DATA EVALUATION RECORD

3/20/01

MESOTRIONE (ZA1296)

Study Type: §81-8a, Neurotoxicity Screening Battery in Rats

Work Assignment No. 2-01-52O (MRIDs 44505017 and 44505018)

Prepared for
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MESOTRIONE (ZA1296) 3/20/2001

Acute oral neurotoxicity (§81-8[a])

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DATA EVALUATION RECORD

STUDY TYPE: Acute Oral Neurotoxicity [Gavage] - rat
OPPTS Number: 870.6200 OPP Guideline Number: §81-8a

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: Acute Neurotoxicity Study in Rats. Central Toxicology Laboratory, Cheshire, UK. Laboratory Project Identification Number AR6196. October 30, 1997. MRID 44505017. Unpublished.

Horner, S.A. (1997) Trimethyltin chloride: Acute Neurotoxicity Study in Rats. Central Toxicology Laboratory, Cheshire, UK. Laboratory Project Identification Number AR6406. October 28, 1997. MRID 44505018. Unpublished.

SPONSOR: Zeneca Ag Products, Wilmington, Delaware

EXECUTIVE SUMMARY: In this acute oral neurotoxicity study (MRID 44505017), mesotrione (ZA1296; 97.6% a.i., Lot/batch # P22) was administered in a single dose by gavage to 10 Alpk:AP$_f$SD rats/sex/dose at doses of 0, 20, 200, or 2000 mg/kg. After two weeks, five animals/sex/group were perfused for neurohistological examination and animals from the control and high-dose groups were examined microscopically. Functional observational battery (FOB) and motor activity were evaluated during week -1 and on days 1 (approximately 2 hours post-dosing), 8, and 15. No treatment-related deaths occurred. Clinical signs, FOB parameters, motor activity, body weights, body weight gains, food consumption, gross pathology, histopathology, and brain weights and dimensions were unaffected by the test substance.

The LOAEL is >2000 mg/kg, based upon lack of any systemic effects. The NOAEL for this study is 2000 mg/kg.

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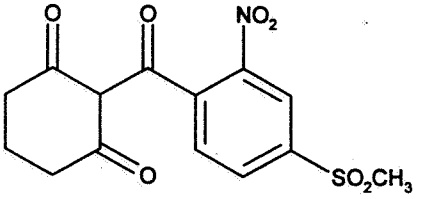
Although no effects were noted at the high dose, the substance was tested at the limit dose, so the high dose is acceptable. This study is classified as **acceptable/guideline** and satisfies the requirements for an acute neurotoxicity screening battery in rats (§81-8a; 870.6200).

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

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Mesotrione
   Description: Light beige solid
   Lot/Batch #: P22
   Purity (w/w): 97.6% a.i.
   Stability of compound: Stable up to 8 days at room temperature
   CAS #: 104206-82-8
   Structure:



2. Vehicle: Deionized water

3. Test animals: Species: Rat
   Strain: Alpk:AP$_f$SD
   Age and weight at the start of dosing: Approximately 42 days old; 171.6-176.3 g (males), 136.2-139.5 g (females)
   Source: Rodent Breeding Unit, Zeneca Pharmaceuticals, Cheshire, UK
   Housing: 5/cage in rat racks suitable for animals of this strain and weight range
   Diet: CT1 diet (Special Diet Services, Ltd., Essex, UK), *ad libitum*, except for 16-18 hours prior to dosing
   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: 2 weeks

B. STUDY DESIGN

1. In life dates - start: 01/28/97 end: 02/21/97

2. Animal assignment - The rats were randomly assigned (stratified by weight) to the test groups shown in Table 1. Four replicates or subgroups were used, with one replicate run at a time.

Table 1. Study design [a]

Test Group	Dose (mg/kg)	Animals Assigned	
		Male	Female
Control	0	10	10
Low [b]	20	10	10
Mid	200	10	10
High	2000	10	10

a Data obtained from the study report, page 17.
b Dosing formulations for the low-dose group were not homogeneous. Residue analysis was performed after dosing to confirm the dose levels received by the animals.

