

CONTAINS NO CEN

ETHYL CORPORATION
Health and Environment Department

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Director, Toxicology
and Regulatory Affairs

2,000-PP

Ethyl Tower
451 Florida Street
Baton Rouge, LA 70801

July 2, 1992

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Attention: Section 8(e) Coordinator

Dear Sir:

RE: 8EHQ 1286-0648

This is a follow-up to an 8(e) submission (8EHQ 1286-0648) Ethyl Corporation made in December 1986. Pursuant to the Agency's letter dated January 16, 1987, Ethyl Corporation is submitting the final report on a two-year bioassay on diethyltoluene diamine in Sprague Dawley rats.

If you have any questions, please call me at (504) 388-7608.

Sincerely,
ETHYL CORPORATION

R. L. Smith

R. L. Smith, PhD
Director
Toxicology and Regulatory Affairs

RLS/MLH:dhs
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PHARMAKON
RESEARCH
INTERNATIONAL INC.

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**Oncogenicity Study - Rat
24 Month Study**

PH 474-ET-001-88

Diethyl Toluene Diamine (DETD)

Lot #6581-14

Submitted to

**Ethyl Corporation
Baton Rouge, Louisiana**

Dennis James Margitich
Dennis J. Margitich, B.S., RLAT
Principal Investigator

4/1/92
Date

Vincent B. Ciofalo
Vincent B. Ciofalo, Ph.D.
Study Director

4/1/92
Date

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Oncogenicity Study - Rat
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Diethyl Toluene Diamine
(DETDA)

SUMMARY

The test article, Diethyl Toluene Diamine (DETDA), Lot #6581-14, was incorporated into certified commercial laboratory rat diet and fed *ad libitum* to four groups of Sprague Dawley rats (50 animals/sex/group) seven days per week for 731 days at dose levels of 0 (control), 10 (low), 35 (mid) or 70 (high) ppm. At the end of the feeding period, all surviving animals were sacrificed. During the study, animals were observed daily and weighed weekly. Food consumption was measured twice weekly when the diet was replenished. Blood was collected at approximately 6 month intervals for hematology exams (differential counts). Indirect ophthalmoscopy was performed on all rats prior to study initiation and at approximately 6, 12, 18 and 24 months. A complete necropsy was performed on all rats. Approximately 40 tissues were examined histologically from all rats in the control and high dose groups and all rats that died or were sacrificed during the study. The adrenal glands, eyes, liver, pancreas, pituitary and thyroid glands were evaluated in the mid and low dose groups. The mammary glands and any tissue mass suspected of being a mammary gland tumor were evaluated in the low and mid dose females.

The majority of clinical signs observed were related to aging. These signs were not considered related to the administration of DETDA as they were distributed across the four groups in a non-dose-related manner. Ocular opacity was noted in a few high dose males. This change is considered the only clinical sign which may have been related to the administration of the test article.

Mean body weight of the male high dose group was statistically decreased from the control male body weight beginning on week 43 and continuing throughout the majority of the remainder of the study. Control and mid dose mean body weights were statistically comparable throughout the study except on one occasion. However, a tendency for lower mean body weight in the mid dose males was seen beginning at about month 15 and continuing to the conclusion of the study. No effect, statistical or otherwise, was found on the body weight of low dose males compared to the control males.

The high dose female mean body weight was statistically comparable to the control female body weight except during four weeks of the study (weeks 80 - 83). During these 4 weeks, mean body weight of the high dose females was statistically lower than the female control mean. A tendency for a lower body weight in the high dose females compared to the control mean was seen in the last 3 months of the study. No statistical differences between the control and mid dose female body weight were found. A tendency for a lower body weight in the mid dose was seen during the last four

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(DETDA)

SUMMARY (continued)

weeks of the study. The low dose female body weight was not affected.

Group mean food consumption was not affected by the administration of DETDA. Only sporadic, non-dose-dependent statistical differences in food consumption were detected between control and treated animals of either sex. However, a few male high dose DETDA rats consistently exhibited a marked increase in daily food consumption.

No ocular lesions related to the administration of DETDA were detected on indirect ophthalmoscopy with the exception of bilateral cataracts in several high dose male rats. A correlation between animals affected with ocular cataracts and blood glucose levels greater than 300 mg/dL was observed at the 18 month blood collection. No treatment related effect on white blood cell differential count or red cell morphology was found at any time point.

Survival was comparable between treated and control animals of each sex except in the low dose male rats. Survival in this group was significantly greater than the control males. Per cent survival was maintained above 50% until week 97, 104, 96 and 92 for males in the control, low, mid and high dose groups, respectively. In females, 50% survival was maintained up to week 103, 102, 101 and 98 for the control, low, mid and high dose groups, respectively. Early deaths or moribund sacrifices occurred during the study. Lesions considered to be the cause of death or moribund sacrifice included pituitary tumors, other large and/or malignant neoplasms, severe chronic renal disease and severe lesions of the hock/foot (suppurative and ulcerative dermatitis). Rats in this study reached high body weights early in the course of the study. This factor may have contributed to the development of certain of the changes leading to the early deaths or moribund sacrifices.

The majority of gross macroscopic observations at terminal necropsy were associated with aging and not considered manifestations of toxicity due to treatment. Certain gross changes in the liver may have been related to the test article.

Histopathology results showed a statistically increased incidence in comparison to the control animals of: hepatocellular carcinomas and proliferative lesions in the liver of the high dose males, hepatocellular adenomas and proliferative lesions in the liver of the high dose females, thyroid follicular cell adenomas

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(DETD)

SUMMARY (continued)

and follicular cell hypertrophy in high dose males and fibroadenomas of the mammary glands of mid and high dose females. Hepatic proliferative lesions (basophilic foci and eosinophilic foci) were significantly increased in all three male dose groups. All three male dose groups had an increased incidence and severity of degeneration changes in the liver (focal and multifocal cystic degeneration and multifocal necrosis). Basophilic foci in the liver of high dose females were increased relative to controls. Eosinophilic foci in the liver of low dose, but not mid or high dose, females were increased compared to control females. Pancreatic acinar atrophy accompanied by interstitial fibrosis and fatty infiltration (fatty atrophy) was present in the high dose male rats. Bilateral cataracts were detected in six high dose male rats. The presence of moderate to severe keratitis in half these animals complicates the interpretation of the significance of the cataracts. However, based on the early detection of cataracts by indirect ophthalmoscopy coupled with elevated blood glucose levels, it appears likely these changes were related to administration of test article. Renal cysts and focal areas of tubular hyperplasia were increased in high dose males compared to the control male rats. The relevance of treatment to this effect is equivocal, since some degree of age-associated chronic nephropathy was present in nearly all of the kidneys examined and cysts and tubular hyperplasia are components of chronic nephropathy. No treatment-related neoplastic or nonneoplastic changes were noted in any of the other tissues examined. A variety of spontaneous neoplasms and incidental lesions were present in both the control and DETDA treated rats.

The results of this study should be evaluated in conjunction with the earlier DETDA studies conducted in this laboratory. The 90 day subchronic and 28 day progression/reversibility studies identified the pancreas as the target organ in the rat. Male rats were more severely affected and at an earlier time and lower dose than females. Histologically, pancreatic acinar cells were affected first; islet cell involvement followed afterward in a time and dose dependent manner. Pathology in other organs occurred after or in conjunction with islet cell involvement and appeared secondary to the metabolic changes induced by the pancreatic toxicity. Secondary effects included ocular cataracts in animals with histologic evidence of islet cell toxicity. It was postulated these cataracts were associated with and related to hyperglycemia and thus the pancreatic toxicity. Histologic evidence of liver or thyroid toxicity was not seen.

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Diethyl Toluene Diamine
(DETDA)

SUMMARY (continued)

The greater DETDA sensitivity of male, compared to female, rats was also observed in the two year study. This response holds true for both toxicity and tumorigenicity. However, effects on the liver and thyroid, not seen in the subchronic studies, were detected in the two year study. Based on the absence of these effects in shorter term studies, liver and thyroid effects appear related to chronic, long term exposure.

In the two year study, histologic evidence of pancreatic toxicity was restricted to the pancreatic acinar cells of high dose male rats. A functional aberration was suggested in individual rats by the hyperglycemia, increased food consumption and ocular cataracts observed in a few high dose male rats. No histologic or suggestive evidence of pancreatic toxicity was seen in the mid and low male dose groups or in females at any dose level. Given these results, the no effect level for pancreatic toxicity in the male rat for two years was 35 ppm; for females the no effect level was 70 ppm.

Unlike pancreatic toxicity, hepatic proliferative and degenerative changes were found in all male dose groups. Proliferative lesions only were found in the females. A clear no effect level was not achieved. However, hepatotoxicity would appear related to long term chronic exposure as this effect was not observed in studies less than two years in length. A no effect level of 320 ppm for hepatotoxicity is available from the 90 day subchronic study.

Evidence for thyroid hyperplasia or hypertrophy was found in high dose males and females. Lower dose levels were not affected. Like hepatotoxicity, thyroid effects were not seen in subchronic studies and are an effect of chronic long term exposure. A no effect level for both males and females in this study is 35 ppm.

With respect to tumorigenicity, hepatocellular carcinomas were increased in the male high dose group. The incidence of a benign tumor of the thyroid, follicular cell adenoma, was also increased in high dose males. Hepatocellular adenoma, a benign tumor of the liver, was increased in high dose female rats. Mammary gland fibroadenoma, a benign tumor, were increased in mid and high dose females. The effect on mammary gland tumors is the only instance where the female rats appeared affected at a lower level than the male. Since the incidence of malignant mammary gland tumors

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Diethyl Toluene Diamine
(DETDA)

SUMMARY (continued)

(adenocarcinomas) was high in the control females, the relevance of the increase in fibroadenomas is unknown. A clear no effect level for liver and thyroid tumors can be established at 35 ppm. The questionable significance of the mammary tumors in the mid and high dose females complicates determination of a no effect level in this organ. At a minimum, the no effect level in this organ is 10 ppm and may be higher.

STUDY DESCRIPTION

Oncogenicity Study - Rat
24 Month Study

PH 474-ET-001-88

Sponsor: Ethyl Corporation
451 Florida
Baton Rouge, LA 70801

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

Study No.: PH 474-ET-001-88

Purpose of the Study: The objective of this long-term Oncogenicity Study was to observe the test animals for a major portion of their life span for the development of neoplastic lesions during exposure to various doses of diethyl toluene diamine (DETD) from dietary administration.

Ownership of the Study: The sponsor owns the study. All raw data, wet tissue, analysis and reports are the property of the sponsor.

