DATA EVALUATION RECORD

7/19/2000

MESOTRIONE (ZA1296)

Study Type: §82-1(a), 90 Day Feeding Study in Rats

Work Assignment No. 2-01-52P (MRID 44505019)

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

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

Primary Reviewer Kelley Van Vreede, M.S.	Signature: Kelley Van Vreede Date: 4/18/00
Secondary Reviewer Mary L. Menetrez, Ph.D.	Signature: Mary L Menetrez Date: 4/18/00
Program Manager Mary L. Menetrez, Ph.D.	Signature: Mary L Menetrez Date: 4/18/00
Quality Assurance Steve Brecher, Ph.D.	Signature: Steven Brecher Date: 4/18/00

Disclaimer

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

165

EPA Reviewer: David Nixon, D.V.M.
Registration Action Branch 1/HED (7509C)

Work Assignment Manager: Marion Copley, D.V.M., D.A.B.T.
Registration Action Branch 1/HED (7509C)

DATA EVALUATION RECORD

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

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

TEST MATERIAL (PURITY): Mesotrione (93.3% a.i.)

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

CITATION: Horner, J. M. (1995) ZA1296: 90 Day Dietary Toxicity Study in Rats. Zeneca Central Toxicology Laboratory, Cheshire, UK. Laboratory Report No. CTL/P/4456, February 8, 1995. MRID 44505019. Unpublished.

SPONSOR: Zeneca, Inc. Agricultural Products, Wilmington, DE

EXECUTIVE SUMMARY: In this subchronic oral toxicity study (MRID 44505019), mesotrione (93.3% a.i., Lot/batch # P8) was administered for 90 days to 12 Alpk:AP$_f$SD rats/sex/dose at dietary concentrations of 0, 1, 125, 1250, or 12500 ppm (equivalent to [M/F] 0/0, 0.09/0.10, 11/13, 112/126, and 1111/1213 mg/kg/day, respectively).

No treatment-related findings were observed in the 1 ppm group. No mortalities occurred during the study. Hematology, clinical chemistry, and urinalysis parameters were unaffected by the test substance.

The eye was the main target organ. At the clinical, ophthalmoscopic, and gross pathological examinations, corneal lesions (eye opacity and vascularization) were observed in both sexes of the 125, 1250, and 12500 ppm dose groups. Upon histological examination, the corneal lesions were characterized as keratitis.

In the 125 ppm males, mean body weights (adjusted for week 1 body weight) were decreased ($p \leq 0.01$ or 0.05) sporadically throughout the first half of the study (↓2-5%) and from week 10 through study termination (↓6-8%). Body weights in the 125 and 1250 ppm females were also decreased ($p \leq 0.05$) sporadically throughout the study. Overall (weeks 1-14) body weight gains

were decreased in both sexes of the 125, 1250, and 12500 ppm dose groups (↓4-23%, calculated by reviewers). Biological significance was noted at ≥ 125 ppm in males and at 12500 in females. Overall food utilization (weeks 1-13) was decreased ($p \leq 0.01$) in the males (↓7-13%) at ≥ 125 ppm, but was only considered biologically relevant at 1250 and 12500 ppm.

Additionally in the 12500 ppm group, decreased ($p \leq 0.01$ or 0.05) mean body weights (↓6-16%) and food consumption (↓9-18%) were observed throughout the study.

In the 125, 1250, and 12500 ppm animals, increased ($p \leq 0.01$ or 0.05) adjusted (for final body weight) liver (↑11-19%) and kidney (↑10-14%, males only) weights were observed. There were no corroborative histological findings to indicate an adverse effect on these organs.

The LOAEL for this study is 125 ppm (equivalent to 11 mg/kg/day in males and 13 mg/kg/day in females) based on corneal abnormalities observed during the clinical, ophthalmoscopic, gross pathological, and histopathological examinations in both sexes, and decreases in body weight gain in males.

The NOAEL for this study is 1 ppm (equivalent to 0.09 mg/kg/day in males and 0.10 mg/kg/day in females).

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

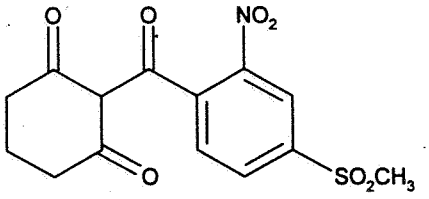
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

3

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Mesotrione (ZA1296)
   Description: Beige solid
   Lot/Batch #: P8
   Purity (w/w): 93.3% a.i.
   Stability of compound: The test substance was stable for 6 months when stored at ambient temperatures in the dark.
   CAS #: 104206-82-8
   Structure:



