UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: ETHYLENE THIOUREA--
Tox. Data Submitted Under MRID 42338101

EPA ID # 014504

Chemical: 913A (014504)/443AA(600016)
RD Record: S-420054
HED Project: D179609

FROM: Irving Mauer, Ph.D., Geneticist
Toxicology Branch-I
Health Effects Division (H7509C)

12-03-92

TO: Walter Waldrop/Terri Stowe, PM #71
Reregistration Branch
Special Review and Reregistration Division (H7509W)

THRU: Karl P. Baetcke, Ph.D., Chief
Toxicology Branch-I
Health Effects Division (H7509C)

12/4/92

Registrant: ETU Task Force (Rohm and Haas, du Pont, BASF, Atochem NA)

Request: Review and evaluate the following chronic (12 month) toxicity study of the subject chemical employing dogs:

ETU: 52-Week Oral (Dietary) Toxicity Study in the Beagle Dog, performed by Hazleton France, Laboratory Project 616/505, Final Report dated May 20, 1992 (EPA MRID 42338101).

TB CONCLUSION: The study is judged CORE MINIMUM, providing the following parameters:

Doses tested: 0, 5, 50, 500 ppm in the diet for 52 weeks.
Systemic NOEL = 5 ppm (0.185 mg/kg/day)
Systemic LOEL = 50 ppm (1.89 mg/kg/day):
slightly decreased body weight gains; enlargement of thyroids, accompanied by follicular dilatation and

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hypertrophy; increased total protein and serum globulin, with decreased alb/glb ratio ; accumulation of pigment in liver cells.

[In addition, at the HDT, 500 ppm (20.14 mg/kg/day): death in males and females, manifesting severe anemia; body weight loss, reductions in both T4 and T3; liver necrosis; increased serum triglycerides and cholesterol.]

ATTACHMENT (DER)

DOC 930220

FINAL

009982

DATA EVALUATION REPORT

ETU

Study Title:
ETU, 52 Week Oral (Dietary) Toxicity
Study in the Beagle Dog

Prepared for:

Office of Pesticide Programs
Health Effects Division
U.S. Environmental Protection Agency
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Prepared by:

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November 13, 1992

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Independent Reviewer: John Liccione Date Nov 13, 1992
John Liccione, Ph.D.

QA/QC Manager: Sharon Segal Date 11/13/92
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 2-004
Clement Number: 2-04-20
Project Officer: James E. Scott

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009382

Guideline Series 83-1: Chronic Toxicity Study in Dogs

EPA Reviewer: Irving Mauer, Ph.D.
Toxicology Branch I,
Health Effects Division

Signature:
Date: 11-16-92

Karl Baetcke, Ph.D.
Branch Chief, Toxicology Branch I,
Health Effects Division

Signature:
Date: 10/9/92

DATA EVALUATION REPORT

STUDY TYPE: Chronic toxicity study in dogs. HED Project: D179609

TEST MATERIAL: Ethylene thiourea (ETU)

Tox Chem. Number: 913A (014504)// 443AA (600016)

MRID Number: 423381-01

SYNONYMS: 2-Imidazolidinethione

STUDY NUMBER: 616/505

SPONSOR: ETU Task Force (Rohm and Haas, du Pont, BASF, and Atochem North America)

TESTING FACILITY: Hazleton France
Les Oncins
France

TITLE OF REPORT: ETU, 52 Week Oral (Dietary) Toxicity Study in the Beagle Dog

AUTHOR: J.P. Briffaux

REPORT ISSUED: May 20, 1992

CONCLUSIONS: Groups of four dogs/sex were fed ethylene thiourea in the diet for 52 weeks at levels of 0, 5, 50, and 500 ppm, equivalent to average test material intake levels of 0.18, 1.99, or 20.13 mg/kg/day in males and 0.19, 1.79, or 20.15 mg/kg/day in females based on nominal dietary levels. Three high-dose dogs died or were sacrificed moribund; one female (during week 8) and two males (during weeks 17 and 18). These dogs had severe anemia, weight loss, reduction of T3/T4 hormones, and liver necrosis.

Mean body weight gains in survivors were decreased slightly in mid-dose males and were reduced moderately in high-dose males and high-dose females. Anemia was observed in one surviving high-dose male, but no corresponding change was seen for females. Serum triglyceride levels were increased compared to controls in high-dose males and females throughout the study, and slight elevations in cholesterol levels were observed at some intervals in high-dose

dogs. An increase in total protein and serum globulin was observed in mid- and high-dose females and high-dose males, and in the males, a decrease in the albumin/globulin ratio was also seen. Decreased thyroid hormone levels (T3 and T4) were observed in the three dogs that died or were sacrificed moribund, and also in one of the two surviving high-dose males; this male had moderately severe anemia. Mean absolute thyroid weights and the ratios of thyroid weight to body or brain weight were markedly increased in surviving high-dose males and high-dose females. Slight increases were seen in mid-dose males, and moderate increases were observed in mid-dose females. When data were combined for both sexes and compared with controls, the thyroid weight increases were significant at the mid- and high-doses ($p<0.05$), thyroid-to-brain weight increases were significant at the mid- ($p<0.001$) and high-dose ($p<0.01$) levels, and thyroid-to-body weight ratios were significantly increased at the mid- ($p<0.001$) and high-dose ($p<0,01$) levels. Histologic changes of the thyroid that accompanied weight changes were follicular dilatation with colloid and follicular hypertrophy (in two out of eight mid-dose and four out of six high-dose dogs) and a slight increase in the number of follicles (in two dogs in the mid-dose group). An increased incidence of slight pigment accumulation in Kuppfer cells and hepatocytes was observed in the livers of surviving mid- and high-dose dogs, and slight hepatocyte hypertrophy was observed in three out of eight mid-dose dogs. Decedent dogs showed necrosis of the liver.

