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Chemical Category	CARBAMODITHIOLIC ACID, DIMETHYL-, SODIUM SALT; 1,3-BUTADIENE		

OFFICE OF TOXIC SUBSTANCES
CODING FORM FOR GLOBAL INDEXING

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EXXON CHEMICAL COMPANY



8EHQ - 0698 - 14115

Safety and Environmental Affairs Department
David J. Johnson
MANAGER, SAFETY PROGRAMS

IDLN: 8898000084

June 12, 1998

Document Control Office (7407)
U. S. Environmental Protection Agency
ATTN: TSCA Section 8(e) Coordinator
Office of Pollution Prevention and Toxics
401 M Street, S. W.
Washington, D. C. 20460

Contains No CBI

Re: TSCA Section 8(e) Document Control Number 8EHQ-98-14115

Dear Sir or Madam:

On January 29, 1998, Exxon Chemical Company submitted a notification of substantial risk under the provisions of TSCA Section 8(e). The initial submission (TSCA Section 8(e) Document Control Number 8EHQ-98-14115) described the results of a bone marrow micronucleus assay in mice on a substance identified as Carbamodithioc acid, dimethyl-, sodium salt (CAS Registry Number 128-04-1). The purpose of this submission is to complete the record on this study by providing a copy of the final report.

The results from this study were reported in summary form in the submission referenced above. Consequently, a summary of results has not been provided with this submission, the purpose of which is only to provide a copy of the final report.

If you have any questions or need additional information, please feel free to contact me on (281) 870-6874.

Sincerely yours,

Steven G. Hentges



8EHQ-98-14115

SGH/jad
Enclosure

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EXXON BIOMEDICAL SCIENCES, INC.

FINAL REPORT

PROJECT NUMBER: 112530

**TEST SUBSTANCE:
DIMETHYLDITHIOCARBAMIC ACID SODIUM SALT (MRD-97-125),
1,3-BUTADIENE (MRD-97-126)**

***IN VIVO* MAMMALIAN BONE MARROW AND BLOOD
MICRONUCLEUS ASSAY-
DERMAL AND INHALATION EXPOSURE**

PERFORMED FOR:

**EXXON CHEMICAL AMERICAS
13501 Katy Freeway
Houston, Texas 77079-1398**

PERFORMED AT:

**EXXON BIOMEDICAL SCIENCES, INC. (EBSI)
Toxicology Laboratory
Mettlers Road
CN 2350
East Millstone, New Jersey 08875-2350**

COMPLETION DATE: April 30, 1998

**IN VIVO MAMMALIAN MICRONUCLEUS ASSAY - DERMAL AND INHALATION EXPOSURE
WITH MDC & BUTADIENE, 112530**

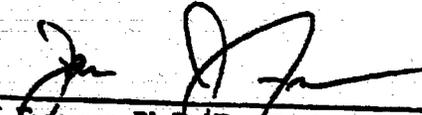
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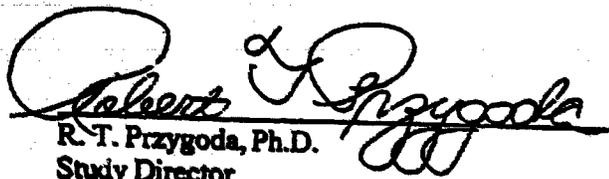
**IN VIVO MAMMALLY MICRONUCLEUS ASSAY - DERMAL AND INHALATION EXPOSURE
WITH DMDC & BUTADIENE: 112530**

APPROVAL SIGNATURES



J. J. Freeman, Ph.D., D.A.B.T.
Director: Laboratory Operations

30 Apr 98
Date



R. T. Przygoda, Ph.D.
Study Director

30 Apr 98
Date

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**IN VIVO MAMMALIAN MICRONUCLEUS ASSAY - DERMAL AND INHALATION EXPOSURE
WITH DMOTC & BUTADIENE: 112530**

**EXXON BIOMEDICAL SCIENCES, INC.
METTLERS ROAD
CN 2350
EAST MILLSTONE, NEW JERSEY 08875-2350**

QUALITY ASSURANCE STATEMENT

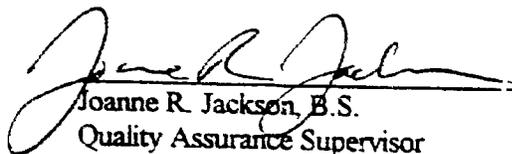
NON-REGULATORY STUDY

STUDY NUMBER: 112530

TEST SUBSTANCE/ARTICLE: MRD-97-125 & MRD-97-126

STUDY SPONSOR: Exxon Chemical Americas

All QA audits (including this final report) have been processed.


