DATA EVALUATION RECORD

MESOTRIONE (ZA 1296) 5/30/2000

Study Type: §82-1(b), Subchronic Oral Toxicity Study in Mice

Work Assignment No. 2-1-52R (MRID 44505022)

Prepared for
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DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity feeding - mice
OPPTS Number: 870.3100 OPP Guideline Number: §82-1(a)

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

TEST MATERIAL (PURITY): Mesotrione (96.8% a.i.)

SYNONYMS: ZA 1296; 2-[4-(methylsulfonyl)-2-nitrobenzoyl]-1,3-cyclohexanedione; 2-(4-mesyl-2-nitrobenzoyl)-cyclohexamne-1,3-dione

CITATION: Pinto, P.J. (1997) ZA 1296: 90 Day Dietary Toxicity Study in Mice. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire. Laboratory Project Report No. CTL/P/5488. Laboratory Project Study No. PM1062. November 5, 1997. MRID 44505022. Unpublished.

SPONSOR: Zeneca Ag Products, Wilmington, DE

EXECUTIVE SUMMARY: In this subchronic oral toxicity study (MRID 44505022), mesotrione (ZA 1296; 96.8% a.i.; Batch No. P17) was administered for 13 weeks to 20 C57BL/10J$_f$CD-1 mice/sex/dose at dietary concentrations of 10, 50, 350, or 7000 ppm (equivalent to 1.7, 8.4, 61.5, and 1212.4 mg/kg/day in males and 2.4, 12.4, 80.1, and 1537.1 mg/kg/day in females). Two control groups of 20 C57BL/10J$_f$CD-1 mice/sex (40/sex) were fed untreated diet during the study.

Mortality, clinical observations, body weight, body weight gain, food consumption and utilization, ophthalmoscopic observations, hematology and clinical chemistry parameters, organ weights, and gross and microscopic pathological findings were unaffected by the test substance.

The NOAEL for this study is 7000 ppm (equivalent to 1212/1537 mg/kg/day [M/F]). The LOAEL was not observed.

The submitted study is classified as **acceptable/guideline (§82-1)** and satisfies the requirements for a subchronic oral toxicity study in rodents. Although a LOAEL was not observed, the study was tested up to the limit dose.

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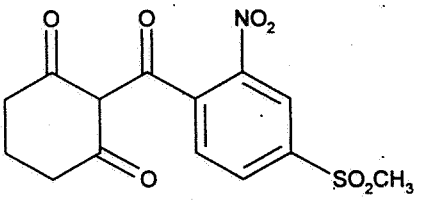
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Mesotrione (ZA 1296)
   Description: Light beige solid
   Lot/Batch #: P17
   Purity: 96.8% a.i.
   Stability of compound: The test substance was stable when stored at ambient temperatures in the dark for at least 2 years. It was stated that the sample was used within the stated expiration date (not reported), and its stability confirmed by re-analysis after the in-life phase of the study.
   CAS #: 104206-82-8
   Structure:



2. Vehicle: Diet

3. Test animals: Species: Mouse
   Strain: C57BL/10J$_f$CD-1
   Age and weight at study initiation: Approximately 5 weeks old; 15.6-23.3 g (males), 14.9-19.2 g (females)
   Source: Barriered Animal Breeding Unit, Zeneca Pharmaceuticals, Alderley Park
   Housing: In multiple mouse racks, five mice per cage, separated by sex.
   Diet: CT1 Diet (Special Diet Services Ltd., Stepfield, Witham, Essex, UK), ad libitum
   Water: Tap water, ad libitum
   Environmental conditions:
   Temperature: 22 ± 3°C
   Humidity: 30-70%
   Air changes: At least 15/hour
   Photoperiod: 12 hours light:12 hours dark
   Acclimation period: At least 5 days

B. STUDY DESIGN:

1. In life dates - start: 08/15/96 end: 11/21/96

2. Animal assignment - Animals were randomly assigned (stratified by body weight) to treatment groups as indicated in Table 1.

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Table 1. Study design

Test Group	Conc. in Diet (ppm)	Achieved Dose[a] (mg/kg/day) [M/F]	Males	Females
Control	0	0/0	20	20
Control	0	0/0	20	20
Low	10	1.7/2.4	20	20
Low-mid	50	8.4/12.4	20	20
Mid	350	61.5/80.1	20	20
High	7000	1212/1537	20	20

a Achieved dosages (mg/kg/day) were obtained from the study report Appendix F, pages 98 and 99.