Based on slightly decreased body weight gains, enlargement of the thyroids accompanied by follicular dilatation and hypertrophy, pigment accumulation in the liver, and a transient increase in serum globulin (females), the LOEL is 50 ppm (a combined intake of 1.89 mg/kg/day for both males and females) and the NOEL is 5 ppm (a combined intake of 0.185 mg/kg/day).

CORE CLASSIFICATION: The study is judged Core Minimum. Although mortality was observed in three high-dose dogs, a LOEL could be established at the mid-dose and a NOEL was established at the lowest dose tested. Further, analysis of test compound in diets was variable, ranging between 80-90% of the target levels of ETU.

A. MATERIALS AND METHODS

1. Test Article Description

Name: Ethylene thiourea (ETU)

Lot number: CW No. 02506 EV

Purity: 98.0%

Physical property: A white crystalline solid

Stability: Information on file with the supplier

2. Diet preparation

A weighed amount of ETU was mixed with a small amount of powdered diet in a Braun® mixer to produce a homogeneous mixture. This was diluted with basic powdered diet to achieve the desired concentration of ETU and mixed in a TM 100 blender for 12 minutes. Diets were prepared weekly and stored at room temperature. Reserve samples from each batch were retained deep frozen. Stability of ETU in diets was determined in a previous 13-week study. Homogeneity was determined on 100-g samples collected from the top, middle, and bottom of the diet mixes prior to study initiation and at weeks 1 and 26.

Samples of diets were collected monthly from all groups and were retained frozen for analysis of levels of test material. A detailed analytical report was provided (MRID No. 423381-02).

Results: The test material was reported to be stable in diets for 18 days at room temperature. Homogeneity was poor for samples analyzed at weeks 1 and 26. At week 1, the coefficients of variation for homogeneity were 18%, 18%, and 14% for the low-, mid-, and high-dose diets, respectively; at week 26, the mean homogeneity values (±SD) for 8 samples were 89.6±5.2% of target at the low dose, 98.6±32% of target at the middle dose, and 100.6±11.7% of target at the high doses. The overall means for concentrations of duplicate samples of diets at 11 study intervals were 4.3±1.3 ppm, 40.4±15.7 ppm, and 449±61.4 ppm, which correspond to 86±26%, 81±31%, and 90±12% of the target levels at the low, middle, and high dose. The data were corrected for recovery with spiked samples.

3. Animals

Species: Dog

Strain: Beagle

Age: 5 months

Weight at initiation: Males ranged from 5.9 to 8.3 kg, and females ranged from 5.0 to 7.9 kg.

Source: Hazleton Research Products, Cumberland, VA

Group assignment: Animals were acclimated to laboratory conditions for 11 weeks and assigned to the following groups using a stratified body weight procedure:

Group number	Group	Dietary level (ppm)	Number of Animals Males	Females
1	Control	0	4	4
2	Low-dose (LDT	5	4	4
3	Mid-dose (MDT)	50	4	4
4	High-dose (HDT)	500	4	4

Dogs were vaccinated against Bordetella, parainfluenza, and canine parvovirus, as well as distemper, hepatitis, leptospirosis, and rabies. They were examined for ill health at arrival and received a veterinary examination and ophthalmologic examination during the acclimation period. The dogs were housed singly in pens (1.5 x 0.9 m) on a stainless steel floor in an air conditioned building with a temperature range of 19-25°C, relative humidity at >50%, and a minimum of eight air changes per hour. There was a 12-hour light/dark cycle. Pairs of dogs of the same sex were allowed to run together overnight in a larger area.

Rationale for dose selection: The doses were selected on the basis of a 13-week toxicity study in beagle dogs (Hazleton, France; Study No. 616/504; MRID No. 421742-01; DER 1-111, dated May 7, 1992).

4. Statistics

Means and standard deviations were calculated for continuous data (body weights, food consumption, hematology, clinical chemistry, quantitative urinary parameters, and organ weights). Variables were analyzed using two-way analysis of variance (ANOVA) except for ovary and testis weights which were analyzed with one-way ANOVA. Levene's test was applied for equality of variances across groups and between sexes. If the Levene's test had a p value of >0.01 for both group and interaction variance (between sexes), and the ANOVA for the interaction was $p>0.05$, a parametric test for dose response was carried out for pairwise comparisons. If p values for group and interactive variances were >0.01 (Levene's test), but the p value for the interactive effect was <0.05 using ANOVA, data for dose-response and pairwise comparisons were analyzed separately for the sexes. For nonparametric data, the Kruskal-Wallis and Wilcoxon's rank sum test were used followed by the Terpstra-Jonkheere test for pairwise comparison.