Joanne R. Jackson, B.S.
Quality Assurance Supervisor

30 Apr 98
Date

**IN VIVO MAMMALIAN MICRONUCLEUS ASSAY - DERMAL AND INHALATION EXPOSURE
WITH DMPYC & BUTADIENE: 112530**

PERSONNEL

Study Director:	R. T. Przygoda, Ph.D.
Sponsor Representative:	S. Frick-Miranda, B.S.
Sponsor:	Exxon Chemical Americas 13501 Katy Freeway Houston, Texas 77079-1398
Director: Laboratory Operations:	J. J. Freeman, Ph.D., D.A.B.T.
Inhalation Toxicology Supervisor:	F. T. Whitman, B.A.
Report Preparation Supervisor:	E. R. Frank, B.A.
Genetic Toxicology/Compound Preparation Supervisor:	M. A. Elliott, B.S.
Quality Assurance/Archives Supervisor:	J. R. Jackson, B.S.
Animal Care Supervisor:	R. C. Forgash, B.S.
Veterinarian:	R. L. Harris, D.V.M.
Maintenance Supervisor:	J. L. McGrath, A.S.

**IN VIVO MAMMALIAN MICRONUCLEUS ASSAY - DERMAL AND INHALATION EXPOSURE
WITH DMDTC & BUTADIENE: 112530**

SUMMARY

This study was conducted to evaluate the potential of dimethyldithiocarbamic acid sodium salt, DMDTC, (MRD-97-125) to induce micronuclei in polychromatic erythrocytes (PCE's) from the bone marrow and blood of B₆C₃F₁ mice. Also, this study evaluated the potential of DMDTC to effect the induction of micronuclei by 1,3-butadiene, BD, (MRD-97-125). This damage may be the result of chromosomal aberrations or damage to the mitotic apparatus.

Eight different groups of 5 male and 5 female mice received dermal exposures to DMDTC (300 mg/kg), inhalation exposures to BD (10 or 200 ppm; 22 mg/m³ or 442 mg/m³, respectively), or co-exposures to both materials. Two additional negative control groups (5/sex/group) were exposed to either air or water (carrier controls). The group designations and their exposure regimens are listed on page 18.

Clinical observations were recorded daily prior to exposure. Animals were weighed once prior to dosing, on Day 1 for Groups 1, 2, and 6 through 9, on Day 5 for Groups 6 through 9, and on the day of their scheduled sacrifice. Animals were sacrificed on Day 6 (Groups 1 through 5) or Day 9 (Groups 6 through 10), and both femurs were immediately removed and processed. Blood samples were collected from the Vena Cava and were prepared for flow cytometry analysis. Smears from bone marrow were prepared (2 slides per animal) and stained with Acridine Orange. For bone marrow samples, the percentage of polychromatic erythrocytes (PCEs) in the total population of erythrocytes was determined by counting 1000 PCEs and nonchromatic erythrocytes (NCEs). Two thousand PCEs from each animal were examined for the presence of micronuclei (MNEs). For blood samples, the percentage of PCEs (reticulocytes) in the total population of erythrocytes was determined by counting 100,000 erythrocytes. Ten thousand PCEs from each animal were examined for the presence of micronuclei (MNEs). Liver and spleen weights were collected on all animals at study termination.

All animals survived to scheduled study termination and there were no adverse effects with respect to clinical signs or body weights.

For both blood and bone marrow, butadiene at 200 ppm induced cytotoxic effects and DMDTC induced evidence of hypererythropoiesis. DMDTC reduced the cytotoxic effects of BD when administered either before or after BD treatment.

Butadiene did induce a statistically significant increase in micronucleated PCEs. DMDTC did not induce any increases in micronucleated PCEs. DMDTC inhibited the induction of micronucleated PCEs by BD.

There were statistically significant increases in mean absolute (17%) and relative (18%) liver weights of the DMDTC/BD high treated male mice compared with controls. In addition, there were statistically significant increases in mean absolute (28%) and relative (22%) spleen weight of the DMDTC treated female mice compared with controls. These findings were considered treatment-related.

**IN VIVO MAMMALIAN MICRONUCLEUS ASSAY - DERMAL AND INHALATION EXPOSURE
WITH DMDTC & BUTADIENE: 112530**

SUMMARY (CONT'D)

In conclusion, BD was considered cytotoxic and clastogenic in mouse polychromatic erythrocytes in both blood and bone marrow. DMDTC was considered to induce hypererythropoiesis. These results indicate that DMDTC penetrated the mouse skin and produced an effect on the bone marrow erythropoiesis. It was not considered clastogenic in mouse polychromatic erythrocytes. DMDTC inhibited both cytotoxicity and clastogenicity of BD in mouse polychromatic erythrocytes.

**IN VIVO MAMMALIAN MICRONUCLEUS ASSAY - DERMAL AND INHALATION EXPOSURE
WITH DMDTC & BUTADIENE: 112530**

INTRODUCTION

This study was conducted for Exxon Chemicals Americas, 13501 Katy Freeway, Houston, Texas 77079-1398 (subsequently referred to as the Sponsor).

The study was conducted by Exxon Biomedical Sciences, Inc. (EBSI), Toxicology Laboratory, Meriters Road, CN 2350, East Millstone, N.J. 08875-2350. The EBSI Toxicology Laboratory is an American Association for Accreditation of Laboratory Animal Care (AAALAC) accredited facility and a Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF) certified facility.

