DATA EVALUATION REPORT

5/31/2000

MESOTRIONE

Study Type: §82-2; 21-Day Dermal Toxicity Study - Rabbit

Work Assignment No. 2-01-52T (MRID 44505024)

Prepared for
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DATA EVALUATION RECORD

STUDY TYPE: 21-Day Repeated Dose Dermal Toxicity Study - Rabbit
OPPTS Number: 870.3200 OPP Guideline Number: §82-2b

DP BARCODE: D259369 SUBMISSION CODE: S541375
P.C. CODE: 122990 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: Lees, D. (1997) ZA1296: 21 Day Dermal Toxicity Study in Rabbits. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Laboratory Study Number LB0575. Report #CTL/P/5035. October 23, 1997. MRID 44505024. Unpublished.

SPONSOR: Zeneca AG Products, Wilmington, DE

EXECUTIVE SUMMARY: In a repeated-dose dermal toxicity study (MRID 44505024), mesotrione (96.8% purity, Lot/Batch #: P17 [WRC 15213-17-1]) was applied to the clipped intact skin of five New Zealand White albino rabbits/sex/dose at nominal doses of 0 (vehicle control), 10, 500, or 1,000 mg/kg/day (limit dose) for 6 hours/day, 5 days/week, for a total of 15 applications during a 21-day period.

No rabbits died during the course of the study as a result of treatment and no treatment-related clinical signs of systemic toxicity were observed. Slight erythema was observed in 4/10 mid-dose animals and 10/10 high-dose animals; however, all irritation subsided by 22 days (study termination) and no gross or microscopic treatment-related dermal abnormalities were observed upon necropsy. No other treatment-related differences in toxicity, body weights, food consumption, hematology, ophthalmology, clinical chemistry, organ weights, or gross or microscopic pathology were observed between the control and treated groups.

The LOAEL was not established.
The NOAEL is ≥1,000 mg/kg/day (limit dose).

This study is classified **acceptable (§82-2)/Guideline** and does satisfy the requirement for a

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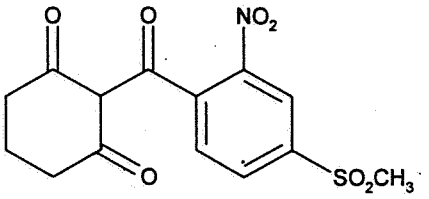
repeated-dose dermal toxicity study. Although a LOAEL was not observed, the test substance was tested up to the limit dose.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Mesotrione
   Description: Light beige solid
   Lot/Batch #: P17 (WRC 15213-17-1)
   Purity: 96.8% a.i.
   Stability of compound: Not monitored, but dosing formulations were prepared daily
   CAS #: 104206-82-8
   Structure:



2. Vehicle - The test material was made into a paste by adding a small amount of deionized water immediately prior to dosing; volumes ranged from 0.05 mL/application for the low-dose to 3 mL/application for the high-dose.

3. Test animals - Species: Albino rabbit
   Strain: New Zealand White
   Age: Young adult
   Body weight (at day 0): males - 2027-2511 g; females - 2162-2629 g
   Source: Charles River UK Limited, Margate, Kent, UK
   Housing: Individually in "suitable" cages
   Diet: STANRAB SQC (Special Diet Services Limited, Witham, Essex, UK), ad libitum
   Water: Tap water, ad libitum
   Environmental conditions:
   Temperature: 17 ± 2 °C
   Humidity: 40-70%
   Air changes: ≥15 changes/hour
   Photoperiod: 12-hour light/dark cycle
   Acclimation period: At least 5 days, during which time they were familiarized with plastic collars

B. STUDY DESIGN

1. In-life dates - start: 02/21/96 end: 03/14/96

2. Animal assignment - Following the acclimation period, rabbits were randomly assigned (stratified by body weight) to the test groups noted in Table 1.

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Table 1. Study design.

Test Group	Dose to animal (mg/kg/day)	Animals assigned	
		Male	Female
Control	0[a]	5	5
Low	10	5	5
Mid	500	5	5
High	1,000[b]	5	5

a Vehicle only
b Limit dose

3. Dose selection rationale - Dose selection was based on the results of a preliminary subacute dermal study conducted prior to the definitive study. The highest dose level was selected because it is the limit dose and the lower doses were chosen to represent a suitable range for the assessment of any effects. No further information was provided.

4. Preparation and treatment of animal skin - Approximately 16-32 hours prior to testing and as necessary thereafter, the fur of each animal was clipped from the dorso-lumbar region (approximately 15 x 13 cm). At each application, the applied quantities of the test substance were adjusted to individual body weight. The test substance was prepared into a paste and evenly applied to the clipped skin. Each site was covered with 4-ply gauze and two overlapping pieces of rubber sheeting and the coverings were secured with polyethylene tape. The rabbits were exposed to the test compound for 6 hours/day, 5 days each week, for 3 weeks (15 days of exposure over a 21-day period). Animals in the control group were treated with deionized water. After each exposure, the dressings were carefully removed and the application areas were cleaned using warm water and blotted dry. For 18 hours following patch removal, each animal was fitted with plastic collars to prevent oral contamination.