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

Study Director: Vincent B. Ciofalo, Ph.D. Pharmakon Research International, Inc.

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

Study Pathologist: Larry J. Ackerman, V.M., Experimental Pathology Laboratories.

Study Ophthalmologist: Thomas Kern, D.V.M., DACVO, Cornell University

Technical Performance: Dennis J. Margitich, B.S., RLAT, Yvonne Coccetti, LAT, Alberta Miller, B.S., Susan A. Schirick, A.S., LAT, Paul Jones, Karen H. Lantzsch, RLAT, Gary G. Bolus, B.S., LATG, Bonita Russell, B.S., MT (ASCP), Susan Ayers, CLT, Nira Madison, Karl J. Field, D.V.M., and George Southwick, Jaime Rodriguez and Shahid Sultan (Experimental Pathology Laboratories).

Oncogenicity Study - Rat
PH 474-ET-001-88

Q.A.U.
Responsible
Personnel:

Douglas B. Hay, Ph.D. and Leslie J. Pinnell,
M.S., Pharmakon Research International, Inc.

Date Protocol
Signed:

May 26, 1988

Dates of
Performance:

July 18, 1988 through July 26, 1990 (In life)
Necropsy Dates: July 24, 1990, July 25, 1990
and July 26, 1990

Good Laboratory
Practices:

This study was conducted in compliance with the Good Laboratory Practice 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 report, documentation, and the protocol and amendments will be maintained in the Pharmakon Archives.

Notebook
Reference:

Notebooks #820-863, #1220 and #1260

Computer and
Statistical
Analysis:

Raw data were collected and/or summarized and statistically evaluated using IABCAT modules designed by Innovative Programming Associates, Inc., 303 Wall Street, Princeton, NJ 08540. Evaluation of equality of means (body weight, food consumption, hematology and blood glucose) 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. Statistical analyses (time-to-tumor) were evaluated using Systat (Version 4.1) by Systat Inc., Evanston, IL. Estimates of the probabilities of survival for rats fed diets containing DETDA were calculated using Kaplan and Meier statistics evaluated by SAS (Version 6.03), SAS Institute Inc., Cary, North Carolina. Statistical analysis of histopathological results were performed using the Fisher Exact Test and Cochran/Armitage Linear Trend Test.

Raw Data:

Standard Pharmakon Research Notebook
Computer generated Pharmakon Research Study
Forms

Genotoxicity Study - R
474-EM-001-80

Archive Reception: All raw data, samples of the test article and unreated diet, study file and the final report will be maintained in the Pharmacokin Research Archives. Wet tissues, paraffin blocks and slides will be archived at Experimental Pathology Laboratories.

TEST ARTICLE

Compound Description: Diethyl toluene diamine (DETDA) -- clear yellow liquid

Lot Number: 6531-14

Amount Received: 200 clear glass vials

Date Received: May 10, 1988

Special Handling Instructions: Stored in original glass containers at -20°C (±10°C). Avoid skin and eye contact and breathing vapors.

Test Article Preparation: Preparation of the test article for administration to the rats consisted of incorporating the test article in the diet utilizing liquid-solid blender, (Twin-Shell Intensifier Blender, Patterson Kelly Company East Stroudsburg, Pennsylvania) according to the Standard Operating Procedure PH-0938 on file at Pharmacokin Research International, Inc. The diet mixtures were stored in stainless steel containers in freezers.

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, 9000 GSRI Avenue, Baton Rouge, Louisiana, 70820. The container was labeled with the following information.

1. Study number
2. Date of sampling
3. Dose level of compound in the feed
4. 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 (±10°C) until the results of the analyses were received.

Correctness of Concentration and Homogeneity: Each time the diet/test article mixtures were prepared, tests were conducted by the sponsor prior to their use in the study to verify the

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correctness of concentration and homogeneity of the test article mixture in the feed. A total of four samples per mixing level (top left, top right and bottom - homogeneity; random correctness of concentration), 12 samples for three dose levels, were collected and submitted to the sponsor each time the diet was prepared. Untreated diet samples were also submitted for purpose of comparison. Samples of test article/diet mixtures and untreated diet were submitted to the sponsor to verify homogeneity and accuracy of dose concentrations. All test article/diet mixtures fed to rats during the study were within acceptable limits. The results of these test article/diet mixtures are presented in Appendix II, Volume 1.

Stability in Diet Under the condition of Storage:

The stability of the test article concentrations in the diet under conditions of storage was determined by the sponsor to be 43 days. Test article/diet mixtures were stored for no longer than 43 days prior to use.

Stability Under Test Conditions:

Stability under test conditions was determined by the sponsor. The diet was renewed in the animal feeders twice weekly to maintain test article dietary concentrations.

Authenticity and Purity of Test Article:

The purity, identity, strength and stability of the test article were 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 samples of the test article were sent to the sponsor on July 18, 1988, July 26, 1988, February 7, 1989, July 11, 1989, January 3, 1990 and October 9, 1990 (termination) to determine stability under storage conditions (Nitrogen blanketed at -20°C (±10°C)). The results of these analyses are presented in Appendix II, Volume 1. The test article remained stable during storage.

TEST SYSTEM

Species:

Rat

Strain:

Sprague Dawley

Supplier (Source):

Charles River Laboratories, Wilmington, Massachusetts

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Purchase Order

Number: 061688ATOX

Total Number Received/Date of Receipt: Received two hundred sixty seven males (267) and two hundred sixty four females (264) on June 30, 1988.

Sex: Male and female

Age at Initiation: 47 days old

No. on Study: Four hundred and twenty (420) - 210 males, 210 females
On test - four hundred (400) (200 males, 200 females); Baseline - Twenty (20) (10 males, 10 females)

Method of Justification for Randomization:

Before technical initiation, 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 had a similar distribution according to body weight. An analysis of variance was performed to ensure that no statistically significant differences in body weight was present between groups of the same sex at study initiation.

Acclimation Period:

The rats were acclimated for seventeen days. During this conditioning period, the rats were weighed and observed for any clinical signs of disease or inadequate weight gain. All rats with evidence of disease or physical abnormalities were discarded. Prior to shipment of these animals, serological samples were obtained from 10 males and 10 females by the breeder (Charles River). A serological profile of latent viruses in these samples was performed by Charles River Professional Services. The results of these analyses are maintained in the study file.

Hematologic and blood chemistry evaluations and necropsy were performed on ten (10) males and ten (10) females during acclimation. The lungs, liver, spleen, nasal structure, pancreas, eyes, kidneys and entire intestinal tract from the necropsied rats were preserved in 10% neutral buffered formalin. Histopathology was performed on these tissues and the results are maintained in the study file.

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System of Identification:

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

HUSBANDRY

Research Facility Registration:

U.S.D.A. Registration No. 23-R-107 under the Animal Welfare Act 75: 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 (66°F to 77°F) and a humidity of 30 to 70%.
Excursions beyond these ranges were of brief duration and small magnitude and did not adversely affect the validity or integrity of this study.

Housing:

Treatment groups were housed by vertical cage positioning. Rats were housed individually in stainless steel ½" wire mesh cages sized in accordance with the "Guide for the Care and Use of Laboratory Animals" of the Institute of Laboratory Animal Resources, National Research Council.

Sanitization:

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

Food:

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

Food Analysis:

Certified food was used in the study. Contaminant certification profiles will be maintained in the Pharmakon Research Central Files. Purina certified Rodent Meal has had extensive use and, to the best of our knowledge, has had no adverse effects on this study.

Water:

Availability - fresh tap water, ad libitum.

Water Analysis:

Water is monitored for contaminants at periodic intervals according to Standard Operating Procedure PH-018. The quality of water did not produce an adverse effect on the study.

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METHODS

Rationale for
Test System:

Rats have historically been used for establishing safety criteria for human exposure and is the species of choice for oncogenicity studies.

Compound
Preparation:

Test article treated diet was prepared as deemed necessary. The dates of preparation are presented in Appendix II, Volume 1.

Rationale for
Dose Selection:

Dose levels were selected by the sponsor and were based upon a 28 Day Toxicity/Reversibility Study and a 90 Day Subchronic Oral Toxicity Study.

Dose Administration:

| Group | Number of Animals | | ppm | % of Diet |
|-------|----------------------|--------|-----|--------------|
| | Male | Female | | |
| * | 10 | 10 | - | - |
| I | 50 | 50 | 0 | 0.0 |
| II | 50 | 50 | 10 | 0.001 |
| III | 50 | 50 | 35 | 0.0035 |
| IV | 50 | 50 | 70 | 0.0070 |

*Baseline data for hematology and clinical chemistries.

Control:

Untreated diet

Route of
Administration:

Oral, in diet

Rationale for
Route of
Administration:

To determine the long term effects of the test article by oral ingestion in diet.

Frequency and
Duration of
Administration:

Daily seven days per week for twenty-four (24) months.

Length of Study:

Twenty-four (24) months

Methods of Study
Performance:

The animals were fed room temperature diet/ test article mixture seven days per week to 50 males and 50 females in each dose group for 24 months. A similar group of animals were administered untreated diet and served as the control. Each animal was handled and its physical condition appraised at least once daily. All pharmacological and toxicological signs were recorded daily including their

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and duration. Such signs included, but were not limited to changes in skin and circulatory, autonomic and central nervous system, somatomotor activity and behavior pattern. Additional observations were made daily with appropriate actions taken to minimize loss of animals to the study. Mortality checks were made and recorded daily for all animals. Special attention was paid to tumor development. The time of onset, location, dimensions, appearance and progression of each grossly visible or palpable tumors was recorded. Body weights were recorded individually, for all animals at least once a week. Food consumption was determined twice weekly. At the end of the study period all surviving rats were sacrificed. Moribund animals, at the discretion of the study director or principal investigator, were removed and sacrificed, examined macroscopically and their tissues collected and preserved in 10% neutral buffered formalin (NBF). At the discretion of the study director or principal investigator animals displaying advanced clinical signs were sacrificed, examined macroscopically and their tissues collected in 10% NBF to preclude loss of specimens by autolysis.

Hematological
Examinations:

At 6 months, 12 months, 18 months and at sacrifice, a blood differential smear was obtained from all surviving animals. The method of blood sampling was performed by tail bleeding (at 6, 12 and 18 months) or by cardiac puncture on moribund sacrificed animals and the animals sacrificed at study termination. A differential blood count was performed on blood smears from those animals in the highest dosage group and the control group at 6 months. Differential blood counts were performed on all animals at 12 months, 18 months, and at terminal sacrifice, and when possible, from the animals moribund sacrificed during the study.