Study Initiation (Protocol Signature Date)

November 18, 1997

Inlife Test Period

November 30, 1997 to December 8, 1997

Justification for Selection of Test System

This assay was designed to determine the capacity of a test substance to induce cytogenetic abnormalities in mammals by analysis of bone marrow and blood cells of treated animals for the presence of elevated levels of micronucleated PCEs (Schmid, 1975; Schmid, 1976; Kliesch et al., 1981).

Justification of Dosing Method

Dermal application of DMDTC is the route of test substance administration, which would most closely resemble human exposure. Dermal administration of a similar material was demonstrated to have significant immunological effects in mice (Pruett et al., 1992). Inhalation exposure is the route of test substance administration for butadiene. The literature has shown this route to be effective in the detection of certain clastogenic agents (Choy et al., 1986; Cunningham et al., 1986; Leavens et al., 1997).

**IN VIVO MAMMALIAN MICRONUCLEUS ASSAY - DERMAL AND INHALATION EXPOSURE
WITH DMDTC & BUTADIENE: 112538**

INTRODUCTION (CONT'D)

Justification of Dose / Exposure

Prior to the start of the test, a rangefinding study was performed. DMDTC rangefinding doses of 50, 100, 200, and 300 mg/kg/day were based on published data (Pruet et. al., 1992). Since no dose elicited signs of toxicity, 300 mg/kg of DMDTC was selected for the main study.

For butadiene, the high dose of 200 ppm was based on published data (Leavens et. al., 1997). It was expected to induce micronuclei in polychromatic erythrocytes from the bone marrow. This dose was confirmed by a rangefinding study.

Compliance

This study was conducted in compliance with the following standards:

OECD, Organization for Economic Cooperation and Development, Principles of Good Laboratory Practice, C(81)30 Annex 2, 1981.

This study was conducted in general agreement with the following guidelines and standards:

Animal Welfare Act of 1966 (P.L. 89-544), as amended in 1970, 1976, and 1985. Code of Federal Regulations, Title 9 [Animals and Animal Products], Subchapter A-Animal Welfare Parts 1, 2, and 3.

Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, National Academy Press, Washington, D.C., 1996.

OECD, Organization for Economic Cooperation and Development, Guidelines for Testing of Chemicals, Test Guideline 474, 1983.

IN VIVO MAMMALIAN MICRONUCLEUS ASSAY - DERMAL AND INHALATION EXPOSURE
WITH DMDTC & BUTADIENE: 112530

MATERIALS AND METHODS

TEST MATERIAL

Material Identification

EBSI Identification: MRD-97-125
Sponsor Identification: dimethyldithiocarbamic acid, sodium salt (DMDTC)

Supplier: Aldrich Chemical
Date Received: October 22, 1997
Expiration Date: October 2002
Description: White powder
Storage Condition: Room temperature

EBSI Identification: MRD-97-126
Sponsor Identification: 1,3-butadiene (BD)

Supplier: Scott Specialty Gases
Date Received: November 18, 1997
Expiration Date: November 1998
Description: Gas
Storage Condition: Room temperature

EBSI Identification: Acetone
Sponsor Identification: Acetone

Supplier: EM Science
Date Received: October 8, 1996
Expiration Date: August 1998
Description: Liquid
Storage Condition: Room temperature

Reverse osmosis water from dispensary tap was used as the carrier for MRD-97-125.

Dilutions of the test substance were not adjusted to correct for the purity of the test substance. The test substance, as received, was considered the "pure" substance

**IN VIVO MAMMALIAN MICRONUCLEUS ASSAY - DERMAL AND INHALATION EXPOSURE
WITH DMDTC & BUTADIENE: 112539**

TEST MATERIAL (CONT'D)

Characterization of Test Material

Analysis for the stability, identity, strength, purity and composition or other characteristics which appropriately identify the test substance are documented by the supplier. It is unknown if this characterization was performed in a GLP (Good Laboratory Practice Standards) compliant manner. This is a deviation from the GLPs.

Analysis of Test Substance Carrier Mixtures

Analysis of test substance mixtures for DMDTC was not performed. This is a deviation from the GLPs. Analytical exposure concentrations of butadiene were determined approximately hourly during each exposure by a photoionization detector.

Characterization of Positive Control Substance

For purposes of this study, 200 ppm of 1,3-butadiene will be considered the positive control group. The stability, identity, strength, and composition or other characteristics, which will appropriately identify the positive control substance, are documented by the supplier. It is unknown if this characterization was performed in a GLP compliant manner. This is a deviation from the GLPs.

Carrier

Water (for DMDTC)
Air (for Butadiene)

Vehicle

None.

Pretreatment Agent

For dermal applications, acetone was used to disrupt surface tension of the dosing solution and to prevent loss of test substance from the dorsal surface.

***IN VIVO* MAMMALIAN MICRONUCLEUS ASSAY - DERMAL AND INHALATION EXPOSURE
WITH DMDTC & BUTADIENE: 112530**

TEST MATERIAL (CONT'D)

Solubility

DMDTC is soluble in water at the concentration required for this study.

