B

DATA EVALUATION RECORD

ETOFENPROX

Study Type: §82-1b, 90-Day Oral Toxicity Study in Mice

Work Assignment No. 3-02-126E (MRID 40449702)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
Rockville, MD 20850-3268

Primary Reviewer
Ronnie J. Bever Jr., Ph.D.

Signature: Ronnie J. Bever Jr
Date: 5/23/01

Secondary Reviewer
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 5/23/01

Program Manager
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 5/23/01

Quality Assurance
Steve Brecher, Ph.D.

Signature: Steven Brecher
Date: 5/24/01

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Waheeda Mani Tehseen, Ph. D.
Registration Action Branch 3 (7509C)
EPA Secondary Reviewer: Steve Dapson, Ph. D.
Registration Action Branch 3 (7509C)
TXR #: 0050128

Date 7/18/2001
Date 07/27/2001

DATA EVALUATION RECORD (DER)

This DER is an upgrade to previously written executive summary and DER (TXR no. 006852 and 014167).

STUDY TYPE: Subchronic Oral Toxicity in mice (feeding); OPPTS 870.3100 (rodent), §82-1

DP BARCODE: D239026
P.C. CODE: 128965

SUBMISSION CODE: S530012

TEST MATERIAL (PURITY): Ethofenprox, MTI-500, (96.3 %)

SYNONYMS: 2-(4-ethoxyphenyl)-2-methylpropyl-3-phenoxybenzylether

CITATION: Owen, P., et. al. (1986). Assessment of the toxicity of MTI-500 to mice by dietary administration for 13 weeks. Huntingdon Research Center Ltd., Huntingdon, Cambridgeshire, England. MTC 55/821112/2 April 2, 1986. MRID no. 40449702. Unpublished.

SPONSOR: Mitsui Toatsu Chemicals, Inc. 2-5 Kasumigaseki 3-chome, Chiyoda-Ku, Tokyo, Japan.

EXECUTIVE SUMMARY:

In a subchronic toxicity study (MRID 40449702), MTI-500 (96.3%) was administered to 20 CD-1 mice/sex/dose in the diet at dose levels of 0, 50, 500, 3000, and 15000 ppm (0, 6.1, 60, 375, and 1975 mg/kg/day males; 0, 6.9, 71, 390, and 2192 mg/kg/day females) for 90 days. Four animals were housed per cage.

One male each in the 50 ppm group and 3000 ppm group died from human error in blood withdrawal. One female and one male in the 15000 ppm group died spontaneously at weeks 6 and 9, respectively. Five females and one male of the 15000 ppm group were sacrificed for humane reasons during week 13. Piloerection (19/20 M, 20/20 F), hunched posture (7/20 M, 12/20 F), emaciated appearance (5/20 M, 10/20 F), anemic appearance (2/20 M, 5/20 F), lethargy

(10/20 F), body tremors (1/20 M, 4/20 F), unsteady gait (3/20 F), and respiratory distress (1/20 M, 4/20 F) were noted in males and females of the 15000 ppm group. Alopecia of the skin was observed in 8/18 males of the 15000 ppm group.

During the first 12 weeks, food consumption (-9% males and -8% females) and body weight gain (-70% males and -83% females) were significantly decreased at 15000 ppm.. During week 5 animals showed a significant decrease in blood glucose levels at 15000 ppm group. Additionally at week 12, males of the 15000 ppm group and females of the 3000 and 15000 ppm groups exhibited statistically decreased blood glucose. Significant increase in water consumption (51% - 98%) was observed during weeks 4, 7, and 10 at 15000 ppm.

Variable significant changes in blood parameters were observed during the treatment period. There were significant decreases in blood parameters (RBC, Hb, hematocrit, MCHC), but increases in WBC parameters (total WBC) at the highest dose level (15000 ppm) in both sexes. Decreases in blood parameters (RBC, Hb, hematocrit, MCHC) were also observed in males at 3000 ppm at week 6 and 13. Significant changes observed in other blood parameters (platelets, neutrophils, leukocytes, and monocytes) in both sexes at week 6 and/or 13 are ambiguous.

Substantial effects on the kidney were observed at 15000 ppm. These effects include: cortical scarring (17/18 M, 16/19 F), pale kidney (7/18 M, 12/19 F), enlarged kidney (3/18 M, 8/19 F); cysts (2/18 M, 1/19 F), misshapened kidney (2/19 F), and increase in relative kidney weights (54% M, 80% F). Histological findings were also observed in the kidney at the highest dose, which include widespread tubular basophilia (16/18 M, 16/19 F), extensive tubular dilatation (14/18 M, 16/19 F) and dilatation of renal pelvis (5/18 M, 5/19 F). Additionally, treatment related effects on the kidney were observed in clinical chemistry and urinalysis. At week 5, males of the 15000 and females of the 3000 and 15000 ppm group showed significant increased in blood urea nitrogen (BUN). At week 12 females of the 15000 group showed a significant increase in BUN. In males of the 15000 group at week 5 and females of the 15000 group at week 5 and 12, cholesterol was statistically increased. At week 5 and 12, globulin was significantly increased in both sexes at high dose. In females at weeks 6 and 13, and in males at week 13 of the 15000 ppm group, the urine specific gravity was statistically decreased. Blood protein in females of the 500, 3000, and 15000 ppm groups at week 5 and males of the 15000 ppm group, at week 12, was significantly increased.

Treatment-related increases in relative liver weights (15% M, 16% F) and corresponding centrilobular hepatocyte enlargement (12/18 M, 10/19 F, control 3/20 M, control 0/20 F) were observed in both sexes at the highest dose (15000 ppm).

At 15000 ppm animals showed small or not visible thymus glands (9/18 M, 9/19 F; Controls 1/20 M, 0/20 F). Histological findings were observed in the lymphoreticular system in males and females of at 15000 ppm. These findings included increased cellularity of splenic white pulp (9/18 M, 7/19 F, control 3/20 M, control 2/20 F) and reduced thymic cellularity (4/18 M, 2/19 F,

control 0/20 M and F). The incidence of reactive changes in lymph nodes, which apparently included lymphoid hyperplasia hystiocytosis, were increased in males of the 500 and 3000 ppm group (5/20 and 7/19, respectively) and also in males and females of the 15000 ppm (8/18 M, 5/19 F, control 2/20 M, control 1/20 F).

