FYI-0395-1257
FYI MN2960-RS FR 79-36
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FINAL REPORT

ON

Two-Year Chronic Feeding of Diethylaminoethanol To Albino Rats

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Pennsalt Chemicals Corporation
King of Prussia, Pennsylvania

FYI-94-001257
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I. SYNOPSIS:

A. Three groups of albino rats (initially consisting of 35 males and 35 females each) were fed Diethylaminoethanol for 24 months at dietary levels of 200, 500 and 1,000 ppm (Test High Level periodically increased to 10,000 ppm during study), respectively. An additional group of rats (60 of each sex), which was maintained under similar laboratory environmental conditions and was fed the same basal diet without the test preparation, served as a control group. At six (6) and twelve (12) months, each, five (5) animals of each sex from each group were sacrificed, necropsied and selected tissues from the control and Test High Level (tissues at other levels where considered necessary) groups were examined micropathologically. Remaining animals at termination (24 months) were sacrificed and selected tissues from ten (10) animals of each sex from the control and Test High Level groups (tissues at other levels where considered necessary) were examined micropathologically. Target tissues from animals which succumbed or which were sacrificed due to a moribund condition during the study (all groups) and tissues from all tumorous lesions (all groups throughout the study) were micropathologically examined.

B. None of the test group animals during the entire 24 months of feeding displayed gross signs of ill effects which were considered related to the feeding of the test preparation in the diet. Incidental adverse signs which occurred predominately during the final six months of the study and which were generally associated with the effects of the ageing process in rats, were noted with equal frequency within the control and test groups.

C. Body weight and feed consumption records at each of the three test levels (both sexes) appeared normal and comparable to their respective controls. Instances of anorexia were noted sporadically throughout the experimental period, however, all groups generally showed a vigorous appetite and the animals of the test groups exhibited ready acceptance of the test preparation within the diet.

D. With the exception of one female rat at the Test High Level (No. 33-180 day test interval), no significant hematological changes were evident in the test groups as compared to the controls and the findings generally were considered to be within normal limits. Scattered instances of slightly lower than normal leucocyte counts were recorded for animals within most groups (test and control) at the 90, 360 and 540 day intervals. This finding was considered to be unrelated to ingestion of the test preparation. Slightly lower hematocrit and hemoglobin values than those obtained for the control groups were noted in the Test High Level animals (both sexes) at 720 days, but this finding was probably associated with the animals' age. There was no evidence that the test preparation produced any alterations of the measured blood parameters during the study.

E. Urine analyses showed no significant differences between the test and control groups throughout the study. Scattered instances of slightly positive findings for occult blood, bilirubin and acetone were evident at the 30, 45, 60, 90, 180 and 360 day test intervals in test and control groups. Albuminuria was noted in all groups (control and test) at each interval and bacterial presence was noted at the 45, 60, 90 and 180 day intervals. Since these findings occurred with similar frequency and severity within the control and test groups (both sexes), it was considered that the feeding program did not produce any abnormal changes that could be detected by means of urine analyses.

F. Terminal organ weights (brain, thyroid, heart, liver, spleen, kidneys and gonads) showed values which differed significantly from the controls for the following:

Interval Sacrifice - 6 months

Organ	Dosage Level	Sex
Heart	Test High	Males
Heart	Test High	Females
Liver	Test Low	Females
Kidneys	Test Low	Males
Kidneys	Test Intermediate	Males
Kidneys	Test High	Males
Spleen	Test Low	Males
Spleen	Test High	Females

Interval Sacrifice - 12 months

Organ	Dosage Level	Sex
Gonad	Test High	Females

Since none of the organs from groups which showed significant differences appeared grossly enlarged or were outside of the accepted normal weight ranges of their respective organs, it appeared that these findings were incidental and unrelated to the ingestion of the test compound.

Gross inspection of the group averages and ranges of the terminal organ weight and organ/body weight ratios of remaining animals sacrificed at termination (24 months) showed no significant differences between the control and test groups (compared to their respective sex).

G. Gross necropsy of the animals sacrificed at six (6) and twelve (12) months showed findings which were unremarkable. Gross necropsy of animals which succumbed during the study, which were sacrificed due to a moribund condition, or which were sacrificed at termination (24 months) showed lesions of varying types and numbers. The necropsy findings of the control and test groups were essentially similar. Since all of the lesions encountered were characteristic of ageing laboratory rats, no gross evidence of compound toxicity was detected.

H. Tumorous masses were detected in all groups (control and test), especially during the final six months of the study. The onset, frequency and type of tumor formations encountered showed no differences between the test and control groups. No evidence of tumor formation could be related to the feeding of the test preparation at the dosage levels employed for this study.