5. Quality Assurance

A quality assurance statement was signed and dated May 21, 1992.

B. METHODS AND RESULTS

1. General Observations

Animals were examined twice daily for moribundity and toxic signs. A veterinary clinical examination was performed during pretest and monthly during the study.

Results: Two high-dose males and one high-dose female died or were sacrificed moribund during the study. The cause of death in all of these dogs was severe anemia.

Male #25 had no clinical signs of toxicity up to day 91. Thereafter, it showed pale mucous membranes, subdued behavior, rough coat, yellow-orange feces, dyspnea, and vomiting. A weight loss of 2.4 kg was observed between day 91 and day 113, when death occurred. Because of decreased food consumption, food was given by gavage of powdered diet in water from day 102.

Male #27 was sacrificed moribund on day 47. This dog had no clinical signs up to day 45. Diarrhea was observed on day 46 followed by subdued behavior and marked pallor. The dog was anorexic and dypsneic and prior to sacrifice had a heart rate of 148 beats/minute. A weight loss of 0.8 kg was observed prior to sacrifice.

Female #29 had no clinical signs up to day 36. On day 37, it had liquid diarrhea, severe pallor, subdued behavior, swelling on the head, rough coat, and dypsnea. Prior to sacrifice, its heart rate was 152 beats/minute, and the respiration rate was 24/minute. A weight loss of 0.5 kg was seen between day 36 and day 37 when it was sacrificed.

Pale mucous membranes were seen in the two high-dose males that survived as well as in the three dogs (two males and one female) that became moribund. High-dose male #26 that survived had pallor and yellowish feces from weeks 17 to 20, but the signs disappeared. No other clinical signs were considered related to dosing. In surviving dogs, the frequency and incidence of diarrhea and vomiting were similar in all groups including controls. One low-dose female had cutaneous lesions that did not respond to treatment with an antiparasitic. A mid-dose female had a cervical mass associated with an infectious syndrome; the dog was treated with antibiotic for 2 weeks and recovered.

2. Body Weights/Food Consumption/Test Material Intake

Body weights were recorded weekly beginning 2 weeks before initiation of dosing. Food consumption was recorded daily and reported weekly. Food efficiency was calculated biweekly for 16 weeks and monthly thereafter. Test material intake was calculated weekly.

Body weights

Table 1 summarizes data for mean body weights and Table 2 for weight gains at selected intervals for surviving dogs.

Body weight losses were observed in the two high-dose dogs that were sacrificed moribund and in the high-dose dog that died. Male #25 had a 2.4-kg weight loss from week 13 until death on day 113. Male #27 had a weight loss of 0.8 kg between day 43 and day 48 when it was sacrificed, and female #29 had a weight loss of 0.5 kg between days 36 and 37.

Mean body weights and body weight gains of surviving dogs were lower in mid- and high-dose males and in high-dose females than in controls, but the decreases did not reach a level of statistical significance. One low-dose male (#12) had several periods of anorexia/dysorexia during the study. Weight gain for this dog was 2 kg for the first 13 weeks, but its overall weight gain for 52 weeks was 1 kg. The decreased mean value of weight gain for the low-dose males at 52 weeks is attributed to this dog and is not considered related to dosing. Weight gains of the three other low-dose males were within the range of controls.

Food consumption and efficiency

No significant or biologically important changes in food consumption were seen for surviving dogs in the treated groups. Food efficiency values had considerable variability, but no trends with dosing were apparent.

Test article intake

Average test compound intake values were 0.18±0.03, 1.99±0.21, and 20.13±3.2 mg/kg/day for males in groups 2, 3, and 4, respectively, and 0.19±0.05, 1.79±0.22, and 20.15±2.19 for females in the same dose groups.

3. Ophthalmoscopic Examination

All animals were examined prior to initiation and at weeks 26 and 52. Examinations were conducted using an indirect ophthalmoscope and fundus photography after topical administration of a mydriatic agent.

Results: No treatment-related changes were observed. Local opacity of the lens of both eyes was observed in one low-dose male at pretest, and it persisted throughout the study. Local opacity of the lens of one eye was observed at weeks 26 and 52 for one low-dose male, one mid-dose male, and one low-dose female. Prolapse of the nictating membrane was observed at week 52 in one high-dose female. These findings were not considered related to dosing.

4. Clinical Pathology

Blood was collected from the jugular vein of unanesthetized fasted animals. For hematology, samples were collected in EDTA. Samples were taken for all dogs twice at pretest, and at weeks 13, 26, and 52. The parameters checked (X) below were examined. Additional blood samples were collected as follows: mid-dose female #21 on day 24; high-dose female #29 on day 37; high-dose males #27 on day 48, #25 on days 98, 101, and 111, and #26 on day 139.