5. Statistics - An analysis of variance was applied to the food consumption, hematology, clinical chemistry, and organ weight data. An analysis of covariance was applied to the body weight and organ/body weight data. Each of these tests was followed by a student t-test as needed.

C. METHODS

1. Observations - Detailed clinical observations were conducted once daily following patch removal. In addition, cage-side observations were conducted twice daily, once

following dosing and once towards the end of the day.

2. Body weight - Body weights were recorded once daily and prior to termination on day 22.

3. Food consumption - Food consumption for each rabbit was recorded continuously throughout the study and calculated as a weekly mean of g food/rabbit/day.

4. Ophthalmoscopic examination - Ophthalmoscopy exams were conducted on all animals prior to the start of the study and during the 2 days prior to termination.

5. Blood - Blood was collected from the central ear artery of each animal pre-experimentally and from all females at the end of the treatment period (day 22). It was not specified if the animals were fasted prior to blood collection. The checked (X) parameters were examined in all samples analyzed. Terminal blood collected from male animals (bled by cardiac puncture following lethal injection of Euthatal) was unsuitable for analysis due to contamination of the blood from Euthatal and hemolysis in the majority of samples.

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	Erythrocyte morphology		Reticulocyte count
X	Leukocyte morphology	X	Hemoglobin conc. distr. width (HDW)
X	Platelet count (Thrombocytes)*	X	RBC volume distr. width (RDW)
	Blood clotting measurements*		Methemoglobin (MetHb)
X	(Partial thromboplastin time)		Absolute lymphocytes
	(Capillary clotting time)		Absolute segmented neutrophils
X	(Prothrombin time)	X	RBC morphology
	(Kaolin-cephalin time)		

* Recommended for repeated dose dermal toxicity studies based on Subdivision F guidelines (1998).

b. Clinical chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride*	X	Creatinine*
	Magnesium	X	Urea*
X	Phosphorus	X	Total cholesterol*
X	Potassium*		Globulin
X	Sodium*	X	Glucose*
		X	Total bilirubin
		X	Total protein (TP)*
	ENZYMES* (at least three)	X	Triglycerides
			A/G Ratio
X	Alkaline phosphatase		
	Cholinesterase (ChE)		
X	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (GPT)		
X	Aspartate aminotransferase (GOT)		
X	Gamma glutamyl transferase (GGT)		
	Sorbitol dehydrogenase		

* Recommended for repeated dose dermal toxicity studies based on Subdivision F guidelines (1998).

6. Sacrifice and pathology - On day 22, all rabbits were killed by a lethal injection of Euthatal. The bodies were subjected to gross pathological examination. The checked tissues of all control and high-dose animals were examined histologically and the (XX) organs were weighed.

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	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue		Aorta*		Brain* (with stem)
	Salivary glands*		Heart*		Periph.nerve*
	Esophagus*		Bone marrow*		Spinal cord (3 levels)*
	Stomach*		Lymph nodes*		Pituitary*
	Duodenum*		Spleen*	X	Eyes (optic n.)*
	Jejunum*		Thymus*		
	Ileum*				GLANDULAR
	Cecum*		UROGENITAL	XX	Adrenal gland*
	Colon*	XX	Kidneys*		Lacrimal gland
	Rectum*		Urinary bladder*		Mammary gland*
XX	Liver*	XX	Testes*		Parathyroid*
	Gall bladder*		Epididymides*		Thyroid*
	Pancreas*		Prostate*		
			Seminal vesicle*		OTHER
	RESPIRATORY		Ovaries*		Bone (femur and sternum)
	Trachea*		Uterus*		Skeletal muscle (thigh)
	Lung*		Oviducts	X	Skin* (treated and untreated)
	Nose*		Vagina	X	All gross lesions*
	Pharynx*				
	Larynx*				
	Tonsils				

* Required for control and high-dose groups in repeated dose dermal toxicity studies based on Subdivision F Guidelines (1998).

II. RESULTS

A. Observations

1. Mortality - No treatment-related mortality occurred. One high-dose female was humanely sacrificed on day 12 due to a spinal injury. All remaining animals survived to the end of the study.

2. Toxicity - No treatment-related systemic toxicity was observed during the study. One high-dose female exhibited reduced hindlimb function, urinary incontinence, and diarrhea; necropsy of this animal on day 12 revealed a fractured spine.

   Application areas of all test group animals were stained light brown by the test material. No signs of skin irritation were observed in control and low-dose animals. Slight erythema was observed in one mid-dose male and three mid-dose females and all high-dose animals between days 5 and 21. It was stated that all irritation had completely subsided by the end of the study.

B. Body weight and weight gain - No treatment-related differences in body weights or adjusted (for day 1 body weight) body weights were observed between the treated and control groups.