2. Vehicle: Diet

3. Test animals: Species: Rat
   Strain: Alpk:AP$_f$SD
   Age at start of dosing and mean weight at week 1: At least 46 days old; 149.8-154.7 g (males), 130.7-133.9 g (females)
   Source: Barriered Animal Breeding Unit, Zeneca Pharmaceuticals, Cheshire, UK
   Housing: 3/cage in stainless steel wire mesh cages
   Diet: CT1 diet (Special Diet Services, Ltd., Essex, UK), ad libitum, except during urine collection
   Water: Tap water, ad libitum, except during urine collection
   Environmental conditions:
   Temperature: 17-27° C
   Humidity: 18-70%
   Air changes: At least 15/hour
   Photoperiod: 12 hours light/12 hours dark
   Acclimation period: Approximately 2 weeks

B. STUDY DESIGN:

1. In life dates: Start: 12/20/93 End: 3/18/94 (assumed by reviewer)

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

145 4

Table 1. Study design

Test Group	Dietary Concentration (ppm)	Achieved Dose [a] (mg/kg/day) [M/F]	Males	Females
Control	0	0/0	12	12
Low	1	0.09/0.10	12	12
Mid	125	11/13	12	12
Mid-high	1250	112/126	12	12
High	12500	1111/1213	12	12

a Mean achieved dosages (mg/kg/day) were obtained from the study report page 96 and were rounded to the nearest whole number by the reviewer.

3. Dose selection rationale - The doses chosen for the current study were based on the results of a 28-day feeding study in Alpk:AP$_f$SD rats in this laboratory (no further information provided).

4. Diet preparation and analysis - Diets were prepared by mixing the test substance with food to obtain a premix and then further diluting the premix with food to obtain the desired concentrations. The frequency of diet preparations was not provided; however all test diets were stored at -20°C. Homogeneity was assessed by testing samples (top, middle, bottom) from the 1 and 12500 dose formulations. Stability of the test substance in the diet stored at room temperature and -20°C for 68 days was determined for the 12500 ppm dose formulation only. Stability of the 1 ppm dose formulation was not possible due to analytical problems. Concentration analysis was performed on samples from the 1, 125, 1250, and 12500 ppm dose formulations prepared on three separate occasions; however, all diets were not analyzed on each occasion.

Results -
Homogeneity analysis (range as-mean % of nominal):
Samples (1 ppm and 12500 ppm) prepared on 12/14/93: 76-105%
Samples (1 ppm only) prepared on 2/17/94: 62-73%

Stability analysis (range as mean % of day 0):
Stored at room temperature: 96.5-103%
Stored at -20°C: 94.9-102.9%

Concentration analysis (range as mean % of nominal): 96.8-108%

5
449

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

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

C. METHODS:

1. Observations - Animals were inspected once daily for mortality and clinical signs of toxicity. Detailed clinical examinations were performed weekly.

2. Body weight - Each animal was weighed immediately prior to dosing, weekly throughout the study, and before necropsy.

3. Food consumption - Food consumption was measured continuously throughout the study and calculated weekly (g/rat/day). Food utilization was calculated as the body weight gained/cage/100 g food consumed.

4. Water consumption - Water consumption was not reported.

5. Ophthalmoscopic examination - Ophthalmoscopic examinations were performed on all test animals prior to the start of treatment and within one week of study termination.

6. Blood - Upon study termination, blood was collected from all rats via cardiac puncture. 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)		
X	(Prothrombin time)		
X	(Partial thromboplastin time with kaolin)		

150 6

b. Clinical Chemistry

	ELECTROLYTES
X	Calcium
X	Chloride
	Magnesium
X	Phosphorus
X	Potassium
X	Sodium
	ENZYMES
X	Alkaline phosphatase (ALK)
	Cholinesterase (ChE)
X	Creatine phosphokinase
	Lactic acid dehydrogenase (LDH)
X	Serum alanine aminotransferase (ALT)
X	Serum aspartate aminotransferase (AST)
X	Gamma glutamyl transferase (GGT)
	Glutamate dehydrogenase

	OTHER
X	Albumin
X	Blood creatinine
X	Blood urea nitrogen
X	Total cholesterol
	Globulin
X	Glucose
	Direct bilirubin
X	Total bilirubin
X	Total serum protein (TP)
X	Triglycerides
	Electrophoretic protein fractions

7. Urinalysis - During the last week of the study, urine was collected from all animals over a 16-18 hour period. During urine collection, rats were housed in metabolism cages and fasted. The checked (X) parameters were examined.

