DATA EVALUATION RECORD

MESOTRIONE (ZA1296) 16/12/2000

Study Type: §83-1(b), One Year Chronic Toxicity Study in Dogs

Work Assignment No. 2-01-52W (MRID 44505027)

Prepared for
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U.S. Environmental Protection Agency
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DATA EVALUATION RECORD

STUDY TYPE: One Year Chronic Toxicity - dog
OPPTS Number: 870.4100 OPP Guideline Number: §83-1b

DP BARCODE: D259369 SUBMISSION CODE: S541375
P.C. CODE: 122990 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Mesotrione (97.6% a.i.)

SYNONYMS: ZA1296; 2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione; 2-(4-mesyl-2-nitrobenzoyl)-cyclohexane-1,3-dione

CITATION: Brammer, A. (1997) ZA1296: One Year Oral Toxicity Study in Dogs. Central Toxicology Laboratory, Cheshire, UK. Laboratory Report No. CTL/P/5511, November 6, 1997. MRID 44505027. Unpublished.

SPONSOR: Zeneca Ag Products, Wilmington, DE

EXECUTIVE SUMMARY: In this chronic oral toxicity study (MRID 44505027), mesotrione (ZA1296; 97.6% a.i., Lot/batch # P22) was administered via gelatin capsule for 1 year to 4 Beagle dogs/sex/dose at concentrations of 0, 10, 100, or 600 mg/kg/day. Body weights, body weight gain, food consumption, hematology, clinical chemistry and urinalysis parameters, and organ weights were not adversely affected by the test substance.

At 10, 100, and 600 mg/kg/day, plasma tyrosine was dose-dependently increased ($p \leq 0.01$) relative to controls (males-↑168, 708, and 915%, respectively; females-↑151, 1213, and 1470%, respectively). During the clinical observations, cloudy eyes were observed in a single high-dose male (1/4). At the ophthalmological examination, lenticular opacities were noted in one high-dose male and one high-dose female. On gross pathology, one high-dose male had corneal and lenticular opacities with ocular discharge with a corresponding keratitis seen on microscopic evaluation. One high-dose female also had a corneal opacity with corneal erosion seen microscopically. A different high-dose male had red spots in the eye corresponding to peri-orbital hemorrhage. The corneal and lenticular effects are considered treatment related. No ocular effects were noted at the low and mid-doses. Interdigital cysts were observed in the mid- (males-2/4, females-1/4) and high- (males-2/4, females-3/4) dose animals and in the low-dose males (1/4) and control females (1/4). There also appeared to be an increase in dry skin sores in

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treated animals, especially males. These effects corresponded with an increase in staining of the hair, thorax, limbs, and paws, and green-colored urine as the dose increased. The skin effects were most likely due to the irritant properties of the phenolic acid metabolites in the urine that contacted the skin and may not be toxicologically relevant. Dermatitis/folliculitis was noted in the histopathology report in areas where dry sores or interdigital cysts occurred. Other areas that were grossly normal showed no abnormalities. Minimal to moderate erythrophagocytosis in the mesenteric lymph nodes was observed during the histopathological examination in the 10 (3/4), 100 (2/4), and 600 (2/4) mg/kg/day females and in the 100 and 600 mg/kg/day males (1/4 and 2/4, respectively). This effect was considered treatment related.

At 600 mg/kg/day, one female was humanely killed during week 47 due to adverse clinical signs including convulsions, hypothermia, a slow, weak pulse, and rapid weight loss. Pathological examination of this animal indicated generalized lymphocytolysis. This death was considered treatment related.

The LOAEL for this study is 10 mg/kg/day based on evidence of tyrosinemia in both sexes and increased incidence of erythrophagocytosis in the mesenteric lymph nodes of females.

No NOAEL was determined for this study.