I. Micropathological examination of the animals sacrificed at six (6) and twelve (12) months revealed no significant alterations associated with ingestion of the test compound during their respective time periods.

With the exception of testicular atrophy, all lesions encountered within the final twelve months of the study and all tumorous lesions from all groups throughout the entire study are naturally-occurring disease processes common to ageing laboratory rats. No specific evidence of drug toxicity or abnormality attributable to the experimental feeding was apparent.

Since testicular atrophy was found in none of the control group rats (0/34), three (3) of eight in Test Low Level group rats, two (2) of seventeen (17) of the Intermediate Test Level group rats and four (4) of fifteen (15) Test High Level group rats, it appears that testicular atrophy detected within each of the test groups is a result of the ingestion of the test preparation, associated with the ageing of the animals since this finding was not encountered at the six (6) and twelve (12) month sacrifices. Statistical treatment of this data by means of a "t" test showed the findings to be significant at each of the three test levels. The test preparation does not appear to produce a dose-related effect at the levels employed for this study.

II. INTRODUCTION:

The purpose of this study was to evaluate and characterize the oral toxicity of diethylaminoethanol when fed to albino rats for a period of 2 years.

The study was initiated on May 12, 1965 and terminated on May 10, 1967.

Progress reports on this study were submitted to the Pennsalt Chemicals Corporation, King of Prussia, Pennsylvania after six, twelve and eighteen months of compound feeding, respectively.

This final report includes and supplements the data presented in the progress reports and provides an overall evaluation of the results obtained.

III. MATERIAL:

The sample of test material used for the first six months of this study was received from the Pennsalt Chemicals Corporation, King of Prussia, Pennsylvania on May 7, 1965.

The analysis of the compound, according to the inscription and as used in this study, was as follows:

CONCENTRATION - 54.3% DEAE* by weight
71.1% DEAE* HCl by weight

1.000 ml. contains 0.585 gms. DEAE or 0.766 gms. DEAE. HCl

The sample of test material used for the final eighteen months of the study was received from the Pennsalt Chemicals Corporation, King of Prussia, Pennsylvania on October 11, 1965.

The analysis of the compound according to the inscription and as used in this phase of the study, was as follows:

CONCENTRATION - 53.5% DEAE* by weight
70.2% DEAE* HCl by weight

1.000 ml. contains 0.577 gms. DEAE or 0.758 gms. DEAE. HCl

The aqueous solution for each of the lots of material was of a light brown coloration, slightly more viscous than water, with a sweet pungent odor.

IV. METHODS:

A. General Procedure:

1. Specific Maintenance and Selection of Groups:

A total of 330 (165 male and 165 female) healthy young, pathogen free albino rats from the Charles River Breeding Laboratories, Inc. were used on this study to evaluate and characterize the potential long-term (chronic) toxicity of diethylaminoethanol.

The animals were allowed a one-week period to become acclimated to laboratory conditions and were then selected at random and divided into three test groups of 35 males and 35 females each. One additional group composed of 60 males and 60 female rats served as controls. The individual members of each group were selected so that the overall average body weight of each sex group was similar to that of the other sex groups. The rats were housed individually in wire-mesh cages supported above the droppings. Temperature and humidity were controlled within narrow limits in the animal room.

* Diethylaminoethanol

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Water and appropriate diet were freely available at all times during the entire study, i.e., seven days a week. The basal laboratory diet consisted of Purina Laboratory Chow. The test compound was added to the basal diet on a weight/weight basis and thoroughly mixed. Fresh diets were prepared every week.

2. Compound Administration:

The following groups were established:

Group No. I (Control) 60 males and 60 females received only basal laboratory diet.

Group No. II (Test Low Level) 35 males and 35 females received the basal laboratory diet to which 200 ppm of diethylaminoethanol was added.

Group No. III (Test Intermediate Level) 35 males and 35 females received the basal laboratory diet to which 500 ppm of diethylaminoethanol was added.

Group No. IV (Test High Level) 35 males and 35 females received the basal laboratory diet to which increasing amounts of diethylaminoethanol were added, as follows:

1,000 ppm for weeks 1 through 47
1,500 ppm for weeks 48 through 56
2,500 ppm for weeks 57 through 64
3,500 ppm for weeks 65 through 72
5,000 ppm for weeks 73 through 80
7,500 ppm for weeks 81 through 84
10,000 ppm for weeks 85 through 104

The concentration of test material for each of the three groups of test animals was calculated according to the free base.