In conclusion, the highest dose (15000 ppm) clearly caused changes in the kidney, including scarring of the cortex, increased weight, widespread microscopic changes, increased BUN and occasionally hemoglobin in the urine. At this dose liver weight accompanied by centrilobular hepatocyte enlargement was increased. There were decreases in RBC, Hb, hematocrit, and MCHC, and increases in WBC. There was a persistent decrease in glucose and increase in cholesterol. The highest dose also affected thymus glands and lymph nodes.

The LOAEL is 3000 ppm (males, 375 mg/kg/day and females, 390 mg/kg/day) based on clinical effects (increased blood urea nitrogen) and effects on hematology (red blood cell count, hemoglobin, and hematocrit). The NOAEL is 500 ppm (males, 60 mg/kg/day and females, 71 mg/kg/day).

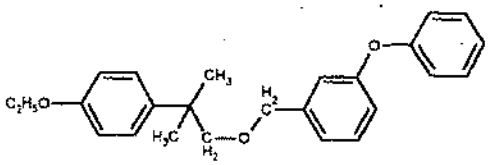
This subchronic toxicity study is classified **acceptable-guideline** and does satisfy the guideline requirement for a subchronic oral study (82-1) in mice.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. A Flagging statement was not provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Etofenprox
   Description: Brown crystalline solid
   Lot/Batch #: ST-101
   Purity: 96% a.i.
   Stability of compound: The compound was stable in the diet for up to 18 days at room temperature.
   CAS #: 80844-07-1
   Structure:



2. Vehicle: Diet

3. Test animals: Species: Mouse
Strain: CD-1 (Swiss)
Age and group mean weight at the start of dosing: 39 days old; 24-26 g males; 23-24 g females
Source: Charles River Laboratories, Manston, Kent, England
Housing: Polypropylene cage with sawdust bedding (animals/cage was illegible)
Diet: Spratt's Laboratory Animal Diet No. 2 (source not reported), *ad libitum*
Water: Tap water, *ad libitum*
Environmental conditions:
Temperature: 22°C
Humidity: 50%
Air changes: Not reported
Photoperiod: 12 hours light/12 hours dark
Acclimation period: 11 days

B. STUDY DESIGN

1. In life dates - Not reported.

2. Animal assignment - The animals were randomly assigned (stratified by weight) to the test groups shown in Table 1.

Table 1. Study design [a]

Nominal Dose (ppm)	Achieved Dose (mg/kg/day) [M/F]	# of Assigned Animals	
		Males	Females
0	0/0	20	20
50	6.1/6.9	20	20
500	60/71	20	20
3000	375/390	20	20
15,000	1975/2192	20	20

a Data obtained from the study report, pages 18 and 44.

3. Dose-selection rationale - Dose-selection was based upon a 4-week dietary study in mice performed at Huntingdon Research Center (HRC Report No. MTC/53/82542). Details of this 4-week study were not reported.

4. Treatment preparation and analysis - Diet formulations were prepared weekly. Etofenprox was melted at ≤40°C, weighed and dispersed in corn oil. This

suspension was added to the diet to form a premix with 2% corn oil by weight. The premix was blended with more diet to yield the desired concentrations. The control diet also contained 2% corn oil but without etofenprox. Concentration analyses were performed on all diet formulations at Weeks 1 and 13. Prior to the initiation of treatment, homogeneity (top, middle, bottom) and stability were determined for dietary formulations at 20 and 20,000 ppm. Stability of the test substance in the diet was determined for up to 18 days at room temperature.

Results - Homogeneity analysis (as % coefficient of variation, calculated by the reviewers): 1.8-1.9%

Stability analysis (range of means as % of day 0): 100-105%

Concentration analysis (range of means as % of nominal): 94.2-105.8%

The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the study animals was acceptable.

5. Statistics - Body weight gain, food and water consumption, and organ weight data were analyzed by using analysis of variance and Student's t-test. Clinical pathology parameters were analyzed using Bartlett's test, followed by analysis of variance when there was homogeneous group variance or Kruskal-Wallis analysis otherwise. Further analysis was performed using Student's t-test (or the non-parametric equivalent) and William's test. For those parameters in which the relative frequency of the mode was 75% or more, the number of animals in each group with values different from the mode was analyzed using Fisher's exact test and Mantel's test. Dose-related trends were detected using Mantel's test. Organ weights were analyzed using analysis of variance techniques.

C. METHODS

1. Observations - The animals were monitored for mortality and moribundity twice daily. Detailed clinical observations were noted daily (except on weekends) for the first four weeks and then weekly.

2. Body weight and body weight gains - Each animal was weighed prior to treatment, weekly throughout the study and at necropsy. Overall (Weeks 0-12) body weight gain (g) was calculated rather than overall (Weeks 0-13) body weight gain because the sacrifice of six females during Week 13 led to "a falsely elevated weight gain."

3. Food consumption/efficiency and compound intake - Food consumption was recorded weekly and reported as mean g/mouse/week. Total (Weeks 0-12) food

consumption (g/mouse) was calculated rather than total (Weeks 0-13) food consumption because the sacrifice of six females during Week 13 led to "a falsely elevated intake." Group mean food conversion ratios and test substance intake values (mg/kg/day) were calculated using the food consumption and body weight data. Food conversion ratios are calculated as weight of food consumed per unit gain in body weight; therefore, food efficiency decreases as food conversion ratios increase.

4. Water consumption - Water consumption (g) was recorded daily for Week 4 in the controls and 15,000 ppm groups and in all groups during Weeks 7 and 10.

5. Ophthalmoscopic examination - Ophthalmoscopic examinations were performed on all surviving animals in the control and 15,000 ppm groups prior to dosing and during Week 4 and 13.