The submitted study is classified as **acceptable/guideline (§83-1b)** and satisfies the requirements for a chronic oral toxicity study in dogs.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

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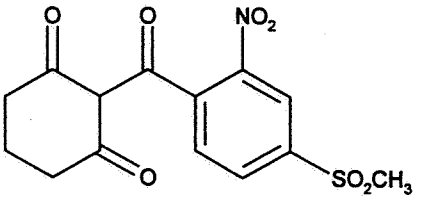
I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Mesotrione (ZA1296)
   Description: Light beige solid
   Lot/Batch #: P22
   Purity (w/w): 97.6% a.i.
   Stability of compound: The test substance was used prior to the expiration date and stability was confirmed after the in-life phase of the study. It is stable for at least 1½ years when stored at ambient temperatures in the dark.
   CAS #: 104206-82-8
   Structure:



2. Vehicle: Gelatin capsules (TORPAC Inc., East Hanover, NJ, USA)

3. Test animals: Species: Dog
   Strain: Beagle
   Age at start of dosing and range of mean weights at week 1: Approximately 28-29 weeks old; 10.18-10.53 kg (males), 8.35-8.65 kg (females)
   Source: Animal Breeding Unit, Zeneca Pharmaceuticals, Alderley Park
   Housing: Housed by treatment level in indoor pens with interlinking gates, allowing the dogs to be separated for feeding and dosing.
   Diet: Laboratory Diet A (Special Diet Services, Ltd., Essex, UK), 350-400 g/day
   Water: Tap water, ad libitum
   Environmental conditions:
   Temperature: 19±2° C
   Humidity: 45-65%
   Air changes: Approximately 15/hour
   Photoperiod: 12 hours light/12 hours dark
   Acclimation period: Approximately 5 weeks

B. STUDY DESIGN:

1. In life dates: Start: 3/19/96 End: 3/21/97

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

Table 1. Study design [a]

Test Group	Dietary Concentration (mg/kg/day)	Males	Females
Control	0	4	4
Low	10	4	4
Mid	100	4	4
High	600	4	4

a Data obtained from the study report, page 18.

3. Dose selection rationale - The doses chosen for the current study were based on the results of previous studies carried out in the performing laboratory (no further information provided). The doses for the current study are presented in Table 1.

4. Dose preparation, administration, and analysis - Gelatin capsules (9 mL capacity) were filled with the appropriate amount of test substance based on the most recent body weight (frequency of capsule preparation was not provided). The neat test substance was used. A certificate of analysis was provided. Dogs were dosed orally immediately prior to feeding every day for one year.

5. Statistics - Body weight, food consumption, hematology, clinical chemistry, urine chemistry, plasma tyrosine, urine phenolic acid, and organ weight data were evaluated by analysis of variance (ANOVA) and/or covariance followed by Student's t-test as necessary.

C. METHODS:

1. Observations - All animals were observed at least three times daily for clinical and behavioral abnormalities. Detailed clinical examinations were performed weekly. Individual daily assessment of fecal consistency was made up to 5 hours post-dosing. All dogs were subjected to a full veterinary examination, including cardiac and pulmonary auscultation and indirect ophthalmoscopy, during pre-study, weeks 13, 26, and 39, and prior to study termination.

2. Body weight - Each animal was weighed at least 2 weeks pre-study, on day 1 and then weekly before feeding.

3. Food consumption - Food consumption was measured daily approximately 4 hours after feeding and was reported as g/dog/day. Measurements started at least 2 weeks pre-study and continued throughout the treatment period.

4. Water consumption - Water consumption was not reported.

5. Ophthalmoscopic examination - Ophthalmoscopic examinations were performed during the veterinary examinations during pre-study, weeks 13, 26, and 39, and prior to study termination.

6. Blood - Blood was collected from the jugular vein of all dogs before feeding during weeks -1, 4, 13, 26, and/or prior to termination. The checked (X) hematology and clinical blood chemistry parameters were examined. An additional 1.3 mL blood was collected from each dog at termination and analyzed for plasma tyrosine.

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		Reticulocyte count
X	Blood clotting measurements	X	Erythrocyte distribution width
	(Thromboplastin time)	X	Blood cell morphology
X	(Activated partial thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin
X	Chloride	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen
X	Phosphorus (as phosphate)	X	Total cholesterol
X	Potassium		Globulin
X	Sodium	X	Glucose
			Direct bilirubin
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total serum protein (TP)
	Cholinesterase (ChE)	X	Triglycerides
X	Creatine phosphokinase		Electrophoretic protein fractions
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine aminotransferase (ALT)		
X	Serum aspartate aminotransferase (AST)		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

7. Urinalysis - Urine samples were collected via catheterization from all dogs pre-study and during weeks 26 and 52. The checked (X) parameters were examined. In addition, samples of urine obtained during week 51 were analyzed for free and conjugated phenolic acids by nuclear magnetic resonance (NMR).