X	Appearance
X	Volume
X	Specific gravity
X	pH
X	Sediment (microscopic)
	Protein

X	Glucose
X	Ketones
	Bilirubin
X	Occult blood
	Nitrites
X	Urobilinogen
	Leukocytes
	Sodium
	Potassium
	Chloride

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; the tissues (except for the nasal passages and oral cavity) from the control and high-dose animals were examined microscopically. Furthermore, the kidneys (males only) and eyes from the intermediate groups were examined microscopically. Kidney sections from all male rats were stained with the Martius Scarlet Blue technique to evaluate the kidney tubules for the presence of hyaline droplets. 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	X	Thyroids w/ parathyroids
X	Pancreas	X	Epididymides		
		X	Prostate		OTHER
	RESPIRATORY	X	Seminal vesicle	X	Bone (Femur and sternum)
X	Trachea	X	Ovaries	X	Skeletal muscle
X	Lung	X	Uterus	X	Skin
X	Pharynx		Vagina	X	All gross lesions and masses
X	Larynx	X	Cervix	X	Nose
				X	Harderian gland
				X	Oral cavity

II. RESULTS

A. Observations

1. Mortality - No mortalities occurred during the study.

2. Clinical signs - Selected clinical signs are presented in Table 2. Eye opacity was observed during weeks 10-14 in the 125, 1250, and 12500 ppm males (11/12, 10/12, and 7/12 animals, respectively) and in the 1250 and 12500 ppm females (8/12, each). Although the incidence of eye opacity did not appear to be dose-dependent, it was considered to be treatment-related because of corneal effects observed during the ophthalmoscopic and pathological examinations.

Table 2. Selected clinical observations noted during weeks 10-14 in rats treated with mesotrione for 90 days. [a]

Observation	Males					Females				
	Dose (ppm)					Dose (ppm)				
	0	1	125	1250	12500	0	1	125	1250	12500
Eye Opacity	0	0	11(45)	10(45)	7(33)	0	0	0	8(29)	8(38)

a Data obtained from the study report Table 6, pages 41 and 43; n=12. Data presented as number of affected animals. Number of observations is listed parenthetically.

B. Body weight and body weight gain - Mean body weights (adjusted for week 1 body weight) were decreased ($p \leq 0.01$) compared to concurrent controls in the 12500 males (↓7-16%) and females (↓6-10%) throughout the study (Table 3). In the 1250 ppm males, adjusted body weights were decreased ($p \leq 0.01$ or 0.05) in the males at week 2 (↓2%) and from week 6 until the end of the study (↓5-8%). In the 125 ppm males, adjusted body weights were decreased ($p \leq 0.01$ or 0.05) sporadically throughout the first half of the study (↓2-5%) and from week 10 through study termination (↓6-8%). Adjusted body weights in the 125 and 1250 ppm females were decreased ($p \leq 0.05$) sporadically throughout the study (↓4-5%); however, there was no sustained decrease until the last two weeks of the study in the 1250 ppm females (↓5%). Overall (weeks 1-14) body weight gains were decreased in all treated groups (↓4-23%, calculated by reviewers) except for the 1 ppm females.

9

Table 3. Selected mean body weights (adjusted for week 1 body weights) and overall body weight gains (g) in rats treated with mesotrione for 90 days.[a]

Week	Dose (ppm)				
	0	1	125	1250	12500
Males					
1	150.3±10.2	150.2±10.4	150.6±11.3	154.7±13.4	149.8±12.3
2	204.4±11.1	204.0±12.0	201.0**±13.0 (↓2)	200.8**±14.8 (↓2)	189.7**±14.7 (↓7)
6	360.0±29.6	363.2±16.9	342.4*±20.8(↓5)	341.9*±23.4(↓5)	318.1**±25.9(↓12)
8	406.4±36.0	406.1±22.4	389.7±24.9	383.1*±33.2(↓6)	354.2**±30.0(↓13)
10	445.7±43.0	441.7±23.2	418.3*±28.9(↓6)	417.0*±34.6(↓6)	381.2**±32.6(↓14)
11	464.2±43.6	454.3±24.7	433.3*±28.4(↓7)	430.6**±37.0(↓7)	392.0**±32.4(↓16)
14	491.0±49.4	478.6±25.2	450.9**±27.7(↓8)	449.9**±35.2(↓8)	414.6**±34.2(↓16)
Overall (weeks 1-14) Body Weight Gain	339.4	326.8	299.4 (↓12)	301.2 (↓11)	262.5 (↓23)
Females					
1	131.6±7.6	131.4±9.9	132.3±8.6	133.9±8.2	130.7±7.9
2	160.2±8.4	160.7±8.9	157.8±9.2	156.5±10.2	150.5**±8.3(↓6)
6	228.1±12.9	229.4±15.9	220.7±15.2	219.3±18.1	208.6**±11.8(↓9)
8	244.5±12.2	243.8±15.4	234.2*±16.3(↓4)	235.0±16.6	224.6**±12.1(↓8)
10	255.1±12.0	259.7±18.7	248.5±16.3	247.4±19.5	234.3**±13.8 (↓8)
11	260.6±12.9	266.9±17.2	252.2±15.8	252.6±16.0	234.4**±12.1 (↓10)
14	272.2±11.6	280.1±17.4	266.0±16.0	259.4*±16.1(↓5)	246.6**±14.4 (↓9)
Overall (weeks 1-14) Body Weight Gain	140.2	148.1	134.0 (↓4)	127.5 (↓9)	114.6 (↓18)

a Data obtained from the study report Table 7, pages 46 through 49; n=12. 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 $p \leq 0.01$.