Sample Retention

Archival retention samples of neat substance MRD-97-125 was taken by the Compound Preparation Department and was stored in the EBSI Archives. No sample of test substance MRD-97-126 was taken due to the practical and safety considerations of storing a mixed gas/liquid phase material under pressure.

pH Determination:

pH = 11

A 30% (w/v) mixture (to simulate a dosing mixture to be used) of test material in reverse osmosis (RO) water was prepared by weighing 1.2 grams of MRD-97-125 and bringing the level to 5 mL using RO water. The test material/water mixture was shaken by hand in a closed jar and the pH was measured by immersing a ColorpHast pH 0-14 paper strip. The pH was again performed using a pH Tester 2 and was determined to be 10.3.

IN VIVO MAMMALIAN MICRONUCLEUS ASSAY - DERMAL AND INHALATION EXPOSURE WITH DMDTC & BUTADIENE: 112530

TEST SYSTEM

Test Animal

Species:	Mouse
Strain/stock:	B ₆ C ₃ F ₁
Supplier:	Charles River Laboratories Inc. Raleigh, N.C.

Animal Receipt Information

Receipt Date:	November 13, 1997
Purchase Order Number:	97GWT1141

Quarantine and Acclimation Period

17 days; animals were examined for viability at least once daily.

Number and Sex

50 males and 50 females per group (5/sex/group)

Age at Initiation of Dosing

Approximately 8 weeks

Animal Identification

Ear tags and corresponding cage identification.

Selection

More animals than required for the conduct of the study were purchased and acclimated. Animals determined to be unsuitable for inclusion on this study because of poor health, outlying body weight, or other abnormalities were excluded from the selection by the Study Director, attending veterinarian, and/or technical staff. Study animals were selected from the remaining animals using a computer-generated body weight sorting program. Weight variation for individual animals was within 20% of the mean body weight of their sex.

**IN VIVO MAMMALIAN MICRONUCLEUS ASSAY - DERMAL AND INHALATION EXPOSURE
WITH DMDTC & BUTADIENE: 112530**

TEST SYSTEM (CONT'D)

Housing

Room: 505, 513
Housing: Single housed during the test period.
Caging: Suspended stainless steel and wire mesh with absorbent paper below cages.

Feed

PMI Certified Rodent Diet 5002, ad libitum during non-exposure periods.
Animals were without food while in chambers.

Manufacturer: PMI Feeds, Inc., Richmond, Indiana.
Analysis: Performed and provided by PMI Feeds, Inc. Copies of the feed analyses are maintained in the EBSI Toxicology Laboratory.
Contaminants: There were no known contaminants in the feed believed to have been present at levels that may have interfered with this study.

The availability of feed was checked at least once daily for all animals.

Water

Automatic Watering System. ad libitum during non-exposure periods.
Animals were without water while in chambers.

Supplier: Elizabethtown Water Company
Analysis: Periodic analysis will be performed by EBSI and will be maintained in the EBSI Toxicology Laboratory.
Contaminants: There were no known contaminants in the water believed to have been present at levels that may have interfered with this study.

The availability of water was checked at least once daily for all animals.

**IN VIVO MAMMALIAN MICRONUCLEUS ASSAY - DERMAL AND INHALATION EXPOSURE
WITH DMDTC & BUTADIENE: 112538**

TEST SYSTEM (CONT'D)

Animal Room: Environmental Conditions

Temperature: 64 to 72 degrees Fahrenheit
Humidity: 30 to 70 percent relative humidity
Lighting: Approximately 12 hours light (0700 to 1900 hours) and 12
hours dark (1900 to 0700 hours) by automatic timer.

Monitored continuously.

Experimental Chamber: Environmental Conditions

Temperature: 68 to 75 degrees Fahrenheit
Humidity: 40 to 69 percent relative humidity

Chamber monitored continuously and recorded every 30 minutes during exposure.

**IN VIVO MAMMALIAN MICRONUCLEUS ASSAY - DERMAL AND INHALATION EXPOSURE
WITH DMDTC & BUTADIENE: 112579**

EXPERIMENTAL DESIGN

Preparation of Test Material

DMDTC was diluted with water and was administered at a constant volume of 1.0 mL/kg. Fresh dosing mixtures were prepared daily. Undiluted BD was administered as received by inhalation exposure.

Preparation of Animals

During the week prior to test substance administration for dermal exposure groups, the upper dorsal region of the appropriate mice was clipped. This procedure was repeated as needed during the study. To disrupt surface tension of the dosing solution and to prevent loss of the test substance from the dorsal surface, 20 μ L of acetone was applied to the shaved skin prior to the administration of DMDTC or water.