3. Observations:

a. A daily record of general physical appearance, gross signs of systemic toxicity and/or pharmacological effect, behavior, and mortality was maintained (6 days/week).

b. Body weights and feed consumption were recorded weekly during the first 26 weeks and biweekly from the 26th through the 52nd weeks of the study. During the second year (weeks 53 through 104) body weights and feed consumption were recorded every four weeks.

c. Records were maintained on the incidence, identification, size and disposition of tumors throughout the study.

B. Laboratory Tests:

1. Hematological Studies:

Microhematocrits, hemoglobin determinations and total and differential leucocyte counts were obtained from five (5) rats of each sex from each group at 30, 45, 90, 180, 360, 540 and 720 days.

Complete blood counts were performed on five (5) additional female rats of the Control and Test High Level groups at 180 days and total white counts only were performed on five (5) additional rats of each sex from all groups (control and test) at 360 days.

2. Urine Analyses:

Urine analyses were performed at 30, 45, 60, 90, 180, 360, 540 and 720 days on pooled samples from the same five (5) rats of each sex from each group which were selected for the initial blood test at the corresponding time interval. Each analysis included determinations of albumin, acetone, bilirubin, color, occult blood, sugar, pH, appearance and microscopic examination of the sediment.

C. Pathological Studies:

1. Necropsy Examination:

a. Interval Sacrifice - 6 and 12 months

At 6 and 12 months, five males and five females from each group, including the controls, were sacrificed and complete necropsies performed.

Representative tissues from each animal were taken at necropsy and placed in 10% formalin solution for fixation.

b. Terminal Sacrifice - 24 months

Complete necropsy examinations were performed on all remaining rats sacrificed at the termination of the two-year feeding period.

Representative tissues from each animal were taken at necropsy and placed in 10% formalin solution for fixation.

c. Moribund Animals And Animals Which Succumbed

Complete necropsies were performed on all animals which succumbed during the course of the study or which were sacrificed due to a moribund condition. Representative tissues from each animal (with exception of instances of advanced post-mortem autolysis) were taken at necropsy and placed in ten percent formalin solution for fixation.

2. Organ Weights:

Organ weight and organ/terminal body weights were recorded from all sacrificed rats (excluding sacrificed moribund animals) for brain, thyroid, heart, liver, spleen, kidneys, adrenals and gonads. Organ weights were not recorded for animals which succumbed. For animals sacrificed after 6 and 12 months, a Chi-Square test utilizing a 2 x 2 or 2 x 3 table was performed on the ratio of organ weight to body weight in order to compare these values for each test group with the values for the control group at the respective sacrifice interval. In order to minimize variation the male animals in the control group were compared with male animals of the test groups and the female animals of the control group were compared with female animals of the test groups.

Ratio of organ weight to body weight for animals sacrificed at termination (24 months)were evaluated by gross inspection of the average values and the ranges of each organ of each of the test groups as compared to the respective organ of their control.

3. Micropathological Examination:

The following tissues from each sacrificed rat and from animals which succumbed that did not exhibit advanced post-mortem autolysis were taken and preserved in 10 percent neutral formalin for possible microscopic examination:

brain (3 levels)	pancreas
pituitary	small intestine (2 levels)
thyroid	large intestine
heart	urinary bladder
lung	gonads (ovary or testis)
liver	bone
spleen	bone marrow
kidney	any unusual lesion
adrenal	
stomach (2 levels)	

From five (5) control rats of each sex and five (5) rats of each sex from the Test High Level all of the above tissues were examined microscopically at the six and twelve month interval sacrifices. At termination (24 months) all of the above tissues from ten (10) control rats and ten (10) rats of the Test High Level and the gonads (ovary or testis) of all animals (sacrificed at 24 months) of the control group and the three test levels (low, medium and high) were examined microscopically.

All tissues which were grossly tumorous in nature and those which showed potential qualities of such were submitted for micropathological evaluation. These tissues were from animals of both sexes from control and test low, intermediate and high level groups which succumbed during the study, which were sacrificed during the study due to a moribund condition and which were sacrificed at termination (24 months).

Micropathological evaluation was performed on target tissues of miscellaneous rats from the control and each test group at various times throughout the study where indicated to substantiate or clarify gross findings.

Individual necropsy examination of all rats and microscopic examination of tissues of the above cited animals are presented in Table V.

V. RESULTS:

A. Appearance, Behavior, Body Weights, Feed Consumption and Mortalities:

Two animals from the control group, one male and one female, succumbed during the 4th and 7th week of the study, respectively. Both animals were substituted at the time of death with additional control rats of similar size and appropriate sex. No other mortalities occurred within this group during the first six months of the study. Three animals from the control group (one male and two females) succumbed during the second six months of the study. Four moribund animals (all males) from the control group were sacrificed during the third six months of the study and two animals (both males) succumbed during this period. During the final six months of the study eight moribund animals (four males and four females) were sacrificed and twelve rats (five males and seven females) succumbed.

