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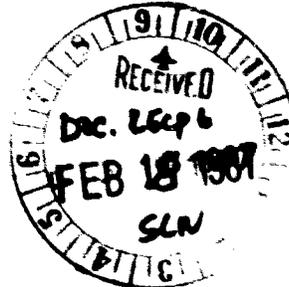
ETHYL CORPORATION
TOXICOLOGY AND INDUSTRIAL HYGIENE DEPARTMENT
ETHYL TOWER, 451 FLORIDA
BATON ROUGE, LOUISIANA 70801

February 9, 1987

CONTAINS NO CBI

Certified Mail - Return Receipt Requested

Document Control Officer (TS-790)
Attn: Section 8(e) Coordinator
Office of Toxic Substances
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, DC 20460



EPA-OTS



000281362M

RE: 8EHQ-1286-0648

Dear Sir:

Please find enclosed the experimental protocol and final report from the 90-day DETDA feeding study cited in the Section 8(e) submission 8EHQ-1286-0648. I have also attached a summary of the toxicology studies conducted on DETDA by Ethyl Corporation and those toxicology studies either planned or in progress. As you can see from these attachments, Ethyl Corporation is aware of carcinogenicity studies conducted on DETDA in Europe. If you desire more detailed information on these studies, I suggest you contact Mobay Corporation.

I trust this information satisfies your request delineated in your January 16th letter. If I can be of further assistance, please contact me at (504) 388-7617.

Sincerely,

ETHYL CORPORATION

T. G. Pullin, Ph.D.
Director
Regulatory Affairs

TGP:cmc/1210

— Attache

16025
186
104
Total 30682

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SUMMARY OF TOXICOLOGY STUDIES
DIETHYLTOLUENE DIAMINE

Acute Toxicity

Diethyltoluene diamine (DETDA) has a moderate to low degree of acute toxicity. The rat oral LD₅₀ is 485 mg/kg. The rabbit dermal LD₅₀ is approximately 700 mg/kg. Slight irritation was induced following skin application to rabbits. DETDA was moderately to severely irritating to the eyes of rabbits. Exposure to a nominal air concentration of 2.45 mg/l for one hour produced no mortality in rats.

Sensitization

DETDA did not induce skin sensitization in guinea pigs following dermal exposure.

Subchronic Toxicity

A subchronic 21 day dermal toxicity study was conducted in rabbits with DETDA. Repeated dermal applications of DETDA at 1, 10 and 100 mg/kg for three weeks (five days/week) resulted in mild to moderate local irritation at the 10 and 100 mg/kg dosages. No local effects were observed at the 1 mg/kg dose. There were no biologically significant systemic effects in any of the rabbits in this study.

A subchronic oral 28 day dose range finding study was conducted in rats in preparation for a 90 day feeding trial. Male and female rats were administered DETDA in the diet at 0, 40, 400, 800, 1200, 1600 or 3200 ppm for 28 days. Rats in the 3200 ppm group were sacrificed on day 17 due to excessive weight loss. Animals in the 0-1600 ppm treatment groups were sacrificed on day 29 as planned. Dose and time dependent changes in body weight and food consumption were observed. No specific lesions attributable to the test article were found on necropsy. No microscopic examinations of tissues were performed. The results of this study were used to select doses for the 90 day feeding trial.

A 90 day feeding trial was conducted in rats to establish doses for a two year carcinogenesis bioassay. Male and female rats were administered DETDA in the diet at 0, 50, 125 and 320 ppm for 90 days. High dose rats experienced a high mortality rate, body weight loss, histopathologic lesions in a number of organs and alterations in serum chemistry and hematology values. Doses of 50 or 125 ppm partially or totally reduced the incidence and severity of the above changes. Based on histopathologic results, the initial or primary effect of DETDA appeared to occur in the pancreas. Other organ effects were thought to be secondary to that of the pancreas. (Report enclosed.)

Distribution

A distribution study was conducted following one or ten daily oral doses of ¹⁴C-DETDA in the rat. Preliminary studies showed that

a single oral dose of ^{14}C -DETDA was slowly absorbed and distributed to various organs and tissues. The compound and/or its metabolites were eliminated primarily in the urine and to a lesser extent in the feces. Excretion via expired air was negligible. Residual radioactivity in tissues was highest in the thyroid followed by liver, kidney and adrenal gland. Repeated dosing did not appear to enhance tissue sequestration.

Genetic Toxicology

An initial battery of mutagenicity assays was conducted by Ethyl Corporation on DETDA. The battery included the Ames, Saccharomyces cerevisiae, E. coli DNA repair, rodent dominant lethal, in vivo cytogenetics, micronucleus, and BALB/3T3 cell point mutation tests. Tests were conducted with and without exogenous metabolic activation, where appropriate. All tests in the above battery, with one exception, produced negative results. The BALB/3TC cell point mutation, when performed without metabolic activation, was the sole positive response. The positive response could not, however, be reproduced in an independent test. A test for unscheduled DNA repair in rat hepatocyte cultures was found negative as well.

Subsequent to the completion of the above battery, a customer reported a DETDA Ames positive result. To resolve the conflicting Ames results, additional studies were conducted by Ethyl Corporation. Positive results were found in two different lots of Ethyl-produced commercial DETDA when tested with enzymatic activation. The purified isomers (99%) 3,5-diethyl-2,6-diaminotoluene and 3,5-diethyl-2,4-diaminotoluene produced positive and negative results, respectively. The positive activity of the 2,6-isomer was weak and difficult to reproduce. In addition, the 2,6-isomer was not effective in inducing unscheduled DNA repair in rat hepatocyte cultures. An Ames-positive commercial DETDA sample also did not induce unscheduled DNA repair.

Ethyl Corporation is aware of several groups across the country who are studying DETDA in a variety of tests. Detailed information concerning those tests or their results is not available.

Carcinogenicity

A DETDA in vitro mammalian cell transformation assay, conducted with and without exogenous metabolic activation, has produced negative results. In addition, Ethyl Corporation is aware of several European studies conducted to investigate the potential carcinogenic activity of DETDA. In a lifetime pilot study, a limited number of rats were administered DETDA by subcutaneous injection. Doses and timing were adjusted, as needed, to prevent life-threatening toxicity. It was concluded DETDA did not increase the incidence of tumors over control animals. DETDA was also administered to rats in the diet or via

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gavage. Test article administration was continued throughout the animals' natural life span in the case of the feeding trial or for two years when dosed via gavage. Dose levels were in the maximum tolerated to toxic range as dose reduction was required to achieve survivability. The sponsors considered no DETDA-induced carcinogenicity was evident.

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DIETHYLTOLUENE DIAMINE TOXICOLOGY STUDIES
PLANNED OR IN PROGRESS

A diethyltoluene diamine (DETDA) two year carcinogenesis bioassay, for which the 28 and 90 day feeding trials were preliminary phases, is planned. It was originally intended that the two year study would be initiated immediately following the completion of the 90 day trial. However, the results of the 90 day study indicate further research on the effects of DETDA is required to properly conduct a two year bioassay. A subchronic feeding trial in two species is planned to further delineate the toxicity of DETDA. Rats and hamsters will be fed DETDA in the diet and the results will be compared between species and with those of previous studies.

Other studies planned or in progress are an investigation of DETDA urinary metabolites in the rat, the compound's potential for covalent binding to DNA, and further characterization of its reaction in the Ames test. Preliminary results of the metabolism study indicate DETDA is excreted in the urine as several products. The covalent binding assay is a continuation of studies investigating the interaction of alkylated anilines and biological macromolecules.

PHARMAKON RESEARCH INTERNATIONAL, INC.

WAVERLY, PENNSYLVANIA 18471

Protocol-470

PHONE
(717) 586-2411

Subchronic 90 Day Oral Toxicity Study - Rat

Sponsor: Ethyl Corporation
451 Florida
Baton Rouge, LA 70801

Testing Facility: Pharmakon Research International, Inc.
Waverly, Pennsylvania 18471

Test Facility
S.O.P. No.: PH-470

Study No.: PH 470-ET-001-86

Purpose of the Study: To provide information on possible health hazards likely to arise from repeated oral exposures of diethyl toluene diamine (DETDA) for a period of 90 days and provide an estimate of a no-effect level of exposure which can be used in chronic studies and for establishing safety criteria for human exposure.

Ownership of the Study: The sponsor owns the study. All raw data, wet tissue, analysis and reports are the property of the sponsor. All wet tissue, paraffin blocks and slides will be maintained by Experimental Pathology Laboratories.