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

* Assessed semi-quantitatively

8. Sacrifice and pathology - At study termination, all animals were anaesthetized, exsanguinated, and subjected to a gross pathological examination. The following CHECKED (X) tissues were collected from all animals; all tissues (except for the femur) were examined microscopically. Bone marrow samples were taken from a rib of all dogs. Additionally, the (XX) organs were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta	XX	Brain (3 regions)
X	Salivary glands	X	Heart	X	Periph. nerve
X	Esophagus	X	Bone marrow (w/sternum)	X	Spinal cord (3 levels)
X	Stomach	X	Lymph nodes	X	Pituitary
X	Duodenum	X	Spleen	X	Eyes
X	Jejunum	X	Thymus		
X	Ileum				GLANDULAR
X	Cecum		UROGENITAL	XX	Adrenal gland
X	Colon	XX	Kidneys		Lacrimal gland
X	Rectum	X	Urinary bladder	X	Mammary gland
XX	Liver	XX	Testes	XX	Thyroids w/ parathyroids
X	Pancreas	XX	Epididymides		
X	Gall bladder	X	Prostate		OTHER
			Seminal vesicle	X	Bone (Femur and stifle joint)
	RESPIRATORY	X	Ovaries	X	Skeletal muscle
X	Trachea	X	Uterus	X	Skin
X	Lung		Vagina	X	All gross lesions and masses
	Pharynx	X	Cervix		
	Larynx				

II. RESULTS

A. Observations

1. Mortality - One high-dose female was humanely killed during week 47 due to adverse clinical signs including convulsions, hypothermia, a slow, weak pulse, and rapid weight loss. Pathological examination of this animal indicated generalized lymphocytolysis.

2. Clinical signs - Interdigital cysts were observed in the mid- (males-2/4, females-1/4) and high- (males-2/4, females-3/4) dose animals and in the low-dose males (1/4). During the veterinary exam at week 52, one interdigital cyst was observed in a female control animal. Cloudy eyes were observed in a single high-dose male (1/4). Lime-green discoloration of the urine was observed in all mid- and high-dose animals; however, this treatment-related effect was considered not to be adverse. Skin lesions, mainly dry sores, were observed in most treatment groups, and the incidences increased with dose.

Table 2. Selected clinical observations (number of affected animals) noted in dogs treated with mesotrione for 1 year.[a]

Observation	Males				Females			
	Dose (mg/kg/day)				Dose (mg/kg/day)			
	0	10	100	600	0	10	100	600
Interdigital cyst	0	1	2	2	0	0	1	3
Cloudy eye(s)	0	0	0	1	0	0	0	0

a Data obtained from the study report Table 1, pages 42-53 and Table 2, page 54; n=4

B. Body weight and body weight gain - No treatment-related differences in body weights were observed. In the females, mean adjusted (covariately for week 1 body weights) body weights were sporadically decreased at the high-dose (↓5-10%, $p \leq 0.05$). No differences from concurrent controls were observed in the mid- or low-dose females. In the males, adjusted body weights were comparable between control and treated animals throughout the study. Overall (weeks 1-53) body weight gain (calculated by reviewers) was decreased in the mid- and high-dose females (↓26 and 48%, respectively; Table 3). During the first 13 weeks of the study, body weight gain was reduced by 13% and 25% in the mid- and high-dose females, respectively. In the next 13 weeks, body weight gain was reduced in the high-dose females only by 48% as compared to the controls. By 6 to 7 months into the study, body weights had leveled out and any changes in body weight gain were probably random fluctuations. Although the percent differences between control and treated body weight gain appear large, the actual body weight gain differs by approximately 1 kg; therefore, the differences in body weight gains are of equivocal toxicological significance.

Table 3. Body weight gains (kg) in dogs treated with mesotrione for one year.[a]

Weeks	Dose (mg/kg/day)			
	0	10	100	600
	Males			
1 - 53	2.60	1.95	2.22	3.30
	Females			
1 - 13	1.58	1.50	1.38 (↓13)	1.18 (↓25)
13 - 26	0.62	0.68	0.70	0.32 (↓48)
26 - 53	0.15	0.07	-0.33	-0.27
1 - 53	2.35	2.25	1.75 (↓26)	1.23 (↓48)

a Body weight gains were calculated by the reviewers from information provided in the study report, Table 6, pages 67-80; n=4. Percent difference from controls is listed parenthetically.

