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DATA EVALUATION RECORD

3/20/2001

MESOTRIONE (ZA1296)

Study Type: §82-7a, Subchronic Neurotoxicity Screening Battery in Rats

Work Assignment No. 2-01-52U (MRID 44505025)

Prepared for

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DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Neurotoxicity [Feeding] - rat
OPPTS Number: 870.6200 OPP Guideline Number: §82-7a

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

TEST MATERIAL (PURITY): Mesotrione (97.6% a.i.)

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

CITATION: Horner, S.A. (1997) ZA1296: Subchronic Neurotoxicity Study in Rats. Central Toxicology Laboratory, Cheshire, UK. Laboratory Project Identification Number PR1043. October 31, 1997. MRID 44505025. Unpublished.

SPONSOR: Zeneca Ag Products, Wilmington, Delaware

EXECUTIVE SUMMARY: In this subchronic neurotoxicity screening battery, mesotrione (ZA1296, 97.6% a.i., Lot/Batch P22) (MRID 44505025) was administered continuously in the diet for 90 days to 12 Alpk:AP$_f$SD rats/sex/group at doses of 0, 2.5, 100, or 5000 ppm (equivalent to [M/F] 0/0, 0.20/0.23, 8.25/9.29, or 402.8/466.6 mg/kg/day). Five animals/sex/group were perfused for neurohistological examination and animals from the control and high-dose groups were examined microscopically. The functional observational battery (FOB) and motor activity were evaluated during weeks -1, 5, 9, and 14.

No treatment-related deaths occurred. Food consumption and utilization, FOB parameters, motor activity, brain dimensions, and neuropathology were unaffected by the test substance.

Corneal opacities and/or vascularization of the cornea were seen in 3/11 mid-dose males and 1/12 mid-dose females. Corneal opacities and/or vascularization of the cornea were seen in 10/12 high dose males, and 7/12 high dose females. Overall (weeks 1-14) body weight gains were decreased in the high-dose females (↓18%). High-dose females displayed decreased body weights (adjusted for initial body weight) from week 2 until study termination (↓5-9%).in the high-dose females at week 14 (↑37%). No treatment-related findings were observed in the 2.5

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ppm group.

The LOAEL for this study is 100 ppm (equivalent to 8.25 mg/kg/day in males and 9.29 mg/kg/day in females) based on corneal opacities and/or vascularization of the cornea of the eye. The NOAEL for this study is 2.5 ppm (equivalent to 0.20 mg/kg/day in males and 0.23 mg/kg/day in females).

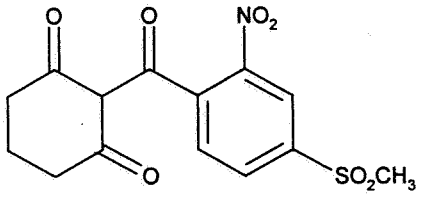
The submitted study is classified as **acceptable/guideline (§82-7[a])** and satisfies the guideline requirements for a subchronic neurotoxicity screening battery in rats.

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 #: P22
   Purity (w/w): 97.6% a.i.
   Stability of compound: Stable in the diet for up to 10 days at room temperature or up to 57 days at -20°C
   CAS #: 104206-82-8
   Structure:



2. Vehicle: Diet

3. Test animals: Species: Rat
   Strain: Alpk:APfSD
   Age and weight at the start of dosing: At least 42 days old; 205.4-210.1 g (males), 166.1-169.8 g (females)
   Source: Rodent Breeding Unit, Zeneca Pharmaceuticals, Cheshire, UK
   Housing: 4/cage in rat 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: 21±2° C
   Humidity: 55±15%
   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: 1/28/97 End: 5/2/97

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

Table 1. Study design.[a]

Test Groups	Dose (ppm)	Mean Achieved Dose (mg/kg/day) [M/F]	Number of Animals	
			Males	Females
Control	0	0/0	12	12
Low	2.5	0.20/0.23	12	12
Mid	100	8.25/9.29	12	12
High	5000	402.8/466.6	12	12

a Data were obtained from study report, page 18, and Appendix G, pages 122-123.