Experimental Dose Groups

DOSE GROUP	TEST MATERIAL
1	DMDTC [ⓐ] /BD Low [ⓑ]
2	DMDTC [ⓐ] /BD High [ⓑ]
3*	Air
4	BD Low [ⓑ]
5+	BD High [ⓑ]
6	BD Low [ⓑ] /DMDTC [ⓐ]
7	BD High [ⓑ] /DMDTC [ⓐ]
8	DMDTC [ⓐ]
9*	Water
10	BD High [ⓑ]

* - Negative control group

+ - Positive control group

- ⓐ - DMDTC = 300 mg/kg
- ⓑ - Low BD = 22 mg/m³ (10 ppm)
- ⓑ - High BD = 442 mg/m³ (200 ppm)

EXPERIMENTAL DESIGN (CONT'D)

Administration of Test Substance

Eight different groups of 10 mice each (5 male, 5 female) received dermal exposures to DMDTC, inhalation exposures to butadiene (10 or 200 ppm), or co-exposures to both materials. Two additional negative control groups were exposed to either air only or water (carrier control).

DMDTC mixtures and/or the carrier (water) control were administered once a day for 4 consecutive days applied dermally at similar times each day (± 2.5 hours). To disrupt surface tension of the dosing solution and to prevent loss of test substance, 20 μ L of acetone was applied to the shaved skin prior to dosing. When a group was administered both DMDTC and butadiene on the same day, DMDTC was applied prior to the inhalation exposure to butadiene.

The animals scheduled for inhalation exposure to butadiene were placed into whole-body inhalation chambers operated under dynamic conditions. The test material was administered in the breathing air of the animals as a gas. The exposure period was 6 hours/day plus time for chamber equilibration (theoretical T99 = 23 minutes).

The chambers used for exposure had a total volume of approximately 1000 liters. They operated at a flow rate (approximately 12 air changes per hour) sufficient to ensure timely equilibration and adequate oxygen content. Chamber airflow, temperature, and relative humidity were monitored continuously using a calibrated flow measuring device and recorded approximately every 30 minutes. All chambers were maintained at a slight negative pressure.

Analytical air concentrations of butadiene were determined approximately every hour during each exposure using a Toxi Rae photoionization detector Model PGM-30 by placing the unit completely into the chamber and sampling the test atmosphere.

**IN VIVO MAMMALIAN MICRONUCLEUS ASSAY - DERMAL AND INHALATION EXPOSURE
WITH DMDTC & BUTADIENE: 112530**

EXPERIMENTAL DESIGN (CONT'D)

Administration of Test Substance (cont'd)

Group	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
1	DMDTC	DMDTC	DMDTC	DMDTC + BD low	BD low	Sacrifice			
2	DMDTC	DMDTC	DMDTC	DMDTC + BD high	BD high	Sacrifice			
3*				Air	Air	Sacrifice			
4				BD low	BD low	Sacrifice			
5+				BD high	BD high	Sacrifice			
6				BD low	BD low + DMDTC	DMDTC	DMDTC	DMDTC	Sacrifice
7				BD high	BD high + DMDTC	DMDTC	DMDTC	DMDTC	Sacrifice
8					DMDTC	DMDTC	DMDTC	DMDTC	Sacrifice
9*					Water	Water	Water	Water	Sacrifice
10				BD high	BD high				Sacrifice

Notes: * - Negative control group
 + - Positive control group
 DMDTC - Dimethyldithiocarbamic acid, sodium salt - 300 mg/kg
 BD low - 1,3-butadiene - 10 ppm
 BD high - 1,3-butadiene - 200 ppm

IN VIVO MAMMALIAN MICRONUCLEUS ASSAY - DERMAL AND INHALATION EXPOSURE WITH DMDTC & BUTADIENE: 112539

EXPERIMENTAL DESIGN (CONT'D)

Dose Initiation

Group 1 and 2	November 30, 1997 (Day 1)
Group 3, 4, 5, 6, 7, and 10	December 3, 1997 (Day 4)
Group 8 and 9	December 4, 1997 (Day 5)

Dose Termination

Group 1, 2, 3, 4, 5, and 10	December 4, 1997 (Day 5)
Group 6, 7, 8, and 9	December 7, 1997 (Day 8)

Sacrifice

Group 1, 2, 3, 4, and 5	December 5, 1997 (Day 6)
Group 6, 7, 8, 9, and 10	December 8, 1997 (Day 9)

Experimental Evaluation

The animals were checked once daily for viability Monday through Friday and once daily on Saturdays and Sundays.

The animals were observed at least daily for signs of toxicity (before exposure and on the day of sacrifice). The observations included the nature, onset, severity, and duration of these effects.

Animals were weighed once prior to dosing, on Day 1 for Groups 1, 2, and 6 through 9, on Day 5 for Groups 6 through 9, and on the day of their scheduled sacrifice.

Necropsy

One day following the last test substance administration, animals were sacrificed by CO₂ asphyxiation and exsanguination. Both femurs were removed and blood samples were collected from the Vena Cava. Livers and spleens were weighed. Carcasses were discarded without further examination.