(a) Hematology

X Packed cell volume (PCV)*	X Leukocyte differential count
X Hemoglobin (HGB)*	X Mean corpuscular HGB (MCH)
X Leukocyte count (WBC)*	X Mean corpuscular HGB concentration (MCHC)
X Erythrocyte count (RBC)*	X Mean corpuscular volume (MCV)
X Platelet count*	Coagulation: thromboplastin time (PT)
X Reticulocyte count (RETIC)	
X Red cell morphology	

* = Recommended by Subdivision F (November 1984) Guidelines

Results: Severe effects on hematology parameters were observed for high-dose dogs that died or were sacrificed moribund. Male #25 had a pretest HGB value of 13.4 g/dL, and the levels decreased to 9.7, 6.0, 4.1, and 1.5 g/dL at days 90, 98, 101, and 111 (day of sacrifice). Similar decreases were observed for PCV and RBC with values, on day 111, that were 15% and 13% of the pretest values. Similarly in male #27, decreases in HGB (2.2 g/dL), RBC ($0.67 \times 10^6/mm^3$), and PCV (6.2%) were seen on day 48. On day 37, female #29 had values of 3.1 g/dL, $0.84 \times 10^6/mm^3$, and 8.7% for HGB, RBC, and PCV, respectively. Values for platelet counts and total WBC count were also markedly decreased compared to controls prior to the moribund sacrifice of this high-dose female.

Table 3 summarizes data for selected hematology parameters in surviving male dogs. One high-dose male (#28) that survived to 52 weeks had marked changes in hematology parameters. At 13, 26, and 52 weeks, HGB values were decreased 14%, 6%, and 40% compared to the pretest value of 12.4 g/dL. The PCV and RBC levels were decreased to 24.6% (32% lower than pretest) and $2.91 \times 10^6/mm^3$, respectively, at 52 weeks. RETIC was 5/1000 at 13 weeks indicating a compensatory response to the anemia. The second surviving high-dose male had no marked effects on red cell parameters. Platelet counts and total WBC levels were markedly reduced at 13 weeks; however, the effects were reversed toward normal by 26 weeks. Mean WBC levels were significantly decreased in high-dose males at week 13 as compared to controls and decreases in platelet counts were significant in both sexes combined at week 13. A female dog in the mid-dose group (#21) had decreased HGB and PCV at day 24, but there was an increase in RETIC and recovery by week 13.

(b) Blood (clinical) chemistry

Electrolytes	Other
X Calcium*	X Albumin*
X Chloride*	X Albumin/globulin ratio
Magnesium	X Blood creatinine*
X Phosphorus*	X Blood urea nitrogen*
X Potassium*	X Cholesterol (total)*
X Sodium*	X Globulins
	X Glucose*
Enzymes	X Total bilirubin*
	Direct bilirubin
X Alkaline phosphatase (ALP)	X Total protein*
Cholinesterase	X Triglycerides
X Creatine phosphokinase	X Thyroid hormones (T3, T4)
Lactic acid dehydrogenase	(radioimmunoassay)
X Serum alanine aminotransferase (SGPT)*	
X Serum aspartate aminotransferase (SGOT)*	
Gamma glutamyltransferase (GGT)	

* = Recommended by Subdivision F (November 1984) Guidelines

Results: Table 4 summarizes mean data for serum cholesterol and triglyceride levels. Mean cholesterol levels tended to be increased in high-dose males and females, but none of the increases were statistically significant when compared to controls. High-dose male #28 had levels of 2.34 mg/dL (70% above its pretest level) at week 13 (this level remained high), 1.72 mg/dL at week 26, and 1.82 mg/dL at week 52, respectively. The triglyceride levels for this male were also elevated at weeks 26 and 52 (0.86 and 0.57 mg/dL, respectively). This dog (#28) had developed an anemia (see above) and also had an elevated total bilirubin level at week 52. The increased cholesterol level in high-dose females at week 13 was caused to some extent by an abnormally high value for female #31 (2.43 mg/dL). Triglyceride levels in high-dose dogs were significantly higher than controls at week 13 in males ($p<0.05$) and females ($p<0.01$) and at weeks 26 and 52 in group 4 (high-dose) males and females combined. The biological importance of this finding is difficult to interpret since the pretest values at week -2 for the high-dose dogs were higher than those in the control group (e.g., 0.46 and 0.47 mg/dL, for group 4 males and females, respectively).

For the three decedent animals, levels of total bilirubin were increased above the normal range. A value of 3.1 mg/dL was recorded for high-dose male #25, and at the day before death the value was 2.7 mg/dL. For high-dose male #27, and for female #29, the levels of bilirubin the day before death were 3.4 and 4.9 mg/dL, respectively. A mid-dose male (#17) had a

bilirubin level of 3.9 mg/dL at week 13, but normal levels were found at weeks 26 and 52.