3. Dose selection rationale - Dose levels for the current study were chosen based on the results of a previous study. No further information was provided.

4. Treatment preparation and analysis: The appropriate amount of test substance was mixed with the diet to obtain a premix, and the premix was diluted with additional feed to obtain the appropriate dose. All diets were frozen for up to 35 days. Homogeneity analysis was not performed for the current study; however, homogeneity data (top, middle, bottom) for 2.5 and 7000 ppm samples from a previous study were provided (CTL Study Nos. PR1001 and PM0983). Stability was determined for the 2.5 and 5000 ppm dose preparations stored at room temperature and at -20°C for a period of up to 10 days. Actual experimental diets for the current study were stored at -20°C for up to 35 days; therefore, stability data for 1 and 7000 ppm samples stored frozen for up to 57 days in previous studies (CTL Study No. PR1001 and PM0983) were provided. Concentration analyses were performed on all dose preparations from samples collected twice during the current study.

   Results:
   Homogeneity (range as mean % of nominal): 78-101%
   Stability (range as mean % of day 0): 96-101% after 10 days at room temperature; 106-113% after 10 days at -20°C; 96-115% after 57 days at -20°C
   Concentration (range as mean % of nominal): 96-101%

   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, food utilization, motor activity, time to tail-flick, landing foot splay, grip strength, and brain weight and dimension data were evaluated by analysis of variance (ANOVA) or covariance followed by Student's t-test as necessary.

C. METHODS:

1. Observations

a. Clinical signs - All animals were observed daily for mortality and clinical signs of toxicity. Detailed clinical observations were recorded weekly.

b. Functional observational battery and motor activity - All animals were subjected to functional observational battery (FOB) and motor activity measurements during weeks -1, 5, 9, and 14. The FOB assessment included the following parameters:

Clinical Assessments	
Lacrimation	Auditory response
Salivation	Abnormal behavior
Piloerection	Stereotypes
Exophthalmus	Emaciation
Urinary incontinence	Dehydration
Diarrhea	Hypotonia/Hypertonia
Pupillary response	Fur appearance
Ptosis	Red or crusty deposits
Convulsions	Quantitative Assessments
Tremors	Forelimb grip strength
Abnormal motor movements	Hindlimb grip strength
Removal from cage/Handling	Landing foot-splay
Arousal/Alertness	Tail-flick test
Posture	
Gait	

Motor activity was measured by an automated activity recording apparatus on the same day the FOB was conducted. The number of movements was tabulated for 10 intervals, each lasting 5 minutes.

c. Positive controls - Positive control data were provided from an acute neurotoxicity study (MRID 44505018), but this data are judged to be inadequate to validate the functional tests or assessment of the peripheral nervous system. Trimethyltin chloride (TMT) was administered in a single oral dose of 6 or 8 mg/kg to Alpk:AP$_f$SD rats (10/sex/dose). It produced CNS pathology at 8 mg/kg consistent with TMT's known pattern of toxicity, including neuronal cell necrosis in the hippocampus, piriform/entorhinal cortex, and amygdaloid body, and some functional effects, but not those associated with trimethyltin (tremors, increased reactivity, and hyperactivity).

2. Body weight - All animals were weighed weekly during the study.

3. Food consumption - Food consumption was measured continuously throughout the study and these data were used to calculate food utilization.

4. Ophthalmoscopic examination - All animals were subjected ophthalmoscopic examinations prior to study initiation and during week 13.

5. Sacrifice and pathology - At study termination, five animals/sex/group were sacrificed by perfusion fixation and submitted for neuropathological examination. The brains were removed from each of these animals and the brain lengths and widths were recorded. The following tissues were collected from each perfused animal, embedded in paraffin or ARALDITE, sectioned, and stained with toluidine blue or hematoxylin-eosin. Only tissues from the control and high-dose animals were examined microscopically.

