1/3/97

DATA EVALUATION REPORT

AZOXYSTROBIN

STUDY TYPE: ACUTE ORAL NEUROTOXICITY - RAT (81-8)

Prepared for

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

Prepared by

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YOA 8/22/96

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MSO 12/31/96

DATA EVALUATION RECORD

STUDY TYPE: Acute Oral Neurotoxicity - Rat [§81-8]

DP BARCODE: D218319 SUBMISSION CODE:S489692
P.C. CODE: 128810 TOX. CHEM. NO.: None (new chemical)

TEST MATERIAL (PURITY): ICIA5504 (Azoxystrobin, 96.2%)

SYNONYMS: None

CITATION: Horner, S.A. (1994) ICIA5504: Acute Neurotoxicity Study in Rats. Zeneca Central Toxicology Laboratory Cheshire, UK. Report No. CTL/P/4313 (Study No. AR5648), June 22, 1994. MRID 43678134. Unpublished.

SPONSOR: Zeneca Inc., Agricultural Products, Wilmington, DE 19897

EXECUTIVE SUMMARY: In an acute neurotoxicity study (MRID 43678134), ICIA5504 (Azoxystrobin, 96.2% a.i.) was administered once in corn oil (10 ml/kg body wt) by gavage to 3 groups of 10 Alpk:ApfSD rats/sex/dose at doses of 0, 200, 600 or 2000 mg/kg. All animals were evaluated in functional observational battery (FOB) and motor activity (MA) testing on Days -7, 1 (2 hr post-dosing), 8 and 15. Five control and high dose animals/sex perfused in situ were evaluated for microscopic neuropathology.

At 200 mg/kg and higher, diarrhea/signs of diarrhea were observed at 2 hr post-dosing in both sexes (males, 1, 4, 5 and 10; females, 0, 9, 9 and 6). Tip-toe gait and upwardly curved spine at 2 hr were also observed in treated but not control animals (no dose-response observed). No treatment-related effects on survival, food consumption, motor activity, brain weight/dimensions, or gross/microscopic pathology were observed. Body weights of males at 2000 mg/kg were slightly decreased (2.9% and 2.6% at day 8 and 15). Statistically significant increases in landing foot splay on Day 8 in females at 600 and 2000 mg/kg are noted (23.7% and 20.5% higher than controls, respectively; on Day 1 females at 600 and 2000 mg/kg had nonstatistically significantly increased values of 11.8 and 12.5%, respectively). These were not considered indicative of neurotoxicity due to lack of effect on day of dosing (only marginal non-significant increase seen) and to lack of a clear dose-response and indications of other effects. These effects are also exacerbated by the observed gastric distress, a non-neurotoxic

76

response. **The systemic toxicity LOEL is 200 mg/kg, based on occurrence of transient diarrhea in both sexes. The systemic toxicity NOEL is < 200 mg/kg. There was no indication of neurotoxicity at the doses tested.**

This acute neurotoxicity study in the rat is classified as **Supplementary (upgradable)** and does not satisfy the guideline requirement for an acute oral study (81-8). The study may be upgraded to Acceptable pending submission of: (1) validation studies demonstrating proficiency of the testing laboratory in conduct of neurobehavioral testing procedures, (2) provision of data supporting selection of 2 hr post-dosing as the time of peak effect and (3) clarification of parameters evaluated in the FOB (see "Discussion" for details).

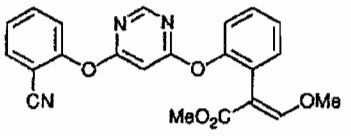
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: ICIA5504

Description: light brown solid
Lot/Batch #: P49/D7534/46
CTL reference number Y06654/014
Purity: 96.2% w/w
Stability of compound: stable for the duration of the study (reported shelf life of at least 2 hrs at room temperature)
CAS #: 131860-33-8



2. Vehicle and/or positive control

Kraft Wesson Corn Oil Lot/Batch #CTL Reference number Y00790/004

3. Test animals

Species: rat
Strain: Alpk:APfSD
Age and weight at study initiation: approximately 42 days; 146-185 g (males), 114-150 g (females)
Source: Barriered Animal Breeding Unit at Zeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK

Housing: 5 animals/cage and sexes separated, in multiple rat racks made of stainless steel with stainless steel mesh bottoms
Diet: CT1 diet (Special Diets Services Limited, Stepfield, Witham, Essex, UK) ad libitum
Water: tap water via an automatic watering system, ad libitum
Environmental conditions:
Temperature: 19-23°C
Humidity: 40-70%
Air changes: 25-30 changes/hr
Photoperiod: 12 hrs light/12 hrs dark
Acclimation period: approximately 2 weeks

B. STUDY DESIGN

1. In-life dates

Start: November 22, 1993 (based on arrival date and 14 day acclimation period); end: December 6, 1993

2. Animal assignment

Animals were assigned randomly to the test groups as presented in Table 1. Four single-sex replicate groups balanced for all dose groups were randomly selected for the neurobehavioral testing.

