DATA EVALUATION RECORD

10/12/2000

MESOTRIONE (ZA1296)

Study Type: §82-1(b), 90 Day Feeding Study in Dogs

Work Assignment No. 2-01-52S (MRID 44505023)

Prepared for
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Office of Pesticide Programs
U.S. Environmental Protection Agency
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This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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David Nixon 10/3/2000

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Marion Copley 10/12/2000

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity - dogs
OPPTS Number: 870.3150

OPP Guideline Number: §82-1b

DP BARCODE: D259369
P.C. CODE: 122990

SUBMISSION CODE: S541375
TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): Mesotrione (96.8% 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: 90 Day Oral Toxicity Study in Dogs. Central Toxicology Laboratory, Cheshire, UK. Laboratory Report No. CTL/P/4945, October 1, 1997. MRID 44505023. Unpublished.

SPONSOR: Zeneca Ag Products, Wilmington, DE

EXECUTIVE SUMMARY: In this subchronic oral toxicity study (MRID 44505023), mesotrione (ZA1296; 96.8% a.i., Lot/batch # P17) was administered via gelatin capsule for 90 days to 4 Beagle dogs/sex/dose at dietary concentrations of 0, 100, 600, or 1000 mg/kg/day.

No mortalities occurred during the study. Clinical signs, body weight, food consumption, hematology and clinical chemistry parameters, organ weights, and gross and microscopic pathological findings were not adversely affected by the test substance.

The incidence of reddened ears increased at 600 (1/4 males, 2/4 females) and 1000 (3/4 males, 3/4 females) mg/kg in both sexes as compared to the controls (0/4 males, 1/4 females). However, since no associated adverse effects were noted with this clinical observation, it is not considered toxicologically relevant.

The NOAEL for this study is 1000 mg/kg/day based on the lack of any adverse effects up to the limit dose. The LOAEL is > 1000 mg/kg/day.

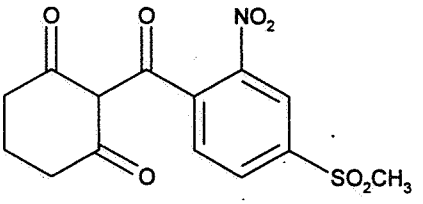
The submitted study is classified as **acceptable/guideline (§82-1b)** and satisfies the requirements for a subchronic oral toxicity study in dogs.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Mesotrione (ZA1296)
   Description: Light beige solid
   Lot/Batch #: P17
   Purity (w/w): 96.8% 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 a year 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: At least 20-29 weeks old; 9.1-12.3 kg (males), 8.3-11.8 kg (females)
   Source: Animal Breeding Unit, Zeneca Pharmaceuticals, Alderley Park
   Housing: 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: 40-70%
   Air changes: Approximately 15/hour
   Photoperiod: 12 hours light/12 hours dark
   Acclimation period: Approximately 4-5 weeks

B. STUDY DESIGN:

1. In life dates: Start: 9/12/95 End: 12/15/95

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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	100	4	4
Mid	600	4	4
High	1000	4	4

a Data obtained from the study report, page 16.

3. Dose selection rationale - The doses chosen for the current study were based on the results of a 6 week oral range-finding study carried out in the performing laboratory; no additional information was 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). A certificate of analysis was provided; the neat test substance was used. Dogs were dosed orally immediately prior to feeding for at least 90 consecutive days.

5. Statistics - Body weights, hematology, clinical chemistry, and organ weight data were evaluated by analysis of variance (ANOVA) and/or covariance followed by Student's t-test.

C. METHODS:

1. Observations - All animals were observed at least three times daily for clinical and behavioral abnormalities. Detailed clinical examinations were performed weekly.

2. Body weight - Each animal was weighed weekly before feeding.

3. Food consumption - Food consumption was measured daily and was reported as g/dog/day.

4. Water consumption - Water consumption was not reported.

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5. Ophthalmoscopic examination - Ophthalmoscopic examinations were performed during the full veterinary examinations pre-dosing and prior to study termination.

6. Blood - Blood was collected from the jugular vein of all dogs before feeding during weeks -1, 4, 8, and prior to termination. 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		Reticulocyte count
	Blood clotting measurements	X	Erythrocyte distribution width
	(Thromboplastin time)		
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	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 - No urinalysis was performed.