EXPERIMENTAL DESIGN (CONT'D)

Necropsy (cont'd)

The bone marrow was aspirated from the femur, pooled, flushed in 30% fetal bovine serum in 1% sodium citrate and centrifuged. After decanting the supernatant, the resulting cell pellet was resuspended in the remaining supernatant. Smears, 2 slides per animal, were prepared from the resuspended cell pellet. For blood samples, a drop of blood was placed on each of two slides. From the remaining blood, 200 μ L of blood from each mouse was placed into 500 μ L heparin (500 USP units/mL). 180 μ L of the blood/heparin suspension was retrieved and added to 2 mL of absolute methanol kept at $<-70^{\circ}\text{C}$. The tube holding the blood cells in cold absolute methanol was tapped to break up any aggregates and then quickly returned to $<-70^{\circ}\text{C}$. Frozen blood samples were shipped to Litron Laboratories for analysis by flow cytometry. The analysis was performed according to procedures published by Dertinger et al., (1996). Since animals were assigned to groups by weight, the animal numbers in each group were essentially random and were used as a blind code. Slides from bone marrow were stained using acridine orange, wet mounted, and blind coded. 2000 PCEs from each animal were examined for the presence of micronuclei. The percent of PCE's in the total population of erythrocytes was determined for each animal by counting a total of 1000 erythrocytes. For blood samples, the percentage of PCEs (reticulocytes) in the total population of erythrocytes was determined by counting 100,000 erythrocytes. Ten thousand PCEs from each animal were examined for the presence of micronuclei (MNEs).

Bone marrow PCE's stain fluorescent red/orange, normochromatic erythrocytes are unstained or stain dull green, and micronuclei stain fluorescent bright yellow. Additional criteria for microscopic identification of micronuclei were a circular appearance and a diameter between 1/20 and 1/5 of a cell's diameter (Schmid, 1975). All slides were retained in the EBSI Archives.

The following parameters were recorded for each animal during bone marrow and blood cell analysis:

Number of bone marrow and blood cell polychromatic and normochromatic erythrocytes in a total of 1000 erythrocytes (bone) or 100,000 erythrocytes (blood)

Number of bone marrow and blood cell polychromatic erythrocytes with micronuclei

Number of bone marrow and blood cell polychromatic erythrocytes scored

EXPERIMENTAL DESIGN (CONT'D)

Statistical Analysis

Statistical analyses included means and standard deviations of the micronuclei data.

The MN and PCE data were log transformed to have the residuals normally distributed. The data were first analyzed by standard two-way analysis of variance (ANOVA), with gender, dose group and their interactions as independent variables (Snedecor and Cochran, 1989). Four separate analyses were done for the blood/bone marrow combinations of MN and PCE measures. The data were then analyzed by standard one-way ANOVA with dose group as the independent variable. Eight separate analyses were done for the blood/bone marrow combinations of MN and PCE measures, male and female. In all analyses, where the differences in group means were statistically significant at the 5% or 1% level, the dose groups were compared to the vehicle by Dunnett's test (Snedecor and Cochran, 1989).

Assay Validity

The assay is considered valid if (1) the mean incidence of micronucleated PCEs per 1000 PCEs (calculated from the evaluation of 2000 PCEs) does not exceed 4 (or 0.4%) in the carrier control, and (2) the mean incidence of micronucleated PCEs for the positive control is significantly greater than that of the carrier control group.

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EXPERIMENTAL DESIGN (CONTD)

Evaluation of Data

The criteria for a test substance to be considered as inducing a positive response when compared to the carrier control are as follows:

- 1) A dose-related statistical increase in the mean number of micronucleated PCEs, including at least one dose point that is statistically different from the mean number of micronucleated PCEs of the carrier control. This value also must be outside the normal range of the mean number of micronucleated PCEs of the carrier control; i.e. the value must be greater than 4; or
- 2) A single dose point that is statistically different from the mean number of micronucleated PCEs of the carrier control, and greater than the normal range of the mean number of micronucleated PCEs of the carrier control; i.e. the values must be greater than 4.

A dose point is considered negative if the mean value for a statistically significant increase in micronuclei is within the normal range of the carrier control (0-4 micronuclei per 1000 polychromatic erythrocytes).

A comparison of the results from different treatment groups may be performed to examine possible synergistic effects caused by the two test substances.

Records

The protocol, all raw data, slides, computer generated listings of raw data, samples of the test substance, the final report, and supporting documentation are maintained on file in the EBSi Toxicology Laboratory Archives.

**IN VIVO MAMMALIAN MICRONUCLEUS ASSAY - DERMAL AND INHALATION EXPOSURE
WITH DMDTC & BUTADIENE: 112539****RESULTS****1. RANGEFINDING**

Prior to the start of the test, a rangefinding study was performed. DMDTC rangefinding doses of 50, 100, 200, and 300 mg/kg/day were based on published data (Pruet et. al., 1992). Since all animals survived to termination and there were no signs of overt toxicity during the inlife period or at necropsy, 300 mg/kg of DMDTC was selected for the main study.

For butadiene, the high dose of 200 ppm was based on published data (Leavens et. al., 1997). It was expected to induce micronuclei in polychromatic erythrocytes from the bone marrow. This dose was confirmed by a rangefinding study.

Rangefinding data is not presented in this final report, but is maintained in the laboratory archives.

2. INHALATION EXPOSURE CONCENTRATIONS

Summary of Inhalation Data: Table 1

Low dose BD concentrations ranged between 9 to 11 ppm with a mean of 10 ppm (22mg/m³) and high dose BD concentrations ranged between 195 to 206 ppm with a mean of 200 ppm (442 mg/m³).