C. Food consumption - No treatment-related differences in food consumption were observed.

D. Ophthalmoscopic examination - Lenticular opacities were seen in one male and one female dog at the high dose. Lenticular opacity in one male control dog was attributed to the remnant of the hyaline blood vessel and noted at the pre-study examination. No ocular lesions were noted in any other control animal or in any of the treated animals in the pre-study examination. The corneal opacities observed in the male control dog at week 52 and in three low-dose male dogs at weeks 26-39 were not seen during the weekly examinations nor at termination, either at gross necropsy or in the histopathology. No other treatment-related ophthalmoscopic observations were noted.

E. Blood analyses

1. Hematology - No treatment-related hematology findings were observed. The following decreases ($p \leq 0.01$ or 0.05) in adjusted (covariately for pre-experimental values) hematology parameters relative to concurrent controls were minor, not dose-dependent, and/or not sustained over time: (i) hemoglobin in the low-dose males and females at weeks 13 and 52, respectively (↓6-7%) and in the mid-dose males at week 13 (↓8%); (ii) hematocrit in the low-dose females at week 52 (↓7%); (iii) mean cell volume in the high-dose males at week 13 (↓6%) and in the high-dose females throughout the study (↓3-10%); (iv) mean cell hemoglobin in the high-dose males at week 13 (↓6%); (v) mean cell

hemoglobin in the high-dose females at weeks 4-26 (↓3-12%); (vi) red cell distribution width in the high-dose males at week 4 (↓7%); (vii) white blood cell count in all treated males at week 4 (↓27-33%); (viii) neutrophil count in all treated males at week 4 (↓28-44%); (ix) lymphocyte count in the low- and mid-dose males at week 4 (↓24-25%); (x) monocyte count in all treated males (↓44-58%); (xi) basophil count in the mid- and high-dose females at week 26 (↓55% each); (xii) large unstained cells in all treated males at week 4 (↓52-61%); (xiii) prothrombin time in the low- and mid- dose females at week 4 (↓4-6%); and (xiv) activated partial thromboplastin time in the mid- and high-dose females at week 13 (↓5% each) and in the mid-dose females at week 52 (↓10%). The following increases ($p \leq 0.01$ or 0.05) in hematology parameters relative to concurrent controls were minor, not dose-dependent, and/or not sustained over time: (i) eosinophil count in the mid-dose males (↑107%) and in the low- and mid-dose females (↑114-116%) at week 52; (ii) basophil count in the mid-dose males at week 26 (↑175%); and (iii) prothrombin time in the high-dose males at week 4 (↑4%); and (iv) erythrocyte count in the high-dose females at week 13 (↑11%). In addition, minor changes in mean cell hemoglobin concentration were observed in the mid- and high-dose females during weeks 13 (↑2%) and 26 (↓4%).

2. Clinical chemistry - No treatment-related changes were noted in the clinical chemistry parameters. The following differences ($p \leq 0.01$ or 0.05) in adjusted mean clinical chemistry parameters were minor, not dose-dependent, and/or not sustained over time: (i) decreased urea in the low- and high-dose males at week 13 (↓22-24%), in the mid- and high-dose males at week 26 (↓19-27%), in the low- and mid-dose females at week 13 (↓18% each), and in the mid-dose females at week 52 (↓19%); (ii) decreased creatinine in the high-dose males at week 26 and in the mid-dose females at week 13 (↓12% each); (iii) decreased albumin in the low-dose males at week 26 (↓7%); (iv) decreased total protein in the low-dose males at weeks 26 and 52 (↓7-8%); (v) decreased cholesterol in the high-dose males at weeks 13 and 52 (↓10-19%) and increased cholesterol in the low-dose females at week 52 (↑22%); (vi) decreased triglycerides in the low- and mid-dose males at week 4 (↓24-27%), increased triglycerides in the high-dose males at week 13 (↑41%) and in the mid-dose females at week 52 (↑107%); (vii) decreased total bilirubin in the high-dose males at weeks 26 and 52 (↓39-43%) and in the high-dose females at weeks 4 and 26 (↓32-36%); (viii) increased alkaline phosphatase in the mid-dose females at weeks 4, 26, and 52 (↑37-97%); (xiv) decreased gamma-glutamyl transferase in the low-dose females at week 4 (↓27%); (xv) increased alanine aminotransferase in the mid- and high-dose males at week 4 (↑46-59%); (xvi) increased aspartate aminotransferase in the high-dose females at week 13 (↑153%); (xvii) increased creatine kinase in the high-dose females at week 4 (↑30%); (xviii) increased sodium in the low- and high-dose males at week 4 (↑1-2%) and decreased sodium in the mid- and high-dose females at week 4 high-dose females at week 52 (↓1-2%); (xix) increased potassium in the mid-dose males at week 26 (↑13%); (xx) increased calcium in the high-dose males at week 13 (↑5%); and (xxi) increased phosphorus in the high-dose males at week 26 (↑25%), in the low-dose