Table 5 summarizes data for total serum protein, globulin, and albumin/globulin ratios. The levels of total protein and globulin were increased and the A/G ratios were decreased compared to controls in high-dose males at weeks 13, 26, and 52. The study author reported that globulin was significantly higher than control levels in high-dose males and females combined at weeks 13 and 52 ($p<0.05$). Values of globulin were above the normal range (>32 g/L) in two mid-dose females at week 26, in one high-dose female at weeks 13 and 26, and in one high-dose male (#26) at weeks 13, 26, and 52. SGPT and SGOT activities were elevated in the two males that were sacrificed moribund and in high-dose male #26 at weeks 13 and 26. Other variations in clinical chemistry parameters were not of biological importance.

Thyroid hormone assays: Table 6 presents mean levels for triiodothyronine (T3) and thyroxine (T4) in surviving males and females at pretest and at weeks 13, 26, and 52. No statistically significant changes were seen in any of the treated groups when compared to controls. In decedent animals, decreases in T3 and T4 compared to pretest values were observed just prior to death or moribund sacrifice. In decedent male #25, the levels at pretest, day 93, and day 94 were 83.0, 51.9, and 39.0 ng/dL, respectively, for T3 and 2.9, 1.4, and 0.9 µg/dL, respectively, for T4. For high-dose male #27, the value for T3 at day 48 was 47.3 ng/dL compared to 89.2 ng/dL at pretest, and the value for T4 was 0.8 µg/dL compared to 3.8 µg/dL at pretest. For female #29, the value for T3 was 33.4 ng/dL at day 37 compared to 68.3 ng/dL at pretest, and the value for T4 was 0.9 µg/dL compared to 1.7 µg/dL at pretest. The decreases were probably due to the poor health condition of the animals and a secondary effect of ETU administration.

Abnormally low values for T3 were occasionally seen in dogs that survived. High-dose male #28 had a value of 38.9 ng/dL at 52 weeks. This dog also had a severe anemia. Values of 36.1 and 35.4 ng/dL were seen for one low-dose male and low-dose female at week 52. The mean historical value for the laboratory was 73.5 ng/dL, and the two SD range was 43.5-103.5 ng/dL (n=56). The laboratory historical mean value for T4 was about 2.7 µg/dL (two SD range of 0.8-4.8). A value of less than 1.0 µg/dL was seen for only one surviving dog, high-dose male #28 (0.6 µg/dL at week 13); the level was 1.4 µg/dL at weeks 26 and 52.

(c) Urinalysis

X Appearance*	X Sediment (microscopic)	X Bilirubin*
X Volume*	X Protein*	X Blood
X Specific gravity*	X Glucose*	Nitrate
X pH*	X Ketones	X Urobilinogen

* = Recommended by Subdivision F (November 1984) Guidelines

Results: No effects on volume, specific gravity, or pH of urine were observed in treated groups. No other dose-related effects were observed in surviving dogs.

5. Sacrifice and Pathology

All animals, including those sacrificed moribund, received a full necropsy, including examination of the external surface, all orifices, body cavities, and viscera. Cranial and nasal cavities, sinuses, and cut surfaces of the brain, liver, and spleen were also examined. The tissues checked (X) below were examined histologically. The double-checked (XX) tissues were also weighed.

Digestive System	Cardiovascular/Hematologic	Neurologic
X Tongue	X Aorta*	XX Brain (3 levels)*
X Salivary glands*	XX Heart*	X Peripheral nerve* (sciatic nerve)
X Esophagus*	X Bone marrow*	X Spinal cord* (3 levels)
X Stomach*	X Lymph nodes*	XX Pituitary*
X Duodenum*	XX Spleen*	X Eyes* (optic nerve)
X Jejunum*	XX Thymus*	
X Ileum*		
X Cecum*	Urogenital	
X Colon*		
X Rectum*	XX Kidneys*	Glandular
XX Liver*	X Urinary bladder*	
X Gallbladder*	XX Testes*	XX Adrenals*
X Pancreas*	X Epididymides	Lacrimal gland
	X Prostate	X Mammary gland
Respiratory	X Seminal vesicle	XX Thyroids*
	XX Ovaries*	X Parathyroids*
X Trachea*	X Uterus*	Harderian glands
X Lung*		

Other

X Bone (sternum and femur)*
X Skeletal muscle*
X Skin*
X All gross lesions and masses*

* = Recommended by Subdivision F (November 1984) Guidelines

(a) Organ weights: Table 7 summarizes data for absolute and relative thyroid weights of surviving dogs. A marked increase in mean values for thyroid weight and thyroid-to-body weight or brain weight ratios were observed for high-dose males and females. Statistical analysis of data for males and females combined indicated p values of <0.05 for absolute thyroid weight, $p<0.01$ for thyroid-to-body weight ratio, and $p<0.001$ for thyroid-to-brain weight ratio. In the mid-dose groups, absolute thyroid weight was increased above the concurrent control mean in one male and all females; the p value was >0.05 for both sexes combined. For the sexes combined, the increase in thyroid-to-body weight ratios had a reported p value of 0.001 when compared to controls. The thyroid-to-brain weight ratios in both mid-dose females and males were slightly increased compared to controls; the increases were not significant ($p>0.05$).

An increase in absolute and relative adrenal weights was observed in mid-dose females ($p<0.05$), but there was no corresponding effect in males. Since the values in high-dose females were decreased compared to controls, the increases in adrenal weights at the mid-dose were not considered related to dosing. No effects on the weights of other organs were observed. Organ weights were not determined on animals necropsied before study termination.

