9 activation. A total of six concentrations were tested in the later mutation tests because the prescreening results suggested the existence of a probable steep dose-response effect.

C. Determination of Mutation Induction

1. Survival (Cytotoxicity)

Table 1 presents the cytotoxicity data for CHO cells treated with TETA-linear in the presence and absence of a liver S9 metabolic activation system. A steep dose-response effect with the test agent was observed from the > 99% cell killing obtained with the top concentration (80 x 10^{-2}%) of TETA-linear, in tests without S9 activation, in comparison to the markedly lower cytotoxicity obtained at only one-half the top dose-level. The same concentration was not cytotoxic in tests with the addition of a metabolic activation system which was probably a result of the buffering of the alkaline test chemical by the relatively acidic liver S9 homogenate.

2. Mutation

Table 2 presents the data for induction of mutants by TETA-linear and control agents. TETA-linear did not produce a dose-related increase in the frequency of mutants/10^6 viable cells over the 16- to 32-fold range of concentrations tested either with or without the presence of an S9 metabolic activation system. No concentration of TETA-linear produced an increase in the mutation frequency which was statistically significant from the concurrent solvent control. Small numerical increases in the mutant

frequency obtained at some dose levels were not considered to be biologically significant because occasional increases of similar magnitude have been obtained in previous tests with solvent or negative controls. The results of this study were considered inconclusive because: 1) a cytotoxic concentration was not attained in the test with S9 metabolic activation and 2) the extremely steep cytotoxic response observed in the test without S9 did not result in the testing of a range of concentrations which produced a moderate degree of cell killing. The latest recommendations for testing suggest that a moderate cytotoxic response should be included and the possibility of a narrow effective dose range cannot be excluded in the results obtained in this test.

Mutation frequencies for the solvent controls for tests both with and without S9 activation were higher than obtained based on historical control values. However, these mutation frequencies were not considered sufficiently high to justify a repeat test because occasional increases in the solvent and negative controls have attained similar values. Statistically significant increases in the mutation frequencies were obtained for the DMN and EMS positive controls for both experiments and these values were within the expected range of values observed in historical control data.

D. Deviations from Standard Procedures

1. A concentration of 0.075% trypsin was used rather than 0.05% as stated in the concurrent SOP in order to improve cell dissociation.
2. Culture flasks were equilibrated with 10% CO_2 in air to attempt to minimize the alkaline effect of the test agent upon the cell culture media.
3. F12 medium was purchased from Kansas City Biological Co. rather than from Grand Island Biological Co.

E. Conclusions

TETA-linear was consistently inactive as a mutagenic agent for CHO cells when tested with and without the incorporation of an S9 metabolic activation system over a 16- to 32-fold range of concentrations. No statistically significant increase above the concurrent solvent control was produced at any dose level tested. However, the extremely sharp cytotoxic effects of this test chemical did not result in testing of dose levels with intermediate cytotoxicity following a routine test protocol and the negative results must be considered inconclusive.

SECTION II - SCE TEST - Triethylenetetramine-Linear (TETA-Linear)

A. Test Dates - Initiated: April 21, 1980
Completed: December 2, 1980

B. Selection of Test Concentrations

A maximum concentration of 40 x 10^{-2}% of TETA-linear was chosen as the top dose level for testing with and without S9 activation respectively, based on cytotoxicity data from the CHO mutation test. Higher concentrations were expected to produce delays in the mitotic cycle and to decrease the number of cells with SCE staining. A 32-fold range of TETA-linear concentrations from 40 x 10^{-2}% to 1.25 x 10^{-2}% was examined in SCE experiment #1 without S9 activation. A 16-fold range of concentrations of TETA-linear from 40 x 10^{-2}% to 2.5 x 10^{-2}% (by volume) was examined in SCE experiment #2 with S9 activation. The highest concentration (40 x 10^{-2}%) tested without S9 activation was cytotoxic to the CHO cells and prevented examination of SCE.