3. Dose selection rationale - Dose selection for the current study was based on the results of a 6-week dietary toxicity study in the mouse conducted in this laboratory (no further information provided). The highest dose level of 7000 ppm (equivalent to 1050 mg/kg/day) selected for the current study is considered to be the limit dose for this strain of mouse.

4. Diet preparation and analysis - Diets were prepared by mixing the appropriate amounts of test substance with the feed and stored in plastic bins at -20°C. The diets were thawed for approximately one hour prior to use. Duplicate samples (top, middle, bottom) of the 10 ppm and 7000 ppm diet formulations were analyzed for homogeneity 9 days prior to study initiation and during study week 2. Stability of ZA 1296 in the diet was tested during previous studies (PR 1001 and PM0983). In those studies, samples of 1 ppm and 7000 ppm diet formulations were analyzed for stability following storage at room temperature for up to 9 and 16 days, respectively, or at -20°C for up to 57 and 40 days, respectively. Concentration was analyzed in all diets prepared for the current study 9 days prior to study initiation and during study week 10.

Concentration analysis (range as mean % of nominal): 99.2-106.0%

Homogeneity (range as mean % of nominal): 93.4-108.6%

Stability (% of day 0):
Room temperature: 84.8-107.4%
-20°C: 87.5-114.6%

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

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4. Statistics - Body weight data were analyzed for each sex by analysis of covariance. Food consumption and food utilization data were analyzed by analysis of variance (ANOVA). Male and female blood chemistry data were analyzed together by ANOVA. All analysis of variance and analysis of covariance were followed by a Student's t-test.

C. METHODS:

1. Observations - Animals were observed daily for changes in clinical condition or behavior. Detailed clinical observations were performed weekly.

2. Body weight - Animals were weighed prior to randomization and weekly throughout the study. Cumulative body weight gains for each weekly interval were calculated for individual animals.

3. Food consumption and compound intake - Food consumption was measured for each cage of mice at weekly intervals throughout the treatment period and a weekly mean (g/animal/day) was calculated for each cage. Food utilization was calculated as body weight gained per cage/100 g food consumed. Mean compound intake was calculated as mg/kg/day using nominal test concentration, food consumption, and body weight data.

4. Ophthalmoscopic examination - Ophthalmoscopic examinations were performed on all control and high-dose animals at the end of the dosing period.

5. Blood - Blood, obtained by cardiac puncture, was collected from 10 animals/sex/group at the end of the dosing period. It was not reported if the animals were fasted prior to blood collection. 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		
	(Thromboplastin time)		
	(Activated partial thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

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b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin
X	Chloride	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen
X	Phosphorus		Total Cholesterol
X	Potassium		Globulins (Calculated)
X	Sodium	X	Glucose
			Direct bilirubin
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total serum protein (TP)
	Cholinesterase (ChE)		
X	Creatine kinase		
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine aminotransferase (also SGPT)		
X	Serum aspartate aminotransferase (also SGOT)		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

6. Urinalysis - Urinalysis was not performed and is not required based on Subdivision F guidelines for subchronic studies.

7. Sacrifice and Pathology - All mice were killed by exsanguination upon study termination. Ten mice/sex/group were subjected to a gross pathological examination. The following CHECKED (X) tissues were collected. Additionally, the (XX) organs were weighed. All collected tissues, except the oral and nasopharyngeal cavities, from the control and high-dose animals and animals killed prematurely were examined microscopically. In addition, lungs, liver, and kidneys from all animals were examined microscopically.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta	XX	Brain (cerebrum, cerebellum, and brainstem)
X	Salivary glands	X	Heart		
X	Esophagus	X	Bone marrow	X	Periph. nerve
X	Stomach	X	Lymph nodes	X	Spinal cord (cervical, thoracic, lumbar)
X	Duodenum	X	Spleen		
X	Jejunum	X	Thymus	X	Pituitary gland
X	Ileum			X	Eyes
X	Cecum		UROGENITAL		
X	Colon	XX	Kidneys		GLANDULAR
X	Rectum	X	Urinary bladder	XX	Adrenal gland
XX	Liver	XX	Testes	X	Harderian gland
X	Gall bladder	X	Epididymides		Lacrimal gland
X	Pancreas	X	Prostate	X	Mammary gland
X	Oral cavity	X	Seminal vesicle	X	Thyroids
		XX	Ovaries	X	Parathyroids
	RESPIRATORY	X	Oviduct	X	Prepuptial gland
X	Trachea	X	Uterus		
X	Lung	X	Cervix		OTHER
	Nose			X	Bone (femur, sternum)
	Pharynx			X	Skeletal muscle
	Larynx			X	Skin
X	Nasopharyngeal cavity			X	All gross lesions and masses