females at week 13 (↑19%), in the high-dose females at week 26 (↑31%), and in the mid- and high-dose females at week 52 (↑27-29%). Adjusted mean alanine aminotransferase was increased in the high-dose females at week 52 (↑151%, $p \leq 0.01$); however the unadjusted mean was associated with a large standard deviation and therefore this value was considered not to be treatment-related.

3. Plasma tyrosine - Plasma tyrosine was dose-dependently increased ($p \leq 0.01$) in all treated animals relative to controls (males-↑168-915%, females-↑151-1470%; Table 4).

Table 4. Plasma tyrosine levels (nmol/mL) in dogs treated with mesotrione for one year.[a]

Dose (mg/kg/day)			
0	10	100	600
Males			
112	300**(↑168)	905**(↑708)	1137**(↑915)
Females			
83	208**(↑151)	1090**(↑1213)	1303**(↑1470)

a Data obtained from the study report, Table 13 page 173; n=4. Percent difference from controls is listed parenthetically.

** Statistically significant at $p \leq 0.01$

F. Urinalysis - No treatment-related differences from concurrent controls were observed in any urinalysis parameter. Urine pH was increased ($p \leq 0.05$) in the low-dose males (6.75 treated vs. 5.25 controls) and decreased in the mid-dose females (5.00 treated vs. 6.25 controls) at week 26; however, these differences were neither dose-dependent nor sustained over time. Large quantities of free phenolic acids (4-hydroxyphenyl pyruvate and 4-hydroxyphenyl lactate) were detected in the urine of all treated animals at week 51; however, no dose relationship was evident.

G. Sacrifice and pathology:

1. Organ weight - No treatment-related changes in organ weights were observed. In the mid-dose males, absolute and adjusted (covariately for final body weight) epididymides weights were decreased (↓32 and 27%, respectively; $p \leq 0.05$) and adjusted kidney weights were increased (↑32%, $p \leq 0.05$); however, neither of these differences was dose-dependent. No differences from concurrent controls were observed in relative (to body) organ weights.

2. Gross pathology - The following pathological abnormalities were observed in the high-dose female sacrificed at week 47 (Table 5): (i) mucosal dark striations in the colon; (ii) red mucosa in the duodenum; (iii) red spots on the epicardium; (iv) prominent Peyer's patches and red mucosa in the ileum; (v) red spots on the mucosa and red mucosa in the jejunum; (vi) pale liver; (vii) dark spots on the lung; (viii) discolored pre-scapular lymph node; (ix) erosions of the stomach; (x) interdigital cysts; and (xi) opaque cornea. Gross pathological observations noted in animals surviving until study termination included the following: (i) prominent Peyer's patches in the ileum of a single high-dose male; (ii) red spots on the stomach of a single high-dose male; (iii) interdigital cysts in the low- (1/4), mid- (2/4), and high- (1/4) dose males and a single high-dose female (1/3); and (iv) corneal and lens opacity and ocular discharge in a single high-dose male and red spots in the eye of another single high-dose male. None of the gross pathological abnormalities were observed in any control animal. Other pathological abnormalities which were not dose-dependent and considered not to be treatment-related included red mucosa in the duodenum, ileum, and jejunum of a single low-dose male and an enlarged popliteal lymph node in a single mid-dose male.

Table 5. Selected gross pathological observations noted in dogs treated with mesotrione for 1 year.[a]