C. Determinations of SCE Induction

1. The data for SCE induction in CHO cells treated with various dose levels of TETA-linear or with positive, negative or solvent control agents without an S9 metabolic activation system are summarized in Table 3. A highly statistically significant increase in the SCE frequency was produced by two dose levels of TETA-linear tested for direct action in the absence of a metabolic activation system. The data suggested that TETA-linear produced a positive effect on the SCE frequency but none of the values attained a two-fold increase over the control (which has been proposed as one guideline for defining a positive effect). A linear regression analysis of the SCE data indicated that the results were significantly dose-related. Additional concentrations between 20 x 10^{-2}% and 40 x 10^{-2}% would have to be tested to verify the reproducibility of the results but the response of CHO cells to treatments with TETA-linear was considered a positive effect with the available data.

The number of SCE produced by the concurrent EMS positive control was highly statistically significant from the concurrent solvent control and these data indicated an appropriate sensitivity of the test system comparable to our historical positive control data. The numbers of SCE obtained with the solvent and negative 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 TETA-linear in the presence of an S9 metabolic activation system are presented in Table 4. Only one statistically significant increase in the SCE values was observed at the highest tested concentrations of TETA-linear. However, there was no definitive evidence for dose-response effect of exposure to the test agent. The production of a highly statistically significant effect at the highest dose level should be investigated with additional tests at the same and higher concentrations to determine the reproducibility of the data. The extremely steep cytotoxicity responses following treatments with the test chemical did not allow the exclusion of the possibility of a narrow range of effective concentrations. The results of this test must be considered inconclusive, but suggestive of a positive mutagenic effect of exposure to TETA-linear. The positive results obtained in the SCE test without metabolic activation indicated that the single positive result was probably a significant effect without additional testing.

The SCE values for the solvent control in the test with S9 activation were in an acceptable range of variability as encountered in previous experiments with this test system. The SCE values for the DMSO (negative control were low and outside the range of usual values from historical control) Highly statistically significant numbers of SCE were produced by the DMN positive control tested which indicated that the metabolic activation system was suitably active.

D. Deviations from Standard Procedures - Several minor changes in the procedure for this test were employed to attempt to improve the sensitivity and reproducibility of SCE staining. These changes are deviations from the concurrent SOP, but which will become part of the revised routine procedures:

1. Medium was purchased from Kansas City Biological Co. rather than from Grand Island Biological Co. as stated in the SOP.
2. A concentration of 0.075% trypsin rather than 0.05% was used to improve cell dissociation.
3. Culture flasks were equilibrated with 10% CO_2 in air to attempt to minimize the alkaline effect upon of the medium by the test agent.
4. Flasks were inoculated with 9×10^5 cells approximately 38 hours prior to treatment to improve the mitotic index.
5. Harvest of cells for chromosome preparation was extended to 33 hours from the time of treatment to improve the mitotic index.
6. A concentration of 0.05 ug/ml of Colchicine was added at 1 hour prior to harvest to increase the mitotic index in the experiment without S9 Colcemid® (0.1 ug/ml) was used for this purpose in the experiment with S9.
7. Staining of chromosomes for SCE in the experiment without S9 used 25 ug/ml of the Hoechst® stain to improve chromatid differentiation.

E. Conclusions

TETA-linear produced statistically significant effects on the SCE frequency at two of five concentrations tested without metabolic activation and at only the highest dose level in tests with metabolic activation. Linear-regression analyses indicated that the effects observed in the test without metabolic activation were significantly dose-related. TETA-linear was considered a positive mutagenic agent in the test without activation and the test with metabolic activation was considered inconclusive.