Study Monitor: Marcia Hardy, D.V.M., Ph.D., Ethyl Corporation

Study Director: Robert W. Naismith, Ph.D., Pharmakon Research International, Inc.

Principal Investigator: Dennis J. Margitich, B.S., RLAT, Pharmakon Research International, Inc.

Study Pathologist: William Busey, D.V.M., Ph.D., Experimental Pathology Labs

Ophthalmologist: Thomas Kern, D.V.M., Ph.D., DACVO or Ronald Riis, D.V.M., M.S., DACVO, Cornell University

Q.A.U. Responsible Personnel: Leslie Maas, B.S.

Dates of Performance: To be determined upon finalization of the protocol.

Good Laboratory Practices Statement. This study will be conducted in compliance with the Good Laboratory Practices Regulations as stated in the EPA Good Laboratory Practice Standards [Subpart I, Part 792, Chapter I of Title 40, Code of Federal Regulations], as well as the Organization for Economic Co-operation and Development (OECD) Guidelines for Testing Chemicals, ISBN 92-64-12221-4, adopted by the council at its 535th meeting on 12 May, 1981.

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Subchronic Oral Toxicity - Rat (90 Day)

IACUC Statement:

This protocol has been reviewed by the Institutional Animal Care and Use Committee (IACUC) at Pharmakon and complies with acceptable standard animal welfare and humane care.

Records Maintained:

All raw data, final reports, documentation and the protocol and amendments will be maintained in the Pharmakon Research Central File.
Body weights (initial, weekly, final or as appropriate)
Food consumption, twice weekly
Animal Health Certificate (Purchase Order)
Schedule of events
Water and food analysis
Feed Lot Number
Formalin Lot No. and Supplier
Temperature and humidity recordings
Test article preparation
Samples of test article
Identification sample of ear markings
Observed mortality
Pharmacological and toxicological signs
Necropsy findings
Organ Weights
Hematology reports
Ophthalmologic findings
Clinical biochemistry reports
Histopathological reports

Statistical Analysis:

Evaluation of equality of means will be made by the one way analysis of variance using the F distribution to assess significance. If significant differences among the means are indicated, Dunnett's test will be used to determine significant differences from control means. Analysis of discrete data where appropriate will be conducted using non-parametric procedures.

Raw Data:

Standard Pharmakon Research Notebook
Computer generated Pharmakon Research Study Forms

Archive Retention:

All raw data
Sample of test article
Wet tissue, paraffin blocks and slides will be maintained at Experimental Pathology Laboratories.

TEST ARTICLE

Test Article Preparation:

Preparation of the test articles for administration to the rats consists of incorporating the test article in the diet utilizing a liquid-solids blender, (Twin-Shell Intensifier Blender, Patterson-Kelly Company, East Stroudsburg, Pennsylvania) according to the Standard Operating Procedure PH-036A on file at Pharmakon Research International, Inc. Appropriate

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samples of the diet test article mixture will be retained for chemical analysis. Dietary analysis of test article concentrations will be performed by the sponsor.

Sampling of
Test Diet:

Samples 100-200 g are usually shipped and/or stored in Nalgene[®] plastic jars. The label on each container will include the following information:

1. Study number.
2. Date of sampling.
3. Day into study.
4. Level of compound in the feed.
5. Test article or code for test article.

The samples will be packed in dry ice in a styrofoam container and shipped to the analytical chemistry laboratory at Ethyl Corporation, 8000 GSRI Avenue, Baton Rouge, Louisiana, 70820 by overnight carrier. An additional set of all samples will be retained and stored at -20°C until the results of the analysis have been received.

Homogeneity:

Samples of the test diet will be analyzed by the sponsor prior to initiation of the study to verify the homogeneity of mixing of the test article in the feed. Three different levels (top left, top right and bottom) from the blender will be sampled. A total of three samples per mixing level, 9 samplings for three levels, will be collected and submitted to the sponsor each time the diet is prepared. In addition, an untreated sample of diet will also be shipped for comparative analysis.

Dose Verification:

A sample of the diet at each level as well as control feed will be submitted to the sponsor for analysis prior to the study's initiation and at each time the diet is prepared. If the results verify that the diet is within $\pm 15\%$ or ± 15 ppm (whichever is greater) of target concentration the diet will be deemed acceptable. All samples will be evaluated prior to feeding.

Stability in Diet:

The stability in diet has been determined by the sponsor to be 36 days. A single sampling for each level will be submitted to the sponsor for analysis and analyzed at the appropriate intervals.

Stability Under
Test Conditions:

Stability under test conditions has been determined by the sponsor and the diet will be renewed twice a week.

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Authenticity and
& Purity of
Test Article:

Responsibility of and supplied by the sponsor.

Stability of Test
Article under
Conditions
of Storage:

Stability under conditions of storage (Nitrogen blanketed at room temperature) will be determined for length of the study. Samples will be submitted to the sponsor for analysis on Days 0, 45 and at termination of the study.

TEST SYSTEM

Species:

Rat

Strain:

Sprague Dawley

Supplier
(Source):

Charles River Laboratories, Wilmington, Massachusetts

Sex:

Male and female

Age at
Initiation:

5 to 7 weeks

No. on Study:

One hundred and eighty (180) (90 males, 90 females)

Method and
Justification for
Randomization:

Treatment groups will be housed by vertical cage positioning. Randomization is carried out by use of a random number table. Prior to treatment commences, all rats will be weighed, ranked according to body weight and assigned to treatment groups using a table of random numbers so that each treatment group will have a similar distribution according to body weight. Rats beyond the extremes of the body weight range will not be assigned to treatment groups. If there is a statistically significant difference in mean body weight between any two groups of the same sex after allocation to the treatment groups, substitution from spare animals will be used to correct the situation.

Acclimation
Period:

Minimum of 1 week. During this conditioning period the rats will be weighed and observed for any clinical signs of disease or inadequate weight gain. All rats with any evidence of disease or physical abnormalities will be discarded.

System of
Identification:

Rats will be individually identified by ear tags (Gey Bard). Individual cages will be marked with the rat number. The first cage card for each group and sex will contain a legend of compound and dose level as well as rat number.

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Subchronic Oral Toxicity - Rat (90 Day)

HUSBANDRY

<u>Research Facility Registration:</u>	U.S.D.A. Registration No. 23-107 under the Animal Welfare Act 74: SC 2131 <u>et seq.</u>
<u>Animal Rooms:</u>	Separate isolation by test system. Light cycle - 12 hours light, 12 hours dark. Temperature/Humidity - Maintained at a temperature of 22°C ± 3°C, and a humidity of 30 to 70%.
<u>Housing:</u>	Rats housed individually in stainless steel ½" wire mesh cages. Size in accordance with the "Guide for the Care and Use of Laboratory Animals" of the Institute of Laboratory Resources, National Research Council.
<u>Sanitization:</u>	Waste material is removed three times a week. Cages and feeders are sanitized every two weeks.
<u>Food:</u>	Purina Certified Rodent Lab Meal ^R , <u>ad libitum</u> . Feeders are designed to reduce soiling, bridging and scattering.
<u>Food Analysis:</u>	Certified food will be used in the study. A contaminant certification profile will be maintained in Pharmakon Research Central Files.
<u>Water Analysis:</u>	Availability - fresh tap water, <u>ad libitum</u> . Water is monitored for contaminants at periodic intervals according to Standard Operating Procedure PH-018.

METHODS

<u>Rationale for Test System:</u>	Rats have historically been used for establishing safety criteria for human exposure.
<u>Compound Preparation:</u>	Incorporated in the diet as a percent of diet and prepared as necessary.
<u>Rationale for Dose Selection:</u>	Based upon a 28 Day Dose-Range-Finding Study. The highest dose level in rodents should result in toxic effects but not produce an incidence of fatalities which would prevent a meaningful evaluation. The lowest dose level should not produce any evidence of toxicity. Where there is an usable estimation of human exposure the lowest dose level should exceed this. Ideally, the intermediate dose level should produce minimal observable toxic effects.

Subchronic Oral Toxicity - Rat (90 Day)

Dose Administration:

Group	No. of Animals ¹	Concentration		
		ppm	% of Diet	Estimated mg/kg/rat/day
1	40 (20M, 20F)	0	0.0	0
2	40 (20M, 20F)	50	0.005	5
3	40 (20M, 20F)	125	0.0125	12.5
4	40 (20M, 20F)	320	0.0320	32

¹ 10 males and 10 females will be sacrificed pre-study (baseline data).