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. Additionally, the (XX) organs were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta	XX	Brain
X	Salivary glands	X	Heart	X	Periph. nerve
X	Esophagus	X	Bone marrow	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, stifle joint, and sternum)
	RESPIRATORY	X	Ovaries		
X	Trachea	X	Uterus	X	Skeletal muscle
X	Lung		Vagina	X	Skin
	Pharynx	X	Cervix	X	All gross lesions and masses
	Larynx				

II. RESULTS

A. Observations

1. Mortality - No mortalities occurred during the study.

2. Clinical signs - Green urine and feces were observed in the mid- and high-dose males and females. Although these effects were considered to be treatment-related, they were not adverse. Faint corneal opacities were observed in one high-dose female and minimal corneal opacities were observed in one control male. Salivation and reddened ears were observed in all animals, but were more prevalent in the animals that received higher doses of the test substance. The salivation appeared to be a learned response that occurred in all groups at the time of dosing. The incidence of reddened ears, however, did increase at 600 and 1000 mg/kg in both sexes and may be treatment related. No other abnormalities were reported.

B. Body weight and body weight gain - Mean body weights (adjusted for week 1 body weight) were slightly decreased ($p \leq 0.01$ or 0.05) in the high-dose males (↓2-7%, Table 2). Adjusted body weights were also decreased ($p \leq 0.01$ or 0.05) in the mid-dose males at weeks 2 and 14 (↓2-5%) and at all doses in females from weeks 3- 14. Overall (weeks 1-14) body weight gains (calculated by reviewers) were decreased in the mid- and high-dose males (↓37 and 44%, respectively) and in all treated female groups (↓29-35%). Although the percent differences between control and treated body weight gains appear large, the actual body weight gains differ by less than 1 kg; therefore, the differences in body weight gains are of equivocal toxicological significance.

Table 2. Selected mean body weights (adjusted for week 1 body weights) and overall body weight gains (kg) in dogs treated with mesotrione for 90 days.[a]

Week	Dose (mg/kg/day)			
	0	100	600	1000
Males				
1	10.35	10.78	10.90	10.85
4	11.37	11.45	11.27	11.09**(↓2)
10	12.41	12.48	12.06	11.77**(↓5)
14	12.83	12.93	12.14*(↓5)	11.98**(↓7)
Overall (weeks 1-14) Body Weight Gain	2.2	2.2	1.38(↓37)	1.23 (↓44)
Females				
1	9.50	9.65	9.43	10.35
4	10.30	10.07	10.04	10.10
10	11.13	10.76	10.69	10.79
14	11.39	10.92	10.87	10.95
Overall (weeks 1-14) Body Weight Gain	1.7	1.2(↓29)	1.2(↓29)	1.1 (↓35)

a Data obtained from the study report Table 5, pages 58 through 61; n=4. Percent difference from controls is listed parenthetically. Overall body weight gains were calculated by the reviewers.
* Statistically different from controls at $p \leq 0.05$.
** Statistically different from controls at $p \leq 0.01$.

C. Food consumption - Food consumption was comparable between controls and treated animals throughout the study.

D. Blood analyses

1. Hematology - Increased ($p \leq 0.01$ or 0.05) erythrocyte counts were observed in the mid- and high-dose animals at week 13 (males-↑16% each, females - ↑17-20%), the mid- and high-dose males at week 8 (↑12-15%), the high-dose females at week 8 (↑12%), and the high-dose males at week 4 (↑11%, Table 3). Mean cell hemoglobin was decreased ($p \leq 0.01$ or 0.05) in the mid- and high-dose males during weeks 4, 8, and 13 (↓4-6%, ↓8-11%, and ↓13-16%, respectively) and the mid- and high-dose females during weeks 8 and 13 (↓7-8% and ↓13% each, respectively). In addition, mean cell volume was decreased ($p \leq 0.01$) in the mid- and high-dose animals at weeks 4, 8, and 13 (males - ↓4-5%, ↓8-10%, and ↓14-16%, respectively; females - ↓3% each, ↓6-8%, and ↓12-14%, respectively). Activated partial thromboplastin time was decreased ($p \leq 0.01$ or 0.05) in the mid-dose males at week 8 (↓5%), in the high-dose males at weeks 8 and 13 (↓5-6%), in the low-dose females at week 13 (↓6%), in the mid-dose females at weeks 8 and 13 (↓5-7%), and in the high-dose females at weeks 8 and 13 (↓8-11%). None of these findings were clinically or toxicologically significant. Other changes ($p \leq 0.01$ or 0.05) in hematology parameters were also noted, but were not dose-dependent, and/or not sustained over time and considered not to be treatment-related.