6. Hematology - Blood was collected from the orbital sinus of 10 animals/sex/group at Weeks 6 and 13 for hematology analysis and at Weeks 5 and 12 for biochemistry analysis. The animals were anaesthetized with ether prior to blood collection. Where possible the same mice were used throughout the study. The checked (X) hematology and clinical blood chemistry parameters were examined

a. Hematology

X	Hematocrit (HCT)	X	Leukocyte differential count
X	Hemoglobin (HGB)	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)	X	Mean corpusc. HGB conc. (MCHC)
X	Erythrocyte count (RBC)	X	Mean corpusc. volume (MCV)
X	Platelet count	X	Reticulocyte count
	Blood clotting measurements		Methemoglobin
	(Thromboplastin time)		Large unstained cells
	(Activated partial thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

b. Clinical Chemistry

	ELECTROLYTES		OTHER
	Calcium	X	Albumin
	Chloride		Blood creatinine
	Magnesium	X	Blood urea nitrogen
	Potassium	X	Total Cholesterol
	Sodium	X	Globulin
	Phosphate	X	Glucose
	ENZYMES		Direct bilirubin
X	Alkaline phosphatase (ALK)		Total bilirubin
	Cholinesterase (ChE)	X	Total serum protein (TP)
	Creatine phosphokinase (CPK)		Albumin/globulin ratio (A/G)
	Lactic acid dehydrogenase (LDH)		Triglycerides
	Serum alanine aminotransferase (ALT)		Serum protein electrophores
	Serum aspartate aminotransferase (AST)		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		
X	Glutamic pyruvic transaminase		
X	Glutamic oxaloacetic transaminase		
	Gamma glutamyl transpeptidase (GTP)		

7. Urinalysis - Water and food were withheld while urine from 10 animals/sex/group was collected overnight at Weeks 6 and 13. The checked (X) parameters were examined.

	Appearance	X	Glucose
X	Volume	X	Ketones
X	Specific Gravity	X	Bilirubin
X	pH		Blood
X	Sediment		Nitrate
X	Protein	X	Urobilinogen
X	Reducing substance		Bacteria
X	Hemoglobin		

8. Sacrifice and Pathology - All surviving animals were subjected to a detailed necropsy following carbon dioxide asphyxiation. The following checked (X) tissues were collected and preserved in 10% formalin (except eyes, which were preserved in Davidson's fixative). The (XX) organs were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./ HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta	XX	Brain
X	Salivary glands	XX	Heart	X	Peripheral nerve
X	Esophagus	X	Bone marrow		Spinal cord
X	Stomach	X	Lymph nodes	XX	Pituitary
X	Duodenum	XX	Spleen	X	Eyes
X	Jejunum	XX	Thymus		
X	Ileum				GLANDULAR
X	Cecum		UROGENITAL	XX	Adrenal gland
X	Colon	XX	Kidneys		Lacrimal gland
	Rectum	X	Urinary bladder	X	Mammary gland, female
XX	Liver	XX	Testes	XX	Thyroids (with parathyroids)
X	Pancreas	X	Prostate		
		X	Seminal vesicle		OTHER
	RESPIRATORY	XX	Ovaries	X	Bone
X	Trachea	XX	Uterus	X	Skeletal muscle
X	Lungs		Vagina	X	Skin
	Pharynx		Oviducts	X	All gross lesions
	Larynx				Head
	Diaphragm				Harderian gland
				X	Gall bladder
					Choledochus

All samples except the aorta, cecum, liver, mammary gland, sciatic nerve, seminal vesicles, skin and tongue were embedded in paraffin, stained with hematoxylin and eosin, and evaluated microscopically. Liver was frozen (-70°C), cut on a cryostat (12μ) and stained with Oil Red O or periodic acid schiff reagent.

II. RESULTS

A. Observations

1. Mortality - Mortality data were not provided in tabular form or a figure. The following information was obtained from the narrative. Treatment-related increased mortality relative to the concurrent controls was observed at 15,000 ppm in both sexes (Table 2). One female died at Week 6, and one male died at Week 9. During Week 13, another male and 5 more females were sacrificed *in extremis*. Clinical signs of toxicity were observed before the animals died (or were terminated due to their deteriorating conditions). Macroscopic examination of these animals suggested renal toxicity. One male each in the 50 and 3000 ppm groups died at Week 12 following withdrawal of blood sample; however, no previous clinical signs had been observed in these animals. Therefore, these deaths were not considered treatment-related.

Table 2.Cumulative mortality (# dead/20) in mice dosed with etofenprox for up to 13 weeks.[a]

Study Week	Males					Females				
	Dose Group (ppm)									
	0	50	500	3000	15,000	0	50	500	3000	15,000
6	0	0	0	0	0	0	0	0	0	1
9	0	0	0	0	1	0	0	0	0	1
12	0	1	0	1	1	0	0	0	0	1
13	0	1	0	1	2	0	0	0	0	6

a Data obtained from the narrative of the study report, page 31.

2. Clinical signs - Clinical signs data are presented in Table 3. The narrative specified that clinical signs of a reaction to treatment were only seen at 15,000 ppm. The following observations (# affected/20 treated vs 0/20 controls) were made in the 15,000 ppm groups at Week 13: piloerection in males (19) and females (20), hunched posture in males (7) and females (12), emaciated appearance in males (5) and females (10), anemic appearance in males (2) and females (5), lethargy in females (10), body tremors in males (1) and females (4), unsteady gait in females (3) and respiratory distress in males (1) and females (4). Piloerection was observed from Week 1, hunched posture and emaciated appearance from Week 3, lethargy in females from Week 6, and other clinical signs were observed by Weeks 12 or 13. Other treatment groups were similar to the controls.

Table 3. Selected clinical signs (# affected) observed in mice fed etofenprox for up to 14 weeks.[a]

Clinical sign	Males		Females	
	Dose Group (ppm)			
	0	15,000	0	15,000
Piloerection	0	19	0	20
Hunched posture	0	7	0	12
Emaciated appearance	0	5	0	10
Anemic appearance	0	2	0	5
Lethargy	0	0	0	10
Body tremors	0	1	0	4
Unsteady gait	0	0	0	3
Respiratory distress	0	1	0	4

a) Data were obtained from the study report. Control response is provided in narrative while the 15,000 ppm treatment group response is tabulated, page 30; n=20.