Slight increases (↑6-8%, $p \leq 0.05$) in adjusted body weight were observed in low-dose females on days 19 and 22 and in high-dose females on days 9 and 19; however, these changes were sporadic and not dose-dependent. At the termination of the study, average weights for all test groups were 2817-2940 g for males and 2762-3037 g for females. In addition, no treatment-related effects on body weight changes were apparent, although data were not tabulated by the sponsor.

C. Food consumption - No treatment-related differences in food consumption were observed between the treated and control groups. It was stated that unusually high food consumption values were recorded during the study and considered to be the result of wastage; these values were excluded from the statistical analysis by the sponsor.

D. Ophthalmoscopic examination - No treatment-related ocular abnormalities were noted.

E. Blood work

1. Hematology - No treatment-related effects in hematology parameters were observed at study termination between the treated and control females (male blood was unusable). The red cell distribution width of high-dose females was slightly higher (↑15%, $p \leq 0.05$) than controls; however, no other changes in red blood cell parameters were noted and this effect was considered not to be treatment related. In mid-dose females, increases ($p \leq 0.05$) in neutrophil (↑62%) and white blood cell counts (↑26%) were observed. Since there was no evidence of a dose-response relationship, the increases were considered to be incidental.

2. Clinical Chemistry - No treatment-related differences in clinical chemistry were observed between the treated and control females (male blood was unusable at study termination). The plasma cholesterol levels of high-dose females were higher (↑33%, $p \leq 0.01$) than the control group at study termination; however, the cholesterol value in the high-dose females at termination (1.28 mmol/L) was lower than the value observed prior to dosing (1.66 mmol/L) and therefore the change in this parameter was considered to be not treatment related. Very high plasma creatine kinase activity was observed in high-dose females at study termination (↑229% vs controls); however, this observation was due to a high value in one animal and was considered to be a spurious finding. Minor decreases ($p \leq 0.01$) at study termination that were not dose-related and were considered to be incidental to treatment included: albumin levels in mid- and high-dose females (↓8% each), total protein in mid-dose females (↓8%), and calcium levels in mid-dose females (↓4%).

F. Sacrifice and pathology

1. Organ weight - No treatment-related differences in absolute or relative organ weights were observed between the treated and control groups. The decrease in liver weight observed in the mid-dose females (↓26%, $p \leq 0.05$) was not observed at other levels and was considered to be incidental.

2. Gross pathology - No findings upon necropsy could be attributed to treatment. At the high-dose, necropsy revealed accessory spleens in one male and one female. It was stated that this finding is common in rabbits of this age and strain and was considered to be incidental to treatment; no historical control data were provided. One mid-dose male had prominent surface vessels on the kidney, which was not observed at the high-dose and was considered incidental to treatment. Necropsy of the single high-dose female that was sacrificed after 12 days revealed a fractured spine, hemorrhaging of the spinal cord, and an accessory spleen.

3. Microscopic pathology -

   a) Non-neoplastic - No microscopic findings could be attributed to treatment.
   b) Neoplastic - No neoplastic tissue was observed in the treated or control rabbits.

III. DISCUSSION

A. Investigator's conclusions - The NOAEL for systemic toxicity of mesotrione is the limit dose of 1,000 mg/kg/day, based on the absence of treatment-related effects in animals of both sexes treated dermally. The dermal NOAEL was also considered to be the limit dose of 1,000 mg/kg/day, based on the absence of treatment-related dermal abnormalities upon study termination.

B. Reviewer's discussion - In a 21-day dermal toxicity study, mesotrione (96.8% a.i.) was applied to the skin of five New Zealand White albino rabbits/sex/dose at nominal dose of 0, 10, 500, or 1,000 mg/kg/day (limit dose) for 6 hours/day, 5 days/week, for a total of 15 applications during a 21-day period.

No treatment-related mortality occurred during the study and no treatment-related differences in toxicity, body weights, food consumption, hematology, ophthalmology, clinical chemistry, organ weights, or gross or microscopic pathology were observed. One high-dose female was sacrificed *in extremis* on day 12 due to a fractured spine after exhibiting reduced hindlimb function, urinary incontinence, and diarrhea. Slight erythema was observed in one mid-dose male and three mid-dose females and all high-dose animals between days 5 and 21; however, all dermal irritation subsided by study termination (day 22) and no gross or microscopic dermal abnormalities were observed upon necropsy of treated

skin. Due to the method of euthanasia, male blood was unsuitable for analysis at study termination.

The LOAEL was not established.
The NOAEL is ≥1,000 mg/kg/day (limit dose).

This study is classified **acceptable (§82-2)/Guideline** and does satisfy the requirement for a repeated-dose dermal toxicity study. Although a LOAEL was not observed, the test substance was tested up to the limit dose.

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

- Blood from the males was unsuitable for terminal analysis.
- Not all required (by Subdivision F guidelines) organs were examined.
- No gross pathology historical control data were provided.