(b) Macroscopic pathology: No observations, except pale carcass, in the animals necropsied prior to study termination could be considered related to dosing. Other findings were incidental. No treatment-related gross findings were present at the terminal sacrifice. Hair loss was observed in several dogs, but no dose-response relationship was seen.

(c) Microscopic pathology: No neoplasms were found. In the two high-dose males and one high-dose female that died or were sacrificed moribund, centrilobular hepatocyte necrosis and pigment accumulation in the Kuppfer cells of the liver were observed. The necrosis was minimal in the female and moderately severe in the two males. Pigment accumulation was minimal or slight in all deceased dogs. One deceased male (#25) also had moderately severe multifocal hepatocyte vacuolation. Histologic thyroid changes (slight hypertrophy and dilatation of the follicular cells) were seen only in one of the deceased males. Table 8 summarizes the incidence of histologic findings in the thyroids and livers of dogs that survived to the 52-week sacrifice. One mid-dose male and one mid-dose female showed slight hypertrophy and moderate dilatation of the follicular cells of the thyroid, and a slight increase in the numbers of follicles was seen in both dogs. Two surviving high-dose males and two high-dose females showed hypertrophy and dilatation of the follicular cells of the thyroid. The severity of the findings was greater for the males than for the females. The hypertrophy was graded slight

for both females and moderate or moderately severe in the two males; the dilatation with colloid was graded moderate in both high-dose females and moderately severe in both high-dose males.

Pigment accumulation in the Kuppfer cells was graded slight in the livers of affected dogs (one male and one female at the mid dose and one male and two females at the high dose). Slight hepatocyte hypertrophy was observed in two males and one female in the mid-dose group but in none of the high-dose dogs. Other microscopic findings, considered incidental, included interstitial pneumonia in a high-dose male, congestion of the lungs in a high-dose female, and degeneration of the testis with seminal vesicle atrophy in one surviving high-dose male.

C. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS

Although the study is flawed because of the death of two high-dose males and one high-dose female, a LOEL can be established based on the middle dose (1.9 mg/kg/day) and the NOEL corresponds to the low dose (0.185 mg/kg/day). The study was adequately conducted and reported and the summary tabulations were supported by individual animal data. The statistical analyses were somewhat limited because of small numbers of animals; however, combining data for males and females for certain parameters had a statistically defensible basis and improved the statistical analysis.

A substantial study deficiency was the variability in the analytical data on measurement of test compound in diets. The method appeared valid, and recoveries of spiked samples were reasonable. Since homogeneity data showed a high variance, the poor reproducibility of dietary concentrations may be related to a deficiency in mixing diets.

The rationale for selection of doses was reasonable. It was based on a 13-week study in which the MTD was exceeded at 2000 ppm and a NOEL was the next lower dose, 150 ppm. Similar effects were seen in both studies. In the subchronic study, two high-dose males were sacrificed moribund (week 8) with severe anemia and weight loss prior to sacrifice. No mortalities were seen in females in the subchronic study, whereas a female receiving 500 ppm in the chronic study was moribund during week 5. Decreased weight gains were not observed in survivors receiving 2000 ppm or 150 ppm in the subchronic study, whereas in the chronic study, slightly decreased weight gains were seen during the first 13 weeks in males receiving 50 and 500 ppm and in females receiving 500 ppm. Anemia was observed in all surviving dogs at 2000 ppm in the subchronic study and was minimal at 150 ppm. In the chronic study, anemia was observed in one surviving male but in no surviving females at 500 ppm. Cholesterol levels were increased in dosed dogs in both studies; at 13 weeks, effects were more marked in males than in females. Triglycerides were not measured in the subchronic study. The decreases in thyroid hormone levels (T3 and T4) were dramatic in the survivors receiving 2000 ppm in the subchronic study, and for most dogs the levels were near or below the level of detection. Of the survivors receiving 500 ppm in the chronic

study, a few had transitory decreases in T3 levels but no clear effect on T4 levels. For dogs that were moribund after receiving 500 ppm, the decreases in T3 and T4 were not as severe as in the subchronic study. No effects on T3 or T4 were seen at 150 ppm (subchronic) or at 50 ppm (chronic). Thyroid stimulating hormone (TSH) levels were not measured in either study. The inclusion of this parameter may have provided useful information on the compensatory mechanisms. Similar histologic changes were seen in the thyroids of affected dogs in both studies. No effects on the pituitary were observed in the chronic study, so conclusions cannot be made of thyroid-pituitary interactions resulting after ETU administration.