All of the animals from the Test Low Level (200 ppm) survived the first six months of the study. Two male animals from the Test Low Level succumbed during the second six months of the study. Three animals from the Test Low Level (two males and one female) succumbed during the third six months of the study. One moribund animal (female) was sacrificed during this period. During the final six months of the study three moribund animals (one male and two females) were sacrificed and two rats (both males) succumbed.

One female rat of the Test Intermediate Level (500 ppm) was sacrificed at the end of the 21st week due to a near moribund condition which resulted from a mechanical injury. No other mortalities occurred within this group during the first six months of the study. One female rat from the Test Intermediate Level succumbed during the second six months of the study. Two animals from the Test Intermediate Level (both males) succumbed during the third six months of the study. Three moribund animals (all males) were sacrificed during this time. During the final six months of the study four moribund animals (one male and three females) were sacrificed and ten rats (two males and eight females) succumbed.

One female rat of the Test High Level (1,000 to 10,000 ppm) was sacrificed due to emaciation and depression as a result of missing upper incisors during the first six months of the study. No other mortalities occurred within this group during the first six months of feeding. All of the animals from the Test High Level survived throughout the second six months of the study. Two animals from the Test High Level (both males) succumbed during the third six months of the study. Two moribund animals (one male and one female) were sacrificed during this period of time. During the final six months of the study six moribund animals (two males and four females) were sacrificed and nine rats (five males and four females) succumbed. Since the incidence of mortality of the test groups compared favorably with the control group (according to sex) throughout the study, the test preparation did not produce any earlier or greater numbers of deaths at any of the dosage levels employed for this study.

All deaths in the control and test groups which were not accidental, were considered to be a result of naturally occurring disease processes which are characteristic of rats, and in particular of ageing rats.

During the first 18 months of the study all surviving animals of both sexes, in each group, including the control, ate well and appeared healthy. During the final 6 months of the study, the effects of ageing in the animals was noted in all groups. This was manifested by a general poorer condition of health and an increase in mortality. There was no grossly discernible difference between the general health of the three test groups and the controls during the study. Incidental findings such as nasal and ocular encrustations and poorly aligned teeth occurred in each of the groups of animals with similar frequency and extent.

None of the test animals displayed gross signs of toxicity or pharmacological effects during the 24 months of feeding.

Tumorous structures were observed in both sexes of each of the groups of animals (controls and three test levels). The majority (more than 90%) of the tumors were noted during the final 6 months of the study or were discovered at the time of necropsy. As evidenced by the gross necropsy and micropathological results, the onset, type, size and frequency of the tumors were similar amongst the test and control groups and the development of the neoplastic structures within the test groups could not be related to ingestion of the test preparation in the diet.

The average weekly body weight and feed consumption values for both male and female test animals were generally comparable to the controls at the corresponding interval.

All groups showed weight gains, a maintenance of proper weight at maturation, and feed consumption which are usual for this strain of rat during its lifetime. The average weekly body weight, feed consumption, compound consumption (test groups) and survival records are presented in Table I.

B. Laboratory Tests:

1. Hematological Studies:

The results of the hematological studies are presented in Table II. At the 180 day interval, Female No. 33 of the Test High Level (1,000 to 10,000 ppm) was the only animal with an abnormal blood picture. This animal had low hematocrit and hemoglobin values accompanied by moderate to severe polychromasia, marked hypochromic red blood cells, moderate aniscytosis and many nucleated red blood cells. The condition of this animal at the time of sacrifice is described above under Section V.

Since abnormal findings were noted in this one rat, hematological studies were performed on five additional female rats of the control and Test High Level groups at 180 days. The hematological findings of the ten additional females (5 controls and 5 High Test Level)at 180 days along with the findings from the other test groups (5 males and 5 females each) including the controls at each of the other intervals (30, 45 and 90 days) showed values which were considered to be within normal limits, however, an occasional aberrant value was obtained within each group. Lower than the expected normal low range of leucocyte counts were recorded for some animals within both control and test groups at 45 and 90 days.

The results of the hematological studies at 360 days, showed leucocyte counts which were lower than the expected normal limits for rats in all groups of animals excluding the control males. This finding was evident in from 1 to 4 animals of each group. Repeat total leucocyte counts performed on five additional rats from each of the three test groups (both sexes) and the controls (females only) showed similar findings. The remaining hematological findings in each of the test groups were comparable to the controls and were considered to be within normal limits.