B. Body weight and body weight gains - Standard deviation and p-values for mean body weights were not calculated by the Sponsor. Body weights in the 15,000 ppm treatment groups differed from the controls (↑8%-↓22%). Treatment-related decreases ($p<0.001$) in overall (Weeks 0-12) body weight gains were observed at 15,000 ppm in males (↓70%) and females (↓83%) (Table 4). Treatment-related decreases in body weights or body weight gains were not observed except in the 15,000 ppm groups. An incidental increase in overall (Weeks 0-12) body weight gain was observed in 50 ppm males (↑32%; $p<0.05$).

Table 4. Mean (± SD) body weights (g) at selected intervals and overall (Weeks 0-12) body weight gains (g) in mice fed etofenprox for up to 13 weeks.[a]

Study Week	Dose Group (ppm)				
	0	50	500	3000	15,000
Males					
0	24	25	25	24	26
7	34	38	37	36	30
13	37	41	40	38	29
Overall (Weeks 0-12) body weight gain	12.2±5.19	16.1*±3.70(↑32)	13.5±3.61	13.3±4.18	3.7***±6.12(↓70)
Females					
0	23	23	23	24	24
7	28	30	29	29	25
13	30	31	30	31	27
Overall (Weeks 0-12) body weight gain	6.4±2.37	7.9±2.95	7.3±3.88	7.9±3.00	1.1***±5.91 (↓83)

a These data were obtained from this study report, pages 32 and 43. Numbers listed parenthetically represent the percent difference from controls (calculated by reviewers); n=14-20. Standard deviations were only reported for body weight gain data by the Sponsor.
* or *** Significantly different from controls at $p<0.05$ or 0.001, respectively.

C. Food and water consumption, compound intake and food efficiency:

1. Food consumption - Standard deviation and p-values for food consumption (except totals) were not calculated by the Sponsor. A treatment-related decrease ($p<0.05$ or 0.01) in total (Weeks 0-12) food consumption was observed at 15,000 ppm in males (↓9%) and females (↓8%) (Table 5). Increased ($p<0.05$ or 0.01) total (Weeks 0-12) food consumption observed in 50 ppm males (↑11%), 500 ppm males (↑7%) and 500 ppm females (↑11%) was not considered treatment-related.

Table 5. Mean (± SD) food consumption (g/mouse/week) at selected intervals and total (Weeks 0-12) food consumption (g) in mice fed etofenprox for up to 13 weeks.[a]

Study Week	Dose Group (ppm)				
	0	50	500	3000	15,000
Males					
1	29	33	33	28	22
7	26	31	31	30	27
13	29	30	30	30	29
Total (Weeks 0-12)	340±20.2	376**±7.4(↑11)	364*±14.6(↑7)	354±17.5	310**±16.7(↓9)
Females					
1	27	28	28	25	21
7	26	29	29	26	24
13	28	28	27	26	33
Total (Weeks 0-12)	318±5.0	331±22.1	343*±22.5(↑11)	315±7.6	292*±12.6 (↓8)

a) These data were obtained from pages 31 and 42. Numbers listed parenthetically represent the percent difference from controls (calculated by reviewers); n=14-20. Standard deviations were only reported for total food consumption data by the Sponsor. * or ** = Significantly different from controls at $p<0.05$ or 0.01, respectively.

2. Water consumption - Water consumption data are presented in Table 6. A treatment-related increase ($p<0.05$ or 0.001) in water consumption was observed at 15,000 ppm in males at Weeks 4 (↑68%), 7 (↑98%) and 10 (↑66%), and in females at Weeks 4 (↑68%), 7 (↑60%) and 10 (↑51%). Increased water consumption was also observed in the males at Weeks 7 at 500 (↑11%) and 3000 (↑18%) ppm.

Table 6. Mean (± SD) water consumption (ml/mouse/day) at selected intervals in mice fed etofenprox for up to 13 weeks.[a]

Study Week	0 ppm	50 ppm	500 ppm	3000 ppm	15,000 ppm
0 ppm	50 ppm	500 ppm	3000 ppm	15,000 ppm	
4	6.0±1.75	NM [b]	NM	NM	10.1***±2.23 (↑68)
7	5.7±1.38	5.6±0.88	6.3*±0.86 (↑11)	6.7***±1.04 (↑18)	11.3***±1.52 (↑98)
10	7.3±2.82	6.0±1.48	6.0±1.67	7.1±2.52	12.1***±1.97 (↑66)
Females					
4	5.5±1.12	NM	NM	NM	10.1***±1.24 (↑84)
7	6.3±1.61	6.9±2.79	6.5±1.42	6.5±1.87	10.1***±2.93 (↑60)
10	6.3±2.10	5.9±2.28	6.7±3.07	5.6±2.09	9.5***2.79 (↑51)

a) These data were obtained from pages 45-47. Numbers listed parenthetically represent the % difference from controls; n=14-20. b) Not measured. *or*** = Sig. different from controls at $p<0.05$ or 0.001, respectively.

3. Compound consumption - The mean achieved dosages are shown in Table 1.

4. Food efficiency - Statistical analysis for food efficiency was not reported. Food conversion ratios are calculated as weight of food consumed per unit gain in body weight; therefore, food efficiency decreases as food conversion ratios increase. A treatment-related increase in overall (Weeks 1-12) food conversion ratios was observed at 15,000 ppm in males (↑173%) and females (↑539%) (Table 7). Hence, food efficiency was significantly decreased in both sexes at 15,000 ppm (0-12 weeks).

