DATA EVALUATION RECORD

7/18/2000

MESOTRIONE (ZA1296)

Study Type: Non-Guideline; Ocular Toxicity Development and Reversibility Study in Rats

Work Assignment No. 2-01-52H (amend 1) (MRID 44537104)

Prepared for
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DATA EVALUATION RECORD

STUDY TYPE: Ocular Toxicity Development and Reversibility Study in Rats
OPPTS Number: N/A OPP Guideline Number: non-GDL

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: Tinston, D.J. (1997) ZA1296: Ocular toxicity Development and reversibility Study in Rats. Central Toxicology Laboratory, Cheshire, UK. Laboratory Report/Study No. CTL/P/5001/PR 1022, April 23, 1997. MRID 44537104. Unpublished.

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

EXECUTIVE SUMMARY: In a non-guideline study (MRID 44537104), mesotrione (96.8% a.i.; lot # P17) was administered in the diet at 2500 ppm (equivalent to 272 mg/kg/day) to Alpk:AP$_f$SD male rats (40 rats treated and 16 control rats) for 5 weeks. Any ocular changes in the treated animals were graded. At the end of the treatment period and, all animals were allocated (based on the severity of any ocular lesions) to receive a gross postmortem examination or to undergo an 8 week recovery period.

No mortalities occurred during the study. When compared to concurrent controls, no treatment-related changes were observed in body weights, body weight gains (calculated by reviewers), food consumption, food utilization, or gross pathology.

During clinical observation, cloudy eye(s) were observed in 22/40 treated males; this finding was not observed after week 11. In the treated animals, the ophthalmoscopic examination at week 1 revealed slight corneal opacity (2/80 examined eyes); by week 2, the incidence of this finding had increased (14/80 eyes). During week 3, slight to moderate corneal opacities (24/80 eyes) were observed. During weeks 4 and 5, slight to marked corneal opacities (34 and 39/80 eyes, respectively) and vascularization (3 and 7/80 eyes, respectively) were observed. At week 6 (week 1 of recovery), slight to moderate corneal opacities (11/30 eyes examined), slight to marked hazy opacities (7/30 eyes), and vascularization (9/30 eyes) were noted. During week 7,

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slight corneal opacity (1/30 eyes), marked hazy opacity (1/30 eyes), vascularization (6/30 eyes), and ghost vascularization (9/30 eyes) were observed. At week 10, 1/30 eyes showed vascularization, while 11/30 eyes displayed ghost vascularization; during week 12, ghost vascularization was noted in 11/30 eyes. Histological abnormalities of the cornea were observed in treated males. No abnormalities were noted in the control group. At the end of treatment, the following observations were noted in the eyes of the treated males: (i) minimal (1/25 animals examined), slight (6/25), or moderate (4/25) keratitis; and (ii) minimal (5/25), slight (4/25), or moderate (1/25) polymorphonuclear leukocytic infiltration at filtration angle. At the end of the recovery, the following observations were noted in the eyes of the treated males: (i) minimal (6/15 animals examined) or slight (2/15) corneal vessels; (ii) minimal (6/15) or slight (2/15) corneal stromal fibroblasts; and (iii) minimal (2/15) or slight (1/15) corneal epithelial disruption.

When compared to concurrent controls, plasma tyrosine levels were decreased ($p \leq 0.01$) during week 1 (↓47%) and increased ($p \leq 0.01$) during weeks 2 (↑1967%), 6 (↑1419%), 7 (↑1254%), and 14 (↑16%).

In conclusion, corneal lesions associated with dietary administration of 2500 ppm mesotrione for 5 weeks were resolved ophthalmoscopically and histologically following an 8 week recovery period.

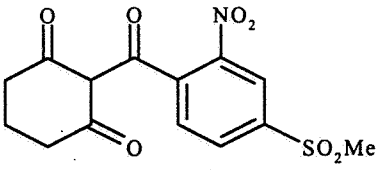
This special study is classified as **acceptable/non-guideline** and satisfies the purpose for which is was intended.

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 (WRC 15213-17-1)
   Purity (w/w): 96.8% a.i.
   Stability of compound: The test substance was stable in the diet for up to 16 days when stored at room temperature or at -20°C for up to 40 days.
   CAS #: 104206-82-8
   Structure:



2. Vehicle: Diet

3. Test animals: Species: Rat
   Strain: Alpk:APfSD
   Age at the start of dosing and mean weight at week 1: 36 days old; 146.0-148.3
   Source: Barriered Animal Breeding Unit, Zeneca Pharmaceuticals, Alderley Park
   Housing: 4/cage in racks suitable for animals of this strain and weight range
   Diet: CT1 diet (Special Diet Services, Ltd., 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: Approximately 2 weeks

B. STUDY DESIGN:

1. In life dates: start: 10/31/95 end: 01/30/96

2. Animal assignment: The rats were randomly assigned to the test groups shown in Table 1.

Table 1. Study design

Test Group	Dietary Concentration (ppm)	Achieved Dose [a] (mg/kg/day)	Males
Control	0	0	16
Treated	2500	272	40

a Mean achieved dose (mg/kg/day) were obtained from the study report page 70.
Note: Eye changes noted in the treated animals were graded as slight, moderate/marked, or vascularized and following 35 days of treatment these animals were allocated to the reversibility phase or for termination as follows: graded as slight (7 animals each to reversibility and termination); graded as moderate/marked (4 animals each to reversibility and termination); and graded as vascularized (4 animals each to reversibility and termination). In the control group, 8 animals were assigned to the reversibility phase and 8 animals were terminated. All remaining treated animals were terminated.