Glucose
Evaluations:

Blood glucose evaluation was performed in selected animals that were moribund sacrificed during the study or as deemed necessary. In addition, blood glucose evaluation was performed at 18 months and on the animals sacrificed on July 26, 1990 (terminal necropsy).

Ophthalmologic:

Indirect ophthalmoscopy was performed on all rats prior to study initiation and at

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approximately 6, 12, 18 and 24 months. Animals displaying ocular lesions prior to study initiation were replaced with normal animals before dosing was initiated.

PATHOLOGY

Gross Nec. opsy:

All animals dying on test or sacrificed for any reason (moribund, advanced clinical signs, or scheduled) were subjected to a complete 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 and tissues, or representative samples thereof, were preserved in 10% neutral buffered formalin:

All gross lesions and tumors
Brain-including sections of medulla/pons,
cerebellar cortex, cerebral cortex
Pituitary
Thyroid/parathyroid
Thymus
Lungs
Trachea
Heart
Sternum with bone marrow
Salivary glands
Liver
Spleen
Kidneys
Adrenals
Pancreas
Nasal Turbinates
Esophagus
Stomach
Duodenum
Jejunum
Ileum
Cecum
Colon
Rectum
Urinary Bladder
Mesenteric Lymph Nodes
Mammary gland
Thigh musculature
Gonads
Uterus
Accessory Genital Organs - Epididymides,
prostate and seminal vesicles)
Aorta
Femur - including articular surface
Skin

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Peripheral nerve
Spinal cord - cervical, midthoracic, and lumbar
Eyes
Zymbals glands (2)
Exorbital lachrymal glands

Histopathology:

Full histopathology was performed on the organs and tissues listed above on all rats in the control and high dose groups and all rats that died or were sacrificed early during the study. In addition, the adrenal glands, eyes, liver pancreas, pituitary gland and thyroids were evaluated from the mid and low dose groups. The mammary glands and any tissue masses suspected of being a mammary gland tumor were evaluated from the low and mid dose females.

RESULTS

Group Mean
Body Weights:

Table 1 of this volume reports weekly group mean body weight and survival data. Figure 1 illustrates the growth curves by dose group and sex. Volume 1 of Appendix 1 provides weekly group mean body weight data with standard deviations and body weight data on individual rats.

Mean body weight of the male high dose group was statistically significantly decreased from the control male body weight beginning on week 43 and continuing throughout the majority of the remainder of the study. (Statistically significant differences in body weight between the control and high dose males were found on weeks 43 through 88, excluding week 57, and on weeks 92, 93 and 101 through study termination.) Control and mid dose mean body weights were statistically comparable throughout the study except on one occasion (a decrease in mid dose body weight was found on week 64). However, a tendency for lower mean body weight in the mid dose males was seen beginning at about month 15 and continuing to the conclusion of the study. No effect, statistical or otherwise, was found on the body weight of low dose males compared to the control males.

The high dose female mean body weight was statistically comparable to the control female body weight except during four weeks of the study. Statistical differences between the control and high dose female body weights were detected on weeks 80 through 83. During this

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time, mean body weight of the high dose females was statistically lower than the female control mean. A tendency for a lower body weight in the high dose females compared to the control mean was seen in the last 3 months of the study. No statistical differences between the control and mid dose female body weight was found. A tendency for a lower body weight in the mid dose was seen during the last four weeks of the study. The low dose female body weight was not affected.

In the table below, mean body weights are expressed as a percentage of control body weight at three month intervals by sex.

| Months | MALES | | | FEMALES | | |
|--------|--------|--------|--------|---------|--------|--------|
| | 10 ppm | 35 ppm | 70 ppm | 10 ppm | 35 ppm | 70 ppm |
| 3 | 99.3 | 98.0 | 98.3 | 100.0 | 101.9 | 98.9 |
| 6 | 98.9 | 97.8 | 97.6 | 100.6 | 101.1 | 96.7 |
| 9 | 98.1 | 97.4 | 94.5 | 100.2 | 102.1 | 96.6 |
| 12 | 98.0 | 95.5 | 91.2 | 100.0 | 100.7 | 93.7 |
| 15 | 99.0 | 94.0 | 88.6 | 101.5 | 104.4 | 94.8 |
| 18 | 99.4 | 93.9 | 83.8 | 101.5 | 104.7 | 91.4 |
| 21 | 102.0 | 87.3 | 87.0 | 96.9 | 99.1 | 90.1 |
| 24 | 98.8 | 88.1 | 75.3 | 96.8 | 89.7 | 86.9 |

Group Mean Food Consumption:

Table 2 of this volume reports by weekly intervals mean daily food consumption and calculated mg/kg dose levels. The daily food consumption in Table 2 was calculated from the twice weekly food consumption measurements collected during the study. Appendix 1, Volume 2 gives both the group mean food consumption at each weighing period in addition to similar data from individual rats.

Group mean food consumption was not affected by treatment. Only sporadic, non-dose-dependent statistical differences in food consumption were detected between control and treated animals of either sex. However, a few male high dose DETDA rats consistently exhibited a marked increase in daily food consumption.

The DETDA dose levels can be expressed on a mg/kg basis using food consumption and body weight data. Over the course of the study, the mean DETDA consumed per day on a mg/kg basis for males in the low, mid and high dose groups was 0.4, 1.4 and 3.2 mg/kg, respectively. The doses consumed by females were 0.5, 1.8 and 3.8

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mg/kg in the low, mid and high dose groups, respectively.

Survival:

Figure 2 illustrates the Kaplan-Meier survival curves by dose group and sex. Survival was comparable between treated and control animals of each sex except in the low dose male rats. Survival in this group was significantly greater than the control males. Percent survival was maintained above 50% until week 97, 104, 96 and 92 for males in the control, low, mid and high dose groups, respectively. In females, greater than 50% survival was maintained up to week 103, 102, 101 and 98 for the control, low, mid and high dose groups, respectively. Early deaths or moribund sacrifices occurred during the study. Cause of death or moribund sacrifice included pituitary tumors, other large and/or malignant neoplasms, severe chronic renal disease and severe lesions of the hock/foot (suppurative and ulcerative dermatitis). Rats in this study reached high body weights early in the course of the study. This factor may have contributed to the development of certain of the changes leading to the early deaths or moribund sacrifices.

The termination (sacrifice) period of the study was week 106. Survival by sex and dose group and estimates of probabilities of survival are presented below. Kaplan and Meier survival curves are shown in Figure 2 of this volume.

| <u>Males</u> | <u>0 ppm</u> | <u>10 ppm</u> | <u>35 ppm</u> | <u>70 ppm</u> |
|--------------------------------|--------------|---------------|---------------|---------------|
| Initially on study | 50 | 50 | 50 | 50 |
| Unscheduled sacrifice | 0 | 0 | 1 | 0 |
| Moribund sacrifice | 10 | 5 | 8 | 9 |
| Found dead | 26 | 21 | 28 | 27 |
| Terminal sacrifice | 14 | 24 | 13 | 14 |
| Survival P values ^a | 0.04 | 0.01 | 0.90 | 0.63 |
| <u>Females</u> | <u>0 ppm</u> | <u>10 ppm</u> | <u>35 ppm</u> | <u>70 ppm</u> |
| Initially on study | 50 | 50 | 50 | 50 |
| Unscheduled sacrifice | 0 | 1 | 3 | 0 |
| Moribund sacrifice | 7 | 6 | 8 | 13 |
| Found dead | 19 | 22 | 16 | 18 |
| Terminal sacrifice | 24 | 21 | 23 | 19 |
| Survival P values ^a | 0.79 | 0.95 | 0.47 | 0.82 |

^a = The results of the life trend test is in the 0 ppm (control) column, the results of the life table pairwise comparisons with the 0 ppm (control) are in the dose columns.

0-0-2-4

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Clinical Signs:

Appendix I, Volume 3 contains clinical observations recorded each day summarized by treatment group and by individual animal. The majority of clinical observations were noted during the latter portion of the study. These were associated with aging and were, in general, similar across dose groups. Clinical signs most frequently noted were chromodacryorrhea, decreased activity, abnormal gait, abnormal stance, flaccid body tone, decubitis ulcer, mass, piloerection, poor grooming, alopecia, scab formation and prostration. Additional incidental clinical signs were noted less frequently. Eye opacity was noted in a few high dose males and is considered to be the only clinical sign recorded that may be related to the administration of the test article.

Ophthalmology:

Table 3 presents individual animal ocular lesions detected during indirect ophthalmoscopy exams at 6, 12, 18 and 24 months. Dense bilateral cataracts, first detected at 18 months in 5 high dose males, were the only ocular lesions considered related to test article administration. Bilateral cataracts were only detected in the high dose male rats. An apparent correlation was found between elevated blood glucose values at 18 months and the appearance of cataracts (see "Blood Glucose" below).

Necropsy:

The incidence of gross necropsy findings at the terminal necropsy is presented in Table 4. The individual terminal necropsy sheets are presented in Appendix I, Volume 4. None to several macroscopic observations were recorded for each animal at the terminal necropsy. The majority of these observations were associated with aging. These included chronic tissue degeneration and/or mass formation. Observations recorded at terminal necropsy that were considered to be test article related were noted for the liver.

There were no gross macroscopic observations on the animals that were sacrificed in moribund condition that were attributed to the administration of DETDA.

Hematology:

Red blood cell morphology and white blood cell differential counts at 6, 12, 18 and 24 months are reported in Table 5 of this volume.

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Individual animal data (at the scheduled intervals and from moribund sacrifices) are reported in Appendix I, Volume 5.

The observed values for all groups were consistent with the age of the animals. A few statistically significant differences were detected between the control and treated groups, but were scattered and without apparent relationship to treatment.

Blood Glucose:

Individual blood glucose data obtained from all animals on test at 18 months and from animals at moribund (where possible) or terminal sacrifice are presented in Table 6 of this volume.

At the 18 month evaluation, blood glucose values were considered within the range of normal for all dose groups with the exception of individual rats in the male high dose group. Six high dose males had nonfasting blood glucose values greater than 300 mg/dl. Five of these males were affected with bilateral cataracts. Four were subsequently diagnosed with multifocal pancreatic acinar cell atrophy, one with fatty infiltration of the pancreas and one with multifocal interstitial fibrosis of the pancreas. These findings suggest a correlation between the cataracts and DETDA's effect on the pancreas. (One of the six males with a high blood glucose value at 18 months was diagnosed with a hepatocellular carcinoma and another male was diagnosed with thyroid hypertrophy. These changes, however, were not considered to be related to the high 18 month blood glucose value in these animals.)

Time to Mammary
Gland Tumor:

The average time of onset (days on test) in female rats for each grossly visible or palpable mammary gland tumor is presented below. The diagnosis of tumor type was made on histologic exam. No statistically or biologically significant difference in time of onset was found between treated and control groups for the three types of mammary gland tumors.