Table 7. Food conversion ratios at selected intervals and overall (Weeks 1-12) in mice fed etofenprox for up to 13 weeks.[a]

Study Weeks	Males					Females				
	Dose Group (ppm)									
	0	50	500	3000	15,000	0	50	500	3000	15,000
1-4	14.8	13.0	12.6	15.0	NC[b]	26.2	22.6	22.8	35.7	NC
5-8	107.0	40.7	40.3	38.3	34.7	103.0	112.0	56.5	52.0	NC
9-12	38.7	42.0	118.0	40.0	105.0	56.0	53.0	115.0	35.0	102.0
Overall (Weeks 1-12)	28.4	23.6	26.1	25.4	77.5 (↑173)	45.7	41.4	42.8	39.5	292.0 (↑539)

a) These data were obtained from this study report, page 33. Numbers listed parenthetically represent the percent difference from controls (calculated by reviewers); n=19-20. Food conversion ratios are calculated as weight of food consumed per unit gain in body weight; therefore, food efficiency decreases as food conversion ratios increase.
b) Not calculated because there was no body weight gain.

D. Ophthalmoscopic examination - No abnormalities were observed.

E. Blood analyses

1. Hematology - Hematology data are presented in Tables 8a and 8b. Hemoglobin and red blood cell counts were statistically decreased in males of all dose groups at week 6. Hematocrit and red blood cell counts were statistically decreased in females of the 15000 ppm group at week 6. Additionally at week 6, MCHC was statistically decreased in males of the 3000 and 15000 ppm groups while MCV was statistically increased in males of the 500, 3000, and 15000 ppm groups. At week 13, hematocrit, hemoglobin, and red blood cells were statistically decreased in males of the 3000 and 15000 ppm groups and in females of the 15000 ppm group. Number of platelets were statistically increased in females of all dose groups at week 6. Platelets were statistically decreased in males and females of the 500, 3000, and 15000 ppm groups at week 13. Total white blood cells were

statistically increased in males and females of the 15000 ppm group at week 6. Neutrophils and leukocytes were statistically increased in females of the 15000 ppm group at week 6. At week 13, total white blood cell counts were statistically increased in the females of the 15000 ppm group along with increases in neutrophils in females of all dose groups. At week 13, neutrophils were statistically decreased in males of the 3000 and 15000 ppm groups while monocytes were statistically decreased in males of the 500, 3000, and 15000 ppm. In conclusion, significant decreases in RBC, Hb, hematocrit, MCHC at mid and high dose in males and/or females and increases in total WBC in both sexes at the high dose were considered treatment-related. Significant changes observed in other blood parameters (platelets, neutrophils, leukocytes, and monocytes) in both sexes at week 6 and/or 13 are ambiguous.

Table 8a. Selected hematological parameters (mean ± SD) in mice fed etofenprox for 6 weeks.[a]

Parameter	Dose (ppm)				
	0	50	500	3000	15,000
Males					
Hemoglobin (g/dl)	14.0±0.53	13.2±0.64* (16)	13.2±0.72* (16)	12.8±0.63** (19)	13.1±1.06** (16)
Erythrocytes ($x10^6/mm^3$)	6.6±0.24	6.1±0.38** (18)	5.9±0.30** (111)	5.9±0.37** (111)	6.2±0.60** (16)
MCHC (pg)	30.5±0.78	30.4±0.84	29.8±0.93	29.3±0.82* (14)	29.9±1.34* (12)
MCV (μg)	69±3.3	72±3.8	75±4.4* (19)	75±2.9* (19)	72±6.9* (14)
WBC, total ($x10^3/mm^3$)	3.5±0.62	4.3±0.70	3.7±0.95	3.9±0.89	3.5±2.18** (157)
Lymphocytes ($x10^3/mm^3$)	3.16±0.57	3.92±0.68	3.44±0.93	3.55±0.84	5.00±2.14** (158)
Females					
Packed cell volume (%)	44±1.9	45±2.8	45±4.1	45±2.9	42±2.8* (15)
WBC, total ($x10^3/mm^3$)	3.2±1.18	4.9±1.61	4.2±2.05	4.3±1.54	6.3±2.09** (197)
Neutrophils ($x10^3/mm^3$)	0.15±0.10	0.34±0.37	0.25±0.13	0.21±0.18	0.42±0.21** (1180)
Lymphocytes ($x10^3/mm^3$)	3.00±1.14	4.57±1.34	3.92±1.94	4.03±1.43	5.85±2.01** (195)
Platelets ($x10^3/mm^3$)	871±52.1	933±66.3	994±90.2* (114)	933±101.8* (17)	953±54.2* (19)

a) Data were extracted from the study report, pages 48 and 49; n =19-20. Percent difference from control is listed parenthetically.

* or ** Significantly different from controls at $p<0.05$ or 0.01, respectively.

Table 8b. Selected hematological parameters (mean ± SD) in mice fed etofenprox for 13 weeks.[a]

Study Week	Dose (ppm)				
	0	50	500	3000	15,000
Males					
Packed cell volume (%)	47±2.5	47±2.8	46±2.0	44±1.8* (↓6)	43±2.6** (↓9)
Hemoglobin (g/dl)	14.1±0.61	14.0±0.73	14.4±0.72	13.3±0.72* (↓6)	12.6±0.60** (↓11)
Erythrocytes ($x10^6/mm^3$)	7.7±0.53	7.5±0.45	7.6±0.55	7.2±0.34* (↓6)	7.0±0.42** (↓9)
MCHC (pg)	30.4±1.00	30.1±0.93	31.3±0.96	30.2±1.08	29.3±0.90* (↓4)
Neutrophils ($x10^3/mm^3$)	1.06±0.68	0.72±0.42	0.87±1.10	0.24±0.14** (↓77)	0.49±0.40** (↓54)
Eosinophils ($x10^3/mm^3$)	0.07±0.08	0.05±0.06	0.04±0.05	0.01±0.02	0.01±0.02* (↓86)
Monocytes ($x10^3/mm^3$)	0.35±0.30	0.28±0.16	0.05±0.06** (↓86)	0.02±0.03** (↓94)	0.03±0.05** (↓91)
Plalelets ($x10^3/mm^3$)	1214±229	1213±121	1015±70.3** (↓16)	964±109** (↓21)	1120±76.0** (↓8)
Females					
Packed cell volume (%)	44±2.3	44±2.7	46±2.2	43±2.4	39±4.1** (↓11)
Hemoglobin (g/dl)	14.0±0.57	14.0±1.71	15.0±0.50	14.0±0.65	12.4±1.55* (↓11)
Erythrocytes ($x10^6/mm^3$)	7.5±0.39	7.6±0.61	7.8±0.20	7.4±0.52	6.8±0.65** (↓9)
WBC, total ($x10^3/mm^3$)	3.1±1.09	4.9±2.20	1.5±1.94	3.4±0.78	5.1±1.49** (↓65)
Neutrophils ($x10^3/mm^3$)	0.06±0.05	0.24±0.16** (↓300)	0.29±0.21** (↓383)	0.28±0.16** (↓367)	0.62±0.36** (↓933)
Lymphocytes ($x10^3/mm^3$)	3.03±1.09	4.63±2.12	4.14±1.76	3.06±0.72	4.45±1.27* (↓47)
Platelets ($x10^3/mm^3$)	890±87.8	864±65.9	789±71.3* (↓11)	732±107.4* (↓18)	829±133.2* (↓7)