10

C. Food consumption/utilization and compound intake

1. Food consumption - Mean food consumption is presented in Table 4. Food consumption was decreased ($p \leq 0.01$ or 0.05) in the 12500 ppm males (↓9-15%) and females (↓11-18%) throughout the study (only the decreases in the males at weeks 5 and 6 were not statistically significant). Other decreases in food consumption were observed in the 125 ppm males and the 125 and 1250 ppm females (↓7-11%, $p \leq 0.05$); however, these differences were either not dose-dependent or not sustained over time. No differences in food consumption relative to concurrent controls were observed in the 1 ppm animals.

Table 4. Food consumption (g/rat/day) in rats treated with mesotrione for 90 days.[a]

Week	Dose (ppm)				
	0	1	125	1250	12500
Males					
1	26.8±0.9	25.9±1.2	25.8±0.3	26.6±0.8	22.7**±0.8(↓15)
2	29.2±0.7	28.4±1.3	28.6±0.8	30.1±0.6	26.5**±0.7(↓9)
5	31.3±1.6	30.6±1.7	29.4±0.7	30.0±2.0	28.4±3.0
9	31.9±1.7	30.0±1.9	29.6*±1.2(↓7)	31.0±0.9	27.6**±1.2(↓13)
13	28.5±1.8	27.4±0.7	26.5*±0.9(↓7)	28.2±0.8	25.9*±1.6(↓9)
Females					
1	21.5±0.9	21.4±1.3	21.0±0.9	20.9±1.5	18.9**±1.5(↓12)
2	24.0±1.9	22.3±1.7	21.7*±0.5(↓10)	21.3*±1.1(↓11)	19.8**±1.3(↓18)
5	23.3±1.4	22.3±0.6	22.8±0.6	22.8±1.0	20.7**±0.6(↓11)
9	22.3±1.1	22.7±0.6	22.4±0.9	22.1±0.9	19.3**±0.8(↓13)
13	21.3±2.2	21.3±1.3	20.8±0.9	20.0±0.5	18.7**±0.5(↓12)

a Data obtained from the study report Table 8, pages 50 through 51; n=12. 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. Food utilization - In the males, food utilization was decreased at 125, 1250, and 12500 ppm throughout the study (↓2-24%). Statistically significant ($p \leq 0.01$ or 0.05, Table 5) decreases were observed as follows: during weeks 1-4 at 12500 ppm (↓8%), weeks 5-8 at 1250 and 12500 ppm (↓13-19%), and weeks 9-13 at 125 and 1250 ppm (↓22-24%). In addition, overall food utilization (weeks 1-13) was decreased ($p \leq 0.01$) in the 125, 1250, and 12500

ppm males (↓7, 10, and 13%). No other differences from concurrent controls were observed in food utilization.

Table 5. Food utilization (g growth/100 g food) in rats treated with mesotrione for 90 days.[a]

Weeks	Dose (ppm)				
	0	1	125	1250	12500
Males					
1-4	21.91	22.96	21.54	20.83	20.09**(↓8)
5-8	10.63	10.55	10.02	9.23*(↓13)	8.60**(↓19)
9-13	5.97	5.14	4.53**(↓24)	4.66*(↓22)	5.02
Overall (1-13)	12.19	12.22	11.39**(↓7)	11.01**(↓10)	10.65**(↓13)

a Data obtained from the study report Table 9, page 52; n=12. Percent difference from controls is listed parenthetically.
* Statistically different from controls at $p \leq 0.05$.
** Statistically different from controls at $p \leq 0.01$.

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

D. Ophthalmoscopic examination - The ophthalmoscopic examination at week 13 revealed the following corneal abnormalities at 12500 ppm (data presented as number of occurrences per 24 eyes, Table 6): (i) slight hazy opacity (1/24, females only); (ii) moderate hazy opacity (4/24, females only); (iii) marked hazy opacity (males-5/24, females-8/24); (iv) slight opacity (males-1/24, females 1/24); (v) moderate opacity (2/24, males only); and (vi) vascularization (males-6/24, females-9/24). Corneal abnormalities at 1250 ppm included the following: (i) slight hazy opacity (1/24, females only); (ii) moderate hazy opacity (males-2/24, females-2/24); (iii) marked hazy opacity (males-9/24, females-3/24); (iv) slight opacity (4/24, females only); (v) moderate opacity (2/24, females only); (vi) marked opacity (1/24, males only); and (vii) vascularization (males-10/24, females-4/24). Corneal abnormalities at 125 ppm included the following: (i) moderate hazy opacity (2/24, males only); (ii) marked hazy opacity (8/24, males only); (iii) slight opacity (1/24, females only); (iv) marked opacity (1/24, males only); and (v) vascularization (10/24, males only). No corneal abnormalities were observed at 1 ppm nor in any control animal.