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| Dose | Average Time of Onset (Days on Test) | | |
|------|--------------------------------------|---------|--------------|
| | Tumor Type | | |
| | Adenocarcinoma | Adenoma | Fibroadenoma |
| 0 | 511 | - | 588 |
| 10 | 455 | - | 511 |
| 35 | 538 | 688 | 532 |
| 70 | 499 | 516 | 556 |

- = Presence of tumor identified histologically

Histopathology:

The dietary administration of 10, 35 and 70 ppm DETDA resulted in a significant increase in neoplastic and proliferative lesions in the liver and thyroids of the high dose male rats and in the liver of the high dose female rats. A significant increase in the number of fibroadenomas of the mammary gland was present in the mid and high dose females. A treatment-related atrophy was present in the pancreas of the high dose male rats.

The livers of the high dose males had a significant increase in hepatocellular carcinomas (nine). Hepatocellular carcinomas were present in three mid dose males, two low dose males and one control male. Proliferative lesions (basophilic foci and eosinophilic foci) were significantly increased in all three dose groups of DETDA treated males. All three groups of DETDA treated males had an increased incidence and severity of degenerative changes in the liver (focal and multifocal cystic degeneration and multifocal necrosis). The livers of the high dose females had a significant increase in hepatocellular adenomas and an increased incidence of basophilic foci. Hepatocellular carcinomas were present in one high dose female and two mid dose females. No hepatocellular carcinomas were present in the livers of the control or low dose females. Several liver changes were diagnosed as nodular regeneration. These areas were usually found in liver lobules containing a marked amount of hepatocellular damage. The specific change was characterized by nodular areas containing some slight disorganization of the hepatic cords combined with an alteration in the staining characteristics of the hepatocytes. In

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general, the nodular expansions did not alter the normal lobular pattern of the liver and were not discretely delineated as observed in the hepatocellular adenoma. A summary of the proliferative lesions of the liver is presented as follows:

| GROUP DOSE (PPM) SEX (NO. EXAMINED) | I 0 | | II 10 | | III 35 | | IV 70 | |
|--|-----------|-----------|-----------------|-----------------|-----------------|-----------|-----------------|-----------------|
| | M (50) | F (50) | M (50) | F (50) | M (50) | F (50) | M (50) | F (50) |
| Hepatocellular Carcinoma ^a | 1 | - | 2 | - | 3 | 2 | 9 ^a | 1 |
| Hepatocellular Adenoma | - | 2 | 1 | - | 3 ^b | 1 | 1 | 8 ^b |
| Basophilic Foci ^{d, e} | 7 | 20 | 15 ^b | 17 | 16 ^b | 28 | 18 ^a | 32 ^b |
| Eosinophilic Foci | - | 4 | 7 ^a | 12 ^b | 10 ^c | 10 | 6 ^b | 3 |
| Nodular Regeneration | - | 3 | 1 | - | 2 | 2 | 2 | 5 |

- a = Significant increase over Controls (same sex); p = 0.01
- b = Significant increase over Controls (same sex); p = 0.05
- c = Significant increase over Controls (same sex); p = 0.001
- d = Males = significant Linear CHI-SQUARE value; p ≤ 0.05
- e = Females = significant Linear CHI-SQUARE value; p ≤ 0.05

The thyroids of the high dose males had a significant increase in follicular cell adenomas (five) and an increased incidence of follicular cell hypertrophy (seven thyroids had diffuse areas of follicles having cuboidal to columnar epithelium). The follicular cell hypertrophy was significantly increased over the mid dose male rats. Follicular cell carcinomas were present in two low dose males. Follicular cell adenomas were present in three low dose males and in four mid dose males. No follicular cell adenomas or carcinomas were present in the control males. Follicular cell hyperplasia/follicular cysts were increased in the thyroids of the mid and high dose males. Two high dose females had follicular cell adenomas and five had follicular cell hyperplasia/follicular cysts. No follicular cell adenomas or carcinomas were present in the control, low dose or mid dose females. Follicular cell hyperplasia was characterized by large nodular cystic lesions containing papillary infoldings of the epithelium and/or foci of hypercellularity. Follicular cysts were similar nodular cystic lesions without any distinct epithelial infoldings or areas of hypercellularity. Since these two were sometimes difficult to discern, the combined diagnosis was used to assure the evaluation of thyroid gland activity. C-cell tumors (adenomas and carcinomas) were present in

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individual rats in the control and DETDA treated rats but there was no significant difference between the DETDA treated rats and the corresponding control rats. A summary of the proliferative lesions of the follicular cells of the thyroids is presented as follows:

| GROUP DOSE (ppm) SEX (NO. EXAMINED) | I 0 | | II 10 | | III 35 | | IV 70 | |
|--|--------|------|----------|------|-----------|------|----------------|----------------|
| | M | F | M | F | M | F | M | F |
| | (50) | (50) | (50) | (50) | (50) | (50) | (50) | (50) |
| Follicular Cell Carcinoma | - | - | 2 | - | - | - | - | - |
| Follicular Cell Adenoma | - | - | 3 | - | 4 | - | 5 ^a | 2 |
| Follicular Cell Hyperplasia/Cysts | 1 | 2 | 1 | 1 | 5 | - | 5 | 5 ^b |
| Follicular Cell Hypertrophy | 1 | 1 | - | 1 | - | 1 | 7 ^a | - |

a = Significant increase over Controls (same sex) p = 0.05
 b = Significant increase over Mid dose (same sex) p = 0.05

The mammary glands of the mid and high dose females had a significant increase in the incidence of fibroadenomas. There was an increase in both the number of tumors and the number of tumor bearing animals. However, the incidence of mammary gland adenocarcinomas was higher in the control females than in the three dose groups of DETDA treated females. A summary of the mammary gland neoplasms in the females is presented as follows:

| GROUP DOSE (ppm) (NO. EXAMINED) Count/No. animals | I 0 (47) Ct./An. | II 10 (50) Ct./An. | III 35 (50) Ct./An. | IV 70 (50) Ct./An. |
|--|---------------------------|-----------------------------|------------------------------|-----------------------------|
| | Adenocarcinoma | 12/10 | 4/4 | 10/7 |
| Adenoma | 1/1 | --- | 2/2 | 3/3 |
| Fibroadenoma ^b | 13/12 | 20/16 | 34/23 ^a | 43/25 ^a |

a = Significant increases over Controls; p = 0.05
 b = Significant Linear CHI-SQUARE value; p ≤ 0.05

The pancreas of the high dose males had a significant increase in multifocal acinar atrophy (p = 0.05) accompanied by interstitial fibrosis and fatty infiltration (replacement of glandular elements by adipose tissue; p = 0.001). Due to the reduction in the acinar glands, the evaluation of the quantity of islet

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cells was difficult but those islet cells present in the sections evaluated appeared to be normal. The microscopic changes in the acinar cells were comparable between the control females and the DETDA treated females. The incidence of islet cell tumors (adenomas and carcinomas) were not significantly different between the corresponding control rats and three groups of DETDA treated male and female rats.

The incidences of cysts and focal areas of tubular hyperplasia of the kidneys were increased in high dose male rats when compared to the male controls. These may have been an exacerbation of lesions seen in chronic nephropathy (discussed below). Six high dose males had bilateral cataracts. Half of these rats had moderate to severe keratitis of the eyes. The relevance of treatment to these cataracts and the cysts and tubular hyperplasia of the kidneys is equivocal.

No treatment-related neoplastic or non-neoplastic changes were noted in any of the other tissues examined from the male and female rats receiving 10, 35 or 70 ppm of DETDA in the diet. A variety of spontaneous neoplasms and incidental lesions were present in both the control and DETDA treated rats.

Pituitary adenoma was the most common neoplasm in either sex and there was a slightly lower incidence in high dose rats than in the control rats. Pituitary carcinomas were of low incidence in all groups. Adrenal cortical neoplasms (carcinomas and adenomas) were present in a few rats, mostly in the females and scattered among the four groups. Medullary tumors (unilateral or bilateral pheochromocytomas) were much more numerous and were found mostly in the males. There was no significant difference between the incidence of these tumors in the control males and three groups of DETDA treated males. Endometrial stromal polyps were present in the uterus of four high dose females and a leiomyoma was present in the uterus of another. Granulosa-theca cell tumors were present in the ovaries of three high dose females. No primary uterine or ovarian neoplasms were diagnosed in the control, low dose or mid dose groups. Interstitial cell tumors (unilateral or

bilateral) were present in the testes of one high dose rat and four control rats. Astrocytoma was diagnosed in the brains of one control female, two low dose males, one high dose male and two high dose females. Malignant lymphoma was present in one female and three male control rats. A variety of other neoplasms occurred infrequently and usually as single entities in various tissues and organs. These are summarized in the Neoplasm Summary Incidence Tables.

Proliferative and degenerative lesions were very common in the adrenals of the male and female rats. Cortical hyperplasia, cortical vacuolation, congestion and hematocysts were very common in both sexes in all four groups. Medullary hyperplasia was more common in the males than in the females. The combined incidence of medullary hyperplasia (focal and multifocal) was fairly proportionate between the control and high dose male rats.

Some degree of age-associated chronic nephropathy was present in nearly all of the kidneys examined. The lesions of chronic nephropathy included multifocal nonsuppurative nephritis (characterized by interstitial fibrosis, mononuclear cell infiltration and glomerular sclerosis), tubular regeneration, tubular dilatation, pigment deposition (tubular and interstitial), cysts and focal areas of tubular hyperplasia. The cysts and focal areas of tubular hyperplasia were increased in high dose male rats when compared to controls. The incidence and severity of the other changes in the kidney were not different between the corresponding control rats and three groups of DETDA treated male and female rats. "End-stage" renal disease was evident in the many of the rats with the more severe lesions of chronic nephropathy. In these rats, the impaired renal function resulted in secondary hyperparathyroidism, characterized by hyperplasia of the parathyroid, resulting in secondary mineralization in the kidney, heart, stomach and media of large arteries. Secondary hyperparathyroidism was considered to be the cause of the osteoporotic changes in the bones of a few rats scattered among the control and DETDA treated male and female rats.

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A number of early deaths or moribund sacrifices occurred during the study. Ventral compression of the brain and dilatation of the ventricles were common in the brains of the rats with large pituitary tumors. In some rats which died these were severe and were considered to have contributed to the death of the animal. Additional lesions considered to be the cause of death or moribund sacrifice were the presence of large and/or malignant neoplasms, severe renal disease and severe lesions of the hock/foot (suppurative and ulcerative dermatitis).