Table 3. Selected hematology parameters in dogs treated with mesotrione for 90 days.[a]

Parameter	Dose (mg/kg/day)							
	Males				Females			
	0	100	600	1000	0	100	600	1000
Week 4								
Red blood cell count (x10^{12}/L)	6.07	6.39	6.48	6.74**(↑11)	6.44	6.85	6.78	6.73
Mean cell volume (fL)	68.9	68.3	66.3**(↓4)	65.3**(↓5)	69.8	69.4	67.9**(↓3)	67.5**(↓3)
Mean cell hemoglobin (pg)	22.7	22.2	21.7*(↓4)	21.3**(↓6)	22.6	22.7	22.1	22.0
Activated partial thromboplastin time (sec)	18.7	17.8	18.3	17.7	17.8	18.4	18.1	17.6
Week 8								
Red blood cell count (x10^{12}/L)	6.26	6.60	6.99*(↑12)	7.21*(↑15)	6.44	6.86	7.04	7.20*(↑12)
Mean cell volume (fL)	67.3	66.4	61.8**(↓8)	60.4**(↓10)	68.2	67.4	64.1**(↓6)	62.7**(↓8)
Mean cell hemoglobin (pg)	22.7	22.3	20.9*(↓8)	20.2**(↓11)	23.0	22.9	21.4*(↓7)	21.2*(↓8)
Activated partial thromboplastin time (sec)	18.4	17.6	17.5*(↓5)	17.5* (↓5)	19.0	19.1	18.1*(↓5)	17.4**(↓8)
Week 13								
Red blood cell count (x10^{12}/L)	6.36	6.88	7.39**(↑16)	7.39**(↑16)	6.59	6.63	7.68**(↑17)	7.89**(↑20)
Mean cell volume (fL)	68.0	65.8	58.5**(↓14)	57.0**(↓16)	68.4	67.6	60.2**(↓12)	59.1**(↓14)
Mean cell hemoglobin (pg)	22.7	22.2	19.7**(↓13)	19.1**(↓16)	23.0	22.8	20.1**(↓13)	20.1**(↓13)
Activated partial thromboplastin time (sec)	17.3	17.0	16.4	16.2* (↓6)	18.9	17.7*(↓6)	17.6*(↓7)	16.9**(↓11)

a Data obtained from the study report Table 7, pages 68 through 82; n=4. Percent difference from controls is listed parenthetically.
* Statistically different from controls at $p \leq 0.05$.
** Statistically different from controls at $p \leq 0.01$.

2. Clinical chemistry - No treatment-related differences from concurrent controls were observed in any clinical chemistry parameter at any dose level. The following differences ($p \leq 0.01$ or 0.05) in adjusted (for pre-experimental values) mean clinical chemistry parameters were not dose-dependent, not sustained over time, and/or not clinically relevant: (i) decreased urea in the high-dose males at weeks 8 and 13 (↓40-43%) and in the low-dose males at week 13 (↓23%); (ii) decreased creatinine in the high-dose males at weeks 8 and 13

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(↓18-20%), in the low-dose males at week 8 (↓16%), and in the low-dose females at week 4 (↓11%); (iii) increased glucose in the high-dose females at week 13 (↓10%); (iv) increased albumin in all treated males (except the high dose at week 13) at all time points (↑5-12%) and in the mid-dose females at all time points (↑6%); (v) increased total protein in the mid- and high-dose males (except the high dose at week 13) at all time points (↑6-9%) and in the high-dose females at week 4 (↑5%); (vi) increased alkaline phosphatase in the mid-dose males during weeks 8 and 13 (↑21-29%) and in the low- and mid- dose females at week 4 (↑21%); (vii) increased creatine kinase in the low-dose females at weeks 8 and 13 (↑46-98%); (viii) decreased potassium in the mid- and high-dose females at week 13 (↓8-10%); (ix) increased calcium in the mid-dose females at week 13 (↑3%); and (x) decreased cholesterol (↓16%, $p \leq 0.05$) relative to concurrent controls in the high-dose males at week 13.

E. Urinalysis - No urinalysis was performed.

F. Sacrifice and Pathology:

1. Organ weight - No treatment-related changes in organ weights were observed. Absolute brain weight and adjusted (for body weight) brain weight were decreased in the high-dose females (↓12% each, $p \leq 0.05$); however, no microscopic abnormalities were noted in any brain tissue from the high-dose group. Adjusted (for body weight) liver weight was increased in the high-dose females (↑7%, $p \leq 0.05$); however this increase was not dose-dependent nor toxicologically significant.