Route of Administration:

Oral, in diet

Rationale for Route of Administration:

To determine the toxicity of the test article by oral ingestion.

Frequency and Duration of Administration:

Daily for ninety (90) days

Length of Study:

Dose phase, 90 days

Methods of Study Performance:

The animals will be fed the diet/compound mixture seven days per week over a period of 90 days. All pharmacological and toxicological signs will be recorded daily including their onset, degree and duration. Such signs will include, but are not limited to changes in skin and fur, eyes and mucous membranes as well as respiratory, circulatory, autonomic and central nervous system, somatomotor activity and behavior pattern. Mortality checks will be made daily and recorded. The animals will be weighed initially and weekly. Measurements will be made of food consumption twice weekly. At the end of the 90 day period all surviving animals will be sacrificed by CO₂ inhalation. Any weak or moribund animals will be removed, sacrificed and necropsied during the study as deemed necessary by the study director or principal investigator.

CLINICAL EXAMINATIONS

Clinical Laboratory Studies:

Blood will be drawn by percutaneous cardiocentesis. All animals will be fasted overnight prior to the time of sacrifice. The following determinations shall be made on twenty rats (10/sex) prior to initiation. In addition, after 90 days of feeding, five (5) animals per sex per group will be chosen by random number tables and the following will be determined.

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Hematology: Hemoglobin
 Hematocrit
 Erythrocyte count
 Total and differential leucocyte counts
 Platelet count

Clinical
 Biochemistry:

Calcium
 Phosphorus
 Chloride
 Sodium
 Potassium
 Fasting Glucose
 Serum alanine aminotransferase
 Serum aspartate aminotransferase
 Gamma glutamyl transpeptidase
 Urea nitrogen
 Albumen
 Blood creatinine
 Total bilirubin
 Total serum protein measurements

Ophthalmologic:

Ophthalmological examinations using an ophthalmoscope will be made prior to the administration of the test substance on all animals. At termination of the study 5/sex/group chosen from a table of random numbers will be evaluated. If changes in the eyes are detected all animals will be examined.

PATHOLOGY

Gross Necropsy:

All animals will be subjected to necropsy which includes examination of the external surface of the body, all orifices, and the cranial, thoracic, abdominal and pelvic cavities and their contents. The following organs will be weighed:

liver	gonads
adrenal glands	brain
kidneys	

The following organs and tissues from the animals at the 90 day sacrifice will be preserved in a suitable medium for future histopathological examination:

All gross lesions
 Brain including sections of medulla/pons
 Cerebral cortex and cerebral cortex
 Pituitary
 Thyroid/parathyroid
 Thymus
 Lungs
 Trachea
 Heart

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Sternum with bone marrow
Salivary glands
Liver
Spleen
Kidneys/adrenals
Pancreas
Gonads
Uterus
Accessory genital organs (epididymides, prostate,
and, if present seminal vesicles)
Aorta
Skin
Esophagus
Nasal turbinates
Stomach
Duodenum
Jejunum
Ileum
Cecum
Colon
Rectum
Urinary bladder
Representative lymph node
Mammary gland
Thigh musculature
Peripheral nerve
Eyes
Femur-including articular surface
Spinal cord at three levels - cervical, mid thoracic
and lumbar
Exorbital lachrymal glands

Histopathology:

A full histopathologic evaluation of the above organs and tissues will be performed in all control and high dose animals sacrificed at 90 days of treatment. All animals which die or are sacrificed in a moribund condition prior to completion of the study shall also be subjected to a full histopathologic evaluation of the above named tissues and organs. All gross lesions in all animals will be examined histologically.

Data and Reporting:

A. Treatment of Results

1. Data shall be summarized in tabular form, showing for each test group the number of animals at the start of the test, the number of animals at the 90 day sacrifice, the number of animals showing lesions, the types of lesions and the percentage of animals displaying each type of lesion.
2. All observed results, quantitative and incidental, shall be evaluated by an appropriate statistical method.

B. Evaluation of the study results

1. The findings of a subchronic oral toxicity study should be evaluated in conjunction with the findings of preceding studies and considered in terms of the toxic effects and the necropsy and histopathological findings. The evaluation will include the relationship between the dose of the test substance and the presence or absence, the incidence and severity, of abnormalities, including behavioral and clinical abnormalities, gross lesions, identified target organs, body weight changes, effects on mortality and any other general or specific toxic effects. A properly conducted subchronic test should provide a satisfactory estimation of a no-effect level.
2. In any study which demonstrates an absence of toxic effects, further investigation to establish absorption and bioavailability of the test substance should be considered.

C. Test report

In addition to the reporting requirements as specified in the EPA Good Laboratory Practice Standards [Subpart J, Part 792, Chapter I of Title 40, Code of Federal Regulations], as well as the Organization for Economic Co-operation and Development (OECD) Guidelines for Testing Chemicals, ISBN 92-64-12221-4, adopted by the council at its 535th meeting on 12 May, 1981, the following specific information shall be reported:

1. Group animal data

Tabulation of toxic response data by species, strain, sex and exposure level for:

- a. Number of animals dying;
- b. Number of animals showing signs of toxicity; and
- c. Number of animals exposed.

2. Individual animal data

- a. Time of death during the study or whether animals survived to termination;
- b. Time of observation of each abnormal sign and its subsequent course;

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Subchronic Oral Toxicity Rat (90 Day)

- c. Body and organ weight data;
- d. Food consumption data;
- e. Hematological tests employed and all results;
- f. Clinical biochemistry tests employed and all results;
- g. Necropsy findings;
- h. Detailed description of all histopathological findings; and
- i. Statistical treatment of results where appropriate.

Protocol - 470
Subchronic Oral Toxicity - Rat 90 Day Study

LEGEND
N/A - not available
N/K - not known

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TEST ARTICLE

Name: Diethyl toluene diamine (DETA)

Chemical Abstract No.
or Code No.: see MSDS supplied

Lot or Batch No.: Identical to that used in the 28 day Dose Range Finding DETA Study
(PH 436-ET-001-86)

Description: amber liquid Strength: Purity:

Amount Submitted:

Expiration Date:

Special Handling or
Storage Instructions: MSDS supplied

Analysis of Purity/Stability: Analysis of the purity and stability of the test article is the responsibility of the sponsor.

Test Article/Carrier Mixtures: Analysis for stability, uniformity and correctness of concentration is the responsibility of the sponsor.

Return Test Article/Carrier Mixtures to the Sponsor

Dispose of Test Article/Carrier Mixtures

Test Article
Disposition:

Test article will be disposed of 3 months following the submission of the final report.

Test article to be returned upon completion of the study.

AMENDMENTS

APPROVAL OF PROTOCOL

Date June 2, 1986 Study Monitor Marcia P. Hagg
Date 5-23-86 Study Director Robert W. Brimmer

010984

PHARMAKON RESEARCH INTERNATIONAL, INC.

WAVERLY, PENNSYLVANIA 18471

PHONE
(717) 586-2411

186103

Subchronic 90 Day Oral Toxicity Study in Rats

PH 470-ET-001-86

diethyl toluene diamine
(DETD)

CONTAINS NO CBI

Submitted to

Ethyl Corporation
Baton Rouge, Louisiana

Robert W. Naismith

Robert W. Naismith, Ph.D.
Director of Toxicology

February 3, 1987

Date

Richard J. Matthews

Test Management Facility

February 3, 1987

Date

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Subchronic 90 Day Oral Toxicity Study - Rat

PH 470-ET-001-86

diethyl toluene diamine (DETDA)

SUMMARY

Test article, diethyl toluene diamine (DETDA), was incorporated into standard certified commercial laboratory rat diet and fed ad libitum to four groups of Sprague Dawley rats, seven days per week for a period of 90 days (except where noted below). Each of the four dose groups, containing twenty males and twenty females per group, were fed the diet mixture at dose levels of 0, 50, 125 or 320 ppm.

A few rats from the control, low and mid dose groups exhibited non-specific pharmacotoxic signs. These included lesions and/or scab formations with alopecia. In addition, piloerection, chromodacryorrhea and poor grooming were observed. The majority of the high dose rats (males and females) exhibited several pharmacotoxic signs. These included abnormal stance, abnormal gait, flaccid body tone, ptosis, decreased activity, chromodacryorrhea, elevated gait, loss of skin elasticity, poor grooming, dyspnea, tremors, ataxia and morbidity. In general, these signs resulted in either moribund sacrifice or death.