A detailed microscopic evaluation is presented in Appendix III from Experimental Pathology Laboratories, Inc.

CONCLUSION

The results of this study should be evaluated in conjunction with subchronic DETDA studies conducted in this laboratory. The 90 day subchronic and 28 day progression/reversibility studies identified the pancreas as the target organ in the rat. Male rats were more severely affected and at an earlier time and lower dose than females. Histopathologically, pancreatic acinar cells were affected first; islet cell involvement followed afterward in a time and dose dependent manner. Pathology in other organs occurred after or in conjunction with islet cell involvement and appeared secondary to the metabolic changes induced by the pancreatic toxicity. Secondary effects included ocular cataracts in animals with histologic evidence of islet cell toxicity. It was postulated these cataracts were associated with and related to hyperglycemia and thus the pancreatic toxicity. Histologic evidence of liver or thyroid toxicity was not seen.

The greater DETDA sensitivity of male, compared to female, rats was also observed in the two year study. This response holds true for both toxicity and tumorigenicity. However, effects on the liver and thyroid, not seen in the subchronic studies, were detected in the two year study. Based on the absence of these effects in shorter term studies, liver and thyroid effect appear related to chronic, long term continuous exposure.

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In the two year study, histologic evidence of pancreatic toxicity was restricted to the pancreatic acinar cells of the high dose male rats. A functional aberration was suggested in individual rats by the hyperglycemia, increased food consumption and ocular cataracts observed in a few high dose male rats. No histologic or suggestive evidence of pancreatic toxicity was seen in the mid and low male dose groups or in females at any dose level. Given these results, the no effect level for pancreatic toxicity in the male rat for two years was 35 ppm; for females the no effect level was 70 ppm.

Unlike pancreatic toxicity, hepatic proliferative and degenerative changes were found in all male dose groups. Proliferative lesions only were found in the females. A clear no effect level was not achieved. However, hepatotoxicity would appear related to long term chronic exposure as this effect was not observed in studies less than two years in length. A no effect level of 320 ppm for hepatotoxicity is available for the 90 day subchronic study.

Evidence for thyroid hyperplasia or hypertrophy was found in high dose males and females in the two year study. Lower dose levels were not affected. Like hepatotoxicity, thyroid effects were not seen in subchronic studies and are an effect of chronic long term exposure. A no effect level for both males and females in this study is 35 ppm.

With respect to tumorigenicity, hepatocellular carcinomas were increased in the male high dose group. The incidence of a benign tumor of the thyroid, follicular cell adenoma, was also increased in high dose males. Hepatocellular adenoma, a benign tumor of the liver, was increased in high dose female rats. Mammary gland fibroadenoma, a benign tumor, were increased in mid and high dose females. The effect on mammary gland tumors is the only instance where the female rats appeared affected at a lower level than the male. Since the incidence of malignant mammary gland tumors (adenocarcinomas) was high in the control females, the relevance of the increase in fibroadenomas in the treated groups is unknown.

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Furthermore, there was no difference between control and treated females in average time of onset of mammary tumors. A clear no effect level for liver and thyroid tumors can be established at 35 ppm. The questionable significance of the mammary tumors in the mid and high dose females complicates determination of a no effect level in this organ. At a minimum, the no effect level in this organ is 10 ppm and may be higher.

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PHARMAKON RESEARCH INTERNATIONAL, INC.
 24 MONTH ONCOGENICITY STUDY
 STUDY NUMBER: PH474-ET-001-88
 TEST ARTICLE: DETDA
 SPONSOR: ETHYL CORPORATION

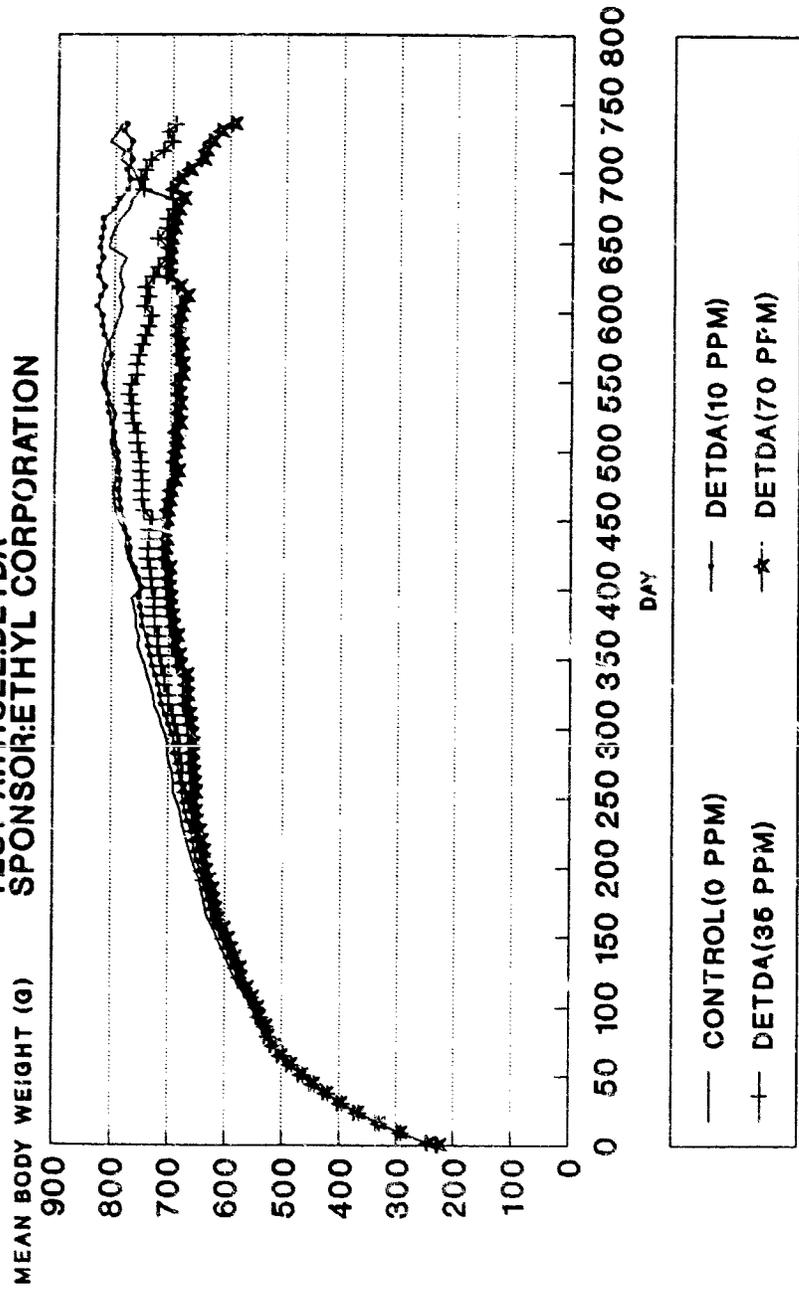


FIGURE 1. GROWTH CURVES FOR MALE RATS FED DIETS CONTAINING DETDA FOR TWO YEARS

PHARMAKON RESEARCH INTERNATIONAL, INC.
24 MONTH ONCOGENICITY STUDY
STUDY NUMBER: PH474-ET-001-88
TEST ARTICLE: DETDA
SPONSOR: ETHYL CORPORATION

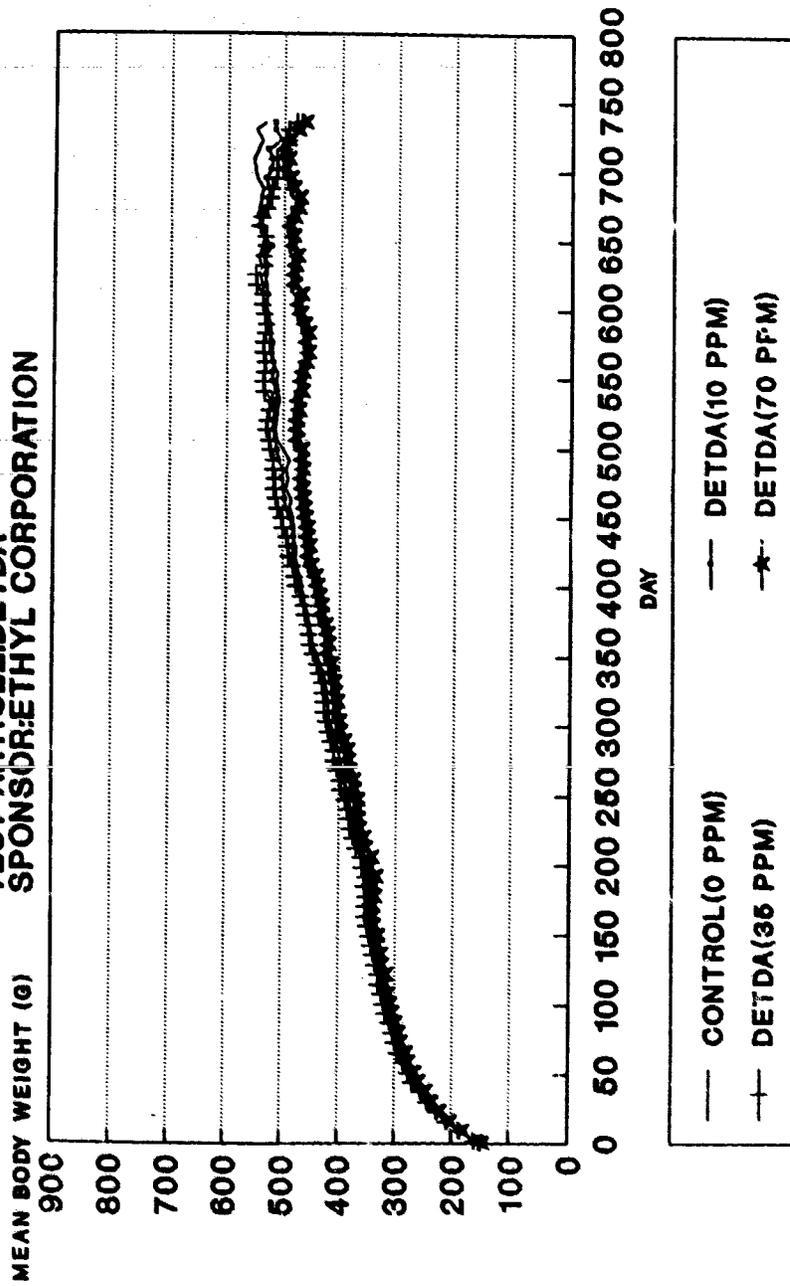


FIGURE 1. GROWTH CURVES FOR FEMALE RATS FED DIETS CONTAINING DETDA FOR TWO YEARS

PHARMAKON RESEARCH INTERNATIONAL, INC.
 24 MONTH ONCOGENICITY STUDY
 STUDY NUMBER: PH474-ET-001-88
 TEST ARTICLE: DETDA
 SPONSOR: ETHYL CORPORATION