Table 6. Ophthalmoscopic observations (# observations/eye) noted in rats treated with mesotrione for 90 days. [a]

Observation	Males					Females				
	Dose (ppm)					Dose (ppm)				
	0	1	125	1250	12500	0	1	125	1250	12500
Number of eyes examined	24	24	24	24	24	24	24	24	24	24
Both eyes normal	24	24	13	12	16	24	24	23	12	10
Cornea										
hazy opacity (total)	0	0	10	11	5	0	0	0	6	13
slight	0	0	0	0	0	0	0	0	1	1
moderate	0	0	2	2	0	0	0	0	2	4
marked	0	0	8	9	5	0	0	0	3	8
opacity (total)	0	0	1	1	3	0	0	1	6	1
slight	0	0	0	0	1	0	0	1	4	1
moderate	0	0	0	0	2	0	0	0	2	0
marked	0	0	1	1	0	0	0	0	0	0
vascularized (total)	0	0	10	10	6	0	0	0	4	9

a Data obtained from the study report Table 10B, page 54.

E. Blood analyses

1. Hematology - No treatment-related differences in hematology parameters were observed in any treated group relative to controls. In the 12500 males, red blood cell count, mean cell volume, and mean cell hemoglobin were slightly variable (↓4-↑6%, $p \leq 0.01$). Platelet counts were decreased in the 1250 ppm males (↓8%, $p \leq 0.05$); however, this decrease was not dose-dependent. In addition, monocytes and eosinophils were decreased (↓23%, each; $p \leq 0.05$) in the 1 ppm males only. In the females, slight increases (↑1-6%, $p \leq 0.01$ or 0.05) in hematology parameters such as hemoglobin, hematocrit, and red blood cells were observed in all treated groups. Platelet counts were increased in the 1 ppm and 12500 ppm females (↓11-↑8, $p \leq 0.05$ or 0.01) relative to concurrent controls; however, these differences were not dose-related. In addition, non dose-dependent decreases in kaolin-cephalin time (↓11-14%, $p \leq 0.05$) were observed in the 125 and 1250 ppm females.

2. Clinical chemistry - No treatment-related differences were observed in clinical chemistry parameters (Table 7). Creatinine was increased in the 125, 1250, and 12500 males (↑18-29, $p \leq 0.01$) and females (↑11-29%, $p \leq 0.05$ or 0.01); however, in the absence of any corroborating histopathological evidence of toxicity, the observed increases in creatinine were of equivocal toxicological importance. Plasma triglycerides were dose-dependently increased in the 125, 1250, and 12500 ppm females (↑30, 48, and 57%, respectively; $p \leq 0.01$

13

or 0.05). These increases may be due to metabolic changes, as further evidenced by decreased body weights, body weight gains, food consumption, and food utilization. Differences ($p \leq 0.01$ or 0.05) in male clinical chemistry parameters that were considered not to be treatment-related included the following: minor increases in albumin (↑4-5%) in all treated groups, and non dose-dependent increases in plasma gamma-glutamyl transferase at 1250 ppm (↑52%), plasma alanine transaminase at 1250 ppm (↑17%), and plasma phosphorus at 12500 ppm (↑13%). In the females, the following differences ($p \leq 0.01$ or 0.05) in clinical chemistry parameters were considered not to be treatment-related: (i) non dose-dependent increases in plasma urea at 125 and 1250 ppm (↑9-11%) and total bilirubin at 1250 and 12500 ppm (↑36%); (ii) minor increases in plasma albumin (↑7%) and plasma calcium (↑5%) at 1250 ppm and total protein at 1, 125, and 1250 ppm (↑4-5%); (iii) non dose-dependent decreases in plasma alkaline phosphatase at 1 and 12500 ppm (↓22%); (iv) and a minor decrease in plasma sodium at 12500 ppm (↓1%). Plasma creatine kinase was increased in the 12500 females (↑53%, $p \leq 0.05$); however, this difference appeared to be due to an inflated value in one animal.

Table 7. Selected clinical chemistry parameters in rats treated with mesotrione for 90 days.[a]

Parameter	Dose (ppm)				
	0	1	125	1250	12500
Males					
Creatinine (mg/100 mL)	0.68±0.06	0.70±0.06	0.81**±0.05(↑19)	0.80**±0.10(↑18)	0.88**±0.09(↑29)
Females					
Creatinine (mg/100 mL)	0.65±0.11	0.64±0.11	0.72*±0.10(↑11)	0.78**±0.09(↑20)	0.84**±0.10(↑29)
Plasma triglycerides (mg/100 mL)	44±13	45±8	57*±16(↑30)	65**±13(↑48)	69**±17(↑57)

a Data obtained from the study report Table 12, pages 59 through 61; n=12. Percent difference from controls is listed parenthetically.

* Statistically different from controls at $p \leq 0.05$.