Statistically significant reductions in mean male and female body weights were observed on study Days 7 through 90 and 14 through 90, respectively, in the high dose (320 ppm) group. The mid dose males (125 ppm) exhibited statistically smaller body weights on Day 77 through Day 90; females on Days 28 and 35, and from Day 49 through Day 90. There was no statistically significant difference in the group mean weights in low dose males or females. The high dose male and female body weight gains were significantly reduced on Day 7 through Day 35. A statistically significant weight loss was recorded on Day 42 until sacrifice for both the high dose males and females. Mid dose male body weight gains were reduced on Days 70 and 84. The mid dose females exhibited a statistically significant smaller body weight gain on Days 21, 28 and 84. In addition, the total gain for the high and mid dose groups were significantly reduced. The amount of feed consumed by the high and mid dose rats was also statistically reduced during the study. The amount of feed consumed by the high dose DETDA treated male rats was significantly reduced on Days 14, 21, 24, 28, 35 and 38. Females from the high dose displayed a statistically significant reduction in food consumption on Day 17 through Day 52. Mid dose male consumption was significantly reduced on Days 45, 59, 63, 73 and 90. Females from this dosage group were significantly reduced on Days 45, 52, 59, 63, 70, 73 and Day 80 through 90. No differences were recorded between control and low dose female consumption. Low dose males, however, exhibited statistically significant increases in the amount of feed consumed on Day 3 through Day 28 and on Day 35.

Twenty-seven rats died from the high dose group during the study. Thirteen were found dead and fourteen were moribund sacrificed. The majority of the necropsy observations were non-specific. These observations included stomach lesions, dark discoloration of the liver, kidneys and/or genital organs and intestines, emaciation and apparent animal dehydration, lung congestion, liver foci and lack of thymic tissue.

Summary (Continued)

A pretermination ocular examination was performed on all the remaining animals. Twenty ocular lesions were noted. Two, one, four and thirteen from control to high dose, respectively. With the exception of a focal cataract observed in one mid dose male, observations recorded on the control, low and additional mid dose animals, were considered inconsequential of treatment. Observations noted in the high dose group included two rats displaying pale fundus, one conjunctivitis, one multifocal cataract, one multifocal anterior cortical and equatorial cataract each from both eyes and three focal cataracts from the right eyes. These noted abnormalities may be considered to be a direct or indirect effect from test article administration.

Terminal necropsy of the surviving rats revealed similar observations in each respective treatment group. These observations included kidneys that appeared mottled, dilated pelvis, subcapsular cystic or presence of calculi. Lobular or small livers, congested lungs, crystalline material in the gastrointestinal tract with or without gas distention were observed in each treatment group. A few high dose rats, however, exhibited small spleens and a larger number of focal or linear stomach lesions. In general, the high dose animals appeared thin and dehydrated.

Statistically significant decreases in the high dose male kidneys, liver, testes and absolute brain weight were observed. Both high dose male and female relative adrenal, kidneys and brain weights was significantly larger. In addition, the mid dose male and female liver, kidney and female brain weights to body weight were statistically larger than the control values. High dose males also displayed significant increases in kidney, liver and testes weight when compared to the percent body weight.

Evaluation of the blood chemistry parameters revealed statistically significant increases in SGPT, SGOT and blood urea nitrogen values in the male and female high dose groups. In addition, high dose males exhibited significant reduction in albumin, globulin, calcium, phosphorus, creatinine and total protein determinations. High dose females exhibited significant increases in GGTP and decreases in total protein content.

High dosed males exhibited statistically significant increases in erythrocyte and hemoglobin counts. High dose male hemoglobin content was also significantly lower than control males. Hematocrit and erythrocyte values were statistically larger for the high dose females. Males from this dosage group displayed significant decreases in leucocyte count, platelet count and mean corpuscular volume. Low and mid dose females exhibited significantly larger hematocrit levels.

Treatment-related microscopic changes were present in all of the male and female rats receiving DETDA at 320 ppm. In these high dose rats there was a high incidence of bilateral cataractous change in the eyes, diffuse atrophy of the acinar cells of the pancreas, bone marrow depletion, tubular vacuolation (hydropic change) of the kidneys and vacuolation of the islet cells of the pancreas, atrophy of many organs, lymphoid depletion of the spleen, thymus and mesenteric lymph node, and increase pigmentation of the liver and spleen.

A minimal to moderate multifocal degeneration of the acinar cells of the pancreas and increased splenic pigmentation in the females were present in the tissues examined from the rats receiving 50 and 125 ppm of DETDA in the diet.

Based upon the results of the Subchronic 90 Day Oral Toxicity Study in Rats with diethyl toluene diamine (DETDA) dose levels will be selected for a 24 month Oncogenecity Study in Rats.

Subchronic 90 Day Oral Toxicity Study - Rat

PH 470-ET-001-86

Sponsor:

Ethyl Corporation
451 Florida
Baton Rouge, LA 70801

Testing Facility:

Pharmakon Research International, Inc.
Waverly, PA 18471

Test Facility
S.O.P. No.:

PH-470

Study No.:

PH 470-ET-001-86

Purpose of
the Study:

To provide information on possible health hazards likely to arise from repeated oral exposures of diethyl toluene diamine (DETDA) for a period of 90 days and provide an estimate of a no-effect level of exposure which can be used in chronic studies and for establishing safety criteria for human exposure.

Ownership of
the Study:

The sponsor owns the study. All raw data, wet tissue, analysis and reports are the property of the sponsor. All wet tissue, paraffin blocks and slides are maintained by Experimental Pathology Laboratories.

Study Monitor:

Marcia Hardy, D.V.M., Ph.D., Ethyl Corporation

Study Director:

Robert W. Naismith, Ph.D., Pharmakon Research International, Inc.

Ophthalmologist:

Thomas J. Kern, D.V.M., DACVO, Cornell University

Study
Pathologist:

Larry Ackerman, V.M.D., Ph.D., Experimental Pathology Laboratories

Principal
Investigator:

Dennis J. Margitich, B.S., RLAT, Pharmakon Research International, Inc.

Technical
Performance:

Dennis J. Margitich, B.S., RLAT, Victor T. Mallory, B.S., RLAT, Karen H. Lantzsich, RLAT, Gary G. Bolus, B.A., LAT, Susan V. Mecca, B.S., LAT, Yvonne Coccetti, LAT, Patricia Giglio, B.S., Susan Ayers and Pamela Barton, B.S.

O.A.U.
Responsible
Personnel:

Douglas B. Hay, Ph.D., Leslie Maas, B.S. and Leslie Pinnell, M.S.

Subchronic 90 Day Oral Toxicity Study
in Rats
PH 470-ET-001-86

Dates of Performance:

June 10, 1986 through September 10, 1986
Necropsy: September 9 and 10, 1986

Good Laboratory Practices Statement:

This study was conducted in compliance with the Good Laboratory Practices Regulations as stated in the EPA Good Laboratory Practice Standards [Subpart I, Part 792, Chapter I of Title 40, Code of Federal Regulations], as well as the Organization for Economic Co-operation and Development (OECD) Guidelines for Testing Chemicals, ISBN 92-64-12221-4, adopted by the council at its 535th meeting on 12 May, 1981.

Records Maintained:

All raw data, final reports, documentation and the protocol and amendments are maintained in the Pharmakon Research Central File.
Body weights (initial, weekly, final or as appropriate)
Food consumption, twice weekly
Animal Health Certificate (Purchase Order)
Schedule of events
Water and food analysis
Feed Lot Number
Formalin Lot No. and Supplier
Temperature and humidity recordings
Test article preparation
Samples of test article
Identification sample of ear markings
Observed mortality
Pharmacological and toxicological signs
Necropsy findings
Organ Weights
Hematology reports
Ophthalmologic findings
Clinical biochemistry reports
Histopathological reports

Notebook Reference:

Notebook #1114

Statistical Analysis:

Evaluation of equality of means was made by the one way analysis of variance using the F distribution to assess significance. If significant differences among the means were indicated, Dunnett's test was used to determine significant differences from control means. Analysis of discrete data where appropriate was conducted using non-parametric procedures.

Computer Analysis:

All raw data were summarized and statistically evaluated using LABCAT modules designed by Innovative Programming Associates, Inc., One Airport Place, Princeton, NJ 08540.