II. RESULTS

A. Observations :

1. Mortality - No treatment-related deaths occurred during the study. Two control (1/sex), two 50-ppm females, one 350-ppm female, and one 7000-ppm male were euthanized during weeks 3, 4, 6, or 13. All of the unscheduled deaths were considered to be incidental and not treatment-related.

2. Clinical signs - No treatment-related clinical signs of toxicity were noted during the study. Although the 7000-ppm males exhibited an increased incidence in hair loss (8/20 vs. 5/39 controls) and reduced numbers of whiskers (14/20 vs. 12/39 controls), these findings were considered not to be toxicologically important.

B. Body weight - No treatment-related changes in body weights were observed in any treatment group. Decreased body weights (Table 2) observed in the 7000-ppm males during 13/14 intervals (↓3-5%, $p \leq 0.01$) were minor and considered not to be toxicologically important. Other minor differences in body weights were observed in all treatment groups at various times throughout the study (↓3-↑6%, $p \leq 0.05$ or 0.01), but none were considered treatment-related.

Body weight gain (as calculated by the reviewers) decreased 17% in males at 7000 ppm; however, decreases of 12.3% at 10 and 50 ppm and 4.9% at 350 ppm did not show any dose-

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related pattern, so the effect was considered not toxicologically significant. Body weight gain in females was comparable between the treated and control groups.

Table 2. Selected mean body weights (g) in mice after treatment with ZA 1296 for 90 days.[a]

Dose (ppm)	Week 1	Week 3	Week 4	Week 6	Week 10	Week 13	Week 14	Overall Body Weight Gain[c] Weeks 1-14
Males								
0[b]	20.6	22.6	23.5	24.9	26.9	28.0	28.7	8.1
10	20.7	22.5	23.3	24.8	26.3	27.4	27.8* (↓3%)	7.1 (↓12.3%)
50	20.9	22.4	23.2	24.7	26.4	27.3	27.8* (↓3%)	7.1 (↓12.3%)
350	20.5	22.6	23.4	25.2	26.8	27.9	28.2	7.7 (↓4.9%)
7000	20.6	21.9** (↓3%)	22.6** (↓4%)	23.9** (↓4%)	25.7** (↓4%)	26.6** (↓5%)	27.3** (↓5%)	6.7 (↓17%)
Females								
0[b]	17.1	18.3	19.0	21.0	22.6	23.6	24.3	7.3
10	16.9	18.5	19.7* (↑4%)	21.3	23.0	24.0	24.9* (↑2%)	7.9
50	16.9	18.8* (↑3%)	20.1** (↑6%)	21.7* (↑3%)	23.3** (↑3%)	23.9	25.1** (↑3%)	8.0
350	16.9	18.9** (↑3%)	20.0** (↑5%)	21.6* (↑3%)	23.3** (↑3%)	24.5** (↑4%)	25.3** (↑4%)	8.4
7000	16.9	18.1	18.8	20.6	22.3	23.3	24.0	7.1

a Data obtained from study report Table 5, pages 47 through 50. Percent difference from controls is listed parenthetically. Adjusted (for initial body weight) mean data were used.
b Data from two control groups (20/group/sex) were averaged by the reviewer.
c Body weight gains were calculated by the reviewers.
* Statistically different from pooled controls at $p \leq 0.05$.
** Statistically different from pooled controls at $p \leq 0.01$.

