3/18/96

DATA EVALUATION REPORT

AZOXYSTROBIN

STUDY TYPE: REPEATED DOSE DERMAL - RAT (82-2)

Prepared for

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Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

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DATA EVALUATION REPORT

STUDY TYPE: Repeated Dose Dermal - Rat
OPPTS 870.3200 [§82-2]

DP BARCODE: D218319
P.C.CODE.: 128810

SUBMISSION CODE: S489692
TOX. CHEM, NO: none

MRID NO.: 43678137

TEST MATERIAL (PURITTY): ICIA5504 (Azoxystrobin) (96.2%)

SYNONYM: None

CITATION: Robinson, P. (1994) ICIA5504: 21-Day Dermal Toxicity Study in the Rat. Zeneca Central Toxicology Laboratory, Alderly Park, Macclesfield, Cheshire, UK. Laboratory project identification: Report No. CTL/P/4360; Study No. LR0561, May 24, 1994. MRID 43678137. Unpublished.

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

EXECUTIVE SUMMARY: In a 21-day repeated dose dermal toxicity study (MRID 43678137), groups of 5 male and 5 female Wistar rats were treated with ICIA5504 (Azoxystrobin) (96.2% w/w) in a deionized water paste by dermal occlusion at doses of 0, 200, 500, or 1000 mg/kg/day, 6 hours/day for 21 days over a 30 day period.

No mortality was observed and there were no significant treatment-related clinical abnormalities. There were no treatment-related effects on bodyweight, food consumption, organ weights, clinical biochemistry, or hematology. There were no treatment-related pathological abnormalities. Abdominal scabs and scabs at the edge of the application area were observed in all groups of females and were attributed to the bandaging method and were not of toxicological significance.

The NOEL for Azoxystrobin was 1000 mg/kg/day; a LOEL was not determined.

This study is classified as **acceptable** and satisfies the guideline requirements for a 21-day dermal study (82-2) in rats.

COMPLIANCE: Signed Quality Assurance (5/17/94), Flagging (5/19/94), and Good Laboratory Practice (5/18/94) Statements were present.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: ICIA5504

Description: light brown solid
Lot/Batch No.: Y06654/014
Purity: 96.2 % w/w
Stability of compound: stable for the duration of the study, from information supplied by the sponsor
CAS No.: unknown
Structure: unknown

2. Vehicle and/or positive control

Vehicle: deionized water
Positive control: none

3. Test animals

Species: rat
Strain: Wistar-derived albino (Alpk:APfSD)
Age and weight at study initiation: males: 8-9 weeks; 249-292 g; females: 9-12 weeks; 219-250 g
Source: Barriered Animal Breeding Unit; Zeneca Pharmaceuticals; Alderley Park, Macclesfield, Cheshire, UK
Housing: individually in stainless steel suspended cages
Diet: Porton Combined Diet, Special Diet Services, Ltd., *ad libitum*
Water: tap water via an automatic watering system, *ad libitum*
Environmental conditions:
- Temperature: 21±2°C
- Humidity: 55±15%
- Air changes: 25-30 changes/hour
- Photoperiod: 12 hour light/dark cycle

Acclimation period: approximately 2 weeks

B. STUDY DESIGN

1. In life dates

Start: November 9, 1993; end: December 9, 1993

2. Animal assignment

Rats were randomly distributed within the experimental groups (Table 1) by a method designed to ensure that unhealthy animals or animals at the extremes of the weight range were excluded. Groups of 5 rats/sex/dose were utilized.

TABLE 1. Study design				
Dose Group	Dose (mg/kg)		No. of Animals	
	Male	Female	Male	Female
Vehicle control (water only)	0	0	5	5
Azoxystrobin (low-dose)	250	250	5	5
Azoxystrobin (mid-dose)	500	500	5	5
Azoxystrobin (high-dose)	1000	1000	5	5

Data taken from p. 13, MRID 43678137.

3. Dose selection rationale

The dose levels were selected from data obtained in a preliminary study in which preparations of 250, 500, or 1000 mg/kg azoxystrobin were applied to groups of two male and two female rats for 5 consecutive days followed by a 2 day observation period. There were no signs of toxicity or skin irritation at any dose level.

4. Test substance preparation and analysis

The appropriate amount of azoxystrobin was weighed out and made into a paste by adding a small amount of deionized water. The amount applied was calculated for each animal according to its weight at the time of dosing.

Results -
Homogeneity analysis - Not applicable.

Stability analysis - Information provided to the testing laboratory by the sponsor indicated test material stability for the duration of the study.

Concentration analysis - Not applicable.

5. Dose application

A 10 cm x 5 cm area of fur was clipped from the dorsal area of the trunk of each animal 16 to 24 hr before application of the test sample. The appropriate amount of test sample paste was applied and kept in contact with the shaved back for 6 hours. Each occlusive dressing consisted of a gauze patch covered by a patch of plastic film and was held in place by PVC tape wrapped around the animal. At the end of the 6-hour exposure period, the dressings were removed and any residual test material was removed using clean swabs of absorbent cotton wool soaked in clean warm water. The area was then dried with tissue paper. A total of 21 six-hour applications were made during a period of 30 days. During this period, there were four two-

day periods when the animals were not dosed. There was an 18-hour period between each application. During the 18-hour period, the rats were fitted with plastic collars to prevent possible test substance ingestion.

6. Statistics

Body weights were analyzed by analysis of covariance. Daily food consumption, hematology, and clinical chemistry were analyzed by analysis of variance. Organ weights were analyzed by analysis of variance and covariance. Test and control data were compared using a two-sided Students t-test.