** Statistically different from controls at $p \leq 0.01$.

F. Urinalysis - No treatment-related differences from concurrent controls were observed in any urinalysis parameter. Urine specific gravity was increased (↑0.6%, $p \leq 0.05$) in the 1250 ppm males and pH was decreased ($p \leq 0.01$ or 0.05) in the 1250 and 12500 ppm males (6.22-6.23 vs. 6.37 controls) and in the 1 and 12500 ppm females (5.97-6.00 vs. 6.10 controls). These differences from concurrent controls were minor and considered not to be treatment-related. In the 12500 ppm females, protein was increased (↑220%, $p \leq 0.01$) relative to concurrent controls; however, the large standard deviation indicated that this parameter was highly

variable among individuals in the high-dose group due to 2 outliers with extremely high values, and the increase was considered not to be treatment-related.

G. Sacrifice and Pathology:

1. Organ weight - Selected organ weight data are presented in Table 8. Liver weights (adjusted covariately for final body weight) were increased ($p \leq 0.01$) in the 125, 1250, and 12500 ppm animals (males-↑17, 18, and 19%, respectively; females-↑11, 13, and 19%, respectively). Increased ($p \leq 0.01$ or 0.05) adjusted (covariately for final body weight) kidney weights in the 125, 1250, and 12500 ppm males (↑10-14%) were also observed. Other differences ($p \leq 0.01$ or 0.05) in organ weights included decreased absolute kidney weight in the 12500 ppm females (↓6%) and decreased absolute brain weights (↓3-6%) in both sexes. These differences were due to slightly decreased body weights in these animals.

Table 8. Adjusted (for final body weight) organ weights (g) in rats treated with mesotrione for 90 days. [a]

Observation	Males					Females				
	Dose (ppm)					Dose (ppm)				
	0	1	125	1250	12500	0	1	125	1250	12500
Liver	18.0	18.2	21.0**(↑17)	21.2**(↑18)	21.4**(↑19)	9.5	9.7	10.5**(↑11)	10.7**(↑13)	11.3**(↑19)
Kidney	3.04	3.15	3.33*(↑10)	3.46** (↑14)	3.37*(↑11)	1.96	1.96	1.98	2.01	2.02

a Data obtained from the study report Table 14, pages 71 and 72; n=12.

2. Gross pathology - Eye opacity was observed in the 125 (2/12), 1250 (1/12), and 12500 (2/12) males (vs. 0/12 controls) and in the 12500 ppm females (1/12 treated vs. 0/12 controls, Table 9). No other treatment-related gross pathological changes were observed in any treated group.

15

Table 9. Pathological observations (# affected animals) noted in rats treated with mesotrione for 90 days. [a]

Observation	Males					Females				
	Dose (ppm)					Dose (ppm)				
	0	1	125	1250	12500	0	1	125	1250	12500
Eye opacity	0	0	2	1	2	0	0	0	0	1

a Data obtained from the study report Table 15, page 74; n=12.

3. Microscopic pathology - Histological abnormalities of the cornea were observed in animals of both sexes at doses ≥125 ppm (Table 10). No abnormalities were noted in the control or 1 ppm groups. At 125 ppm, the following observations were noted in the males: (i) minimal keratitis in the left eye (1/12); (ii) moderate keratitis in the left eye (4/12); (iii) minimal keratitis in the right eye (1/12); (iv) moderate keratitis in the right eye (3/12); (v) moderate keratitis in both eyes (1/12); (vi) minimal corneal vascularization in the left eye (1/12); (vii) slight corneal vascularization in the left eye (2/12); (viii) moderate corneal vascularization in the left eye (2/12); (ix) slight corneal vascularization in the right eye (1/12); (x) moderate corneal vascularization in the right eye (2/12); (xi) and slight corneal vascularization in both eyes (1/12). At 1250 ppm, the following observations were noted in the males: (i) moderate keratitis in the left eye (2/12); (ii) marked keratitis in the left eye (2/12); (iii) slight keratitis in the right eye (2/12); (iv) marked keratitis in the right eye (1/12); (v) slight keratitis in both eyes (1/12); (vi) marked keratitis in both eyes (1/12); (vii) minimal corneal vascularization in the left eye (1/12); (viii) slight corneal vascularization in the left eye (1/12); (ix) moderate corneal vascularization in the left eye (2/12); (x) slight corneal vascularization in the right eye (2/12); moderate corneal vascularization in the right eye (1/12); (xi) slight corneal vascularization in both eyes (1/12); (xii) and moderate corneal vascularization in both eyes (1/12). At 12500 ppm, the following observations were noted in the males: (i) slight keratitis in the left eye (2/12); (ii) moderate keratitis in the left eye (1/12); (iii) marked keratitis in the left eye (1/12); (iv) slight keratitis in the right eye (3/12); (v) slight corneal vascularization in the left eye (3/12); (vi) moderate corneal vascularization in the left eye (1/12); (vii) and slight corneal vascularization in the right eye (3/12).