C. Food and compound intake/water consumption

1. Food consumption and utilization - No treatment-related differences in food consumption were observed in any treatment group. Reduced (↓13%, $p \leq 0.05$) food consumption was observed in the 7000-ppm females during week 3. Increases ($p \leq 0.05$ or 0.01) in food consumption were observed in the following groups: the 350-ppm males during 5 of 13

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weekly intervals (↑5-10%); the 350-ppm females during 2/13 weekly intervals (↑6-10%); the 50-ppm females during 11/13 weekly intervals (↑8-21%); and the 10-ppm females during 8/13 weekly intervals (↑8-13%). The differences in food consumption were not dose-dependent and therefore, considered not to be treatment-related. Decreases ($p \leq 0.05$) in cumulative food utilization which were not dose-dependent and therefore, considered not to be treatment-related, were observed as follows: (i) in the 7000-ppm males during weeks 1-4 (↓30%) and weeks 1-13 (↓17%); (ii) in the 350-ppm males during weeks 5-8 (↓24%); (iii) in the 50-ppm males during weeks 1-13 (↓13%), and (iv) in the 10-ppm males during weeks 9-13 (↓28%) and 1-13 (↓14%). Increased ($p \leq 0.05$ or 0.01) cumulative food utilization was observed in the 7000-ppm females during weeks 9-13 (↑23%), and in the 350-ppm females during weeks 1-13 (↑10%), but was not dose-dependent and therefore, considered not to be treatment-related.

Table 3. Selected food utilization values (g growth/100 g food) in mice after treatment with ZA 1296 for 90 days.[a]

Dose (ppm)	Weeks 1-4	Weeks 5-8	Weeks 9-13	Weeks 1-13
Males				
0[b]	3.30	1.62	1.62	2.11
10	2.94	1.61	1.16* (↓28%)	1.82* (↓14%)
50	2.99	1.46	1.29	1.84* (↓13%)
350	3.35	1.23* (↓24%)	1.33	1.90
7000	2.30* (↓30%)	1.56	1.44	1.75* (↓17%)
Females				
0[b]	2.85	1.42	1.06	1.68
10	2.88	1.39	1.14	1.69
50	3.23	1.21	0.98	1.66
350	3.32	1.36	1.20	1.83** (↑10%)
7000	2.48	1.57	1.29* (↑23%)	1.69

a Data obtained from the study report Table 7, page 55. Percent difference from controls is listed parenthetically.
b Data from 8 control cages/sex (20/group) were averaged by the reviewer.
* Statistically different from controls at $p \leq 0.05$.
** Statistically different from controls at $p \leq 0.01$.

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2. Compound consumption - The achieved mean dosages based on nominal dietary concentrations, actual body weights, and actual food consumption are shown in Table 1.

3. Water consumption - Water consumption was not measured.

D. Ophthalmoscopic examination - No treatment-related ocular effects were observed.

E. Blood analyses

1. Hematology - Decreases in the following hematology parameters were observed in the 7000-, 350-, 50-, and 10-ppm males ($p \leq 0.05$ or 0.01): white blood cells (↓45, 40, 39, and 34%, respectively); lymphocytes (↓47, 40, 40and 36%, respectively); monocytes (↓61, 50, 51, and 38%, respectively); and eosinophils (↓45, 57, 54, and 39%, respectively). It was stated that the decreases observed in these hematology parameters were due to abnormally high concurrent control values, but no historical control data were provided. However, no effects on white blood cell counts or differential counts were seen in male mice in a longer-term carcinogenicity study (MRID 44505028). Therefore, the effects were not considered to be related to administration of the test compound. Other differences ($p \leq 0.05$ or 0.01) that were considered not to be treatment-related because they were not dose-dependent and/or not toxicologically significant were decreased neutrophils (↓47%) in the 350-ppm males, decreased eosinophils (↓69%) in the 350-ppm females, and increased (↑163%) large unstained cells in the 10-ppm females. Minor differences (↓4-↑4%, $p \leq 0.05$ or 0.01) observed sporadically in several hematological parameters in the 7000-, 350-, and 50-ppm groups were considered not to be toxicologically important.