The hematological findings in each of the test groups at 540 days were comparable to the values obtained for the controls and were generally found to be within normal limits, however, lower than normal leucocyte values were obtained in some animals of the control and test groups. With the exception of slightly lower hemoglobin and hematocrit values recorded for the Test High Level animals at the final determination (720 days), the hematological findings of the test group at this interval were comparable to the controls and were generally within normal limits.

No evidence of hematological alterations of the parameters which were measured during this test were produced by the test preparation when added to the diet. The overall blood picture of the three test groups and the controls were considered to be comparable and generally within normal limits.

2. Urine Analyses:

The results of the urine analyses are presented in Table III. The urine analyses showed high albumin values for each of the groups, including the controls at all of the test intervals. Occasional positive findings for acetone, bilirubin and occult blood were also recorded during the first 360 days of the study and bacterial presence was noted at the 45, 60, 90 and 180 day intervals, however, the values for the test groups were comparable to the controls in frequency and severity. The results of the urine analyses throughout the 24 months of feeding showed no indications of adverse effects which could be attributed to the ingestion of the test preparation.

C. Pathological Studies:

1, Necropsy Findings:

Gross necropsy of the animals of each of the three test groups and the controls sacrificed at the 6 and 12 month intervals showed findings which were unremarkable. Gross necropsy of the animals from each of the four groups which succumbed or which were sacrificed due to a moribund condition and of animals at terminal sacrifice (24 months) showed varying numbers and types of lesions. The number, type and severity of lesion was specific to each animal and no evidence of a pattern of gross lesions attributable to ingestion of the test preparation was evident in any of the test groups. All of the lesions which were recorded were considered to be naturally occurring disease processes common to laboratory rats. Tumorous masses and information pertaining to their onset, incidence, size and type are discussed in Section V. A. There was no evidence of tumor formation within any of the test groups which could be related to the ingestion of the test preparation within the diet.

Necropsy findings for individual rats are presented within Table V.

2. Organ Weights:

Terminal body weights, organ weights and organ/terminal body weight ratios are presented in Table IV.

Values which differed significantly from the control animals were obtained for the following organs:

Interval Sacrifice - 6 months

Organ	Dosage Level	Sex
Heart	Test High	Males
Heart	Test High	Females
Liver	Test Low	Females
Kidney	Test Low	Males
Kidney	Test Intermediate	Males
Kidney	Test High	Males
Spleen	Test Low	Males
Spleen	Test High	Females

Interval Sacrifice - 12 Months

Organ	Dosage Level	Sex
Gonad	Test High	Females

All of the organs of the test animals sacrificed at 6 and 12 months grossly appeared to be of normal size and all were within the expected weight limits for the respective organs. Although the above stated organs showed a significant deviation from those for their respective controls, there appeared to be no dose response relationship for these findings.

Gross inspection of the terminal body weights, organ weights and organ terminal body weight ratios of survivors sacrificed at termination (24 months) showed no significant differences between the average values and the ranges of the organs of the test groups and their respective controls. Occasional hypertrophy and atrophy were noted in various organs within each group (both sex, test and controls) but these findings were generally attributed to the age of the animals. Overall findings throughout the study showed no definitive evidence of organ weight alterations which could be attributed to ingestion of the test preparation within the diet.

3. Micropathological Findings:

The results of the micropathological examination of tissues from all of the selected rats are presented in Table V.

These findings are as follows:

a. Interval Sacrifice - 6 months
   - 5 animals of each sex Control Group
   - 5 animals of each sex Test High Level

The histopathological evaluation of tissues of control and Test High Level animals revealed cellular alterations which were within normal limits, and in the case of the Test High Level animals, were considered to be unrelated to the dietary feeding of the test preparation.

The microscopic findings of sections of all listed tissues of one female of the Test Intermediate Level which succumbed before 6 months and sections of lung tissue from one male of the Test Low Level (six (6) months interval sacrifice) are presented within Table V.

Evaluation of 6 months Sacrifice Micropathological Findings:

The changes in the control and Test High Level groups were minimal and were not of pathological nature, therefore, indicating no toxic response related to dietary feeding of the test preparation.

b. Interval Sacrifice - 12 months } 5 animals of each sex Control Group; 5 animals of each sex Test High Level

All animals exhibited negligible, minimal chronic bronchitis. Other abnormalities which were encountered were mild pituitary chromophobe cell hyperplasia in one male and four females of the control group and one male and two females of the Test High Level. One additional control group male showed a tiny pituitary chromophobe cell adenoma. One test group female exhibited mild focal chronic pyelonephritis, and one control group female showed focal calcifications in the mucosa of the renal pelvis. Minimal squamous metaplasia of occasional thyroid follicles was present in one male and one female rat in the control group. Sections of a caecal tumor, which was present in one additional control group male which succumbed between 6 and 12 months showed a fibrosarcoma.