3. Dose selection rationale - Dose levels for the current study were chosen based on the results of another rat study carried out in this laboratory. No further information was provided.

4. Treatment preparation and dosing - The test substance was weighed and diluted with the appropriate volume of deionized water. Doses were prepared one day prior to administration and stored at room temperature in the dark. Dose volumes were 1 ml/100g.

   Prior to treatment, homogeneity (top, middle, bottom) and stability were confirmed using the 2 mg/mL and 200 mg/mL dose formulations. Stability at room temperature was determined for up to 8 days. Concentration analyses were conducted on all dose formulations prior to dosing.

   Results - Homogeneity analysis (range as % of nominal): 2 mg/mL formulation, 78-93%; 200 mg/mL formulation, 100-104%.

   Stability analysis (range as % of day 0): Samples stored for 8 days at room temperature were 93-107% of day 0.

   Concentration analysis (range as % of nominal): 87-105%

   It was stated that the low-dose formulation was not homogeneous; however, residue analysis confirmed that animals received 92.2-102.4% of the target dose. Overall, 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 - All data were analyzed using analysis of variance and/or covariance (procGLM, SAS).

C. METHODS

1. Observations

A. Clinical signs - All animals were examined daily for mortality and clinical signs of toxicity.

B. Functional observational battery and motor activity - All animals were subjected to functional observational battery (FOB) and motor activity measurements during week -1 and on days 1 (approximately 2 hours post-dosing), 8, and 15. 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 on days -7, -1, 1 (immediately prior to dosing and again approximately two hours post-dosing), 8, and 15.

3. Food consumption - Food consumption for each cage was recorded continuously during the study and reported as a weekly mean (g/rat/day)

4. Sacrifice and pathology - Five animals/sex/group were sacrificed by perfusion fixation and subjected to neuropathological examinations. The following control and high-dose tissues were embedded in paraffin or ARALDITE, sectioned, stained with toluidine blue or hematoxylin-eosin, and examined microscopically:

Central Nervous System		
Brain		
Examined in the traverse plane at seven levels		
Spinal cord		
Cervical swelling (C3-C6)	Lumbar swelling (L1-L4)	
Peripheral Nervous System		
Sciatic nerve	Sural nerve	Tibial nerve
Dorsal root ganglion (C3-C6)	Dorsal root ganglion (L1-L4)	Cervical spinal root (C3-C6)
Gasserian ganglia	Lumbar spinal root (L1-L4)	

Gastrocnemius muscle was also examined.

All animals that died during the study or were killed by perfusion fixation were subjected to gross necropsy. The remaining animals (up to 5 rats/sex/group) were killed by exsanguination and discarded.

II. RESULTS

A. Observations

1. Mortality - No treatment-related mortalities occurred. One mid-dose male was found dead on day 5; necropsy revealed dark red, discolored lungs. This death was considered not to be treatment-related.

2. Clinical signs - No treatment-related clinical signs were observed.

3. Functional observational battery - Results of the FOB indicated no treatment-related findings during the clinical or quantitative assessments for most endpoints. Time to tail-flick (See Table 2) was significantly increased in the high-dose males on day 1 (11.4 secs vs. 7.9 secs, controls; 44%). A 27% increase at 200 mg/kg failed to achieve statistical significance. While the study authors cited this value (11.4secs) as within the historical control range (4.73-11.6 seconds), such comparisons are at best secondary to concurrent control group, whose mean (7.9) was in fact higher than 10 of the 12 historical control means cited. Thus, even though this one finding seems to be a treatment-related effect, in conjunction with the other negative FOB findings plus no other neurotoxic effects in this study, the Hazard Identification Assessment Review Committee (March 13, 2001)

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concluded that the weight-of-the-evidence shows that the effect on time to tail-flick is of no biological relevance.