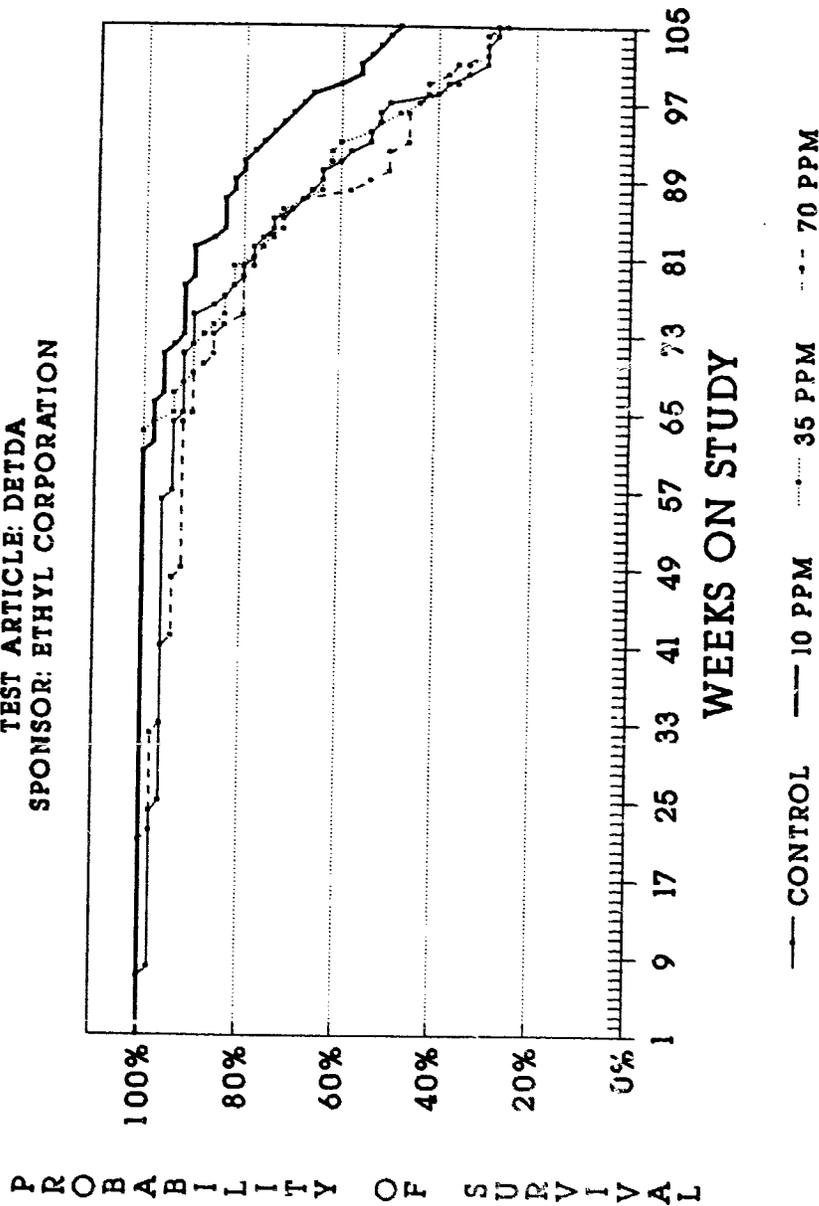


FIGURE 2. KAPLAN-MEIER SURVIVAL CURVES FOR MALE RATS
 FED DIETS CONTAINING DETDA FOR TWO YEARS

PHARMALON RESEARCH INTERNATIONAL, INC.
 24 MONTH ONCOGENICITY STUDY
 STUDY NUMBER: PH474-ET-001-88
 TEST ARTICLE: DETDA
 SPONSOR: ETHYL CORPORATION

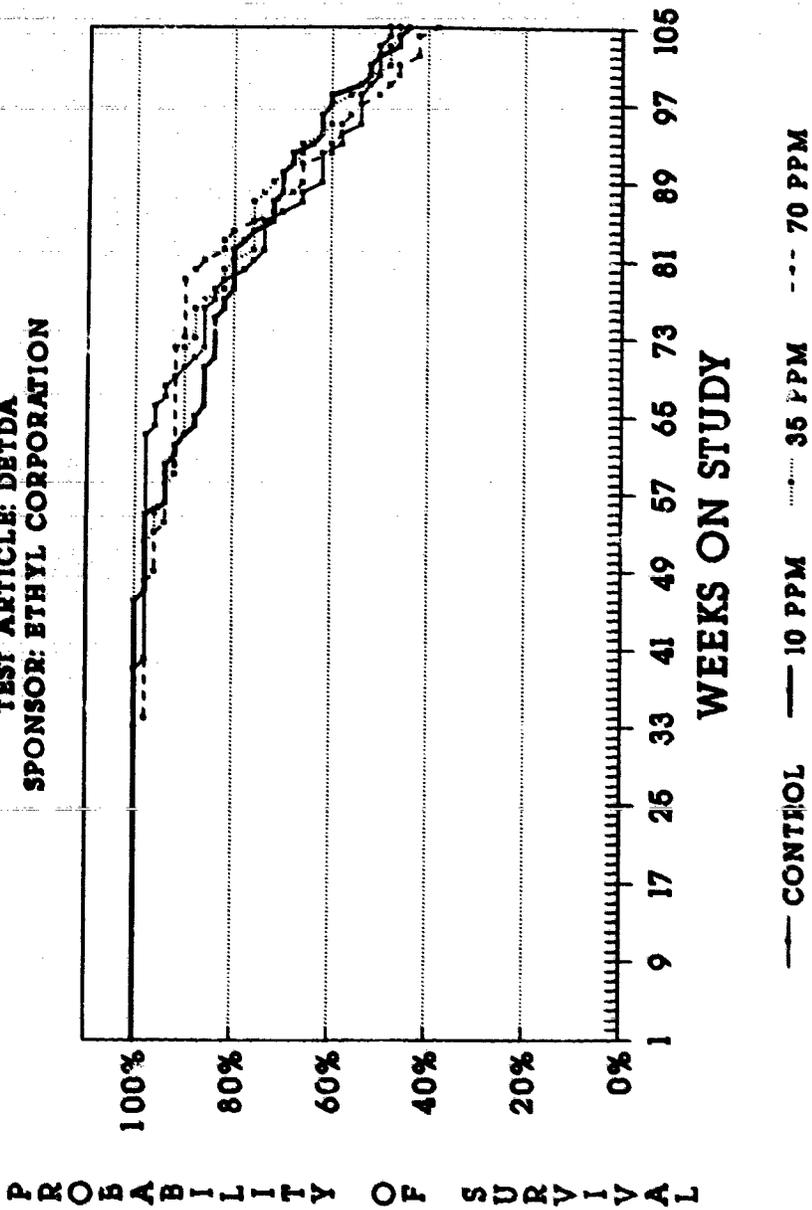


FIGURE 2. KAPLAN-MEIER SURVIVAL CURVES FOR FEMALE RATS
 FED DIETS CONTAINING DETDA FOR TWO YEARS

PHARMAKON RESEARCH INTERNATIONAL, INC.
 24 Month Oncogenicity Study in Rats
 Study Number: 474-ET-001-88
 Test Article: Diethyl Toluene Diamine

Group Mean Male Body Weights and Survival by Weekly Interval

Table 1

| Week | 0 ppm | | 10 ppm | | 35 ppm | | 70 ppm | |
|------|-----------|---------------------|-----------|---------------------|-----------|---------------------|-----------|---------------------|
| | Survivors | Body weight (grams) |
| 1 | 50 | 291.1 | 50 | 290.8 | 50 | 291.3 | 50 | 292.8 |
| 2 | 50 | 332.1 | 50 | 331.8 | 50 | 329.3 | 50 | 331.0 |
| 3 | 50 | 365.2 | 50 | 367.4 | 50 | 365.1 | 50 | 368.1 |
| 4 | 50 | 396.5 | 50 | 398.5 | 50 | 393.5 | 50 | 398.1 |
| 5 | 50 | 418.1 | 50 | 421.5 | 50 | 419.5 | 50 | 422.1 |
| 6 | 50 | 444.3 | 50 | 444.9 | 50 | 442.2 | 50 | 446.5 |
| 7 | 50 | 464.1 | 50 | 465.4 | 50 | 462.3 | 50 | 465.6 |
| 8 | 49 | 483.6 | 50 | 485.1 | 50 | 482.4 | 50 | 485.9 |
| 9 | 49 | 501.4 | 50 | 502.6 | 50 | 497.4 | 50 | 502.9 |
| 10 | 49 | 515.0 | 50 | 515.3 | 50 | 510.1 | 50 | 514.4 |
| 11 | 49 | 527.8 | 50 | 525.6 | 50 | 522.7 | 50 | 525.4 |
| 12 | 49 | 539.5 | 50 | 535.8 | 50 | 528.5 | 50 | 530.1 |
| 13 | 49 | 546.2 | 50 | 541.7 | 50 | 537.5 | 50 | 539.9 |
| 14 | 49 | 553.8 | 50 | 550.9 | 50 | 544.6 | 50 | 544.0 |
| 15 | 49 | 562.1 | 50 | 557.0 | 50 | 553.7 | 50 | 552.5 |
| 16 | 49 | 574.9 | 50 | 569.1 | 50 | 563.6 | 50 | 562.8 |
| 17 | 49 | 583.8 | 50 | 577.4 | 50 | 574.6 | 50 | 573.1 |
| 18 | 49 | 591.1 | 50 | 583.1 | 50 | 580.3 | 50 | 575.3 |
| 19 | 49 | 597.8 | 50 | 589.5 | 50 | 587.6 | 50 | 583.2 |
| 20 | 49 | 605.5 | 50 | 596.8 | 50 | 594.2 | 50 | 589.1 |
| 21 | 49 | 611.9 | 50 | 603.7 | 50 | 601.6 | 50 | 595.7 |
| 22 | 49 | 619.4 | 50 | 613.0 | 50 | 606.9 | 49 | 603.1 |
| 23 | 49 | 629.7 | 50 | 620.6 | 50 | 615.4 | 49 | 614.1 |
| 24 | 49 | 634.3 | 50 | 622.1 | 50 | 618.0 | 49 | 617.7 |
| 25 | 48 | 637.1 | 50 | 629.8 | 50 | 623.2 | 49 | 621.7 |
| 26 | 48 | 641.3 | 50 | 634.1 | 50 | 629.4 | 49 | 623.5 |
| 27 | 48 | 644.2 | 50 | 639.4 | 50 | 635.6 | 49 | 629.6 |
| 28 | 48 | 650.1 | 50 | 643.5 | 50 | 639.7 | 49 | 633.7 |
| 29 | 48 | 656.8 | 50 | 648.8 | 50 | 644.3 | 49 | 638.9 |
| 30 | 48 | 561.1 | 50 | 653.0 | 50 | 644.3 | 49 | 638.0 |
| 31 | 48 | 667.6 | 50 | 655.3 | 50 | 649.7 | 49 | 642.2 |