2. Gross pathology - There was an increased incidence of yellow discolored hair in both sexes at 600 and 1000 mg/kg. This was probably due to the discolored feces and urine seen during clinical observations of the same treatment groups throughout the study. No treatment-related abnormalities were observed.

3. Microscopic pathology - No treatment-related histopathological observations were noted. Minimal to slight focal mesothelial proliferation of the heart atria was observed in 2/4 high-dose males (vs. 0/4 controls, Table 4), but was not considered treatment-related since this finding was not observed in the chronic dog study (MRID 44505027).

Table 4. Selected histopathological observations noted in the hearts of dogs treated with mesotrione for 90 days.[a]

Observation	Males				Females			
	Dose (mg/kg/day)				Dose (mg/kg/day)			
	0	100	600	1000	0	100	600	1000
Focal mesothelial proliferation (total)	0	0	0	2	0	0	0	0
minimal	0	0	0	1	0	0	0	0
slight	0	0	0	1	0	0	0	0

a Data obtained from the study report Table 11, page 110.

III. DISCUSSION

A. Investigator's conclusions - Oral administration of mesotrione for 90 days was minimally toxic at 600 and 1000 mg/kg/day. Reduced body weights and microcytic polycythemia were observed. The NOAEL for this study was 100 mg/kg/day.

B. Reviewer's discussion - In this subchronic oral toxicity study, mesotrione was administered via gelatin capsule for 90 days to 4 Beagle dogs/sex/dose at concentrations of 0, 100, 600, or 1000 mg/kg/day. No analytical data were provided.

No mortalities occurred during the study. Food consumption, hematology and clinical chemistry parameters, organ weights, and gross and microscopic pathological findings were unaffected by the test substance.

During the clinical examinations, green urine and feces were observed in the mid- and high-dose males and females. Although these effects were considered to be treatment related, they were not adverse. Faint corneal opacities were observed in one high-dose female and minimal corneal opacities were observed in one control male. Although this effect is seen in other species when treated with the test compound, the incidence here does not advocate that this finding is treatment related. Salivation and reddened ears were observed in all animals, but were more prevalent in the animals that received higher doses of the test substance. The salivation appeared to be a learned response that occurred in all groups at the time of dosing. The incidence of reddened ears increased at 600 (1/4 males, 2/4 females) and 1000 (3/4 males, 3/4 females) mg/kg in both sexes as compared to the controls (0/4 males, 1/4 females). The incidence not only increased in number of dogs affected, but in number of observations of the effect (600: 2, males; 2 females; 1000: 10, males; 17 females) and may be treatment related. However, since no associated adverse effects were noted with this clinical observation, it is not considered toxicologically relevant.

- Mean adjusted (for week 1 body weight) body weights were slightly decreased ($p \leq 0.01$ or 0.05) in the high-dose males (↓2-7%). Overall (weeks 1-14) body weight gains (calculated by reviewers) were decreased in the mid- and high-dose males (↓37 and 44%, respectively) and in all treated female groups (↓29-35%). Although the percent differences between control and treated body weight gains appear large, the actual body weight gains differ by less than 1 kg; therefore, the differences in body weight gains are of equivocal toxicological significance.

None of the hematological changes were clinically significant. The erythrocyte counts were within normal reference ranges. The decreased MCV values were just below normal values, but the PCV, MCHC, hemoglobin, and RBC counts were not affected. Also, this effect was not seen in the chronic dog study (MRID 44505027) at 600 mg/kg, and, thus, the effect was not considered adverse. Decreased APTT has no clinical significance.

No treatment-related histopathological observations were noted. During the histopathological examination, focal mesothelial proliferation of the heart was observed in 2/4 high-dose males (vs. 0/4 controls). However, this finding was not observed in females or in either sex in the chronic dog study. Although the highest dose tested was 600 mg/kg/day in the chronic study, this effect would probably have been seen at this dose over the longer treatment period. Thus, it is unlikely this finding is treatment related.

The NOAEL for this study is 1000 mg/kg/day based on the lack of any adverse effects up to the limit dose. The LOAEL is > 1000 mg/kg/day.

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

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

- No urinalysis was performed; however, this study was published prior to the 1998 Subdivision F guidelines.