Other FOB changes were considered incidental to treatment. Landing foot-splay was increased in the high-dose males relative to concurrent controls on day 8 (↑20%, $p \leq 0.05$); however, there was a 14% increase observed in the high-dose males during the pretest interval. In the females, landing foot-splay was decreased at the low-, mid-, and high-dose (↓16-24%, $p \leq 0.05$ or 0.01) relative to concurrent controls; however, the decreases were not dose-dependent, and were also similar to decreases observed during the pretest interval (↓17-18%). FOB tables on hind limb grip strength and landing foot splay are attached to this report (Study pages 67, 68, and 70; Tables 9-11).

Table 2 . Time to Tail Flick in seconds (mean ± std. dev.; N=10).

	Controls	20 mg/kg	200 mg/kg	2000 mg/kg
Males				
Day -7	7.2 ± 4.5	8.0± 31.	5.9±2.9	8.7±4.6
Day 1	7.9±1.5	7.3±2.5	10.0±3.5 (27%)	11.4*±5.4 (44%)
Day 8	5.9±2.3	7.5±3.5	6.3±1.8	9.0±5.9
Day 15	4.1±1.4	4.6±1.6	3.6±1.7	4.0±1.6
Females				
Day -7	8.8±4.0	8.2±3.3	7.1±3.7	7.4±2.9
Day 1	6.0±2.4	5.5±2.0	6.8±2.6	5.4±2.2
Day 8	5.7±1.8	4.8±2.4	5.4±3.5	4.6±1.9
Day 15	4.6±1.7	5.5±1.7	3.9±1.6	3.6±1.9

* Statistically significantly different from controls, $p<0.05$; t test.
Adapted form study report Table 10, p68.

4. Motor activity - No treatment-related effects on total motor activity counts or on the pattern of motor activity habituation were observed. Only isolated increases in some sub-sessions were noted, which were not consistent or dose-dependent, and therefore considered not to be treatment-related. Complete motor activity data are presented as an attachment (study report pages 71-78, Table 12).

B. Body weight and body weight gain - No treatment-related differences in body weights or body weight gains were observed.

C. Food consumption - No treatment-related differences were observed in food consumption.

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D. Sacrifice and pathology

1. Gross pathology - No treatment-related gross pathological changes were observed.

2. Brain weights and dimensions - No treatment-related differences in brain weights and dimensions were observed.

3. Histopathology - No treatment-related histopathological changes were observed.

III. DISCUSSION

A. Investigator's conclusions - The study author concluded that mesotrione, administered to rats in a single dose by gavage at doses of 0, 20, 200, or 2000 mg/kg, did not produce neurotoxic effects at any level tested.

B. Reviewer's discussion - In this acute oral neurotoxicity study, mesotrione was administered as a single dose by gavage to 10 Alpk:AP$_f$SD rats/sex/dose at doses of 0, 20, 200, or 2000 mg/kg. 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 for a period of 8 days.

No treatment-related deaths occurred. Clinical signs, FOB parameters, motor activity, body weights, body weight gains, food consumption, gross pathology, histopathology, and brain weights and dimensions were unaffected by the test substance.

The LOAEL is >2000 mg/kg, based upon lack of any systemic effects.
The NOAEL for this study is 2000 mg/kg.

Although no effects were noted at the high dose, the substance was tested at the limit dose, so the high dose is acceptable. This study is classified as **acceptable/guideline** and satisfies the requirements for an acute neurotoxicity screening battery in rats (§81-8a; 870.6200).

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

- No dose rationale was provided; however, the test substance was administered at the limit dose (2000 mg/kg).

- No information supporting the choice of peak effect time was provided.

ATTACHMENTS

1. FOB tables on hind limb grip strength and landing foot splay are attached to this report (Study report pages 67, 68, and 70; Tables 9-11).

2. Complete motor activity data are presented as an attachment (Study report pages 71-78; Table 12).

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ATTACHMENTS ARE NOT AVAILABLE ELECTRONICALLY
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