The microscopic findings of sections of a caecal lesion of a Test Low Level group male which succumbed between 6 and 12 months are presented within Table V.

All of these abnormalities represented naturally occurring disease processes common to ageing laboratory rats.

Evaluation of 12 months Sacrifice Micropathological Findings:

There was no morphologic evidence in the tissues examined of abnormalities attributable to the ingestion of the test compound in the diet.

c. Terminal Sacrifice - 24 months } 10 animals of each sex Control Group; 10 animals of each sex Test High Level

Almost all rats of each sex of the control and Test High Level, which were examined completely, showed mild degrees of chronic bronchitis and/or pneumonitis, occasionally of the aspiration variety, plus a mild chronic interstitial nephritis. Minimal squamous metaplasia of thyroid follicular epithelium was found in one male animal of the control and Test High Level groups. Perivascular iron deposition in macrophages in the pancreas was found to a mild degree in four control males and one Test High Level male. Roundworms were encountered within the colon of two control and three Test High Level rats. Patchy adrenal cortical degeneration was present in five control and eight Test High Level animals. Cystitis was seen in one control and two Test High Level rats. Testicular atrophy was conspicuous in four Test High Level animals but was absent from the control group, and ovarian atrophy was similarly found in one experimental female only. Miscellaneous findings included one animal each with fatty metamorphosis of the liver, a chronic gastric ulcer, chronic sialadenitis, and epicarditis, all within the Test High Level group. Careful examination of the sections of cerebellum in the Test High Level animals showed no degenerative change, cell loss, or other abnormality.

Tumors were numerous in the forty animals which were examined completely. These included pituitary adenomas in nine (9) animals each in the control and Test High Level groups; mammary gland fibromas, adenomas or fibroadenomas in eight (8) control and four (4) Test High Level females; and miscellaneous tumors which included one (1) ganglioneuroma, one (1) pheochromocytoma, and two (2) renal embryomas in the control group; adrenal cortical adenomas in one (1) control and three (3) Test High Level females; and one (1) pancreatic duct adenoma, one (1) hepatoma and three (3) granulosa cell tumors in the Test High Level group.

Evaluation of 24 months Sacrifice Micropathological Findings:

With the exception of gonadal atrophy (Evaluation presented in immediately following section), the lesions encountered in this study are all found as naturally-occurring disease processes common to ageing laboratory rats. No specific evidence of drug toxicity or abnormality specifically attributable to the experimental feeding study was apparent.

d. Study of Gonads (Ovary or Testis) - Terminal Sacrifice - 24 months - All Survivors in Control and Three Test Levels Which Were Not Completely Examined At 24 Months:

Since gonadal atrophy was noted in four of ten male and one of ten female rats on the preliminary examination of the Test High Level and the gonadal tissues of the twenty control animals were found to be within normal limits, additional sections of gonad from all survivors at termination (24 months) were prepared to resolve the question of possible drug toxicity. The additional study comprised tissues from twenty four (24) males and twenty seven (27) females in the control group, eighteen (18) males and twenty one (21) females in the Test Low Level group, seventeen (17) males and twelve (12) females in the Test Intermediate Level group and five (5) males and eight (8) females in the Test High Level group.

All sections of ovary in the additional study in all groups (test and control) were within the limits of expected histologic appearance.

All additional sections of testis in the control and Test High Level groups were similarly normal. Mild to moderate atrophy of the testicular tubules, with decreased cellularity and reduced to absent spermatogenesis was present in three (3) males in the Test Low Level group and two (2) males in the Intermediate Test Level group.

Evaluation of 24 months Sacrifice Micropathological Findings of Additional Gonadal Tissues:

If the findings in the preliminary study (10 animals of each sex from control and Test High Level groups - 24 months) plus the additional sections of gonadal tissues are combined, testicular atrophy was found in none of the control group rats, three (3) of eighteen (18) of the Test Low Level group rats, two (2) of seventeen (17) of the Intermediate Test Level group rats and four (4) of fifteen (15) Test High Level group rats. Only one case of ovarian atrophy was noted in a female rat of the High Test Level and this finding may well be fortuitous.

The findings of testicular atrophy within the three test groups were subjected to statistical confirmation by means of a "t" test.