TABLE 1: Study design

Test Group	Dose to Animal (mg/kg)	Animal/Group	
		Male	Female
Control	0	10	10
Low	200	10	10
Mid	600	10	10
High	2000	10	10

Data taken from Table 1, page 14, MRID 43678134.

3. Dose selection rationale

A limit dose of 2000 mg/kg was the highest dose given. Selection of the low and mid doses was not explained other than being based on results from previous studies conducted at this laboratory in the Alpk:APfSD rat. A basis for selection of 2 hr post-dosing as the time of peak effect was also not provided.

4. Dose preparation and analysis

Doses were prepared once by mixing appropriate amounts of test substance with Kraft Wesson Corn Oil and were stored at ambient temperature in the dark. Homogeneity and chemical stability were tested over a period of 8 days.

Results - The dosing solutions were homogeneous and were stable for at least 8 days, and animals received the appropriate dose (see Tables 2-4, below for summary of analytical results).

TABLE 2: Homogeneity of ICIA5504 dosing solutions

Nominal Concentration (mg/ml)	Sampling Point	Analyzed Concentration (mg/ml)		Mean Concentration (mg/ml)	% Deviation
20.8	Bottom	20.4	20.3	20.4	+0.5
	Middle	20.3	20.4	20.4	+0.5
	Top	20.3	20.0	20.2	-0.5
207.9	Bottom	200	199	200	-1.5
	Middle	204	206	205	+1.0
	Top	205	205	205	+1.0

Data taken from Table 3 of study report, p. 29.

TABLE 3: Stability of ICIA5504 dosing solutions

Nominal Concentration (mg/ml)	Analysis Date	Analyzed Concentration (mg/ml)		Mean Concentration (mg/ml)	% of Initial Concentration
20.8	18/11/93	20.0	20.2	20.1	100.0
	26/11/93	20.8	20.4	20.6	102.5
207.9	18/11/93	203	204	204	100.0
	26/11/93	198	211	205	100.5

Data taken from Table 4 of study report, p. 30.

TABLE 4: Concentration of ICIA5504 dosing solutions

Nominal Concentration (mg/ml)	Analyzed Concentration (mg/ml)		Mean Concentration (mg/ml)	% of Nominal Concentration
20.8	20.0	20.2	20.1	96.6
62.4	60.6	61.1	60.9	97.6
207.9	203	204	204	98.1

Data taken from Table 2 of study report, p. 28

5. Statistics

The body weights were analyzed by covariance; food consumption, motor activity measurements, time to tail-flick, landing foot splay and fore and hind-limb grip strength were analyzed by analysis of variance; and brain weight, brain length, and brain width were analyzed by analysis of covariance. All analyses used the GLM procedure in SAS (SAS/STAT User's Guide, version 6, 4th edition, volume 2, 1989).

6. Validation studies

Studies using positive controls substances to demonstrate proficiency of the testing laboratory in neurobehavioral testing were not provided.

C. METHODS

1. Observations

Animals were inspected daily for signs of toxicity and mortality.

2. Body weight

Animals were weighed on Day -7, immediately before dosing on Day 1, 2 hrs after dosing on Day 1, and on Days 8 and 15.

3. Food consumption and compound intake

Food consumption for each cage of rats was determined and mean weekly diet consumption was calculated as g food/rat/day.

4. Functional observation battery

Detailed clinical observations (during which each rat was removed from its cage and physically examined for changes in general health status) and quantitative assessments of landing foot splay, sensory perception (tail-flick test) and muscle weakness (fore- and hind-limb grip strength) were made in Week -1, on Day 1 (2 hrs after dosing), on Day 8, and on Day 15. The clinical observations included, but were not limited to, the following list of measures: assessment of autonomic function (e.g., lacrimation, salivation, piloerection, exophthalmus, urination, defecation,

80

pupillary function, ptosis); description, incidence and severity of any convulsions, tremors, abnormal motor function, abnormal behavior, etc.; reactivity to stimuli; changes in level of arousal; sensorimotor responses; and alterations in respiration. The observations were made by one observer who was "blind" with respect to animal treatment, and recorded on a computer system by personnel not directly involved in the clinical observations. The observations were carried out in a room separate from that in which the animals were housed, and animals were presented to the observer with no indication of the treatment group. The observations were coded and the degree of condition was noted (slight, moderate, or extreme) where appropriate. This included the recording of no abnormalities detected. The study report did not indicate whether handling observations were performed.