In the 125 ppm females, minimal keratitis was observed in the left eye (1/12). At 1250 ppm, the following observations were noted in the females: (i) minimal keratitis in the right eye (1/12); (ii) slight keratitis in both eyes (3/12); (iii) moderate keratitis in both eyes (1/12); (iv) minimal corneal vascularization in the right eye (1/12); (v) slight corneal vascularization in the right eye (1/12); and (vi) slight corneal vascularization in both eyes (2/12). At 12500 ppm, the following observations were noted in the females: (i) minimal keratitis in the left eye (1/12); (ii) slight keratitis in the left eye (2/12); (iii) slight keratitis in the right eye (1/12); (iv) slight keratitis in both eyes (3/12); (v) moderate keratitis in

both eyes (1/12); (vi) marked keratitis in both eyes (1/12); (vii) minimal corneal vascularization in the left eye (1/12); (viii) slight corneal vascularization in the left eye (1/12); (ix) slight corneal vascularization in the right eye (3/12); (x) slight corneal vascularization in both eyes (2/12); (xi) and moderate corneal vascularization in both eyes (1/12).

Table 10. Selected histopathological observations noted in the eyes of rats treated with mesotrione for 90 days.[a]

Observation	Males					Females				
	Dose (ppm)					Dose (ppm)				
	0	1	125	1250	12500	0	1	125	1250	12500
Number of eyes examined	12	12	12	12	12	12	12	12	12	12
Keratitis, left eye (total)	0	0	5	4	4	0	0	1	0	3
minimal	0	0	1	0	0	0	0	1	0	1
slight	0	0	0	0	2	0	0	0	0	2
moderate	0	0	4	2	1	0	0	0	0	0
marked	0	0	0	2	1	0	0	0	0	0
Keratitis, right eye (total)	0	0	4	3	3	0	0	0	1	1
minimal	0	0	1	0	0	0	0	0	1	0
slight	0	0	0	2	3	0	0	0	0	1
moderate	0	0	3	0	0	0	0	0	0	0
marked	0	0	0	1	0	0	0	0	0	0
Keratitis, both eyes (total)	0	0	1	2	0	0	0	0	4	5
slight	0	0	0	1	0	0	0	0	3	3
moderate	0	0	1	0	0	0	0	0	1	1
marked	0	0	0	1	0	0	0	0	0	1
Corneal vascularization, left eye (total)	0	0	5	4	4	0	0	0	0	2
minimal	0	0	1	1	0	0	0	0	0	1
slight	0	0	2	1	3	0	0	0	0	1
moderate	0	0	2	2	1	0	0	0	0	0
Corneal vascularization, right eye (total)	0	0	3	3	3	0	0	0	2	3
minimal	0	0	0	0	0	0	0	0	1	0
slight	0	0	1	2	3	0	0	0	1	3
moderate	0	0	2	1	0	0	0	0	0	0
Corneal vascularization, both eyes (total)	0	0	1	2	0	0	0	0	2	3
slight	0	0	1	1	0	0	0	0	2	2
moderate	0	0	0	1	0	0	0	0	0	1

a Data obtained from the study report Table 16, page 77-78.

b Although it was stated that 12 eyes/dose were examined, the data indicates that 24 eyes/dose were examined in order to obtain the observations for both eyes.

17

III. DISCUSSION

A. Investigator's conclusions - Oral administration of mesotrione for 90 days in the diet was systemically toxic at doses ≥125 ppm. The eye was the main target organ for toxicity. Additionally, the test compound caused decreased body weights and food consumption, and increased kidney weights (adjusted for body weight). The NOAEL for this study was 1 ppm.

B. Reviewer's discussion - In this subchronic oral toxicity study, mesotrione was administered for 90 days to 12 Alpk:AP$_f$SD rats/sex/dose at dietary concentrations of 0, 1, 125, 1250, or 12500 ppm (equivalent to [M/F] 0/0, 0.09/0.10, 11/13, 112/126, and 1111/1213 mg/kg/day, respectively). The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the study animals was acceptable.

No treatment-related findings were observed in the 1 ppm group. No mortalities occurred during the study. Hematology and urinalysis parameters were unaffected by the test substance.

During the clinical examinations, eye opacity was observed during weeks 10-14 in the 125, 1250, and 12500 ppm males (11/12, 10/12, and 7/12 animals, respectively) and in the 1250 and 12500 ppm females (8/12 each). The ophthalmoscopic examination at week 13 revealed the following corneal abnormalities at 12500 ppm (data presented as number of occurrences per 24 eyes): (i) slight to marked hazy opacity (males-5/24, females-13/24); (ii) slight to moderate opacity (males-3/24, females 1/24); and (iii) vascularization (males-6/24, females-9/24). Corneal abnormalities at 1250 ppm included the following: (i) slight to marked hazy opacity (males-11/24, females-6/24); (ii) slight to marked opacity (males-1/24, females-6/24); and (iii) vascularization (males-10/24, females-4/24). Corneal abnormalities at 125 ppm included the following: (i) moderate to marked hazy opacity (10/24, males only); (ii) slight to marked opacity (males-1/24, females-1/24); and (v) vascularization (10/24, males only).