C. METHODS

1. Observations

Animals were examined for gross signs of toxicity and for signs of irritation at the application site twice daily on days of dosing (prior to dosing and at decontamination) and once daily on the days animals were not dosed.

2. Body weight

Animals were weighed daily prior to dosing.

3. Food consumption

Individual food consumption was calculated daily throughout the study.

4. Ophthalmoscopic examination

No ophthalmoscopic examinations were performed.

5. Blood samples were obtained by cardiac puncture from each rat immediately after death. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
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)		
	(Clotting time)		
X	(Prothrombin time)		
X	(Kaolin-cephlin time)		
X	Erythrocyte morphology		

b. Clinical chemistry

X	ELECTROLYTES	X	ELECTROLYTES
X	Calcium	X	Albumin
X	Chloride	X	Blood creatinine
	Magnesium	X	Blood urea nitrogen
X	Phosphorus	X	Total Cholesterol
X	Potassium		Globulins
X	Sodium	X	Glucose
		X	Total bilirubin
	ENZYMES	X	Total serum protein (TP)
X	Alkaline phosphatase (ALK)	X	Triglycerides
	Cholinesterase (ChE)		Serum protein electrophoresis
X	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine amino-transferase (also SGPT)		
X	Serum aspartate amino-transferase (also SGOT)		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

6. Urinalysis

Urinalysis was not required and was not performed.

7. Sacrifice and pathology

All animals survived until the scheduled termination of the study. Rats were euthanized at the end of the study with halothane vapor and exsanguination. Gross pathological examinations were conducted and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue		Aorta	X	Brain
	Salivary glands		Heart		Periph. nerve
	Esophagus		Bone marrow		Spinal cord (3 levels)
	Stomach		Lymph nodes		Pituitary
	Duodenum		Spleen		Eyes (optic n.)
	Jejunum		Thymus		
	Ileum				GLANDULAR
	Cecum		UROGENITAL	X	Adrenal gland
	Colon	XX	Kidneys*		Lacrimal gland
	Rectum		Urinary bladder		Mammary gland
XX	Liver*	XX	Testes		Parathyroids
	Gall bladder	X	Epididymides		Thyroids
	Pancreas		Prostate		
			Seminal vesicle		OTHER
	RESPIRATORY		Ovaries		Bone
	Trachea		Uterus		Skeletal muscle
	Lung*			X	Skin (treated & untreated)
	Nose				All gross lesions and masses
	Pharynx				
	Larynx				

*Required in Repeated Dose Dermal study.

II. **RESULTS**

A. Observations

No treatment-related mortality or clinical signs of toxicity were seen in any rats. The fur of some males and most females was removed by the bandages during the study and abdominal scabs and scabs on the edge of the application area (not on the treated areas) were observed on some females in all groups. The authors attributed these effects to bandaging or chewing of the bandaged areas and did not consider them toxicologically significant. Slight erythema was observed on two high-dose males during days 7-14, and slight desquamation was observed in one 500 mg/kg female on days 11 and 12 and in three 1000 mg/kg females from days 10 to 12. These effects are not considered toxicologically relevant due to their transitory nature.

B. Bodyweight and weight gain

No significant differences in group body weight means occurred for any treated group as compared to controls.

C. Food consumption

1. Food consumption

Sporadic significant ($p<0.05$) differences in food consumption were observed. However, in the absence of a dose-related response and any effects on body weight, the differences are considered biologically insignificant.

2. Food efficiency

Feed efficiency ({body weight gain [kg]/food consumption [kg per unit time]} x 100) values were not calculated by the study authors. Because there were no toxicologically relevant changes in food intake or body weight, food efficiency was not considered to provide any additional information.

D. Opthalmoscopic examination

No opthalmoscopic examinations were performed.

E. Blood work

No biologically significant hematological effects were observed. Statistically significant, however minor, changes were observed in MCH, MCHC, and lymphocyte counts in males and in MCV, MCHC, and lymphocyte counts in females. These changes were considered toxicologically insignificant since they were small, sporadic, and did not exhibit a dose-response. Statistically significant changes in plasma phosphorus, calcium, potassium, glucose, triglycerides, total protein, and GGT were observed. However, due to the lack of a dose-response

and/or small magnitude of change, these effects are not considered biologically relevant.

F. Urinalysis

Urinalysis was not required and was not performed.

G. Sacrifice and pathology

1. Organ weight

There were no compound-related effects on organ weight.

2. Gross pathology

No toxicologically significant effects were observed. The scabbing and erythema on the skin were attributed to self trauma or an artifact of the bandaging procedure.

3. Microscopic pathology

a) Non-neoplastic - Minimal acanthosis and inflammatory cell infiltration were observed in a treated skin section of one high-dose male and an abdominal skin section of one high-dose female.

b) Neoplastic - A nephroblastoma was observed in one high-dose female. It is not considered treatment-related due to its isolated incidence, and no supporting treatment-related kidney histopathology.

III. **DISCUSSION**

A. Male and female Wistar rats were treated dermally with 0, 200, 500, or 1000 mg/kg/day of Azoxystrobin 6 hours/day for 21 days over a 30 day period. No statistically significant differences in group body weight means or cumulative body weight gains occurred for any treated group of either sex as compared to controls. No treatment-related mortality or clinical signs were observed. No biologically significant clinical chemistry or hematological effects were observed. No significant gross or microscopic treatment-related pathology was observed.

Under conditions of this study, the NOEL for azoxystrobin is 1000 mg/kg/day; a LOEL is not identified.

B. Study deficiencies

Lung histopathology was not examined. This is not thought to significantly compromise this study.