Central Nervous System		
Brain		
Cerebral cortex	Hippocampus	Cerebellum
Pons	Medulla	
Spinal cord		
Cervical swellings C3-C6	Lumbar swellings L1-L4	
Peripheral Nervous System		
Gasserian ganglia	Sural nerve	
Spinal root ganglia at C3-C6	Dorsal root ganglia at C3-C6	Sciatic nerve
Spinal root ganglia at L1-L4	Dorsal root ganglia at L1-L4	Tibial nerve

gastrocnemius muscle

All animals that died during the study or were killed by perfusion fixation were subjected to gross necropsy. The remaining animals (up to 7 rats/sex/group) were killed by exsanguination and discarded.

II. RESULTS

A. Observations

1. Mortality - No treatment-related deaths occurred. One 100 ppm male was sacrificed *in extremis* during week 7 following body weight loss during week 6. Clinical signs prior to sacrifice included abnormal respiratory noise, eye discharge, irregular breathing, and piloerection; however, this mortality was considered not to be treatment-related due to the absence of mortality at the high dose.

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2. Clinical signs - Eye opacities were observed during weeks 8 to 14 in the 100 ppm males (3/11), 5000 ppm males (10/12), and 5000 females (4/12). Signs of eye opacity were not observed in any of the controls.

Table 2. Clinical observations noted in rats treated with mesotrione for 90 days. [a]

Observation	Males				Females			
	Dose (ppm)				Dose (ppm)			
	0	2.5	100	5000	0	2.5	100	5000
Eye opacities	0	0	3/11	10/12	0	0	0	4/12

a Data obtained from the study report Table 5, pages 52 and 55; n=11 or 12.

B. Body weight and body weight gains: High-dose females displayed decreased body weights (adjusted for initial body weight) from week 2 until study termination (↓5-9%, $p \leq 0.01$). Overall (weeks 1-14) body weight gains were decreased in the high-dose females (↓18%, calculated by reviewers; Table 3). In the mid and low dose females, decreased body weights were observed in the low- and mid-dose females only during week 2 (↓3%, $p \leq 0.05$ or 0.01) and at the mid dose on week. Other than at the high dose, these differences were not considered large enough or consistent enough to be considered toxicologically significant. Female body weight data are presented as an attachment (study report pages 59-60, Table 6).

Table 3. Overall body weight gains (g) in rats after treatment with mesotrione for 90 days.[a]

Dose (ppm)							
Males				Females			
0	2.5	100	5000	0	2.5	100	5000
276.8	284.3	271.4	257.4 (-7%)	103.9	100.8	96.8 (-7%)	85.5 (-18%)

a Body weight gains calculated by reviewers from data obtained from the study report Table 6, pages 57-60; n=11 or 12.

C. Food consumption/food utilization: No consistent treatment-related differences in food consumption were observed in any treated group relative to concurrent controls. High-dose males displayed decreased food consumption during week 7 only (↓17%, $p \leq 0.05$). High-dose females displayed decreased food consumption during weeks 4, 9, and 10 (↓7-11%, $p \leq 0.05$ or 0.01); however, these decreases were inconsistent and considered not to be treatment-related. In addition, food consumption was sporadically decreased in the low- and mid-dose females (↓5-8%, $p \leq 0.05$ or 0.01), but these decreases were minor and inconsistent and

generally lacked dose-dependence. Food utilization was decreased ($p \leq 0.01$ or 0.05) in the mid- (↓14%) and high- (↓24%) dose females during weeks 5-8; however, this decrease was not sustained over time, and therefore, considered not to be treatment-related. Food consumption and utilization data are presented as an attachment (study report pages 61-65, Tables 7 and 8).

D. Ophthalmoscopic examination: Corneal opacities and/or vascularization of the cornea were seen in 10/12 high dose males, 7/12 high dose females, in 3/11 mid-dose males and 1/12 mid-dose females. No corneal effects were seen at the low dose. None of these corneal abnormalities were observed in any control animal. No other findings considered treatment-related were noted.