During the gross pathological examination, eye opacity was observed in the 125 (2/12), 1250 (1/12), and 12500 (2/12) males (vs. 0/12 controls) and in the 12500 ppm females (1/12 treated vs. 0/12 controls). Histological abnormalities of the cornea were observed in animals of both sexes at doses ≥125 ppm. At 125 ppm, the following observations were noted: (i) keratitis in the left eye (males-5/12, females 1/12); (ii) keratitis in the right eye (4/12 males); (iii) keratitis in both eyes (1/12 males); (iv) corneal vascularization in the left eye (5/12 males); (v) corneal vascularization in the right eye (3/12 males); and (vi) corneal vascularization in both eyes (1/12 males). At 1250 ppm, the following observations were noted: (i) keratitis in the left eye (4/12 males); (ii) keratitis in the right eye (males-3/12, females1/12); (iii) keratitis in both eyes (males-2/12, females-4/12); (iv) corneal vascularization in the left eye (4/12 males); (v) corneal vascularization in the right eye (males-3/12, females-2/12); and (vi) corneal vascularization in both eyes (males-2/12, females-2/12). At 12500 ppm, the following observations were noted: (i) keratitis in the left eye (males-4/12, females-3/12); (ii) keratitis in the right eye (males-3/12, females-1/12); (iii)

18

keratitis in both eyes (5/12 females); (iv) corneal vascularization in the left eye (males-4/12, females-2/12); (v) corneal vascularization in the right eye (males-3/12, females-3/12); and (vi) corneal vascularization in both eyes (3/12 females).

Mean adjusted body weights (for week 1 body weights) were decreased ($p \leq 0.01$) in the 12500 males (↓7-16%) and females (↓6-10%) throughout the study. In the 1250 ppm males, adjusted body weights were decreased ($p \leq 0.01$ or 0.05) in the males at week 2 (↓2%) and from week 6 until the end of the study (↓5-8%). In the 125 ppm males, adjusted body weights were decreased ($p \leq 0.01$ or 0.05) sporadically throughout the first half of the study (↓2-5%) and from week 10 through study termination (↓6-8%). Adjusted body weights in the 125 and 1250 ppm females were decreased sporadically throughout the study (↓4-5%, $p \leq 0.05$); however, there was no sustained decrease until the last two weeks of the study (↓5% at 1250 ppm only). Overall (weeks 1-14) body weight gains were decreased in all treated groups (↓4-23%, calculated by reviewers) except for the 1 ppm females, but were only biologically relevant in males at $\geq$ 125 ppm and in females at 12500 ppm.

Food consumption was decreased ($p \leq 0.01$ or 0.05) in the 12500 ppm males (↓9-15%) and females (↓11-18%) throughout the study (only the decreases in the males at weeks 5 and 6 were not statistically significant).

In the males, food utilization was decreased at 125, 1250, and 12500 ppm throughout the study (↓2-24%). Statistically significant ($p \leq 0.01$ or 0.05) decreases were observed as follows: during weeks 1-4 at 12500 ppm (↓8%), weeks 5-8 at 1250 and 12500 ppm (↓13-19%), and weeks 9-13 at 125 and 1250 ppm (↓22-24%). In addition, overall food utilization (weeks 1-13) was decreased in the 125, 1250, and 12500 ppm males (↓7, 10, and 13%, respectively; $p \leq 0.01$).

Creatinine was increased in the 125, 1250, and 12500 males (↑18-29, $p \leq 0.01$) and females (↑11-29%, $p \leq 0.05$ or 0.01); however, in the absence of any corroborating histopathological evidence of toxicity, the observed increases in creatinine were not of toxicological importance. Plasma triglycerides were dose-dependently increased in the 125, 1250, and 12500 ppm females (↑30, 48, and 57%, respectively; $p \leq 0.01$ or 0.05), but the increase was not considere clinically relevant.

Liver weights (adjusted covariately for final body weight) were increased ($p \leq 0.01$) in the 125, 1250, and 12500 ppm animals (males-↑17, 18, and 19%, respectively; females-↑11, 13, and 19%, respectively). Increased ($p \leq 0.01$ or 0.05) adjusted (covariately for final body weight) kidney weights in the 125, 1250, and 12500 ppm males (↑10-14%) were also observed.

The LOAEL for this study is 125 ppm (equivalent to 11 mg/kg/day in males and 13 mg/kg/day in females) based on corneal abnormalities observed during the clinical,

19

ophthalmoscopic, gross pathological, and histopathological examinations in both sexes, and decreases in body weight gain in males.

The NOAEL for this study is 1 ppm (equivalent to 0.09 mg/kg/day in males and 0.10 mg/kg/day in females).

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

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

- The temperature and humidity ranges reported in this study were not within acceptable limits.

20