The calculations are as follows:

Dose Level	Lesions in Testis		Difference	Deviation	Squared Deviation
	Control X_1	Preparation X_2	$D=X_1-X_2$	$d=D-\bar{d}$	d^2
Low	0	3	-3	0	0
Int.	0	2	-2	1	1
High	0	4	-4	-1	1
Total	0	9	-9		2
Mean	0	3	$\bar{d}=-3$		$s_D^2=1$

$s_D = \sqrt{1} = 1$

$s_{\bar{d}}^2 \quad 1/3 = 0.333$

$s_{\bar{d}} = \sqrt{.333} = 0.577$

$t = \dfrac{\bar{d} - \mu_D}{s_{\bar{d}}} = \dfrac{-3}{0.577} = -5.20 \qquad \mu_D = 0$

d.f. = N-1=2

With 2 degrees of freedom 5.20 falls between p=.050 and p=.025

$\bar{d}$ =3.00 lesions is significant (p=.025)

Based on the results of the statistical analyses, it appears that the testicular atrophy within each of the test groups is a result of the ingestion of the test preparation within the diet, associated with the ageing of the animals since this finding was not encountered at the 6 or 12 months sacrifices. The test preparation does not appear to produce a dose-related effect at the levels employed for this study.

e. Micropathological Study on Tumorous Lesions (Animals from all group (test or control) throughout entire study excluding those results presented at the scheduled sacrifice periods)

This special study of tumors removed from rats of all groups which were not completely examined microscopically included tissues from eight (8) males and thirteen (13) females in the control group, one (1) male and eight (8) females in the Test Low Level group and three (3) males and nine (9) females in the Test Intermediate Level group, and four (4) males and six (6) females in the High Test Level group. Pituitary adenomas were found in thirteen (13) control rats and six (6) and two (2) rats in the Test Intermediate and Test High Level groups, respectively. Mammary gland fibroadenomas were found in female rats in the control, Test Low Level, Test Intermediate Level and Test High Level groups (two (2), five (5), one (1) and one (1) times, respectively, with malignant stromal change present in one (1) tumor in a control animal. Sarcomas were present within the same groups two (2), one (1), two (2) and two (2) times each. Miscellaneous tumors included a renal hamartoma, an astrocytoma, and a testicular carcinoma with metastases, once each in control animals; a thyroid carcinoma in one (1) Test Low Level group male; undifferentiated carcinoma in one (1) and squamous cell carcinoma of the skin in two (2) Test Intermediate Level group animals, and adenocarcinoma and bronchogenic carcinoma each in one (1), and angioma in each of two (2) Test High Level group rats.

Evaluation of Micropathological Findings of Tumorous Lesions:

The tumorous lesions encountered within this study are naturally-occurring disease processes common to ageing laboratory rats. No specific evidence of drug toxicity or abnormality specifically attributable to the experimental feeding study was apparent.

Diethylaminoethanol: Two-Year Chronic Feeding in Rats

Table I - Male Rats: Body Weight, Grams; Feed Consumption, gm/rat/day and gm/kg/day; Survival

Group I - Controls (0 ppm)

End of Week No.	Body Weight Grams	Feed Consumption gm/rat/day	Feed Consumption gm/kg/day	Survival
Initial	82.6			60/60
1	134.5	16.6	123.4	60/60
2	188.6	20.9	110.8	60/60
3	244.5	24.6	100.6	60/60
4	290.2	24.9	85.8	59/60*
5	329.2	24.3	73.8	60/60
6	358.5	25.1	70.0	60/60
7	377.4	24.9	66.0	60/60
8	394.3	24.7	62.6	60/60
9	406.7	24.3	59.7	60/60
10	419.8	24.6	58.6	60/60
11	435.9	24.3	55.7	60/60
12	447.2	23.6	52.8	60/60
13	444.0	24.1	54.3	60/60
14	456.6	24.4	53.4	60/60
15	461.5	23.4	50.7	60/60
16	466.9	23.4	49.9	60/60
17	473.4	23.5	49.6	60/60
18	480.2	22.9	47.7	60/60
19	474.8	23.1	48.7	60/60
20	479.6	23.3	48.6	60/60
21	479.6	23.7	49.4	60/60
22	494.8	23.5	47.5	60/60
23	489.5	22.2	45.4	60/60
24	484.9	20.9	43.1	60/60
25	488.1	21.8	44.7	60/60
26	493.5	22.4	45.4	60/60

* Rat No. 52 succumbed during 4th week and was replaced with another male

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Diethylaminoethanol: Two-Year Chronic Feeding in Rats

Table I - (continued) Male Rats: Body Weight, Grams; Feed Consumption, gm/rat/day and gm/kg/day; Survival