Table 4. Ophthalmoscopic observations (# animals with a finding in one or both eyes) noted in rats treated with mesotrione for 90 days. [a]

Observation	Males				Females			
	Dose (ppm)				Dose (ppm)			
	0	2.5	100	5000	0	2.5	100	5000
Both eyes normal	11	9	8	2	12	12	11	5
Cornea								
hazy opacity								
slight	0	0	2	3	0	0	0	3
marked	0	0	0	2	0	0	0	0
opacity								
slight	0	0	1	2	0	0	0	4
marked	0	0	0	6	0	0	0	0
vascularized	0	0	2	9	0	0	0	2
ghost vascularization	0	0	0	0	0	0	1	1

a Data obtained from the study report Table 14, page 104; n=11 or 12.

E. Functional observational battery:

1. Clinical observations: No treatment-related clinical observations were noted during the FOB.

2. Quantitative assessments: Decreased landing foot splay was observed in the low- and high-dose females at week 14 (↓26 and 21%, respectively; $p \leq 0.01$ or 0.05); however, these decreases were inconsisent and not dose-dependent and considered to be not treatment-related. Time to tail-flick was increased ($p \leq 0.05$) at week 14 in the low-dose males (↑45%) and week 9 in the low-dose females (↑35%). These increases were also

inconsistent, not dose-dependent, and therefore, not considered treatment-related. All other quantitative parameters were comparable between the treated groups and controls. Landing foot splay and time to tail-flick data are presented as an attachment (study report pages 91-92, Tables 10 and 11).

F. Motor activity: Overall motor activity was significantly increased in the high-dose females at week 14 (↑37%) ($p \leq 0.05$) and in the mid-dose females at week 5 (↑42%). A 35% decrease in the high dose at week 5 did not quite reach statistical significance. A sharp rise in the control mean for females at week 9 eliminated this apparent effect for both the mid and high dose groups at that time, and the effect was not apparent at the mid dose on week 14. The Hazard Identification Assessment Review Committee (March 13, 2001) concluded that the increase in motor activity was not treatment-related since it occurred at the mid-dose only in one sex, there was no dose-response, and an outlier was probably responsible for dramatic increase at that time period. High dose males showed 14-29% increases, but these also failed to achieve statistical significance. Variation in interval motor activity data occurred sporadically throughout the study (↓84-↑174, $p \leq 0.05$ or 0.01), but were not considered to show any treatment-related pattern independent of the overall data. Motor activity data are presented as an attachment (study report pages 95-102, Table 13).

Table 5. Motor activity (counts) in rats treated with mesotrione for 90 days (mean ± standard deviation).[a]

Treatment interval (weeks)	Dose (ppm)			
	0	2.5	100	5000
Males				
Pretest	163.3±49.1	173.6±109.3	169.0±68.9	178.8±42.9
5	352.3±168.5	421.5±139.6	355.3±198.7	453.5±168.5(↑29)
9	378.3±138.8	393.3±163.5	342.5±190.9	431.3±128.1(↑14)
14	270.0±145.3	242.1±111.9	324.9±119.9	328.6±126.7(↑22)
Females				
Pretest	257.9±72.1	208.5±119.5	231.3±101.4	227.3±118.8
5	297.7±131.6	362.6±186.9	422.3*±163.6(↑42)	402.9±146.8(↑35)
9	420.0±167.0	401.0±206.5	451.6±188.6	496.2±150.7(↑18)
14	314.8±174.1	246.1±97.7	279.7±92.7	432.4*±17.7(↑37)

a Data obtained from the study report Table 13, pages 95-102; n=11 or 12. Percent difference from controls is listed parenthetically.

* Statistically significant at $p \leq 0.05$

G. Pathology:

1. Gross pathology - Eye opacities were observed in 3/5 high-dose males, 1/5 high-dose females, and 1/5 mid-dose males (vs. 0/11 controls, Table 6). No other treatment-related gross pathological findings were observed.