3. MICRONUCLEUS DATA

Summary of Micronucleus Data: Table 2
Individual Cell Counts: Appendix A

The MN and PCE data were log transformed to have the residuals normally distributed. The data were first analyzed by standard two-way analysis of variance (ANOVA), with gender, dose group and their interactions as independent variables (Snedecor and Cochran, 1989). Four separate analyses were done for the blood/bone marrow combinations of MN and PCE measures. The data were then analyzed by standard one-way ANOVA with dose group as the independent variable. Eight separate analyses were done for the blood/bone marrow combinations of MN and PCE measures, male and female. In all analyses, where the differences in group means were statistically significant at the 5% or 1% level, the dose groups were compared to the vehicle by Dunnett's test (Snedecor and Cochran, 1989).

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RESULTS (CONT'D)

3. MICRONUCLEUS DATA (CONT'D)

In order to have the residuals normally distributed, four observations were excluded from the analysis:

MN, bone marrow, Male, Group 5, CAA408
MN, blood, Female, Group 5, CAA476
MN, blood, Female, Group 5, CAA462
PCE, bone marrow, Male, Group 8, CAA430

The overall conclusions are equivalent whether the four observations are retained or removed.

In general, the pattern of responses are similar for PCE and MN, in both blood and bone marrow. The magnitude of the response may be different but the relative responses are in the same direction. The females have lower MN and higher PCE values than the males in all conditions.

Dose-related decreases in the mean percentage of bone marrow and blood polychromatic erythrocytes (% PCE) were observed in the BD treated animals (Group 4 males and Group 5 males and females). Additionally, there were statistically significant decreases in the mean % PCE in the bone marrow and blood of the high dose BD treated animals (Group 5) compared with controls. There were dose-related increases in mean percentage of bone marrow and blood micronucleated PCEs (% MN) in the BD treated animals (Groups 4 and 5) of both sexes. Also, there were statistically significant increases in the mean % MN in the bone marrow and blood of the high BD treated animals compared with controls. There was a statistically significant increase in %MN in the blood of males in the low BD treated animals (Group 4). In addition, there was a statistically significant increase in % MN in bone in the BD high (Group 10) group, females only. These changes were considered treatment-related.

In all but one of the DMDTC treated groups, either treated with DMDTC alone or with BD, there was a statistically significant increase in the mean % PCEs in the blood compared to controls. The only exception was in the males of the BD high/DMDTC group. In the bone marrow, there was also a statistically significant increase in the % PCEs in the males and females of the group treated with DMDTC alone (Group 8). Although there were fewer statistically significant increases in the % PCE, the general pattern of response in the bone marrow was similar to that in the blood. Other findings included statistically significant increases in the % MN of the bone marrow in the males and females of the DMDTC/BD high dose group (Group 2). This increase was less than that observed for the BD high (Group 5) group. In addition, there were statistically significant decreases in the blood % PCEs of the group treated with low BD (Group 4 males) and in the bone and blood % PCEs of the high BD group (Group 10 males).

RESULTS (CONT'D)**4. ORGAN WEIGHTS**

Mean Organ and Relative Organ Weights: Table 3 and 4
Individual Organ and Relative Organ Weights: Appendices D and E

There were statistically significant increases in mean absolute (17%) and relative (18%) liver weights of the DMDTC/BD high treated male mice compared with controls. In addition, there were statistically significant increases in mean absolute (28%) and relative (22%) spleen weight of the DMDTC treated female mice compared with controls. These findings were considered treatment-related.

Other organ weight findings included a statistically significant increase in mean absolute liver weight of the BD low/DMDTC and the DMDTC treated males and a decrease in the mean absolute spleen weight of the positive control (BD high) group females compared with controls. However, in the absence of correlating effects in the respective relative body weights and/or a clear dose response, these findings were considered the result of differences in terminal body weights rather than a treatment-related effect. In addition, there was a statistically significant increase in mean relative liver weight of the DMDTC/BD low treated males compared with controls. However, this small (9%) difference was not considered biologically important.

5. INLIFE OBSERVATIONS AND BODY WEIGHTS:

Individual Inlife Observations: Appendix B
Individual Body Weights: Appendix C

All animals were free of adverse clinical signs throughout the study except for one Group 9 female (water control) on Day 6. Two DMDTC/BD high treated animals were observed with dry white material on the dose site on Day 5.

There were no apparent biologically significant differences in body weight between treated and control animals.

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CONCLUSION

For both blood and bone marrow, butadiene at 200 ppm induced cytotoxic effects and DMDTC induced evidence of hypererythropoiesis. DMDTC reduced the cytotoxic effects of BD when administered either before or after BD treatment.

Butadiene did induce a statistically significant increase in micronucleated PCEs. DMDTC did not induce any increases in micronucleated PCEs. DMDTC inhibited the induction of micronucleated PCEs by BD.