Subchronic 90 Day Oral Toxicity Study

In Rats

PH 470-ET-001-86

Raw Data:

Standard Pharmakon Research Notebook
Computer generated Pharmakon Research Study Forms
All raw data

Archive Retention:

All raw data. Sample of test article. Wet tissue, paraffin blocks and slides will be maintained at Experimental Pathology Laboratories, P.O. Box 474, Herndon, VA 22070.

TEST ARTICLE

Compound

Description:

diethyl toluene diamine (DETDA) -- amber liquid

Run Number:

544

Drum Number:

6131

Amount Submitted:

10 gallons

Date Received:

March 27, 1986

Special Handling
Instructions:

Standard precautions

Test Article
Preparation:

Preparation of the test article for administration to the rats consisted of incorporating the test article in the diet utilizing a liquid-solids blender, (Twin-Shell Intensifier Blender, Patterson-Kelly Company, East Stroudsburg, Pennsylvania) according to the Standard Operating Procedure PH-036A on file at Pharmakon Research International, Inc.

Diet Mixture
Samples

Samples of diet mixtures (approximately 200 grams each) were placed into Nalgene[®] jars for shipment to the analytical chemistry laboratory at Ethyl Corporation, 8000 GSRI Avenue, Baton Rouge, Louisiana, 70820. The container was labeled with the following information:

1. Study number
2. Date of sampling
3. Day into study
4. Dose level of compound in the feed
5. Test article or code for test article

The samples were packed in dry ice in a styrofoam container. An additional set of all samples was retained and stored at -20°C until the results of the analysis were received.

Subchronic 90 Day Oral Toxicity Study
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Homogeneity: This test was conducted by the sponsor prior to initiation of the study to verify the homogeneity of the test article mixture in the feed. Three different levels (top left, top right and bottom) from the blender were sampled. A total of three samples per mixing level, 9 samples for three dose levels, were collected and submitted to the sponsor each time the diet was prepared.

Dose Verification: A sample of the diet at each level as well as control feed was submitted for analysis on June 3, June 18, July 2, July 16, August 7 and August 25, 1986.

Stability in Diet: The stability in the diet was determined by the sponsor to be 36 days.

Stability Under Test Conditions: The stability under test conditions was determined by the sponsor and the diet was renewed twice a week.

Authenticity and Purity of Test Article: The purity, identity, strength and stability of the test article is the responsibility of the sponsor. There was no apparent change in the physical state of the test article during administration.

Stability of Test Article under Conditions of Storage: Stability under conditions of storage (nitrogen blanketed at room temperature) was determined at days 0 (June 10, 1986), 45 (July 25, 1986) and at termination (September 10, 1986). These samples were submitted to the sponsor for analysis.

Test Article/Diet Mixtures: Samples of the test article/diet mixtures and untreated diet were submitted to the sponsor on June 3, June 18, July 2, July 16, August 7 and August 25, 1986 for analysis of test article concentration in diet mixtures. The results of these analyses are attached on Appendix 3.

TEST SYSTEM

Species: Rat

Strain: Sprague Dawley

Supplier (Source): Charles River Laboratories, Wilmington, Massachusetts

Sex: Male and female

Age at Initiation: Forty-one (41) days old

No. on Study: One hundred and eighty (180) (90 males and 90 females)

subchronic 90 Day Oral Toxicity Study
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Method and
Justification for
Randomization:

Treatment groups were housed by vertical cage positioning. Randomization was carried out by use of a random number table. One week before treatment commenced all rats were weighed, ranked according to body weight and assigned to treatment groups using a table of random numbers so that each treatment group would have a similar distribution according to body weight. An Analysis of Variance Test was performed to insure that no statistical significance was present.

Acclimation
Period:

The rats were acclimated twelve days. During this conditioning period, the rats were weighed and observed for any pharmacotoxic signs of disease or inadequate weight gain.

System of
Identification:

Individual cages were marked with a rat number. Rats were individually identified by ear tags (Gey Band). The first cage card for each group and sex contained a legend of compound and dose level as well as rat number.

HUSBANDRY

Research Facility
Registration:

U.S.D.A. Registration No. 23-107 under the Animal Welfare Act 74: SC 2131 et seq.

Animal Rooms:

Separate isolation by test system.
Light cycle - 12 hours light, 12 hours dark.
Temperature/Humidity - Every attempt was made to maintain a temperature of 22°C ± 3°C and a humidity of 30 to 70%.

Housing:

Rats were housed individually in stainless steel 1/2" wire mesh cages. Size in accordance with the "Guide for the Care and Use of Laboratory Animals" of the Institute of Laboratory Resources, National Research Council.

Sanitization:

Waste material was removed three times per week. Cages and feeders were sanitized every two weeks.

Food:

Purina Certified Rodent Meal^R, ad libitum. Feeders were designed to reduce soiling, bridging, and scattering.

Food Analysis:

Certified food was used and the analysis from the supplier maintained in the central files.

Water Analysis:

Availability - fresh tap water, ad libitum. Water is monitored for contaminants at periodic intervals according to Standard Operating Procedure PH-018.

subchronic 90 Day Oral Toxicity Study
in Rats
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METHODS

Rationale for
Test System:

Rats have historically been used for establishing safety criteria for human exposure.

Compound
Preparation:

Chemically treated diet was prepared on June 3, June 18, July 2, July 16, August 6 and August 24, 1986.

Rationale for
Dose Selection:

Based upon a 28 Day Dose-Range-Finding Study.

Dose Administration

<u>Group</u>	<u>No. of Animals</u> ¹	<u>ppm</u>	<u>Concentration</u>	
			<u>Estimated % of Diet</u>	<u>mg/kg/rat/day</u>
I	40 (20 male, 20 female)	0	0.0	0
II	40 (20 male, 20 female)	50	0.005	5
III	40 (20 male, 20 female)	125	0.0125	12.5
IV	40 (20 male, 20 female)	320	0.032	32

¹Ten males and 10 females were sacrificed pre-study (baseline data).

Route of
Administration:

Oral, in diet

Rationale for
Route of
Administration:

To determine the toxicity of the test article by oral ingestion.

Frequency and
Duration of
Administration:

Daily for ninety (90) days.

Length of Study:

Dose phase, 90 days.

Methods of Study
Performance:

Four groups of rats (20 males and 20 females per group) were initially fed with the test article at dose levels of 0, 50, 125 and 320 ppm, seven days per week over a period of 90 days. Pharmacotoxic signs were recorded as they were observed, including the time of onset, degree and duration. Cageside observations included, but were not limited to changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system, somatomotor activity and behavior patterns. Measurements were made of food consumption twice weekly, the diet was replenished with fresh freezer stored diet at the time of food consumption and the rats were weighed weekly. At the end of the

Subchronic 90 Day Oral Toxicity Study
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90 day period all surviving animals were sacrificed by CO₂ asphyxiation. Any weak or moribund animals were removed, sacrificed and necropsied during the study as deemed necessary by the study director and principle investigator.

CLINICAL EXAMINATIONS

Clinical
Laboratory
Studies:

Blood was drawn by percutaneous cardiocentesis. All animals were fasted overnight prior to the time of sacrifice. The following determinations were made on twenty rats (10/sex) prior to initiation. In addition, after 90 days of feeding, five (5) animals per sex per group were chosen by random number tables and the following were determined.

Hematology: Hemoglobin
Hematocrit
Erythrocyte count
Total and differential leucocyte counts
Platelet count

Clinical
Biochemistry:

Calcium
Phosphorus
Chloride
Sodium
Potassium
Fasting Glucose
Serum alanine aminotransferase
Serum aspartate aminotransferase
Gamma glutamyl transpeptidase
Urea nitrogen
Albumin
Blood creatinine
Total bilirubin
Total serum protein measurements

Ophthalmologic:

Ophthalmological examinations using an ophthalmoscope were made prior to the administration of the test substance on all animals. At termination of the study 5/sex/group chosen from a table of random numbers were evaluated. If changes in the eyes were detected all the animals would be examined.