5. Motor activity

Locomotor activity was monitored by an automated activity recording apparatus (type not indicated). All animals were tested on Day -7, on Day 1 (2 hrs after dosing), and on Days 8 and 15. Each observation period was divided into ten scans of five-minute durations. Treatment groups were counter balanced across test times and across devices, and when the trials were repeated each animal was returned to the same activity monitor at approximately the same time of day. Motor activity was assessed in a separate room to minimize disturbances. The study report did not indicate whether testing was conducted under dimmed light or with white noise to minimize background disturbances.

6. Sacrifice and pathology

All animals that died and those sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination (see table below). Termination was performed under anesthesia with halothane vapor followed by exsanguination. In addition, at the scheduled termination, 5 animals/sex/group were deeply anesthetized with intraperitoneal sodium pentobarbitone and killed by perfusion fixation with modified Karnovsky's fixation. The previously listed tissues were removed, and the brain weight, length, and width were recorded. All perfused fixed tissues from the control and 2000 mg/kg groups and the perfused fixed brains from the 200 and 600 mg/kg groups were processed for histology.

X	Brain	X	Spinal Cord	X	Peripheral nerves
X	Whole (7 sections)	X	Vertebral column	X	Sciatic nerve
		X	Dorsal root ganglion	X	Sural nerve
		X	Spinal roots	X	Tibial nerve
					Other
				X	Gastrocnemius
				X	Gasserian ganglion

II. RESULTS

A. OBSERVATIONS

1. Toxicity

An increased incidence of diarrhea or signs of diarrhea, tip toe gait, and/or upward curvature of the spine were observed in the treatment groups (Table 5). These signs were observed approximately 2 hrs after administration on day 1, and recovery usually occurred by day 2. No apparent dose-related increase in incidence was noted except for combined diarrhea/signs of diarrhea in males.

TABLE 5: Clinical signs of diarrhea for animals acutely administered ICIA5504

Clinical Sign	Control	200 mg/kg	600 mg/kg	2000 mg/kg
Males				
Diarrhea	1	1	1	6
Signs of diarrhea	0	3	4	4
Upward curved spine	0	1	3	0
Tip-toe gait	0	4	4	5
Females				
Diarrhea	0	1	2	2
Signs of diarrhea	0	8	7	4
Upward curved spine	0	1	1	0
Tip-toe gait	0	6	7	3

Data taken from Table 7, page 35-37, MRID 43678134.

2. Mortality

No deaths occurred prior to the scheduled sacrifice.

B. BODY WEIGHT

In the 2000 mg/kg group, the males had a statistically significantly decreased body weight (adjusted mean) of 2.9 and 2.6% at Days 8 and 15, respectively (Table 6).

Statistically significantly decreased body weight in the males of the 200 mg/kg group on Day 15 were observed. However, a dose response was not evident and the decrease was considered to be incidental.

TABLE 6: Mean (± SD) body weight (g) for animals acutely administered ICIA5504

Study Day	Control	200 mg/kg	600 mg/kg	2000 mg/kg
Males				
Day 1	165.1 ± 10.5	165.6 ± 12.3	166.5 ± 10.9	169.1 ± 9.0
Day 8	242.3 ± 13.2	240.5 ± 15.6	239.3 ± 15.7	235.2** ± 10.2 (2.9%)[a]
Day 15	291.3 ± 13.0	284.7* ± 14.2 (2.3%)	288.3 ± 16.2 (1.0%)	283.8* ± 12.1 (2.6%)
Females				
Day 1	131.9 ± 9.9	131.2 ± 7.0	128.5 ± 10.1	130.6 ± 4.2
Day 8	172.3 ± 14.2	176.2 ± 10.2	169.9 ± 12.6	172.3 ± 6.6
Day 15	193.5 ± 17.8	196.0 ± 13.8	190.3 ± 14.0	189.8 ± 9.0

Data taken from Table 5, page 32, MRID 43678134.
[a]Parenthesis: percent decrease of the treated group body weight as compared to the control body weight (calculated by the reviewer)
*P≤ 0.05
**P≤ 0.01

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

No treatment-related changes were noted.

D. FUNCTIONAL OBSERVATION BATTERY

No consistent evidence of a treatment-related effect on landing foot splay measurements was noted (Table 7). In the females receiving 600 or 2000 mg/kg, the landing foot splay values were statistically significantly higher on Day 8. Since measurements on Days 1 and 15 did not demonstrate

83

any change and no dose-response relationship was observed, this finding was not considered treatment-related by the study author. A non-significant increase of 23.8% was observed in males on Day 1 at 2000 mg/kg.