2. Clinical chemistry - No treatment-related effects in clinical chemistry parameters were observed in any treatment group. Increased ($p \leq 0.05$) phosphorus in the 7000- (↑26%), 50- (↑13%), or 10- (↑30%) ppm males when compared to concurrent controls was not dose-dependent and therefore, considered not to be treatment-related. Increased ($p \leq 0.05$) alanine aminotransferase in the 7000- (↑40%) and 350- (↑31%) ppm males appeared to be the result of low values for two control males and high values for single 7000- and 350-ppm males. The males displayed additional changes ($p \leq 0.05$ or 0.01) considered to be unrelated to treatment due to lack of a dose response: increased alkaline phosphatase (↑18%) at 350 ppm; decreased calcium (↓6%) at 50 ppm; and, increased urea (↑34%) and potassium (↑20%) at 10 ppm. In the female treatment groups, increased ($p \leq 0.01$) phosphorus in the 7000- (↑41%) and 350- (↑22%) ppm groups and decreased urea (↓26%) at 7000 ppm lacked corroborating toxicological evidence to suggest that the increases were due to the test substance. Other differences ($p \leq 0.05$ or 0.01) observed in treated females which were considered unrelated to treatment due to lack of a dose response were decreased alkaline phosphatase (↓8%) at 350 ppm and increased chloride (↑2%) at 10 ppm.

F. Sacrifice and Pathology:

1. Organ weight - No treatment-related differences in absolute or relative organ weights were observed. Increased absolute liver weight (↑10%, $p \leq 0.05$) in the 50-ppm females compared to concurrent controls was not dose-related and therefore, was considered not to be treatment-related. Increased ($p \leq 0.05$ or 0.01) absolute liver weights (adjusted for final body weight) in the 7000-ppm males (↑5%) and in the 7000- and 50-ppm females (each, ↑7%) were minor and considered not to be treatment-related.

2. Gross pathology - No test substance-related findings in gross pathology were observed.

3. Microscopic pathology - No test substance-related findings in microscopic pathology were observed.

III. DISCUSSION

A. Investigator's conclusions - Oral administration of ZA 1296 for 13 weeks was systemically toxic at 7000 ppm. The test compound at this dose caused reduced body weights and food utilization in males. The NOAEL for this study was 350 ppm.

B. Reviewer's discussion - In this subchronic oral toxicity study, mesotrione (ZA 1296) was administered for 13 weeks to 20 C57BL/10J$_f$CD-1 mice/sex/dose at dietary concentrations of 10, 50, 350, or 7000 ppm (equivalent to [M/F] 1.7/2.4, 8.4/12.4, 61.5/80.1, and 1212.4/1537.1 mg/kg/day, respectively). Two groups of 20 C57BL/10J$_f$CD-1 mice/sex (40/sex) fed untreated diet during the study served as controls. Clinical signs, body weight, body weight gain, food consumption, food utilization, ophthalmoscopic observations, clinical chemistry, organ weights, and gross and microscopic pathological findings were unaffected by the test substance.

Two control (1/sex), two 50-ppm females, one 350-ppm female, and one 7000-ppm male were euthanized during weeks 3, 4, 6, or 13. All of the unscheduled deaths were considered to be incidental and not treatment-related. Decreases in the following hematology parameters were observed in the 7000-, 350-, 50-, and 10-ppm males ($p \leq 0.05$ or 0.01): white blood cells (↓45, 40, 39, and 34%, respectively); lymphocytes (↓47, 40, 40, and 36%, respectively); monocytes (↓61, 50, 51, and 38%, respectively); and eosinophils (↓45, 57, 54, and 39%, respectively). It was stated that the decreases observed in these hematology parameters were due to abnormally high concurrent control values, but no historical control data were provided. However, no effects on white blood cell counts or differential counts were seen in male mice in a longer-term carcinogenicity study (MRID 44505028). Therefore, the effects were not considered to be related to administration of the test compound.

The NOAEL for this study is 7000 ppm (equivalent to 1212 mg/kg/day in males and 1537 mg/kg/day in females).

The LOAEL was not observed.

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The submitted study is classified as **acceptable/guideline (§82-1)** and satisfies the requirements for a subchronic oral toxicity study in rodents. Although a LOAEL was not observed, the study was tested up to the limit dose.

C. Study deficiencies - The following deficiencies were noted, but will not change the conclusions of this review. First, not all of the organs and tissues required by §82-1 were collected and weighed. The spleen, heart, thymus and epididymides should have been weighed. In addition, a measure of blood clotting potential, such as prothrombin time or activated partial thromboplastin time, should have been performed. Lastly, no dose rationale was provided; however, the animals were treated at the limit dose.