a) Data were extracted from the study report, pages 50 and 51; n =14-20. Percent difference from control is listed parenthetically.

* or ** Significantly different from controls at $p<0.05$ or 0.01, respectively.

2. Clinical chemistry - Clinical chemistry data are presented in Tables 9a and 9b. During week 5 animals showed a significant decrease in blood glucose levels at 15000 ppm group. Additionally at week 12, males of the 15000 ppm group and females of the 3000 and 15000 ppm groups exhibited statistically decreased blood glucose. At week 5, males of the 15000 and females of the 3000 and 15000 ppm group showed significant increased in blood urea nitrogen (BUN). At week 12 females of the 15000 group showed a significant increase in BUN. In males of the 15000 group at week 5 and females of the 15000 group at week 5 and 12, cholesterol was statistically increased. In females at weeks 6 and 13, and in males at week 13 of the 15000 ppm group, the urine specific gravity was statistically decreased. At week 5 and 12, globulin was significantly increased in both sexes at high dose. Blood protein in females of the 500, 3000, and 15000 ppm groups at week 5 and males of the 15000 ppm group, at week 12, was significantly increased.

Table 9b. Selected clinical chemistry findings (mean ± SD) in mice fed etofenprox for 12 weeks.[a]

Parameter	Dose (ppm)				
	0	50	500	3000	15,000
Males					
Glucose (mg/dl)	185±16.5	196±35.4	183±16.4	228±59.8	140±25.4* (124)
Total Protein(g/dl)	4.8±0.27	5.0±0.24	5.0±0.32	5.0±0.25	5.3±0.33**(110)
Globulin (g/dl)	1.5±0.31	1.5±0.31	1.6±0.32	I.Π. [b]±0.20	2.0±0.25** (133)
Females					
Glucose (mg/dl)	174±15.9	169±27.8	174±21.0	147±10.0** (116)	118±36.3** (132)
Total Protein (g/dl)	5.0±0.10	4.7±0.34* (16)	4.7±0.21* (16)	4.6±0.09*(18)	5.1±0.79*(12)
Globulin (g/dl)	2.0±0.28	1.5±0.43** (125)	1.6±0.15** (120)	1.3±0.25** (135)	1.6±0.81** (120)
BUN (mg/dl)	19±3.2	25±3.6	26±5.7	21±4.2	35±11.7** (184)
Cholesterol (mg/dl)	67±13.9	65±13.5	65±21.2	66±13.9	117±35.0** (175)

a) Data were extracted from the study report, pages 54 and 55; n=19-20. Percent difference from control is listed parenthetically.
b) Illegible.
* or ** Significantly different from controls at $p<0.05$ or 0.01, respectively.

F. Urinalysis - Changes in urine parameters indicating effects on the kidney were observed at 15000 ppm. The following decreases ($p<0.05$ or 0.01) were observed: (i) specific gravity at Week 6 in the 15,000 ppm females (↓2%) and at Week 13 in the 15,000 ppm males (↓2%) and females (↓2%); (ii) pH at Week 6 in all female treatment groups (↓3-8%) and at Week 13 in females at 3000 (↓6%) and 15,000 (↓5%) ppm and in all male treatment groups (↓8-10%); and (iii) protein at Week 6 in males at 3000 (↑33%) and 15,000 (↑31%) ppm and in the 15,000 ppm females (↑91%), and at Week 13 in the 15,000 ppm males (↑78%).

G. Sacrifice and Pathology:

1. Organ weight - Organ weight data are presented in Table 10. Terminal body weights were decreased ($p<0.001$) at 15,000 ppm in males (↓24%) and females (↓24%). Significant treatment-related changes in liver, kidney and thymus were observed at the highest dose. Treatment related changes ($p<0.05$ or 0.001) were observed at 15,000 ppm in the following organ weights: (i) liver, decreased

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absolute in males (↓12%), and increased relative (to body) in males (↑15%) and females (↑16%); (ii) kidney, increased absolute in males (↑12%) and females (↑25%), and increased relative in males (↑54%) and females (↑80%); and (iii) thymus, decreased absolute in females (↓32%). The following differences ($p<0.05$ or 0.01 or 0.001) from the concurrent controls were also observed; however, the differences were minor and were not substantiated by other evidence of toxic effect: (i) brain, decreased absolute in males (↓5%) and females (↓11%); (ii) heart, decreased absolute in males (↓15%) and females (↓20%); and (iii) gonads, decreased absolute in males (↓11%) and females (↓26%). Incidental increases ($p<0.05$ or 0.001) were observed in absolute pituitary weight of females at 500 (↑67%) and 3000 (↑33%) ppm and in absolute ovary weight at 500 (↓37%) ppm.