Table 1 continued

EPL

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

CONTAINS NO CBI

PHARMAKON STUDY NUMBER PH 474-ET-001-88

ONCOGENICITY STUDY - RAT, 24 MONTH STUDY

PATHOLOGY REPORT

VOLUME 1 OF 8

Submitted to:

Pharmakon Research International, Inc.
Waverly, PA 18471

CONTAINS NO CBI

July 8, 1991

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PATHOLOGY SUMMARY

PHARMAKON STUDY NUMBER Ph 474-ET-001-88

ONCOGENICITY STUDY - RAT, 24 MONTH STUDY

PATHOLOGY SUMMARY

A histopathological evaluation was performed on selected tissues from Charles River Sprague-Dawley rats which had received various doses of Diethyltoluene diamine (DETD) in the diet. This study was designed to assess the potential carcinogenicity of the test article when administered to rats via dietary admixture for a major portion of their life span. The experimental design of this study is outlined as follows:

| <u>Group</u> | <u>Dose ppm</u> | <u>Number of Animals</u> | |
|----------------|---------------------|--------------------------|----------------|
| | | <u>Males</u> | <u>Females</u> |
| I (Control) | 0 | 50 | 50 |
| II (Low dose) | 10 | 50 | 50 |
| III (Mid dose) | 35 | 50 | 50 |
| IV (High dose) | 70 | 50 | 50 |

Hematoxylin and eosin stained sections of the following tissues were prepared by Experimental Pathology Laboratories, Inc. and examined from all rats in the control (Group I) and high dose (Group IV) groups and from all rats in the low (Group II) and mid dose (Group III) groups found dead or sacrificed as moribund during the course of the study: brain, pituitary, thyroids, parathyroids, thymus, lungs

(including mainstem bronchi), trachea, heart, salivary glands (submandibular), liver, spleen, kidneys, adrenals, aorta, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, uterus, rectum, urinary bladder, Zymbal's glands, mesenteric lymph node, pancreas, ovaries, testes, prostate, seminal vesicle, epididymides, mammary gland, skin, skeletal muscle (thigh), sciatic nerve, bone (femur, including articular surface), spinal cord (cervical, midthoracic and lumbar), bone (sternum), bone marrow (sternum), eyes, lacrimal gland (exorbital), nasal turbinates and all gross lesions and tissue masses. In addition, the following tissues were examined from the male and female rats in the low (Group II) and mid dose (Group III) groups which were sacrificed at the termination of the study: adrenals, eyes, liver, pancreas, pituitary and thyroids. The mammary glands and any tissue masses suspected of being a mammary gland tumor were evaluated from the low and mid dose females.

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 or lost during histologic processing. Varying degrees of autolysis were evident microscopically in tissues from several of the rats which died prior to sacrifice. In a few rats, autolysis was of such a degree that critical evaluation of the tissues was difficult. However, these factors did not affect the overall evaluation of the study

Microscopic findings for each tissue examined from each animal are listed in the Histopathology Incidence Tables. Inflammatory, degenerative and hyperplastic changes were graded from one to five depending upon severity; neoplasms, cysts and nongradable changes were designated as present (P) in the Histopathology Incidence Tables. All lesions are summarized by sex, treatment group and disposition in the Summary Incidence Tables together with the total number of animals in each group for which the tissues were examined. All neoplasms are also summarized in the same manner in the Neoplasm Summary Incidence Tables. A tabulation of gross lesions observed at the time of necropsy or tissue trimming with the corresponding microscopic change, if any, is provided in the Correlation of Gross and Microscopic Findings Tables. The descriptions of the gross findings on these tables were transcribed from the Individual Animal Necropsy Sheets for the terminal sacrifice animals. For the early sacrifices/deaths the changes were taken from copies of the client's General Observations record. Gross lesions observed at the time of gross processing are labeled as lesions observed at the time of grossing. Within each sex, comparisons were made using the Fisher Exact Test and Cochran/Armitage Linear Trend Test of the rats in the control and treated groups.

RESULTS

The dietary administration of 10, 35 and 70 ppm DETDA resulted in a significant increase in neoplastic and proliferative lesions in the liver and thyroids of the high dose male rats and in the liver of the high dose female rats. A significant increase in fibroadenoma of the mammary gland was present in the mid and high dose females. A treatment-related atrophy was present in the pancreas of the high dose male rats.

The livers of the high dose males had a significant increase in hepatocellular carcinomas (nine). Hepatocellular carcinomas were present in three mid dose males, two low dose males and one control male. Proliferative lesions (basophilic foci and eosinophilic foci) were significantly increased in all three dose groups of DETDA treated males. All three groups of DETDA treated males had an increased incidence and severity of degenerative changes in the liver (focal and multifocal cystic degeneration and multifocal necrosis). The livers of the high dose females had a significant increase in hepatocellular adenomas and an increased incidence of basophilic foci. Hepatocellular carcinomas were present in one high dose female and two mid dose females. No hepatocellular carcinomas were present in the livers of the control or low dose females. Several liver changes were diagnosed as nodular regeneration. These areas were usually found in liver lobules containing a marked amount of hepatocellular damage. The specific change was characterized by nodular areas containing some slight disorganization of the hepatic cords combined with an alteration in the

staining characteristics of the hepatocytes. In general, the nodular expansions did not alter the normal lobular pattern of the liver and were not discretely delineated as observed in the hepatocellular adenoma. A summary of the proliferative lesions of the liver is presented in Table I.

Table I

| GROUP DOSE (ppm) SEX (NO. EXAMINED) | I 0 | | II 10 | | III 35 | | IV 70 | |
|--|-----------|-----------|-----------------|-----------------|-----------------|-----------|-----------------|-----------------|
| | M (50) | F (50) | M (50) | F (50) | M (50) | F (50) | M (50) | F (50) |
| Hepatocellular Carcinoma ^a | 1 | - | 2 | - | 3 | 2 | 9 ^a | 1 |
| Hepatocellular Adenoma | - | 2 | 1 | - | 3 | 1 | 1 | 8 ^b |
| Basophilic Foci ^{d, e} | 7 | 20 | 15 ^b | 17 | 16 ^b | 28 | 18 ^a | 32 ^b |
| Eosinophilic Foci | - | 4 | 7 ^a | 12 ^b | 10 ^c | 10 | 6 ^b | 3 |
| Nodular Regeneration | - | 3 | 1 | - | 2 | 2 | 2 | 5 |

- ^a = Significant increase over Controls (same sex); $p = .01$
^b = Significant increase over Controls (same sex); $p = .05$
^c = Significant increase over Controls (Same sex); $p = .001$
^d = Males = significant Linear CHI-SQUARE value; $p \leq .05$
^e = Females = significant Linear CHI-SQUARE value; $p \leq .05$

The thyroids of the high dose males had a significant increase in follicular cell adenomas (five) and an increased incidence of follicular cell hypertrophy (seven thyroids had diffuse areas of follicles having cuboidal to columnar epithelium). The follicular cell hypertrophy was significantly increased over the mid dose male rats.

Follicular cell carcinomas were present in two low dose males. Follicular cell adenomas were present in three low dose males and in four mid dose males. No follicular cell adenomas or carcinomas were present in the control males. Follicular cell hyperplasia/follicular cysts were increased in the thyroids of the mid and high dose males. Two high dose females had follicular cell adenomas and five had follicular cell hyperplasia/follicular cysts. No follicular cell adenomas or carcinomas were present in the control, low dose or mid dose females. Follicular cell hyperplasia was characterized by large nodular cystic lesions containing papillary infoldings of the epithelium and/or foci of hypercellularity. Follicular cysts were similar nodular cystic lesions without any distinct epithelial infoldings or areas of hypercellularity. Since these two were sometimes difficult to discern, the combined diagnosis was used to assure the evaluation of thyroid gland activity. C-cell tumors (adenomas and carcinomas) were present in individual rats in the control and DETDA treated rats but there was no significant difference between the DETDA treated rats and the corresponding control rats. A summary of the proliferative lesions of the follicular cells of the thyroids is presented in Table II.

Table II

| GROUP DOSE (ppm) SEX (NO. EXAMINED) | I 0 | | II 10 | | III 35 | | IV 70 | |
|--|-----------|-----------|-----------|-----------|-----------|-----------|----------------|----------------|
| | M (50) | F (50) | M (50) | F (50) | M (50) | F (50) | M (50) | F (50) |
| Follicular Cell Carcinoma | - | - | 2 | - | - | - | - | - |
| Follicular Cell Adenoma | - | - | 3 | - | 4 | - | 5 ^a | 2 |
| Follicular Cell Hyperplasia/Cysts | 1 | 2 | 1 | 1 | 5 | - | 5 | 5 ^b |
| Follicular Cell Hypertrophy | 1 | 1 | - | 1 | - | 1 | 7 ^a | - |

^a = Significant increase over Controls (same sex) p = .05

^b = Significant increase over Mid dose (same sex) p = .05

The mammary glands of the mid and high dose females had a significant increase in the incidence of fibroadenomas. There was an increase in both the number of tumors and the number of tumor bearing animals. However, the incidence of mammary gland adenocarcinomas was higher in the control females than in the three dose groups of DETDA treated females. A summary of the mammary gland neoplasms in the females is presented in Table III.

Table III

| GROUP DOSE (ppm) (NO. EXAMINED) Count/No. animals | I 0 (47) Ct./An. | II 10 (50) Ct./An. | III 35 (50) Ct./An. | IV 70 (50) Ct./An. |
|--|---------------------------|-----------------------------|------------------------------|-----------------------------|
| Adenocarcinoma | 12/10 | 4/4 | 10/7 | 6/4 |
| Adenoma | 1/1 | --- | 2/2 | 3/3 |
| Fibroadenoma ^b | 13/12 | 20/16 | 34/23 ^a | 43/25 ^a |

^a = Significant increase over Controls; $p = .05$

^b = Significant Linear CHI-SQUARE value; $p \leq .05$

The pancreas of the high dose males had a significant increase in multifocal acinar atrophy ($p = .05$) accompanied by interstitial fibrosis and fatty infiltration (replacement of glandular elements by adipose tissue; $p = .001$). Due to the reduction in the acinar glands, the evaluation of the quantity of islet cells was difficult but those islet cells present in the sections evaluated appeared to be normal. The microscopic changes in the acinar cells were comparable between the control females and the DETDA treated females. The incidences of islet cell tumors (adenomas and carcinomas) were not significantly different between the corresponding control rats and three groups of DETDA treated male and female rats.