PATHOLOGY

Gross Necropsy:

All animals were subjected to necropsy which included examination of the external surface of the body, all orifices, and the cranial, thoracic, abdominal and pelvic cavities and their contents. The following organs were weighed:

Subchronic 90 Day Oral Toxicity Study
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liver gonads
adrenal glands brain
kidneys

The following organs and tissues from the animals at the 90 day sacrifice were preserved in 10% neutral buffered formalin for future histopathological examination:

All gross lesions
Brain-including sections of medulla/pons
Cerebellar cortex and cerebral cortex
Pituitary
Thyroid/parathyroid
Thymus
Lungs
Trachea
Heart
Sternum with bone marrow
Salivary glands
Liver
Spleen
Kidneys/adrenals
Pancreas
Gonads
Uterus
Accessory genital organs (epididymides, prostate,
and, if present, seminal vesicles)
Aorta
Skin
Esophagus
Nasal turbinates
Stomach
Duodenum
Jejunum
Ileum
Cecum
Colon
Rectum
Urinary bladder
Representative lymph node
Mammary gland
Thigh musculature
Peripheral nerve
Eyes
Femur-including articular surface
Spinal cord at three levels - cervical, midthoracic
and lumbar
Exorbital lachrymal glands

Histopathology:

A full histopathologic evaluation of the above organs and tissues was evaluated in all control and high dose

animals sacrificed at 90 days of treatment. All animals which died or were sacrificed in a moribund condition prior to completion of the study were also subjected to a full histopathologic evaluation of the above named tissues and organs. All gross lesions in all animals were examined histologically.

RESULTS

Body Weight:

The group mean body weight data are presented in Table 1. The summary plots are presented in Figures 1. Individual body weight data are attached in Appendix I. The high dose (320 ppm) group mean male body weights were significantly reduced from study Day 7 through Day 90 when compared to the controls. The mid dose (125 ppm) group mean male body weights were significantly reduced from study Day 77 through Day 90. The group mean high dose female body weights were significantly reduced from Day 14 through Day 90. In addition, the mid dose female weights were significantly reduced on Days 28 and 35, and from Day 49 through Day 90.

Body Weight Gains:

The summary of the group mean body weight gains are set forth in Table 2. The summary plots are presented in Figures 2. The individual data are attached in Appendix I. The high dose male body weight gains were significantly reduced on Day 7 through Day 35. The high dose males also displayed a statistically significant body weight loss on Day 42 through Day 90. The total male high dose gain was also significantly decreased when compared to the controls. The mid dose male body weight gains were significantly reduced on Days 70 and 84. The total mid dose gain was also statistically decreased. A statistical increase in the low dose male body weight gains occurred on study Day 21. In a similar manner, the high dose female body weight gains were significantly reduced on Day 7 through Day 35. A statistically significant weight loss was exhibited by the high dose females on Day 42 through Day 90. The total body weight gain was also significantly decreased. Mid dose females exhibited a statistically significant reduced gain on Days 21, 28 and 84. The total gain was also significantly decreased. No statistically significant changes were observed in the low female dosage group.

Food Consumption:

The group mean food consumption determinations are presented in Table 3. The summary plots are presented in Figures 3. The individual food consumption data

Subchronic 90 Day Oral Toxicity Study
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PH 470-ET-001-86

are attached in Appendix I. The amount of feed consumed by the high dose DETDA treated male rats was statistically reduced on Days 14, 21, 24, 28, 35 and 38. The mid dose male food consumption was significantly reduced on Days 45, 59, 63, 73 and 90. Statistically significant increases in the amount of feed consumed by the low dose male group was recorded on Day 3 through Day 28 and on Day 35. Female high dose food consumption was significantly reduced on Day 17 through Day 52. Mid dose females exhibited a statistically significant reduced feed consumption on Days 45, 52, 59, 63, 70, 73 and Day 80 through 90. No differences were noted between control and low dose female feed consumption. Significant differences in mean food consumption among the four groups were also found on Days 31, 42, 56, 66, 70 and 77, however, no additional significance was noted between control and DETDA treated groups.

Clinical Signs:

Pharmacotoxic signs were observed and recorded for the individual animals and presented in Table 4. Three control males exhibited lesions, alopecia and/or scab formations in the ventral cervical region. Control male #1015, exhibited an opaque, buphthalmic left eye. Inferior, anterior synechia was subsequently diagnosed. This ocular abnormality resulted in chronic keratitis. There were no additional control male or female observations recorded. A similar lesion and alopecia formation were observed in one low dose male rat. Low dose females displayed no pharmacotoxic or abnormal signs. Alopecia and/or lesion formation with or without scab formation was observed from one mid dose male and two mid dose females. In addition, piloerection and chromodacryorrhea were exhibited from one mid dose male. These abnormalities were the result of a dislodged upper incisor. Transient piloerection (2 days) was observed from mid dose male #1087. Transient poor grooming was also observed from one mid dose female. With the exception of four rats (1 male and 3 females), all the high dose animals exhibited several combinations of pharmacotoxic observations. These included abnormal stance, abnormal gait, flaccid body tone, ptosis, decreased activity, chromodacryorrhea, elevated gait, loss of skin elasticity, poor grooming, dyspnea, tremors, ataxia and morbidity. In general, these signs resulted in either moribund sacrifice or death in the majority of these high dose rats.

Mortality:

Scheduled sacrifices, moribund sacrifices and rats dying on test are presented in Table 4. Twenty-seven

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rats from the high dose group died during the study. Thirteen (9 females and 4 males) were found dead and fourteen (7 females and 7 males) were moribund sacrificed. At necropsy, there were no visible lesions recorded for seven females and four males. The majority of the observations recorded for both the males and females were non-specific. These observations recorded included stomach lesions, dark discoloration of the liver, kidneys and/or genital organs, discolored anal region, discoloration and/or distention of the intestines, emaciation and apparent dehydration of the animal, lung congestion, liver foci and lack of thymic tissue. The microscopic correlations of these necropsy observations with the histopathology are found in Appendix II, containing the histopathological evaluation from Experimental Pathology Laboratories.

Necropsy:

The incidence of the gross necropsy findings are summarized in Table 5. The individual observations recorded at necropsy and the individual necropsy sheets are attached in Appendix I. Similar observations, such as kidneys, that appeared mottled, dilated pelvis, subcapsular cystic or presence of calculi. Lobular or small livers, congested lungs, crystalline material in the gastrointestinal tract with or without gas distention were observed in each treatment group. There were, however, a few necropsy findings which occurred with greater frequency in the high dose group. The spleens of a few of the high dosed rats appeared small. There were a larger number of focal or linear stomach lesions. In general, the high dose animals appeared thin and dehydrated. With the exception of these observations, the majority of tissues examined appeared normal.

Absolute Organ Weights:

The absolute organ weight data are presented in Table 6. The individual organ weight raw data may be found in Appendix I. Statistically significant decreases in the high dose group male kidneys, liver, testes and brain weights were observed when compared to the controls. No significant differences were observed in the female organ weights among the four dose groups.

Relative Organ to Body Weights:

The relative organ to body weights are presented in Table 7. The individual organ to body weight data is attached in Appendix I. Males and females from the high dose group exhibited statistically significant increases in adrenal, kidneys and brain weights to percent body weight. In addition, high dose male testes weights and female liver weights were significantly larger. Also, the male and female mid

Subchronic 90 Day Oral Toxicity Study
in Rats
PH 470-ET-001-86

dose liver and kidney weights and female brain weights were statistically larger than control values.

Relative Organ to
Brain Weights:

The relative organ to brain weight data are presented in Table 8. No statistically significant differences for the females were observed among the four groups. High dose males, however, exhibited a statistically significant increases in kidneys, liver and testes weights when compared to percent brain weight. The individual data recorded for the organ weights when compared to the body and brain weights are presented in Appendix I.

Clinical Chemistry:

Results of the terminal blood chemistry evaluations are summarized in Table 9. The individual data are presented in Appendix I. Males in the high dose group exhibited statistically significant decreases in albumin, globulin, calcium, phosphorus, creatinine and total protein. Increases which were statistically significant by the high dose males were observed for the blood urea nitrogen, SGPT and SGOT values. The GGPT male values were markedly larger, although not statistically significant. High dosed females displayed statistically significantly larger blood urea nitrogen, GGTP, SGPT and SGOT values. The total protein values for the high dose females were also statistically reduced.

Hematology:

The results from the hematologic evaluation are summarized in Table 10. The individual data are presented in Appendix I. Statistically significant decreases were observed in the high dose male mean corpuscular volume, leucocyte and platelet counts. The high dose male erythrocyte and hemoglobin values were statistically larger when compared to controls. High dose females exhibited statistically significant increases in erythrocyte values. Hematocrit counts for each DETDA-treated female group were statistically larger than the control group. There were no additional statistically significant changes in any hematology parameters for either males or females when the test-article treatment groups were compared to the controls. However, a few instances of individual hematology values outside the normal range were found in each dosage group.