Table 10. Selected absolute and relative to body (g) organ weights (mean ± SD) in mice fed etofenprox for up to 13 weeks.[a]

Organ		Dose (ppm)				
		0	50	500	3000	15,000
Males						
Terminal Body Weight		38±4.1	40±3.6	39±2.9	38±3.0	29±5.8*** (↓24)
Liver	absolute	2.35±0.44	2.35±0.26	2.31±0.28	2.33±0.22	2.07±0.52* (↓12)
	relative[b]	612	585	589	612	702*** (↑15)
Kidneys	absolute	0.675±0.10	0.714±0.11	0.663±0.12	0.654±0.10	0.753±0.15* (↑12)
	relative	177	177	169	172	272*** (↑54)
Females						
Terminal Body Weight		29±2.1	29±3.4	29±3.5	29±2.8	22±6.7*** (↓24)
Liver	absolute	1.75±0.27	1.68±0.28	1.67±0.21	1.83±0.28	1.61±0.63
	relative	605	572	584	626	704*** (↑16)
Kidneys	absolute	0.463±0.05	0.433±0.05	0.461±0.06	0.428±0.05	0.577±0.139*** (↑25)
	relative	161	149	161	147	290*** (↑80)
Thymus[c]	absolute	0.037±0.14	0.040±0.018	0.036±0.014	0.039±0.013	0.025±0.02* (↓32)

a Data were obtained from the study report, pages 62-64; n=18-20. Percent change from controls is presented parenthetically.

b Standard deviations were not reported for relative organ weights.

c Relative weights were not reported.

* or *** Significantly different from controls $p<0.05$ or 0.001, respectively.

2. Gross pathology - The incidences of selected macroscopic lesions are presented in Table 11. Substantial effects on gross pathology of kidney and thymus were observed at 15,000 ppm. These effects include: cortical scarring, cysts, pale, enlarged and misshapened kidney and small or not visible thymus. The incidence

of other macroscopic lesions were similar to the concurrent controls for all treatment groups.

Table 11. Incidence (%) of selected macroscopic lesions in mice fed etofenprox for up to 13 weeks.[a]

Observation	Dose (ppm)				
	0	50	500	3000	15,000
Males (n=18-20)					
Kidney					
Cortical scarring	0	0	0	0	94
Pale	0	0	0	0	39
Enlarged	0	0	0	0	17
Cysts	0	0	0	0	11
Thymus, small/not visible	5	5	0	0	50
Skin, alopecia	0	11	10	5	22
Adipose tissue, minimal	0	0	0	0	22
Females (n=19-20)					
Kidney					
Cortical scarring	0	0	0	0	84
Pale	0	0	0	0	63
Enlarged	0	0	0	0	42
Misshapen	0	0	0	0	11
Thymus, small/not visible	0	0	5	0	47
Adipose tissue, minimal	0	0	5	0	53

a Data were obtained from the study report, pages 60 and 61.

3. Microscopic pathology - The incidences of selected microscopic lesions are presented in Table 12. Treatment-related histopathological findings were observed in kidney, liver and lymph nodes at the highest dose. This includes increased incidences of widespread tubular basophilia, tubular dilatation in the kidney, dilation of the renal pelvis, centrilobular hepatocyte enlargement, increased cellularity of splenic white pulp, reactive changes in lymph nodes in males and reduced thymic cellularity. All other incidences of abnormalities were similar to controls.

Table 12. Incidence (%) of selected microscopic lesions in mice fed etofenprox for up to 13 weeks.[a]

Observation	Dose (ppm)				
	0	50	500	3000	15,000
Males (n=18-20)					
Kidney					
Widespread tubular basophilia	0	0	0	0	89
Extensive tubular dilatation	0	0	0	0	78
Dilatation of the renal pelvis	0	0	5	0	28
Liver, centrilobular hepatocyte enlargement	15	11	5	11	67
Lymphoreticular system					
Increased cellularity of splenic white pulp	15	15	0	5	50
Reactive changes in lymph nodes	10	5	25	37	44
Reduced thymic cellularity	0	0	0	0	22
Females (n=19-20)					
Kidney					
Widespread tubular basophilia	0	0	0	0	84
Extensive tubular dilatation	0	0	0	0	84
Dilatation of the renal pelvis	0	0	0	0	26
Liver, centrilobular hepatocyte enlargement	0	0	0	0	53
Lymphoreticular system					
Increased cellularity of splenic white pulp	10	10	0	10	37
Reactive changes in lymph nodes	5	5	5	5	26
Reduced thymic cellularity	0	0	5	0	11

a Data were obtained from the study report, page 37.

III. DISCUSSION

A. Investigator's conclusions - Mortality, clinical signs, body weight gain, food and water consumption, food efficiency, hematology, blood chemistry, urinalyses, organ weights, gross pathology and histology differed from the concurrent controls in the 15,000 ppm treatment groups. Minimal changes were noted in the 500 and 3000 ppm treatment

groups, and these changes were considered to be of no toxicological significance.

B. Reviewer's discussion/conclusions - In this subchronic oral study (MRID 40449702), etofenprox was administered in the diet for 13 weeks to 20 CD-1 mice/sex/dose at nominal doses of 0, 50, 500, 3000 and 15,000 ppm (equivalent to 0, 6.1, 60, 375 and 1975 mg/kg/day in males and 0, 6.9, 71, 390 and 2192 mg/kg/day in females). The analytical data indicated that the variation between nominal and actual dosages to the study animals was acceptable.

Ophthalmoscopy results were unaffected by the test substance.

At 15,000 ppm, there were several indications of general toxicity. Mortality increased in males (10% treated vs 0% controls) and females (30% treated vs 0% controls). Several clinical signs were observed (# affected/20 treated vs 0/20 controls) at Week 13 as follows: piloerection in males (19) and females (20), hunched posture in males (7) and females (12), emaciated appearance in males (5) and females (10), anemic appearance in males (2) and females (5), lethargy in females (10), body tremors in males (1) and females (4), unsteady gait in females (3) and respiratory distress in males (1) and females (4). Total (Weeks 0-12) food consumption decreased ($p<0.05$ or 0.01) in males (↓9%) and females (↓8%), while overall (Weeks 0-12) body weight gains decreased ($p<0.001$) even more in males (↓70%) and females (↓83%). Thus, overall (Weeks 1-12) food conversion ratios were increased in males (↑173%) and females (↑539%). Additionally, minimal adipose tissue was found in males (22% treated vs 0% controls) and females (53% treated vs 0% controls). Water consumption was increased ($p<0.05$ or 0.001) in males at Weeks 4 (↑68%), 7 (↑98%) and 10 (↑66%), and in females at Weeks 4 (↑84%), 7 (↑60%) and 10 (↑51%). Alopecia was observed in males (22% treated vs 0% controls).