There were statistically significant increases in mean absolute (17%) and relative (18%) liver weights of the DMDTC/BD high treated male mice compared with controls. In addition, there were statistically significant increases in mean absolute (28%) and relative (22%) spleen weight of the DMDTC treated female mice compared with controls. These findings were considered treatment-related.

In conclusion, BD was considered cytotoxic and clastogenic in mouse polychromatic erythrocytes in both blood and bone marrow. DMDTC was considered to induce evidence of hypererythropoiesis. These results indicate that DMDTC penetrated the mouse skin and produced an effect on the bone marrow. It was not considered clastogenic in mouse polychromatic erythrocytes. DMDTC inhibited both cytotoxicity and clastogenicity of BD in mouse polychromatic erythrocytes.

PROTOCOL EXCEPTIONS

% RELATIVE HUMIDITY: The mean % relative humidity in the chamber during the December 3, 1997 exposure was 18, 20, and 21%RH, lower than the target range specified by protocol (40-69%).

VIABILITY CHECK: Animals were checked once daily Monday through Friday, not twice as specified by protocol.

OBSERVATIONS: All animals were observed daily prior to exposure, not after exposure as specified by protocol.

DAY 1 WEIGHING: Animals in Groups 3, 4, and 5 were not weighed on Day 1 and animals in Group 10 were not weighed on Day 5 as specified by protocol.

SAMPLE RETENTION: A retention sample of 1,3-butadiene was not taken as specified by protocol due to safety considerations of storing a gas/liquid mix under pressure.

It is unlikely that these protocol exceptions adversely affect study results or integrity.

No other circumstances occurred that would have affected the quality or integrity of the data.

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REFERENCES

- Animal Welfare Act of 1966 (P.L. 89-544), as amended in 1970, 1976, and 1985. Code of Federal Regulations, Title 9 [Animals and Animal Products], Subchapter A-Animal Welfare Parts 1, 2, and 3.
- Choy, W.N., Vlachos, D.A., Cunningham, M.J., Arce, G.W. and Sarrif, A.M. Genotoxicity of 1,3-butadiene. Induction of bone marrow micronuclei in $B_6C_3F_1$ mice and Sprague-Dawley rats *in vivo*. *Environ. Mut.* 6:18, 1986.
- Cunningham, M.J., Choy, W.N., Arce, G.T., Rickard, L.B., Vlachos, D.A., Kinney, L.A. and Sarrif, A.M. *In vivo* sister chromatid exchange and micronucleus induction studies with 1,3-butadiene in $B_6C_3F_1$ mice and Sprague-Dawley rats. *Mutagenesis* 1: 449-452, 1986.
- Dertinger, S.D., Torous, D.K., and Tometsko, K.R. Simple and reliable enumeration of micronucleated reticulocytes with a single-laser flow cytometer. *Mutation Research* 371:283-292, 1996.
- Garriot, M.L., Piper, C.E. and Kokkino, A.J. A simplified protocol for the mouse bone marrow micronucleus assay. *Journal of Applied Toxicology* 8:141-144 (1988).
- Guide for the Care and Use of Laboratory Animals. Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, National Academy Press, Washington, D.C., 1996.
- Heddle, J.A., Hite, M., Kirchart, B., Mavourmin, K., MacGregor, J.T., Newell, G.W. and Salamone, M.F. The induction of micronuclei as a measure of genotoxicity - A Report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutation Research* 123:61-118 (1983).
- Hollander and Wolfe. *Nonparametric Statistical Methods*, John Wiley and Sons, New York (1973).
- Kliesch, U., Danford, N., Alder, I.D. Micronucleus test and bone marrow chromosomal analysis. A comparison of 2 methods *in vivo* for evaluating chemically induced chromosomal alterations. *Mutations Research* 80:321-332 (1981).
- Leavens, T.L., Farris, G.M., James, R.A., Shah, R., Wong, V.A., Marshall, M.W., and Bond, J.A. genotoxicity and cytotoxicity in male $B_6C_3F_1$ mice following exposure to mixtures of 1,3-butadiene and Styrene. *Environ. Mol. Mutagen.* 29:335-345, 1997.
- OECD, Organization for Economic Cooperation and Development. Guidelines for Testing of Chemicals, Test Guideline 474, 1983.
- OECD, Organization for Economic Cooperation and Development. Principles of Good Laboratory Practice, C(81)30 Annex 2, 1981.

REFERENCES (CONT'D)

- et, S.B., Barnes, D.B., Han, Y. and Munson, A.e. Immunotoxicological characteristics of sodium methylthiocarbamate. *Fundamental and Applied Toxicology* 18:40-47 (1992).
- oid, W. The micronucleus test for cytogenetic analysis in Chemical Mutagens: Principles and Methods for their Detection. A. Hollander ed. Plenum Press, New York 53 (1976).
- oid, W. "The Micronucleus Test". *Mutation Res.* 31:9-15 (1975).
- ro, S.S., and Wilk, M.B., An analysis of variance test for normality (complete cases), *Biometrika*, 52. 1965.
- score and Cochran, *Statistical Methods*, 8th ed., Iowa State Press, Ames, Iowa, 1989.