3. Dose selection rationale - The dose levels were selected based on the results of a concurrent study conducted in this laboratory on the same strain of rat.

4. Diet preparation and analysis - Diets were prepared by mixing the test substance with feed to obtain a premix and then further diluting the premix with feed 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 2500 dose formulation. Stability of the test substance in the diet was determined in previous studies for 1 and 7000 ppm dietary formulations stored at room temperature and at -20°C for up to 16 days. Concentration analysis was performed on samples from the 2500 ppm dose formulation on two occasions.

   Results -
   Homogeneity analysis (overall mean % of nominal): 103.0%

   Stability analysis (range as mean % of day 0): 84.8-107%

   Concentration analysis (range as mean % of nominal): 103.9-104.6%

   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, and plasma tyrosine 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 clinical signs of toxicity. Detailed clinical observations were recorded at the time of weighing.

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

3. Food consumption - Food consumption measured continuously throughout the study and calculated weekly (g/rat/day). Food utilization was calculated as the body weight gained per cage per 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 once weekly during the study. Animals in the recovery phase were examined in weeks 6, 7, 10, and 12.

6. Blood - Blood analyzed for plasma tyrosine levels was collected from the tail vein, except at termination when animals were bled by cardiac puncture. For animals terminated in week 6, blood samples were taken pre-dosing, during week 2, and at termination. For recovery animals, samples were taken pre-dosing, during weeks 2 and 6, and weeks 7 and 14 of the recovery phase.

7. Sacrifice and pathology - At study termination, all animals were anaesthetized, exsanguinated, and subjected to a gross pathological examination. The eye and Harderian gland were collected from all animals and examined using light microscopy.

II. RESULTS

A. Observations

1. Mortality - No mortalities occurred during the study.

2. Clinical signs - Selected clinical signs are presented in Table 2. Cloudy eye(s) were observed in 22/40 treated males; this finding was not observed after week 11. No other treatment-related clinical observations were made.

Table 2. Selected clinical observations noted in male rats treated with mesotrione for 5 weeks and then allowed an 8 week recovery phase [a]

Observation	Dose (ppm)	
	0	2500
Animals examined	16	40
Eye(s) cloudy	0	22 (77)

a Data obtained from the study report , Table 5, page 37. Data presented as number of affected animals. Number of incidences is listed parenthetically.

B. Body weight and body weight gain - When compared to concurrent controls, no treatment-related changes were observed in body weights or body weight gains (calculated by reviewers). Minor decreases were observed in adjusted (for week 1 body weight) body weight of the treated males (↓2-5%, $p \leq 0.05$ or 0.01) during weeks 2, 4, 5, and 7. Body weight gains are shown in Table 3.

Table 3. Body weight gains (g) in male rats treated with mesotrione for 5 weeks and then allowed an 8 week recovery phase [a]

Interval	Dose (ppm)	
	0	2500
Treatment body weight gain (weeks 1-6)	209.6	197.8
Overall body weight gain (weeks 1-14)	346.6	346.6

a Body weight gains were calculated by reviewers from data obtained from the study report, Table 6, pages 40 and 41.

C. Food consumption/utilization and compound intake - No treatment-related changes were observed in food consumption or food utilization. A minor decrease was observed in food consumption of the treated males during week 2 (↓4%, $p \leq 0.05$). The achieved mean dosages are shown in Table 1.

D. Ophthalmoscopic examination - The ophthalmoscopic examination (Table 4) at week 1 revealed slight corneal opacity (2/80 examined eyes); by week 2, the incidence of this finding had increased (14/80 eyes). During week 3, slight to moderate corneal opacities (24/80 eyes) were observed. During weeks 4 and 5, slight to marked corneal opacities (34 and 39/80 eyes,

respectively) and vascularization (3 and 7/80 eyes, respectively) were observed. At week 6 (week 1 of recovery), slight to moderate corneal opacities (11/30 eyes examined), slight to marked hazy opacities (7/30 eyes), and vascularization (9/30 eyes) were noted. During week 7, slight corneal opacity (1/30 eyes), marked hazy opacity (1/30 eyes), vascularization (6/30 eyes), and ghost vascularization (9/30 eyes) were observed. At week 10, 1/30 eyes showed vascularization, while 11/30 eyes displayed ghost vascularization; during week 12, ghost vascularization was noted in 11/30 eyes. Minor changes of the lens and iris were observed in control and treated animals during pre-study, treatment weeks 1-5, and weeks 7, 10, and 12 of recovery.