Observation	Males				Females			
	Dose (mg/kg/day)				Dose (mg/kg/day)			
	0	10	100	600	0	10	100	600 [b]
Colon								
Dark striations, mucosa	0	0	0	0	0	0	0	1
Duodenum								
Red mucosa	0	1	0	0	0	0	0	1
Heart								
Red spots, epicardium	0	0	0	0	0	0	0	1
Ileum								
Prominent Peyer's patches	0	0	0	1	0	0	0	1
Red mucosa	0	1	0	1	0	0	0	1
Jejunum								
Red spots/areas, mucosa	0	0	0	0	0	0	0	1
Red mucosa	0	1	0	0	0	0	0	1
Liver								
Pale	0	0	0	0	0	0	0	1
Lung								
Dark spots or areas	0	0	0	0	0	0	0	1
Popliteal lymph node								
Enlarged	0	0	1	0	0	0	0	1
Firm	0	0	0	0	0	0	0	1
Pre-scapular lymph node								
Discolored	0	0	0	0	0	0	0	1
Stomach								
Erosions	0	0	0	0	0	0	0	1
Red spots/areas	0	0	0	1	0	0	0	0
Skin								
Interdigital cysts	0	1	2	1	0	0	0	2
Eye								
Opaque cornea	0	0	0	1	0	0	0	1
Red spots	0	0	0	1	0	0	0	0

a Data obtained from the study report Table 16, pages 182-188; n=4

b Data includes animal sacrificed for humane reasons during week 47.

3. Microscopic pathology - The following selected histopathological abnormalities were observed in the high-dose female sacrificed at week 47 (Table 6): (i) minimal lymphocytolysis of the liver; (ii) slight lymphocytolysis of the spleen and thymus; (iii) moderate lymphocytolysis of the cecum, colon, duodenum, ileum, jejunum, mesenteric lymph node, pre-scapular lymph node, and rectum; (iv) minimal congestion of the lamina propria in the cecum; (v) slight congestion of the lamina propria in the colon, duodenum, and rectum; (vi) minimal mucous filled cysts/Peyer's patches in the duodenum; (vii) minimal mucosal congestion in the ileum; (viii) marked reduction of glycogen and slight sinusoidal neutrophilia in the liver; (ix) slight erythrophagocytosis in the mesenteric lymph node; (x) marked blood-filled sinuses and slight lymphadenitis in the pre-scapular lymph node; (xi) generalized lymphocytolysis; (xii) minimal epicardial and slight sub-epicardial hemorrhage in the heart; (xiii) minimal intramural hemorrhage of the coronary arteries; (xiv) minimal unilateral corneal erosion; and (xv) moderate interdigital dermatitis/folliculitis. Histopathological observations noted in animals surviving until study termination included the following: (i) slight erythrophagocytosis in the mesenteric lymph nodes of the mid- and high-dose males (1/4 each) and in the low-(3/4) and high-(1/3) dose females; (ii) moderate erythrophagocytosis in the mesenteric lymph node of one mid-dose female and one high-dose male; (iii) slight blood-filled sinuses in the pre-scapular lymph node of a single high-dose male; (iv) minimal mucous filled cystic spaces in Peyer's patches in the rectum of a single high-dose male; (v) minimal unilateral keratitis and moderate peri-orbital hemorrhage in the high-dose males (1/4 each); and (vi) marked interdigital dermatitis/folliculitis in the low- (1/4), mid- (2/4), and high- (2/4) males. None of these histopathological abnormalities were observed in any control animal.

Table 6. Selected histopathological observations noted in dogs treated with mesotrione for 1 year.[a]

Observation	Males				Females			
	Dose (mg/kg/day)				Dose (mg/kg/day)			
	0	10	100	600	0	10	100	600[b]
Cecum								
Lymphocytolysis (total)	0	0	0	0	0	0	0	1
moderate	0	0	0	0	0	0	0	1
Congestion, lamina propria (total)	0	0	0	0	0	0	0	1
minimal	0	0	0	0	0	0	0	1
Colon								
Lymphocytolysis (total)	0	0	0	0	0	0	0	1
moderate	0	0	0	0	0	0	0	1
Congestion, lamina propria (total)	0	0	0	0	0	0	0	1
slight	0	0	0	0	0	0	0	1
Duodenum								
Lymphocytolysis (total)	0	0	0	0	0	0	0	1
moderate	0	0	0	0	0	0	0	1
Congestion, lamina propria (total)	0	0	0	0	0	0	0	1
slight	0	0	0	0	0	0	0	1
Mucous filled cysts, Peyer's patches (total)	0	0	0	0	0	0	0	1
minimal	0	0	0	0	0	0	0	1
Ileum								
Lymphocytolysis (total)	0	0	0	0	0	0	0	1
moderate	0	0	0	0	0	0	0	1
Mucosal congestion (total)	0	0	0	0	0	0	0	1
minimal	0	0	0	0	0	0	0	1
Jejunum								
Lymphocytolysis (total)	0	0	0	0	0	0	0	1
moderate	0	0	0	0	0	0	0	1
Liver								
Lymphocytolysis (total)	0	0	0	0	0	0	0	1
minimal	0	0	0	0	0	0	0	1
Reduced glycogen (total)	0	0	0	0	0	0	0	1
marked	0	0	0	0	0	0	0	1
Sinusoidal neutrophilia (total)	0	0	0	0	0	0	0	1
slight	0	0	0	0	0	0	0	1