SECTION III - UDS TEST - Triethylenetetramine-Linear (TETA-Linear)

A. Test Dates - Initiated: May 7, 1980
Completed: July 7, 1980

B. Selection of Test Concentrations

Standard procedures were followed and TETA-linear was tested over a 3-log range of concentrations from 100×10^{-2}% to 0.1×10^{-2}% by volume. The maximum dose-level was selected with consideration of the cytotoxicity data obtained in the CHO Mutation test which indicated that higher values would result in excessive cell killing. These dose-levels were considered

to be appropriate for testing because uptake and incorporation of ^{3}H-thymidine into hepatocytes are generally even more sensitive to chemical effects than survival measurements with CHO cells (in which cytotoxicity is measured after a 24 hour recovery period following treatment).

C. Determination of UDS Induction

1. Nuclear-Bound Radioactive Label (Data in Table 5)

Values of "unscheduled" incorporation of ^{3}H-thymidine into nuclei of hepatocytes exposed to TETA-linear or to appropriate positive and negative controls are presented in Table 5. In hepatocytes treated with TETA-linear, three concentrations tested for potential activity induced a highly statistically significant increase in the amount of ^{3}H-thymidine incorporation. The effect on UDS attained a maximal level at the 1×10^{-2}% concentration and declined at higher concentrations; this result was interpreted as a cytotoxic effect of the test agent. In contrast to the data from the SCE and CHO test (where higher concentrations may have shown greater activity) these results suggest that additional, lower concentrations should have been tested to better demonstrate dose-related effects. The available results in this test indicated that TETA-linear produced a positive effect on DNA damage in rat hepatocytes and the test was considered an indication of positive mutagenic activity.

Both of the positive control agents, NQO and DMN, induced numerically elevated and statistically significant increases in UDS over values obtained with the solvent control. With NQO, an inverse dose-response effect was observed which was considered an indication of the cytotoxicity of this alkaline test agent.

2. DNA-Bound Radioactive Label (Data in Table 6)

Analyses of DNA, from aliquots of hepatocyte nuclei used for the UDS studies presented on Table 5, were performed as a second assessment of "unscheduled" incorporation of radioactive thymidine. Values for radioactivity incorporated into the DNA of these hepatocyte nuclei are presented in Table 6.

For hepatocytes treated with TETA-linear, three of the six test concentrations induced levels of UDS which were highly statistically significant from the solvent control. However, we did not observe a definitive dose-related effect upon ^{3}H-thymidine incorporation following treatments with TETA-linear and the increase in the levels of UDS attained a peak at the 1×10^{-2}% concentration. The results of ^{3}H-thymidine incorporation into DNA were consistent with the data obtained in the assessment of UDS measured using nuclei from cells treated with the same range of concentrations. TETA-linear was considered to be active in the induction of DNA damage discernible by UDS activity in the present study.

The pattern of UDS activity produced by varying concentrations of the positive control agents NQO and DMN were similar and consistent with the data obtained in the assessment of ^{3}H-thymidine incorporated into nuclei presented in Table 5. Again, all doses of the positive control agents produced a numerical elevation in the amount of UDS, but the highest concentration of DMN was not statistically significant from the concurrent control, probably resulting from cytotoxic effects of exposure.

D. Deviations from Standard Procedures - None. "Flocculence" in harvest tubes during preparation of nuclei prompted refrigeration for one week in the lysing solution to attempt to alleviate this problem.

E. Conclusion

TETA-linear stimulated a highly statistically significant increase in the incorporation of radioactive thymidine in hepatocytes at three of six dose levels spanning a 1000-fold range of test concentrations. TETA-linear was considered active in producing primary DNA damage in the present test with the hepatocyte test system.

Reviewed and Approved by:

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

Elton R. Hobson, Ph.D.
Associate Director, Toxicology

Fred R. Frank, Ph.D.
Director

Contributors:

Chinese Hamster Ovary test	Peggy J. Guzzie, B.S. Master Technologist
Sister Chromatid Exchange test	Michelle W. Gaunt, B.S. Master Technologist
	Peggy J. Guzzie, B.S. Master Technologist
	Kathy J. Wagner, B.S. Master Technologist
Unscheduled DNA Synthesis Assay	W. Christopher Hengler, M.S. Assistant Scientist

WPC/1128-3