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ETHYL CORPORATION

TOXICOLOGY AND INDUSTRIAL HYGIENE DEPARTMENT

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December 22, 1986

CONFIDENTIAL

Document Control Officer (TS-790)
Office of Toxic Substances
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460

8EHD-1286-0648
88-870000K5

EPA-OTS



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Dear Sir:

This letter is submitted in accordance with Section 8(e) of the Toxic Substances Control Act, 15 USC 2607(e) and the Environmental Protection Agency's "Statement of Interpretation and Enforcement Policy" thereon 43 F.R. 1110, et seq, March 16, 1976. This notice concerns diethyltoluene diamine (DETD), CAS No. 68479-98-1. DETDA is a mixture of 3,5-diethyltoluene-2,4-diamine and 3,5-diethyltoluene-2,6-diamine.

We recently received the draft report of a 90-day DETDA rat feeding study. This 90 day study was done in preparation for a two year bioassay. Doses were chosen to cause toxic effects and help set the dose levels for the two year study. DETDA was fed at 0, 50, 125, and 320 ppm in the diet. At the high dose, changes were detected in a number of organs, including the pancreas. Results of the 50 and 125 ppm treatment groups indicated that the primary effect was initiated in the pancreas. Changes in additional organs at the high dose are thought to be consequences of the effect on the pancreas. Summaries provided by the contract laboratories are attached. We will send the final report on the study when we receive it.

Industrial hygiene monitoring of Ethyl employees engaged in the manufacture of DETDA has shown no detectable airborne DETDA in the workplace. The minimum detection limit of the sampling/analytical method is 50 ppb. DETDA has a very low vapor pressure and we handle it in a closed system. Our workers wear full body protective clothing to prevent skin contact. We are notifying our customers and employees of the results of the 90 day rat study.

We plan several animal studies to further investigate this material. We hope to elucidate the mechanism of DETDA's toxic effect in the pancreas and determine if this effect is species-specific or reversible.

Sincerely,

Robert L. Smith

Robert L. Smith
Manager, Regulatory Affairs

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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.

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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 gland, 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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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

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 any 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 Dose: Control		II Low		III Mid		IV High	
	Sex:							
	M	F	M	F	M	F	M	F
(20) (20)	(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 focal or 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 (DETDA) 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.



LARRY J. ACKERMAN, V.M.D.
Pathologist

December 1, 1986

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