TABLE 7: Mean (± SD) landing foot splay measurements (mm) for animals acutely administered ICIA5504

Study Day	Control	200 mg/kg	600 mg/kg	2000 mg/kg
		Males		
Day -7	47.3 ± 7.9	45.4 ± 9.8	48.6 ± 7.7	46.5 ± 5.9
Day 1	45.9 ± 9.8	47.7 ± 13.8 (3.9%)	48.6 ± 10.1 (5.9%)	56.8 ± 19.2 (23.8%)
Day 8	50.9 ± 14.7	55.3 ± 10.3 (8.6%)	56.0 ± 11.8 (10.0%)	52.8 ± 8.8 (3.7%)
Day 15	52.7 ± 12.8	53.4 ± 7.9 (1.3%)	57.7 ± 10.0 (9.5%)	54.1 ± 15.6 (2.7%)
		Females		
Day -7	42.7 ± 7.5	42.5 ± 8.3	45.1 ± 7.3	43.0 ± 7.0
Day 1	45.7 ± 8.5	44.0 ± 9.9	51.1 ± 4.3 (11.8%)	51.4 ± 14.8 (12.5%)
Day 8	44.3 ± 9.8	49.6 ± 9.8	54.8* ± 8.2 (23.7%)	53.4* ± 11.5 (20.5%)
Day 15	46.8 ± 8.2	46.4 ± 9.5	49.6 ± 3.2 (6.0%)	47.6 ± 13.6 (1.7%)

Data taken from Table 8, page 38, MRID 43678134.
[a]Parenthesis: percent increase of the treated group landing foot splay as compared to the control landing foot splay (calculated by the reviewer)
*P≤ 0.05

Times to tail flick were inconsistent and were considered unaffected by administration of ICIA5504.

Forelimb and hindlimb grip strength had no treatment-related effect.

E. MOTOR ACTIVITY MEASUREMENTS

No treatment-related effect was observed on the motor activity measurements.

F. SACRIFICE AND PATHOLOGY

1. Brain parameters (weight, length, width)

No treatment-related changes were observed in these brain measurements.

2. Gross pathology

No treatment related findings were observed.

3. Microscopic pathology

No treatment related findings were observed. Minimal neuronal cell necrosis in the hippocampal formation/pyriform cortex and sciatic nerve fiber degeneration were observed in both control and high dose animals and were considered background events.

III. DISCUSSION

A. DISCUSSION

The reviewer agreed with the study author that azoxystrobin did not appear to be neurotoxic at the doses tested, but that transient, treatment-related systemic toxicity was observed post-dosing at all dose levels. Treated animals of both sexes in all groups showed increased incidence of either diarrhea or signs of diarrhea; the combined incidence in males, but not females, showed a dose-response. Diarrhea was only observed at the day 1 observation time (2 hr post-dosing) and did not persist, but is considered to be related to administration of the test material (in the rat developmental toxicity study, MRID 43678142, diarrhea was observed in females given 100 and 300 mg/kg/day, and in the acute rat oral LD_{50} study, MRID 43678122, at the only dose administered, 5000 mg/kg). Tip-toe gait and upwardly curved spine did not show a dose-response, but are possibly treatment-related since they were not observed in controls and were observed immediately post-dosing.

In males, a non-significant increase in landing foot splay measurement on day 1 at 2000 mg/kg was observed but was not considered treatment-related due to variability. Landing foot splay measurements in females were slightly, nonstatistically significantly increased by 11.8 and 12.5% on Day 1 in the 600 and 2000 mg/kg groups, respectively. On Day 8 these two dose groups had statistically significantly increased measurements (23.7 and 20.5%, respectively). By Day 15 the measurements from these two groups were approximately equal to controls. The increases at day 8 were not considered to be treatment-related neurotoxic effects because a dose-response was not observed, no other effects were reported and a significant increase was not seen at day 1. These effects are also exacerbated by the observed gastric distress, a non-neurotoxic response.

Based on the transient occurrence of diarrhea post-dosing in both sexes at all dose levels, the LOEL for systemic

toxicity is 200 mg/kg and a LOEL was not determined. The findings did not indicate that azoxystrobin caused neurotoxicity at the doses tested.

B. STUDY DEFICIENCIES

1. Major

FOB and motor activity validation data were not provided in the report.

Support (eg. results of preliminary study) for selection of 2 hr post-dosing as the time of peak effect was not provided in the report.

It was unclear from the study report whether certain standard parameters were evaluated in the FOB, for example handling observations, corneal, pinna or righting reflexes, and muscle tone, or which types of response to stimuli were evaluated.

2. Minor

The study protocol, protocol deviations, protocol amendments should be included.

Classification: Supplementary - Upgradable with submission of acceptable FOB and motor activity validation (positive control) studies, support for the selection of 2 hrs post-dosing as the time of peak effect and a more complete listing of the FOB parameters evaluated in this study.

ATTACHMENTS

THE FOLLOWING ATTACHMENTS ARE NOT AVAILABLE ELECTRONICALLY. SEE THE FILE COPY.

Azoxystrobin

Page _____ is not included in this copy.

Pages 14 through 22 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

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_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

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_____ The product confidential statement of formula.

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