(Table continues, footnotes to follow)

Observation	Males				Females			
	Dose (mg/kg/day)				Dose (mg/kg/day)			
	0	10	100	600	0	10	100	600[b]
Mesenteric lymph node								
Lymphocytolysis (total)	0	0	0	0	0	0	0	1
moderate	0	0	0	0	0	0	0	1
Erythrophagocytosis (total)	0	0	1	2	0	3	2	2
minimal	0	0	0	0	0	0	1	0
slight	0	0	1	1	0	3	0	2
moderate	0	0	0	1	0	0	1	0
Pre-scapular lymph node								
Lymphocytolysis (total)	0	0	0	0	0	0	0	1
moderate	0	0	0	0	0	0	0	1
Blood filled sinuses (total)	0	0	0	1	0	0	0	1
slight	0	0	0	1	0	0	0	0
marked	0	0	0	0	0	0	0	1
Lymphadenitis (total)	0	0	0	0	0	0	0	1
slight	0	0	0	0	0	0	0	1
Lymphoreticular system								
Generalized lymphocytolysis	0	0	0	0	0	0	0	1
Rectum								
Lymphocytolysis (total)	0	0	0	0	0	0	0	1
moderate	0	0	0	0	0	0	0	1
Congestion, lamina propria (total)	0	0	0	0	0	0	0	1
slight	0	0	0	0	0	0	0	1
Mucous filled cystic spaces in Peyer's patches (total)	0	0	0	1	0	0	0	0
minimal	0	0	0	1	0	0	0	0
Spleen								
Lymphocytolysis (total)	0	0	0	0	0	0	0	1
slight	0	0	0	0	0	0	0	1
Thymus								
Lymphocytolysis (total)	0	0	0	0	0	0	0	1
slight	0	0	0	0	0	0	0	1

(Table continues, footnotes to follow)

Observation	Males				Females			
	Dose (mg/kg/day)				Dose (mg/kg/day)			
	0	10	100	600	0	10	100	600[b]
Heart								
Hemorrhage (total)	0	0	0	0	1	0	0	0
slight	0	0	0	0	1	0	0	0
Epicardial hemorrhage (total)	0	0	0	0	0	0	0	1
minimal	0	0	0	0	0	0	0	1
Sub-epicardial hemorrhage (total)	0	0	0	0	0	0	0	1
slight	0	0	0	0	0	0	0	1
Intramural hemorrhage, coronary arteries (total)	0	0	0	0	0	0	0	1
minimal	0	0	0	0	0	0	0	1
Eye								
Unilateral keratitis (total)	0	0	0	1	0	0	0	0
minimal	0	0	0	1	0	0	0	0
Peri-orbital hemorrhage (total)	0	0	0	1	0	0	0	0
moderate	0	0	0	1	0	0	0	0
Unilateral corneal erosion (total)	0	0	0	0	0	0	0	1
minimal	0	0	0	0	0	0	0	1
Skin								
Interdigital dermatitis/folliculitis (total)	0	1	2	2	0	0	0	2
moderate	0	0	0	0	0	0	0	1
marked	0	1	2	2	0	0	0	1

a Data obtained from the study report Table 17, pages 189-201; n=4
b Data includes animal sacrificed for humane reasons during week 47.

III. DISCUSSION

A. Investigator's conclusions - Oral administration of mesotrione for 1 year was minimally toxic at 600 mg/kg/day. Slightly reduced body weight gains and ocular changes were observed. One 600 mg/kg/day female was killed for humane reasons due to generalized lymphocytolysis which was considered treatment related. The NOAEL for this study was 100 mg/kg/day.

B. Reviewer's discussion - In this chronic oral toxicity study, mesotrione was administered via gelatin capsule for 1 year to 4 Beagle dogs/sex/dose at concentrations of 0, 10, 100, or 600 mg/kg/day. The neat test substance was used. Body weights, food consumption, hematology, clinical chemistry and urinalysis parameters, and organ weights were unaffected by the test substance.