Table 1. Mean Body Weights (kg ±SD) at Selected Intervals in Dogs Fed ETU for 1 Year

Dose (ppm)	Week 0	13	26	52
		Males		
0	8.3±1.3	9.4±2.1	9.8±2.1	10.4±2.4
5	8.5±0.8	9.7±1.4	9.9±1.4	9.8±1.6
50	8.4±1.3	9.2±1.3	9.3±1.3	9.6±1.1
500	8.1±1.2	9.2±1.3	9.0±0.5	8.8±0.7[a]
		Females		
0	8.0±0.7	8.9±1.4	9.1±1.1	9.6±1.0
5	8.2±0.8	9.4±1.7	9.4±1.7	9.9±2.0
50	7.9±0.7	9.2±0.6	9.6±0.8	9.5±0.8
500	7.7±0.7	7.9±0.9[b]	8.0±1.1[b]	8.1±1.3[b]

[a]Based on two surviving dogs

[b]Based on three surviving dogs

Table 2. Mean Body Weight Gains (kg ±SD) in Dogs Fed ETU for 1 Year[a]

Dose (ppm)	Weeks 0-13	Weeks 0-26	Weeks 0-52
		Males	
0	1.1±0.8	1.5±0.8	2.1±1.0
5	1.3±0.9	1.5±0.9	1.3±0.9
50	0.8±0.7	1.0±0.9	1.2±0.6
500	0.8±0.4[b]	1.0±0.9[c]	0.8±0.7[c]
		Females	
0	1.0±0.9	2.0±0.8	1.7±0.4
5	1.1±1.0	1.2±1.1	1.4±1.3
50	1.3±0.2	1.7±0.2	1.6±0.1
500	0.5±0.3[b]	0.6±0.6[b]	0.7±0.7[b]

[a]Calculated by the reviewers

[b]Based on three surviving animals

[c]Based on two surviving animals

Table 3. Mean Hemoglobin (HGB), Erythrocyte (RBC), Packed Cell Volume (PCV), and Total Leukocyte (WBC) Levels (±SD) in Male Dogs Fed ETU for 1 Year

Parameter/ Dose (ppm)	Week -2	13	26	52
HGB (g/dL)				
0	13.5±0.7	14.1±0.5	15.1±0.5	14.8±0.5
5	13.6±0.8	13.9±0.7	14.3±0.9	13.9±0.6
50	13.5±0.5	13.7±1.0	13.8±1.4	14.5±0.8
500	13.6±0.9	11.7±2.7	13.0±1.8	11.6±5.9
RBC (10^6/mm^3)				
0	6.10±0.33	6.40±0.34	6.67±0.34	6.59±0.24
5	5.87±0.19	6.14±0.39	6.14±0.32	6.01±0.20
50	5.89±0.42	6.27±0.46	6.03±0.41	6.36±0.36
500	5.81±0.26	5.30±1.04	5.78±0.60	4.91±2.83
PCV (%)				
0	39.3±2.4	41.0±1.9	42.4±2.0	41.9±1.8
5	39.2±1.9	40.5±2.3	40.4±2.6	39.4±0.5
50	38.3±1.5	40.9±3.5	38.8±3.1	40.6±1.7
500	38.8±2.7	34.6±8.2	36.8±4.1	34.8±14.4
Total WBC (10^3/mm^3)				
0	10.6±0.7	10.4±1.5	13.7±2.3	11.5±0.5
5	9.9±1.6	9.2±1.4	12.2±1.0	10.5±0.7
50	12.8±2.6	11.9±2.8	13.4±3.0	11.9±1.7
500	11.1±1.1	5.5±2.4	10.9±6.4	12.6±9.1

Table 4. Mean Cholesterol and Triglyceride Levels (±SD) in Dogs Fed ETU for 1 Year

Parameter/ Dose (ppm)	Week -2	13	26	52
		Males		
Cholesterol (g/L)				
0	1.32±0.05	1.15±0.17	0.86±0.16	0.99±0.20
5	1.47±0.13	1.33±0.15	0.93±0.14	1.10±0.10
50	1.27±0.03	1.40±0.10	1.06±0.10	1.30±0.07
500	1.36±0.07	1.62±0.62	1.27±0.64	1.49±0.47
Triglycerides (g/dL)				
0	0.32±0.07	0.24±0.06	0.24±0.05	0.25±0.06
5	0.34±0.07	0.27±0.04	0.26±0.04	0.27±0.04
50	0.38±0.02	0.27±0.07	0.28±0.05	0.30±0.05
500	0.46±0.09	0.34±0.04	0.58±0.40	0.44±0.19
		Females		
Cholesterol (g/L)				
0	1.36±0.23	1.47±0.39	1.19±0.15	1.53±0.17
5	1.21±0.21	1.15±0.27	0.98±0.21	1.42±0.62
50	1.38±0.20	1.36±0.09	1.13±0.09	1.34±0.08
500	1.19±0.19	1.81±0.54	1.12±0.07	1.79±0.21
Triglycerides (g/L)				
0	0.36±0.02	0.26±0.07	0.27±0.02	0.26±0.04
5	0.40±0.07	0.28±0.04	0.35±0.09	0.44±0.28
50	0.54±0.08	0.32±0.07	0.38±0.09	0.32±0.09
500	0.47±0.09	0.55±0.01	0.52±0.10	0.44±0.07

Table 5. Mean Total Serum Protein, Globulin Levels, and Albumin-to-Globulin Ratios in Dogs Fed ETU for 1 Year