Group I - Controls (0 ppm)

End of Week No.	Body Weight Grams	Feed Consumption gm/rat/day	Feed Consumption gm/kg/day	Survival
28	497.0	23.3	46.9	55/55
30	498.8	23.0	46.1	55/55
32	505.2	22.9	45.3	54/55
34	516.1	23.6	45.7	54/55
36	513.6	24.7	48.1	54/55
38	523.4	23.6	45.1	54/55
40	529.2	24.2	45.7	54/55
42	538.2	24.0	44.6	54/55
44	541.5	23.9	44.1	54/55
46	547.8	23.8	43.4	54/55
48	554.0	23.7	42.8	54/55
50	564.1	22.5	39.9	54/55
52	573.3	22.4	39.1	49/50
56	584.4	21.5	36.8	48/50
60	603.8	21.9	36.3	48/50
64	609.9	21.9	35.9	46/50
68	596.7	23.0	38.5	46/50
72	608.3	22.6	37.2	45/50
76	617.0	22.7	36.8	43/50
80	612.7	22.1	36.1	43/50
84	600.1	23.0	38.3	43/50
88	599.4	22.9	38.2	41/50
92	603.1	23.4	38.8	38/50
96	599.0	22.7	37.9	37/50
100	608.6	23.2	38.1	35/50
104	587.7	21.9	37.3	34/50

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Diethylaminoethanol: Two-Year Chronic Feeding in Rats

Table I - (continued) Female Rats: Body Weight, Grams; Feed Consumption, gm/rat/day and gm/kg/day; Survival

Group I - Controls (0 ppm)

End of Week No.	Body Weight Grams	Feed Consumption gm/rat/day	Feed Consumption gm/kg/day	Survival
Initial	74.8			60/60
1	115.4	15.3	132.6	60/60
2	148.8	17.2	115.6	60/60
3	178.4	18.9	106.1	60/60
4	193.3	18.0	93.1	60/60
5	214.0	18.1	84.6	60/60
6	232.0	18.7	80.6	60/60
7	245.1	19.4	79.2	59/60*
8	256.7	19.6	76.4	60/60
9	265.2	19.4	73.2	60/60
10	274.5	20.0	72.9	60/60
11	281.5	18.9	67.1	60/60
12	283.0	18.1	64.0	60/60
13	284.8	18.0	63.2	60/60
14	296.3	18.8	63.4	60/60
15	297.6	18.1	60.8	60/60
16	303.8	18.0	59.2	60/60
17	305.9	17.5	57.2	60/60
18	307.3	17.9	58.2	60/60
19	308.4	17.9	58.0	60/60
20	313.1	17.4	55.6	60/60
21	313.6	18.6	59.3	60/60
22	318.2	17.8	55.9	60/60
23	320.7	17.0	53.0	60/60
24	327.1	16.8	51.4	60/60
25	324.7	17.1	52.7	60/60
26	326.7	16.7	51.1	60/60

* Rat No. 57 sacrificed due to near moribund condition and replaced with another female.

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Diethylaminoethanol: Two-Year Chronic Feeding in Rats

Table I - (continued) Female Rats: Body Weight, Grams; Feed Consumption, gm/rat/day and gm/kg/day; Survival

Group I - Controls (0 ppm)

End of Week No.	Body Weight Grams	Feed Consumption gm/rat/day	Feed Consumption gm/kg/day	Survival
28	324.2	17.2	53.1	55/55
30	326.8	17.7	54.2	54/55
32	329.8	18.1	54.9	54/55
34	334.2	17.9	53.6	54/55
36	335.6	20.1	59.9	54/55
38	341.2	18.8	55.1	54/55
40	347.0	19.6	56.5	54/55
42	348.2	19.6	56.3	54/55
44	357.9	19.3	53.9	54/55
46	363.2	19.7	54.2	54/55
48	367.6	19.0	51.7	54/55
50	371.3	18.2	49.0	54/55
52	383.4	18.2	47.5	48/50
56	394.4	17.5	44.4	48/50
60	412.6	17.6	42.7	48/50
64	416.5	17.6	42.3	48/50
68	419.7	19.6	46.7	48/50
72	435.9	18.9	43.4	48/50
76	444.2	18.4	41.4	48/50
80	452.0	17.8	39.4	46/50
84	448.8	19.6	43.7	46/50
88	450.3	19.0	42.2	46/50
92	449.9	18.6	41.3	44/50
96	449.9	19.0	42.2	41/50
100	460.0	19.5	42.4	39/50
104	446.6	16.9	37.8	37/50