One high-dose female was humanely killed during week 47 due to adverse clinical signs including convulsions, hypothermia, a slow, weak pulse, and rapid weight loss. Pathological examination of this animal indicated generalized lymphocytolysis. Since this is a rare finding, it could not be ruled out as a treatment-related effect.

Overall (weeks 1-53) body weight gain (calculated by reviewers) was decreased in the mid- and high-dose females (↓26 and 48%, respectively; Table 3). During the first 13 weeks of the study, body weight gain was reduced by 13% and 25% in the mid- and high-dose females, respectively. In the next 13 weeks, body weight gain was reduced in the high-dose females only by 48% as compared to the controls. By 6 to 7 months into the study, body weights had leveled out and any changes in body weight gain were probably random fluctuations. Although the percent differences between control and treated body weight gain appear large, the actual body weight gain differs by approximately 1 kg; therefore, the differences in body weight gain are of equivocal toxicological significance.

During the clinical observations, cloudy eyes were observed in a single high-dose male (1/4). At the ophthalmological examination, lenticular opacities were noted in one high-dose male and one high-dose female. Lenticular opacity in one male control dog was attributed to the remnant of the hyaline blood vessel and noted at the pre-study examination. No ocular lesions were noted in any other control animal or in any of the treated animals in the pre-study examination. On gross pathology, one high-dose male had corneal and lenticular opacities with ocular discharge with a corresponding keratitis seen on microscopic evaluation. One high-dose female also had a corneal opacity with corneal erosion seen microscopically. A different high-dose male had red spots in the eye corresponding to peri-orbital hemorrhage. The corneal and lenticular effects are common pathology seen with tyrosinemia in various species and are considered treatment related. No ocular lesions were observed at the low- or mid-dose in either sex.

Interdigital cysts were observed in the mid- (males-2/4, females-1/4) and high- (males-2/4, females-3/4) dose animals and in the low-dose males (1/4) and control females (1/4). There also appeared to be an increase in dry skin sores in treated animals, especially males. These effects corresponded with an increase in staining of the hair, thorax, limbs, and paws, and green-colored urine as the dose increased. The skin effects were most likely due to the irritant properties of the phenolic acid metabolites in the urine that contacted the skin and may not be toxicologically relevant. Dermatitis/folliculitis was noted in the histopathology report in areas where dry sores or interdigital cysts occurred. Other areas that were grossly normal showed no abnormalities.

In the individual records, most of the staining was not characterized, so the cause of the staining, either from licking or urine stains, could not be determined. The author mentioned yellow staining of the coat in most animals, but the time of duration did not correspond to the amount of staining and duration of general staining that occurred throughout the study. Also, the same was true with the green coloring of the urine, which occurred infrequently in the

low-dose animals. If all the staining was yellow from urine, the question is why was there no staining in any control animal and was there a change in behavior, either in urination or grooming patterns, that allowed greater urine staining in treated animals only?

Plasma tyrosine was dose-dependently increased ($p \leq 0.01$) in the low-, mid-, and high-dose animals relative to controls (males-↑168, 708, and 915%, respectively; females-↑151, 1213, and 1470%, respectively). Normal serum tyrosine levels in dogs range from 10 to 110 nmoles/ml. Urine phenolic acid concentrations also increased, but was not related to dose.

Other histopathological observations of interest noted in animals surviving until study termination included the following: (i) minimal erythrophagocytosis in the mesenteric lymph nodes of a single mid-dose female; (ii) slight erythrophagocytosis in the mesenteric lymph nodes of the mid- and high-dose males (1/4 each) and in the low-(3/4) and high- (1/3) dose females; (iii) moderate erythrophagocytosis in the mesenteric lymph node of one mid-dose female and one high-dose male. The high-dose female that was killed for humane reasons also had slight erythrophagocytosis in the mesenteric lymph nodes. Although not strictly dose-related, like the urine phenolic acid concentration, this effect is considered treatment related.

The LOAEL for this study is 10 mg/kg/day based on evidence of tyrosinemia in both sexes and increased incidence of erythrophagocytosis in the mesenteric lymph nodes of females.

No NOAEL was determined for this study.

The submitted study is classified as **acceptable/guideline (§83-1b)** and satisfies the requirements for a chronic oral toxicity study in dogs.

C. Study deficiencies - The following deficiencies were noted, but do not change the conclusions of this review:

- No dose rationale was provided; however, a subchronic oral toxicity study in dogs carried out by the performing laboratory was provided for review (MRID 44505023).