The kidney was a target organ at 15,000 ppm. Blood-urea-nitrogen was increased ($p<0.01$) in females at Weeks 5 (↑111%) and 12 (↑84%). Kidney weights were increased ($p<0.05$ or 0.01), both absolute in males (↑12%) and females (↑25%) and relative in males (↑54%) and females (↑80%). After 13 weeks of dosing, there were increased incidences (% treated vs 0% controls; n=18-20) of the following macroscopic lesions: (i) cortical scarring in the kidney of males (94%) and females (84%); (ii) pale kidneys in males (39%) and females (63%); (iii) enlarged kidneys in males (17%) and females (42%); (iv) cysts in kidneys in males (11%); and (v) misshapen kidney in females (11%). After 13 weeks of dosing, there were increased incidences (% treated vs 0% controls; n=18-20) of the following microscopic lesions: (i) widespread tubular basophilia in kidneys in males (89%) and females (84%); (ii) extensive tubular dilatation in the kidney in males (78%) and females (84%); and (iii) dilation of the renal pelvis in males (28%) and females (26%).

At 15,000 ppm, there was also evidence of an impact on the immune system. Differences ($p<0.05$ or 0.01) from the concurrent controls were observed in the differential leukocyte

count of the treatment groups as follows: (i) increased leukocytes in females at Weeks 6 (↑97%) and 13 (↑65%); (ii) increased neutrophils in females at Weeks 6 (↑180%) and 13 (↑933%); (iii) decreased neutrophils in males at Week 13 (↓54%); and (iv) increased lymphocytes in females at Weeks 6 (95%) and 13 (↑47%). The absolute thymus weight decreased in females (↓32%), and small thymuses were observed in males (50% treated vs 5% controls) and females (47% treated vs 0% controls). Microscopic lesions were observed (% treated vs % controls) as increased cellularity of splenic white pulp in males (50% vs 15%) and females (37% vs 10%), reactive changes in lymph nodes in males (44% vs 10%) and females (26% vs 5%), and reduced thymic cellularity in males (22%) and females (11%).

The mice were slightly anemic at 15,000 ppm. An anemic appearance was observed in males (10% treated vs 0% controls) and females (25% treated vs 0% controls). Additionally, treatment-related decreases ($p<0.05$ or 0.01) were observed in the following hematological parameters: (i) packed cell volume at Week 6 in females (↓5%) and at Week 13 in males (↓9%) ppm and in females (↓11%) ppm; (ii) hemoglobin at Weeks 6 in males (↓6%) and at Week 13 in males (↓11%) and females (↓11%); (iii) erythrocytes at Week 6 in males (↓6%) and at Week 13 in males (↓9%) and females (↓9%); and (iv) MCHC in males at Weeks 6 (↓2%) and 13 (↓4%).

There was limited evidence of hepatotoxicity at 15,000 ppm. The following blood chemistry parameters differed ($p<0.05$ or 0.01) from concurrent controls: (i) decreased glucose at Week 5 in males (↓15%) and females (↓26%), and at Week 12 in males (↓24%) and females (↓32%); (ii) increased globulin in males at Weeks 5 (↑24%) and 12 (↑33%); and (iii) increased cholesterol in females at Weeks 5 (↑41%) and 12 (↑75%). The specificity of these blood chemistry parameters as indicators of hepatotoxicity is poor. For instance, the decrease in glucose could also have been due to the decreased food consumption and food efficiency. Additionally, absolute liver weight decreased ($p<0.05$) in males (↓12%), but relative (to body) liver weight increased ($p<0.001$) in males (↑15%) and females (↑16%).

Some of the same indicators of toxicity were observed in the 3000 ppm males as follows: (i) reactive changes in lymph nodes (37% vs 10% controls); (ii) decreased packed cell volume at Week 13 (↓6%; $p<0.05$); (iii) decreased ($p<0.05$ or 0.01) hemoglobin at Weeks 6 (↓9%) and 13 (↓6%); (iii) decreased ($p<0.05$ or 0.01) erythrocytes at Weeks 6 (↓11%) and 13 (↓6%); and (iv) decreased neutrophils at Week 13 (↓77%; $p<0.01$). In the 3000 ppm females, increased neutrophils (↑367%; $p<0.01$) ppm were observed at Week 13, and blood glucose levels decreased (↓16%; $p<0.01$) at Week 12. Finally, there were some abnormalities observed at 50 and 500 ppm as follows: (i) reactive changes were observed in lymph nodes in males at 500 ppm (25% treated vs 10% controls); (ii) hemoglobin decreased ($p<0.05$) in males at 50 (↓6%) and 500 (↓6%) ppm at Week 6; and (iii) increased ($p<0.01$) neutrophils in females at 50 (↑300%) and 500 (↑383%) ppm at Week

13.

The LOAEL is 3000 ppm (equivalent to 375 mg/kg/day in males and 390 mg/kg/day in females) based on clinical effects (increased blood urea nitrogen) and effects on hematology (red blood cell count, hemoglobin and packed cell volume). The NOAEL is 500 ppm (equivalent to 60 mg/kg/day in males and 71 mg/kg/day in females).

C. EPA Reviewer's Conclusions - In conclusion, the highest dose (15000 ppm) clearly caused changes in the kidney, including scarring of the cortex, increased weight, widespread microscopic changes, increased BUN and occasionally hemoglobin in the urine. At this dose liver weight accompanied by centrilobular hepatocyte enlargement was increased. There were decreases in RBC, Hb, hematocrit, and MCHC, and increases in WBC. There was a persistent decrease in glucose and increase in cholesterol. The highest dose also affected thymus glands and lymph nodes.

The submitted study is classified as acceptable/guideline (§82-1b) and satisfies the requirements for a subchronic oral toxicity study in mouse.

D. Study deficiencies - Several deficiencies were noted, but do not change the conclusions of this review:

- Tabulated mortality and clinical observation data for all doses were not provided.
- Statistical analysis of body weight data was not reported.
- Only the histology data which the Sponsor felt was treatment-related were presented in a summary table.

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