Parameter/ Dose (ppm)	Week -2	Week 13	Week 26	Week 52	Week -2	Week 13	Week 26	Week 52
	Males				Females			
Total Protein (g/L)								
0	57±1	62±4	60±5	62±6	56±2	62±1	55±1	60±3
5	56±3	61±5	58±5	60±5	57±4	59±3	58±3	62±3
50	55±1	62±4	56±3	63±5	59±2	62±4	61±7	63±5
500	57±2	67±6	64±6	69±6	58±3	66±4	60±6	64±1
Globulin (g/L)								
0	25±1	30±2	28±5	29±7	24±2	29±1	23±1	25±2
5	24±3	29±4	27±5	28±5	25±4	28±3	27±3	27±3
50	24±2	30±4	25±4	29±4	25±3	27±3	28±7	26±5
500	26±2	37±7	36±7	40±6	23±4	33±4	28±7	29±1
A/G Ratio								
0	1.3±0.1	1.1±0.1	1.2±0.3	1.2±0.3	1.4±0.2	1.1±0.1	1.4±0.1	1.4±0.1
5	1.4±0.2	1.1±0.1	1.2±0.2	1.2±0.2	1.4±0.2	1.1±0.2	1.2±0.1	1.3±0.1
50	1.3±0.2	1.1±0.1	1.3±0.3	1.2±0.2	1.3±0.2	1.3±0.2	1.2±0.3	1.5±0.3
500	1.2±0.1	0.8±0.2	0.8±0.1	0.8±0.1	1.4±0.3	1.0±0.2	1.2±0.3	1.2±0.1

Table 6. Mean Thyroid Hormone (T3, T4) Levels (±SD) in Dogs Fed ETU for 1 Year

Hormone/ Dose (ppm)	Week -2	13	26	52
		Males		
T3 (ng/dL)				
0	79.0±17.0	76.5±16.0	57.0±13.2	66.8±29.2
5	72.4±19.3	85.7±24.5	56.8±15.4	54.4±12.7
50	73.8±11.3	90.9±25.3	65.4±25.1	73.6±25.9
500	82.9±6.5	60.4±7.4	64.8±6.4	60.8±30.9
T4 (µg/dL)				
0	2.7±0.9	2.8±0.3	2.1±0.4	2.2±0.3
5	2.4±0.6	2.3±0.8	1.8±0.5	1.9±0.6
50	2.5±0.3	2.6±0.6	2.3±0.7	2.5±0.6
500	3.5±0.5	1.5±0.9	1.7±0.4	1.6±0.2
		Females		
T3 (ng/dL)				
0	78.1±10.0	89.7±18.2	71.3±13.3	124.0±106.2
5	70.0±8.0	72.0±9.4	64.8±13.9	67.3±28.9
50	74.7±6.6	85.8±21.1	68.4±14.5	72.9±17.3
500	88.0±17.3	95.6±14.9	63.1±15.3	94.5±4.6
T4 (µg/dL)				
0	2.5±0.8	3.1±0.5	2.5±0.5	2.5±0.6
5	2.6±0.6	2.7±0.6	2.3±0.6	2.6±1.2
50	2.3±0.5	2.4±0.7	2.4±0.6	2.2±0.6
500	2.4±0.9	2.8±0.3	2.1±0.4	2.7±0.4

Table 7. Mean Absolute Thyroid and Thyroid-to-Body Weight, and Thyroid-to-Brain Weight Ratios in Dogs Fed ETU for 1 Year

Dose (ppm)	Absolute Thyroid (g)	Thyroid-to-Body Weight Ratio (%)	Thyroid-to-Brain Weight Ratio
		Males	
0	0.812±0.148	0.0080±0.0009	0.0108±0.0023
5	0.713±0.183	0.0075±0.0023	0.0089±0.0023
50	0.868±0.147	0.0090±0.0005	0.0110±0.0021
500	3.000±1.283	0.0349±0.0172	0.0416±0.0213
		Females	
0	0.635±0.081	0.0067±0.0008	0.0084±0.0007
5	0.700±0.204	0.0071±0.0009	0.0093±0.0026
50	0.951±0.120	0.0100±0.0012	0.0125±0.0015
500	1.091±0.235	0.0132±0.0010	0.0161±0.0048

Table 8. Incidence of Histologic Findings in Liver and Thyroid of Dogs Fed ETU for 1 Year[a]

Organ/ Lesion	Dietary Level (ppm)							
	Males				Females			
	0	5	50	500	0	5	50	500
Thyroids	(4)	(4)	(4)	(2)	(4)	(4)	(4)	(3)
Hypertrophy of follicular cells	0	0	1	2	0	0	1	2
Dilatation/colloid in follicles	0	0	1	2	0	0	1	2
Increased number of follicles	0	0	1	0	0	0	1	1
Liver	(4)	(4)	(4)	(2)	(4)	(4)	(4)	(3)
Pigment accumulation	0	0	1	1	0	0	1	2
Hepatocyte hypertrophy	0	0	2	0	0	0	1	0

[a]Includes only the surviving dogs. The numbers in parentheses are the numbers of animals with tissues examined.