Table 6. Pathological observations (# affected animals) noted in rats treated with mesotrione for 90 days. [a]

Observation	Males				Females			
	Dose (ppm)				Dose (ppm)			
	0	2.5	100	5000	0	2.5	100	5000
Eye opacity	0	0	1	3	0	0	0	1

a Data obtained from the study report Table 16, page 109; n=5 or 6.

2. Neuropathology - No treatment-related neuropathological effects were observed. At the high dose, minimal degeneration of the sciatic nerve was observed in 2/5 males and 2/5 females (vs. 2/5 and 0/5 controls, respectively). Brain weights were reduced in the high-dose females (↓4%, $p \leq 0.05$) Brain weight data are presented below and as an attachment (study report page 105, Table 15).

Table 7. Brain Weights in grams, (Mean ± standard deviation; N= 5 or 6)

Dose	0 (controls)	2.5 ppm	100 ppm	5000 ppm
Males				
Brain Weight	2.04±0.05	2.07±0.08	1.99±0.08	1.98±0.05
Females				
Brain Weight	1.93±0.06	1.93±0.04	1.89±0.02	1.85±0.07* (↓4%)

* $p<0.05$

While the magnitude of this change was modest, i.e., 4%, given the low variability associated with this measurement, it was statistically significant. Because of the general protection of this organ by the organism, that is, the brain's weight is preserved in the face of nutritional pressure at the expense of other organs, brain weight decreases in general effect are considered biologically significant and independent of body weight changes. Thus, this effect should be considered significant and treatment related.

III. DISCUSSION

A. Investigator's conclusions - Oral administration of 100 or 5000 ppm mesotrione produced decreased growth, reduced food consumption and utilization, and ocular toxicity. There was no evidence of neurotoxicity at any dose tested. The systemic NOAEL for this study was 2.5 ppm. The neurotoxicity NOAEL was 5000 ppm.

B. Reviewer's discussion/conclusions - In this subchronic neurotoxicity screening battery, mesotrione was administered continuously in the diet for 90 days to 12 Alpk:AP$_f$SD rats/sex/group at doses of 0, 2.5, 100, or 5000 ppm (equivalent to [M/F] 0/0, 0.20/0.23, 8.25/9.29, or 402.8/466.6 mg/kg/day). 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. Furthermore, the results confirmed the stability of the test substance in the vehicle for a period of 10 days at room temperature or 57 days at -20°C. No treatment-related findings were observed in the 2.5 ppm group. No treatment-related

deaths occurred. One 100 ppm male was sacrificed *in extremis* during week 7 following body weight loss during week 6; however, this death was considered unrelated to treatment. Food consumption and utilization, FOB parameters, motor activity, brain dimensions, and neuropathology were unaffected by the test substance.

Corneal opacities and/or vascularization of the cornea were seen in 3/11 mid-dose males and 1/12 mid-dose females. Corneal opacities and/or vascularization of the cornea were seen in 10/12 high dose males, and 7/12 high dose females. Overall (weeks 1-14) body weight gains were decreased in the high-dose females (↓18%). High-dose females displayed decreased body weights (adjusted for initial body weight) from week 2 until study termination (↓5-9%, $p \leq 0.01$).in the high-dose females at week 14 (↑37%) ($p \leq 0.05$)

The LOAEL for this study is 100 ppm (equivalent to 8.25 mg/kg/day in males and 9.29 mg/kg/day in females) based on corneal opacities and/or vascularization of the cornea of the eye. The NOAEL for this study is 2.5 ppm (equivalent to 0.20 mg/kg/day in males and 0.23 mg/kg/day in females).

The submitted study is classified as **acceptable/guideline** and satisfies the guideline requirements for a subchronic neurotoxicity screening battery **(§82-7[a]; 870.6200)** in rats.

C. Study deficiencies - Positive control data were provided from an acute neurotoxicity study (MRID 44505018), but this data are judged to be inadequate to validate the functional tests or assessment of the peripheral nervous system.

ATTACHMENT

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