Ophthalmologic
Examination:

All rats in each dose group were examined prior to study initiation and at termination utilizing indirect ophthalmoscopic and biomicroscopic procedures. The examination evaluated adnexa, conjunctiva, sclera, cornea, anterior chamber, lens and posterior segment (vitreous, retina and optic disc). Eleven rats

Subchronic 90 Day Oral Toxicity Study
in Rats
PH 470-ET-001-86

exhibited abnormal ocular defects prior to study initiation. These animals were subsequently replaced with normal animals. On Day 87 of the study, a pretermination ocular examination was performed on all the remaining rats. Twenty ocular lesions were noted. Two, one, four and thirteen from control to high dose, respectively. Striate retinopathy (right eye) and chronic keratitis were observed in two rats (males) from the control group. Striate retinopathy (right eye) was observed from one low dose female. Two males from the mid dose group also displayed striate retinopathy (right eye). In addition, hyaloid remnant (left eye) from one male and focal posterior cataract (left eye) from one female were also observed in the dosage group. With the exception of the focal posterior cataract, the abnormalities noted are inherent of this species' age group. Eleven high dose rats (males and females) exhibited nuclear sclerosis of both eyes. Two displayed pale fundus, one conjunctivitis, one multifocal cataract, one multifocal anterior cortical and equatorial cataract each from both eyes and three focal cataracts from the right eyes. These noted abnormalities may be considered to be a direct or indirect effect from test article administration.

Histopathology:

Treatment-related microscopic changes were present in all of the male and female rats receiving DETDA at 320 ppm. In these high dose rats, there was a high incidence of bilateral cataractous change in the eyes, diffuse atrophy of the acinar cells of the pancreas, bone marrow depletion, tubular vacuolation (hydropic change) of the kidneys and vacuolation of the islet cells of the pancreas, atrophy of many organs, lymphoid depletion of the spleen, thymus and mesenteric lymph node, and increase pigmentation of the liver and spleen.

A minimal to moderate multifocal degeneration of the acinar cells of the pancreas and increased splenic pigmentation in the females were present in the tissues examined from the rats receiving 50 and 125 ppm of DETDA in the diet.

A detailed evaluation of the microscopic changes are presented in Appendix II from Experimental Pathology Laboratories, Inc.

CONCLUSION

Based upon the results of the Subchronic 90 Day Oral Toxicity Study in Rats with diethyl toluene diamine (DETDA) dose levels will be selected for a 24 month Oncogenecity Study in Rats.

PHARMAKON RESEARCH INTERNATIONAL, INC.
SUBCHRONIC 90 DAY ORAL TOXICITY STUDY IN RATS
 STUDY NUMBER: PH-470-ET-001-84 TEST ARTICLE: DETDA
 SPONSOR: ETHYL CORPORATION, BATON ROUGE, LA
 TABLE 1

SUMMARY OF BODY WEIGHTS (gms)

PERIOD	DOSE: (ppm) GROUP:	BODY WEIGHT			
		0	50	125	200
		1-M	2-M	3-M	3-M
DAY 0	MEAN	184	186	187	187
	S.D.	8.6	8.7	10.2	7.4
	N	20	20	20	20
DAY 7	MEAN	206	243	237	226*
	S.D.	11.6	17.9	14.8	9.8
	N	20	20	20	20
DAY 14	MEAN	283	291	277	260**
	S.D.	16.7	19.0	18.9	17.6
	N	20	20	20	20
DAY 21	MEAN	321	337	318	290**
	S.D.	21.3	27.0	22.7	17.0
	N	20	20	20	20
DAY 28	MEAN	352	369	344	306**
	S.D.	27.9	28.4	24.8	24.2
	N	20	20	20	20
DAY 35	MEAN	381	401	369	312**
	S.D.	26.3	30.8	26.8	22.2
	N	20	20	20	20
DAY 42	MEAN	406	428	397	307**
	S.D.	28.6	32.1	28.1	22.7
	N	20	20	20	20
DAY 49	MEAN	431	454	418	291**
	S.D.	31.9	35.6	30.1	21.8
	N	20	20	20	20
DAY 56	MEAN	451	474	432	272**
	S.D.	32.6	40.1	28.9	19.6
	N	20	20	20	19
DAY 63	MEAN	472	495	449	290**
	S.D.	34.7	44.7	37.8	22.8
	N	20	20	20	18

* P Less than .05
 ** P Less than .01

Analysis of Variance Using DUNNETT'S Procedure

REPORT CONTINUED

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APPENDIX II

PH 470-ET-001-86

EXPERIMENTAL PATHOLOGY
LABORATORIES REPORT

CONTAINS NO CD 1

2
EPL

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

PHARMAKON STUDY NUMBER PH 470-ET-001-86
SUBCHRONIC 90 DAY ORAL TOXICITY STUDY IN RATS
PATHOLOGY REPORT

Submitted to:
Pharmakon Research International, Inc.
Waverly, PA 18471

December 1, 1986

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PATHOLOGY SUMMARY

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EPL

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

PHARMAKON STUDY NUMBER PH 470-ET-001-86
SUBCHRONIC 90 DAY ORAL TOXICITY STUDY IN RATS
PATHOLOGY SUMMARY

Microscopic examinations were performed on selected tissues from 160 Sprague-Dawley rats used in a subchronic ninety-day oral toxicity study. The purpose of the study was to provide information on possible health hazards likely to arise from repeated oral exposures of diethyl toluene diamine (DETD) and provide an estimate of a no-effect level of exposure which can be used in chronic studies and for establishing safety criteria for human exposure.

The animals used in this study are outlined in the following table:

Group	No. of Animals	Deaths	Concentration		
			ppm	% of Diet	Estimated mg/kg/rat/day
I	40 (20M, 20F)	-	0	0.0	0
II	40 (20M, 20F)	-	50	0.005	5
III	40 (20M, 20F)	-	125	0.0125	12.5
IV	40 (20M, 20F)	12M, 15F	320	0.0320	32

According to protocol, the following hematoxylin and eosin stained tissues were evaluated from all rats in Groups I and IV: adrenals, aorta, bone marrow (sternum)*, femur, brain (cerebrum, cerebellum, and brain stem), testes with epididymides, esophagus, eyes with lacrimal glands, ovaries, heart, duodenum, jejunum, ileum, cecum, colon, rectum, kidneys, liver, lung with mainstem bronchi, nasal turbinates, mesenteric lymph node, mammary gland, peripheral nerve,

*Animal Number 1180, Group III female, had bone marrow evaluated from femur.

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EPL

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

pancreas, pituitary, prostate, salivary gland, seminal vesicles, skeletal muscle, skin, spinal cord (cervical, thoracic, and lumbar), spleen, stomach, thymus, trachea, thyroid, parathyroid, urinary bladder, cervix, uterus (corpus), vagina and gross lesions.

Based on the microscopic findings found in the high dose (Group IV) rats, the following tissues were evaluated from the rats in Groups II and III: bone marrow (sternum), pancreas, eyes, kidneys, liver, mesenteric lymph node, salivary gland, spleen, stomach, thymus, and all gross lesions.

In general, all tissues to be examined, as called for in the protocol, were represented in the sections. Only a few tissues were apparently inadvertently missed at the time of necropsy. Varying degrees of autolysis were evident microscopically in tissues from many of the rats that died prior to sacrifice. However, these factors did not affect the overall evaluation of the study.

Hematoxylin and eosin stained slides of the protocol-required tissues were prepared by Experimental Pathology Laboratories, Inc. Microscopic findings for each tissue examined from each animal are listed in the Histopathology Incidence Tables. Inflammatory, degenerative, and hyperplastic changes were graded one to five depending upon severity; nongradable changes such as cysts, and developmental changes were designated as present (P) in the Histopathology Incidence Tables. Tissues that were autolyzed to a moderate or moderately severe degree, but could be evaluated for lesions, are tabulated with the diagnosis and

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an "A" to indicate autolysis. Those that were severely autolyzed and could not be evaluated are indicated with an "A" only. All lesions are summarized by treatment groups in the Summary Incidence Tables together with the total number of animals in each group for which the tissues were examined. A tabulation of gross lesions observed at the time of necropsy with the corresponding microscopic change, if appropriate, is given in the Correlation of Gross and Microscopic Findings Tables. The descriptions of gross findings on these tables were transcribed from the Individual Animal Necropsy Sheets prepared at the time the necropsies were performed.