Table 4. Ophthalmoscopic observations (# observations/eye) noted in male rats treated with mesotrione for 5 weeks and then allowed an 8 week recovery phase [a]

Observation	Dose (ppm)			
	0	2500	0	2500
	End of treatment (week 5)		End of recovery (week 12)	
Number of eyes examined	32	80	16	30
Nothing abnormal detected	31	41	13	18
Cornea				
opacity				
slight	0	23	0	0
moderate	0	12	0	0
marked	0	4	0	0
hazy opacity				
slight	0	0	2	1
moderate	0	0	0	0
marked	0	0	0	0
vascularized	0	7	0	0
ghost vascularization	0	0	0	11

a Data obtained from the study report, Table 9, pages 50 and 54.

E. Plasma tyrosine levels - When compared to concurrent controls, plasma tyrosine levels (Table 5) were decreased ($p \leq 0.01$) during week 1 (↓47%) and increased ($p \leq 0.01$) during weeks 2 (↑1967%), 6 (↑1419%), 7 (↑1254%), and 14 (↑16%).

Table 5. Mean plasma tyrosine levels (nmol/mL) in male rats treated with mesotrione for 5 weeks and then allowed an 8 week recovery phase [a]

Parameter	Dose (ppm)	
	0	2500
Treatment		
Number examined	16	38-40
Week 1	422	222** (↓47)
Week 2	143	2956** (↑1967)
Week 6	167	2536** (↑1419)
Recovery		
Number examined	8	15
Week 7	127	1719** (↑1254)
Week 14	97	113** (↑16)

a Data obtained from the study report, Table 10, page 56. Percent difference from controls is listed parenthetically.

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

G. Sacrifice and Pathology:

1. Gross pathology - No treatment-related gross pathological findings were noted.

2. Microscopic pathology - Histological abnormalities of the cornea were observed in treated males (Table 6). No abnormalities were noted in the control group. At the terminal sacrifice, the following observations were noted in the eyes of the treated males: (i) minimal keratitis (1/25 animals examined); (ii) slight keratitis (6/25 animals); (iii) moderate keratitis (4/25 animals); (iv) minimal polymorphonuclear leukocytic infiltration at filtration angle (5/25 animals); (v) slight polymorphonuclear leukocytic infiltration at filtration angle (4/25 animals); (vi) and moderate polymorphonuclear leukocytic infiltration at filtration angle (1/25 animals). At the recovery sacrifice, the following observations were noted in the eyes of the treated males: (i) minimal corneal vessels (6/15 animals examined); (ii) slight corneal vessels (2/15 animals); (iii) minimal corneal stromal fibroblasts (6/15 animals); (iv) slight corneal stromal fibroblasts (2/15 animals); (v) minimal corneal epithelial disruption (2/15 animals); and (vi) slight corneal epithelial disruption (1/15 animals).

Table 6. Selected histopathological observations noted in the eyes of male rats treated with mesotrione for 5 weeks and then allowed an 8 week recovery phase [a]

Observation	Dose (ppm)	
	0	2500
Terminal sacrifice (end of treatment)		
Number of animals examined	8	25
No abnormalities detected	7	12
Keratitis (total)	0	11
minimal	0	1
slight	0	6
moderate	0	4
Polymorphonuclear leukocytic infiltration at filtration angle (total)	0	10
minimal	0	5
slight	0	4
moderate	0	1
Recovery sacrifice		
Number of animals examined	8	15
No abnormalities detected	7	12
Corneal vessels (total)	0	8
minimal	0	6
slight	0	2
Corneal stromal fibroblasts (total)	0	8
minimal	0	6
slight	0	2
Corneal epithelial disruption (total)	0	3
minimal	0	2
slight	0	1

a Data obtained from the study report, Table 12, pages 59 and 60.

III. DISCUSSION

A. Investigator's conclusions - Corneal lesions associated with dietary administration of mesotrione at 2500 ppm for 5 weeks were resolved both ophthalmoscopically and histologically during an 8 week recovery period.

B. Reviewer's discussion - Mesotrione (96.8% a.i.; lot # P17) was administered in the diet at 2500 ppm (equivalent to 272 mg/kg/day) to Alpk:AP$_f$SD male rats (40 rats treated and 16 control rats) for 5 weeks. Any ocular changes in the treated animals were graded. Following treatment, the animals were divided equally between the reversibility phase and termination based on the severity of the ocular lesion. 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.

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In conclusion, corneal lesions associated with dietary administration of 2500 ppm mesotrione for 5 weeks were resolved ophthalmoscopically and histologically following an 8 week recovery period.

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C. Study deficiencies - No deficiencies were noted.

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