The incidences of cysts and focal areas of tubular hyperplasia of the kidneys were increased in high dose male rats when compared to the male controls. These may have been an exacerbation of

lesions seen in chronic nephropathy (discussed below). Six high dose males had bilateral cataracts. Half of these rats had moderate to severe keratitis of the eyes. The relevance of treatment to these cataracts and the cysts and tubular hyperplasia of the kidneys is equivocal.

No treatment-related neoplastic or non-neoplastic changes were noted in any of the other tissues examined from the male and female rats receiving 10, 35 or 70 ppm of DETDA in the diet. A variety of spontaneous neoplasms and incidental lesions were present in both the control and DETDA treated rats.

Pituitary adenoma was the most common neoplasm in either sex and there was a slightly lower incidence in high dose rats than in the control rats. Pituitary carcinomas were of low incidence in all groups. Adrenal cortical neoplasms (carcinomas and adenomas) were present in a few rats, mostly in the females and scattered among the four groups. Medullary tumors (unilateral or bilateral pheochromocytomas) were much more numerous and were found mostly in the males. There was no significant difference between the incidence of these tumors in the control males and three groups of DETDA treated males. Endometrial stromal polyps were present in the uterus of four high dose females and a leiomyoma was present in the uterus of another. Granulosa-theca cell tumors were present in the ovaries of three high dose females. No primary uterine or ovarian neoplasms were diagnosed in the control, low dose or mid dose groups. Interstitial cell tumors

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(unilateral or bilateral) were present in the testes of one high dose rat and four control rats. Astrocytoma was diagnosed in the brains of one control female, two low dose males, one high dose male and two high dose females. Malignant lymphoma was present in one female and three male control rats. A variety of other neoplasms occurred infrequently and usually as single entities in various tissues and organs. These are summarized in the Neoplasm Summary Incidence Tables.

Proliferative and degenerative lesions were very common in the adrenals of the male and female rats. Cortical hyperplasia, cortical vacuolation, congestion and hematocysts were very common in both sexes in all four groups. Medullary hyperplasia was more common in the males than in the females. The combined incidence of medullary hyperplasia (focal and multifocal) was fairly proportionate between the control and high dose male rats.

Some degree of age-associated chronic nephropathy was present in nearly all of the kidneys examined. The lesions of chronic nephropathy included multifocal nonsuppurative nephritis (characterized by interstitial fibrosis, mononuclear nuclear cell infiltration and glomerular sclerosis), tubular regeneration, tubular dilatation, pigment deposition (tubular and interstitial), cysts and focal areas of tubular hyperplasia. The cysts and focal areas of tubular hyperplasia were increased in high dose male rats when compared to controls. The incidence and severity of the other changes in the kidney were not different between the corresponding control rats and three groups of

DTDA treated male and female rats. "End-stage" renal disease was evident in the many of the rats with the more severe lesions of chronic nephropathy. In these rats, the impaired renal function resulted in secondary hyperparathyroidism, characterized by hyperplasia of the parathyroid, resulting in secondary mineralization in the kidney, heart, stomach and media of large arteries. Secondary hyperparathyroidism was considered to be the cause of the osteoporotic changes in the bone of a few rats scattered among the control and DETDA treated male and female rats.

A number of early deaths or moribund sacrifices occurred during the study. Ventral compression of the brain and dilatation of the ventricles were common in the brains of the rats with large pituitary tumors. In some rats which died these were severe and were considered to have contributed to the death of the animal. Additional lesions considered to be the cause of death or moribund sacrifice were the presence of large and/or malignant neoplasms, severe renal disease and severe lesions of the hock/foot (suppurative and ulcerative dermatitis).

Additional spontaneous lesions and incidental findings were noted in both control and treated rats. A complete listing of these lesions is summarized in the Summary Incidence Tables.

CONCLUSIONS

The dietary administration of 10, 35 and 70 ppm of DETDA to male and female Charles River Sprague-Dawley rats for periods up to 24 months resulted in an increased incidence in hepatocellular carcinomas and proliferative lesions in the liver of the high dose males, hepatocellular adenomas and proliferative lesions in the liver of the high dose females, follicular cell adenomas of the thyroids in the high dose males, and fibroadenomas of the mammary glands of the mid and high dose females. Pancreatic acinar atrophy accompanied by fatty infiltration (fatty atrophy) was present in the high dose male rats. Equivocal histomorphologic changes present in the male rats receiving 70 ppm of DETDA consisted of cataracts, renal cysts and renal tubular hyperplasia.

Analysis of the mechanism of action of DETDA in these rats is difficult to ascertain. All three groups of DETDA treated males had an increased incidence and severity of degenerative changes in the liver (focal and multifocal cystic degeneration and multifocal necrosis). The increase in proliferative lesions in the liver suggests previous degenerative changes in the liver. Since the pancreas and liver may sometimes contain very high concentrations of an administered compound, the degenerative changes in the pancreas (acinar atrophy and fatty infiltration) and in the liver may be of similar origin.¹

¹Graves, P. (1990). Histopathology of Preclinical Toxicity Studies: Interpretation and Relevance in Drug Safety Evaluation. Elsevier, New York. (Exocrine Pancreas, p. 447).

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Proliferative lesions of the thyroid can occur in animals with toxic severe liver damage and may be related to liver damage in these rats, but the possibility of a direct effect on the thyroid cannot be excluded. Pituitary neoplasms were the most common neoplasm in either sex. Although there was a slightly lower incidence in high dose rats than in the control rats, the relationship of the thyroid/pituitary feedback mechanism may have further complicated the issues in this study.

The presence of the pituitary tumors on the incidence of mammary gland tumors also complicates the issues. Prolactin from the anterior pituitary may enhance the growth of existing mammary gland carcinomas in rats, but an excess or a deficiency of prolactin at the time of administration of a carcinogen can also inhibit tumor development.² Elevated levels of prolactin can also increase the prevalence of fibroadenomas in Sprague-Dawley rats.³ The presence of ovarian hormones in excess or deficiency can also affect the growth and development of carcinogen-induced mammary neoplasms in rats.⁴ The reported range for fibroadenomas in Charles River CD Sprague-Dawley rats⁵ is 14.6% to 58.1% and for adenocarcinomas is 0.0% to 16.0%.

²Ibid., (Factors Affecting Development and Growth of Mammary Neoplasms, p. 64).

³Ibid.

⁴Ibid.

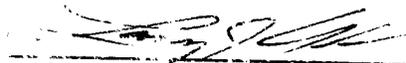
⁵Charles River Monograph "Spontaneous Neoplastic Lesions in the Cr1:CD[®] BR Rat."

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Since the incidence of malignant mammary gland tumors (adenocarcinomas) was high in the control females of this study, the relevance of the incidence of the fibroadenomas in the mammary gland of the high dose females is unknown. The presence of a few uterine and ovarian neoplasms in the high dose females is noteworthy and suggests additional sources of hormonal imbalance. Analysis of the pituitary tumor bearing animals with respect to the occurrence of other endocrine organ tumors within these rats did not show any apparent relationship to the presence of the pituitary tumor.

A number of neoplasms were observed in a variety of tissues and organs without respect to treatment. Spontaneous lesions and incidental findings occurred in both treated and control rats at essentially comparable incidences. The lesions observed were of the usual type and incidence commonly seen in aging Sprague-Dawley rats.


Henry J. Ackerman V.M.D.
Pathologist

July 8, 1991

LJA/au

EPL

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

QUALITY ASSURANCE FINAL CERTIFICATION

Study Title: Oncogenicity Study - Rat, 24 Month Study

Client Study: PH 474-ET-001-88 EPL Project Coordinator: Dr. Larry Ackerman

EPL Project Number: 219-042 EPL Pathologist: Dr. Larry Ackerman

The following aspects of this study were inspected by the Quality Assurance Unit of Experimental Pathology Laboratories, Inc. Dates inspections were performed and findings reported to the Study Director and Management are indicated below.

| Area Inspected | Dates | |
|--------------------|--|---|
| | Inspection | Reporting |
| EPL Project Sheets | 9/19/88; 12/19/88; 1/25/89; 4/5/89; 5/2,31/89; 6/16/89; 7/6/89; 8/8,21/89; 9/8,22/89; 7/19/90; 11/5/90; 12/27, 31/90; 2/4/91 | 1/23/89; 5/31/89; 6/16/89; 7/6/89; 8/8,21/89; 9/8,22/89; 7/19/90; 11/5/90; 12/27/90; 2/4/91 |
| Project Setup | 4/5/89; 9/25/89; 11/8,20/89; 1/4/90; 4/20/90; 7/13/90; 2/4/91 | 4/5/89; 9/25/89; 11/9, 20/89; 12/27/89; 1/4/90; 4/20/90; 7/13/90; 12/31/90; 2/4/91 |
| Histology Setup | 4/5/89; 7/3/89; 9/14/89; 1/4/90; 5/7,10/90; 10/22, 25/90; 12/31/90; 1/2/91; 3/26/91 | 4/5/89; 7/3/89; 9/14/89; 1/9/90; 5/10/90; 8/13/90; 10/22,25/90; 1/2/91; 3/26/91 |
| Histology Complete | 4/13/89; 6/12/89; 7/3,18/89; 10/26/89; 1/4/90; 10/31/90; 11/15,28,29,30/90; 12/6,7, 17,19,21/90; 2/22/91; 3/28/91 | 6/12/89; 7/18/89; 10/26/89; 1/4/90; 11/1,15,28,29, 30/90; 12/6,7,17,19,21/90; 2/22/91; 3/28/91 |
| Rough Draft Report | 4/1,2,3,5,9,10,11,12/91; 5/3/91 | 4/5,12/91; 5/3/91 |
| Final Report | 7/8/91 | 7/8/91 |
| Other | N/A | N/A |

Date of last quarterly facility inspection 6/91

This study is certified to have been performed in compliance with the appropriate Good Laboratory Practice regulations.

Mary Beth Custer
EPL Quality Assurance Unit

7/8/91

Date

SUMMARY INCIDENCE TABLES