RESULTS

Treatment-related microscopic changes were present in the eyes, pancreas, bone marrow, liver, spleen, kidneys, and mesenteric lymph nodes of almost all of the male and female rats receiving 320 ppm of diethyl toluene diamine (DETD) in the diet. In addition, atrophy was present in the salivary gland, thymus, stomach, and genital organs of both sexes in this group. Treatment-related changes were present in the pancreas and spleen from the rats receiving 50 and 125 ppm of DETDA in the diet.

In the high dose rats, there were minimal to moderate bilateral degenerative changes of the lenses of the eyes (cataractous change) in all of the males and fifteen of the twenty females. The changes of the lenses were primarily anterior subcapsular degeneration of the lens

cortical cells sometimes accompanied by a hyperplasia of the anterior epithelium. In more severe cases, there was involvement of the cortical cells of the posterior polar area.

The pancreatic changes in the high dose rats involved both the acinar (exocrine) cells and the islet (endocrine) cells. There was a slight to moderate diffuse atrophy of the acinar cells in all of the males and nineteen of the twenty females. Female Number 1185 had a normal pancreas. The atrophy of the acinar cells was characterized, generally, by small acini surrounded by a thickened interstitial tissue. The interstitial tissue appeared hypercellular due to the presence of atrophic cells but there was no indication of an inflammatory infiltration or interstitial fibrosis. In most of the rats, the pancreas also had a vacuolation and a reduction in the quantity of islet cells. This vacuolation of islet cells was present in fifteen males and thirteen females.

The bone marrow in all of the high dose males and in eighteen females had a reduction in cellularity with a slight to severe hemorrhage and/or congestion of the marrow cavity. The bone marrow of female rats numbered 1186 and 1185 was comparable to the control female rats. The kidneys of all of the high dose males and thirteen females had a minimal to moderate vacuolation of the cytoplasm of the tubules of the cortex. This type of tubular change has been described as a hydropic change or osmotic nephrosis.

Lymphoid depletion was present in many of the high dose male and female rats as demonstrated in the thymus, spleen, and mesenteric lymph node. Moderately severe to severe involution of the thymus was present in nine males and eight females. The thymus was missing from the sections of "thymic area" evaluated in eleven males and ten females (assumed to be completely involuted). The thymus of female Number 1186 was only slightly involuted. In the high dose groups, lymphoid depletion was present in the spleen of thirteen males and twelve females and in the mesenteric lymph nodes of seventeen males and twelve females. Thymic involution and lymphoid depletion of spleen and mesenteric lymph nodes were not observed in any of the control, low, or mid dose rats.

Atrophy of numerous organs was present in the high dose group but not in the low or mid dose groups. These changes were observed grossly as emaciated and/or dehydrated animals and were consistent with the large number of early deaths. This atrophy was most evident in the depletion of periaortic adipose tissue. Diffuse atrophy was present in the salivary glands, glandular mucosa of the stomach, testes and associated male organs, uterus and associated female organs, and in the myopathy (atrophy) of the skeletal muscle.

In the high dose groups, there was an increase in the amount of pigment found in the liver and spleen. A brown pigment was present in the Kupffer cells of the liver in ten males and eleven females. This pigment was probably hemosiderin and similar to the pigment found in increased severity in the spleen of the high dose male and female rats.

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The amount of splenic pigmentation in all of the groups is presented in the following table:

SPLENIC PIGMENTATION (No. Examined)	Group: I		II		III		IV	
	Dose: Control		Low		Mid		High	
	M	F	M	F	M	F	M	F
(No. Examined)	(20)	(20)	(20)	(20)	(20)	(20)	(20)	(19)
Minimal	3	-	-	-	-	-	-	-
Slight	10	6	10	3	10	1	1	-
Moderate	7	13	10	9	10	13	10	4
Moderately severe	-	-	-	8	-	6	9	11
Severe	-	-	-	-	-	-	-	3

The amount of pigment was increased in the spleens of the low and mid dose female groups and pigment not present in the livers of the males or females of these two treated groups of rats.

In the group treated with 125 ppm of DETDA, there was an increase in the incidence and severity of multifocal degeneration of acinar cells of the pancreas when compared to the male and female control rats. In the males, all but one of the rats had a slight to moderate degree of degeneration of acinar cells. In the females, fourteen of twenty had minimal to slight degeneration of acinar cells of the pancreas. The incidence and severity of this change in the female rats receiving 50 ppm of DETDA were similar to the control female rats, while in the low dose males there was an increase in both incidence and severity. The changes found in the pancreas for all groups are summarized in the following table:

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	Group: I		II		III		IV	
	Dose: Control		Low		Mid		High	
	M	F	M	F	M	F	M	F
PANCREAS								
(No. Examined)	(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)
Multifocal								
Acinar Degeneration (Total)	(5)	(4)	(12)	(4)	(19)	(14)	-	-
Minimal	4	4	6	4	-	8	-	-
Slight	1	-	5	-	8	6	-	-
Moderate	-	-	1	-	11	-	-	-
Diffuse Acinar Atrophy (Total)	-	-	-	-	-	-	(20)	(19)
Islet Cell Vacuolation (Total)	-	-	-	-	-	-	(15)	(13)

Inflammatory and/or degenerative lesions were occasionally present in the other tissues of a few rats in the treated and/or control groups. Their incidence and distribution did not suggest a treatment-related effect. The kidneys of individual rats had changes comparable with the early stages of chronic progressive nephropathy. These consisted of foci of multifocal nonsuppurative nephritis, tubular dilatation, and tubular regeneration. Incidental changes present in the liver were multifocal nonsuppurative hepatitis, foci of mononuclear cells and multifocal pericholangitis. A lung change consisted of multifocal pneumonitis. When these inflammatory changes in the high dose group are compared to the control groups, there is a definite negative trend. This negative trend in the inflammatory changes composed of mononuclear cell infiltrates correlates directly with the lymphoid depletion in the high dose animals. A few high dose male or female rats did have suppurative inflammatory reactions composed of infiltrations of

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polymorphonuclear leukocytes. These were exemplified as suppurative bronchopneumonia of the lung, suppurative capsulitis of the adrenal, suppurative pyelonephritis of the kidney, suppurative prostatitis, diffuse cystitis of the urinary bladder and suppurative vaginitis. The acute or subacute inflammatory changes were not considered to be a direct treatment-related effect but may have been secondary to some decreased resistance of the animal.

CONCLUSIONS

Administration of diethyl toluene diamine (DETD) to rats by repeated oral exposure for a period of ninety days at levels of 50, 125, and 320 ppm resulted in a high mortality rate in the high dose animals. Treatment-related microscopic changes were present in all of the male and female rats receiving 320 ppm. In these high dose rats, there was a high incidence of bilateral cataractous change in the eyes, diffuse atrophy of the acinar cells of the pancreas, bone marrow depletion, tubular vacuolation (hydropic change) of the kidneys and vacuolation of the islet cells of the pancreas, atrophy of many organs, lymphoid depletion of the spleen, thymus and mesenteric lymph node, and increased pigmentation of the liver and spleen.

A minimal to moderate multifocal degeneration of the acinar cells of the pancreas and increased splenic pigmentation in the females

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were present in the tissues examined from the rats receiving 50 and 125 ppm of DETDA in the diet.



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Pathologist

December 1, 1986

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QUALITY ASSURANCE
FINAL CERTIFICATION

Client Name Pharmakon Research International, Inc. EPL Project Coordinator Dr. Larry J. Ackerman

Client Study PH 470-ET-001-86 EPL Pathologist Dr. Larry J. Ackerman

Species Rat EPL Project Number 219-026

Study Title Subchronic 90 Day Oral Toxicity Study in Rats
 Test Article Diethyl toluene diamine (DETD)

The following phases of this study (marked by "X") were inspected by Experimental Pathology Laboratories Quality Assurance Unit. The dates of the inspections performed are as indicated below. All findings were reported to the EPL Project Coordinator and to Management.

(X)		Date
X	Part 1 of 6 - Project Sheet Preparation	8/13/86
X	Part 2 of 6 - Master Individual Animal Worksheet	8/15/86
X	Part 3 of 6 - Histology Setup	9/18/86, 9/24/86 11/03/86
X	Part 4 of 6 - Histology Completion	10/07/86, 10/15/86 11/25/86
X	Part 5 of 6 - Rough Draft Report	11/12/86, 12/01/86
X	Part 6 of 6 - Final Report	12/01/86
	Other -	N/A

Quality Assurance Janet Milazzo Date 12/1/86