4/18/96
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DATA EVALUATION REPORT

AZOXYSTROBIN

STUDY TYPE: SUBCHRONIC ORAL TOXICITY FEEDING - RAT (82-1a)

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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DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity Feeding - rat
OPPTS 870.3100 [§82-1a]

DP BARCODE: D218319 SUBMISSION CODE: S489692
P.C. CODE: 128801 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): ICIA5504 (azoxystrobin) (95.2%)

SYNONYMS: Methyl (E)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate.

CITATION: Milburn, G. (1995) ICIA5504: 90 day feeding study in rats. ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK. Report No. CTL/P/3649, July 21, 1992. MRID 43678135. Unpublished.

SPONSOR: ZENECA Ag Products

EXECUTIVE SUMMARY: In a subchronic toxicity study (MRID 43678135), ICIA5504 (95.2% a.i., Lot No. P32) was administered to 12 Alpk:APfSD rats/sex/dose in the diet at concentrations of 0, 200, 2000 or 4000 ppm (0, 20.4, 211.0 or 443.8 mg/kg/day for males and 0, 22.4, 223.0 or 448.6 mg/kg/day for females) for 13 weeks. The 4000 ppm treatment groups were initially administered 6000 ppm in the diet, but this concentration was reduced after 15 days due to reduced food consumption and a marked reduction in growth.

Final body weights of males and females receiving 4000 ppm in the diet were reduced by 32 and 18%, respectively, and final body weights of males and females receiving 2000 ppm in the diet were reduced by 18 and 11%, respectively. Food consumption and food efficiency were reduced in both sexes receiving 4000 ppm, particularly during weeks 1-2 or weeks 1-4. However, by the end of the study, food efficiency of females in the 4000 ppm treatment was not significantly reduced compared with that of controls. In addition to small body size, distended abdomens, attributable to reduced nutritional status, were observed in both sexes in these two exposure groups. Minimal reductions in hemoglobin, MCV, MCH (females) and reduced cholesterol (males), glucose (females), increased triglycerides (both sexes), and some plasma enzyme activities (both sexes) were increased at 4000 ppm were also attributable to reduced nutritional status. Elevated white cell counts and decreased platelets in both sexes may be treatment related, but were not accompanied by histopathological findings,

indicating they were not toxicologically significant. All of these findings were less marked in the groups receiving 2000 ppm and were absent in the groups receiving 200 ppm. Increases in liver and kidney weights adjusted for body weight in the 2000 and 4000 ppm treatment groups were attributable to treatment. Changes in organ weights were accompanied by histopathological findings in two males in the 4000 ppm treatment group. Treatment-related effects in these males included marked elevations in total bilirubin, cholesterol, triglycerides, and plasma enzyme activities. The effect on the liver of these two animals was observed microscopically as proliferation of the intrahepatic bile duct/ductiles and oval cells. Hepatocellular hyperplasia and an enlarged hepatic lymph node was observed in one of the two males. **The LOEL is 2000 ppm (211.0 and 223.0 mg/kg/day for males and females) based on decreased weight gain in both sexes, clinical observations of distended abdomens and reduced body size, and clinical pathology findings attributable to reduced nutritional status. The NOEL is 200 ppm (20.4 and 22.4 mg/kg/day for males and females).**

This subchronic toxicity study is classified acceptable because it generally satisfies the guideline requirement for a subchronic oral study (82-1a) in rats. The study was properly conducted and a NOEL and LOEL were determined. No deficiencies were noted.

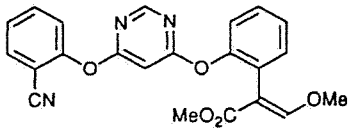
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: ICIA5504

Description: off-white solid
Lot/Batch #: P32, CTL Reference No. YO6654/004
Purity: 95.2% (w/w) a.i.
Stability of compound: stable over duration of test when stored at 15-25°C
CAS #: not available
Structure:



2. Vehicle and/or positive control

Dry test material was mixed with feed; therefore, no vehicle was required. A positive control was not included.

3. Test animals

Species: rat
Strain: Alpk:APfSD (Wistar derived)
Age and weight at study initiation: 30 days of age; males, 76.3 g; females, 72.0 g (study initiation is designated week 1 in the study).
Source: Specific Pathogen Free colony, Barriered Animal Breeding Unit, ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK
Housing: four per cage, sexes separate, in stainless steel/stainless steel mesh cages
Diet: CT1 diet manufactured by Special Diets Services Ltd., Stepfield, Witham, Essex, UK, was available *ad libitum*
Water: Filtered water was available *ad libitum* via an automatic watering system.
Environmental conditions:
Temperature: 17-24°C
Humidity: 29-69%
Air changes: 15 changes per hour
Photoperiod: 12-hour light/dark
Acclimation period: 7-8 days

B. STUDY DESIGN

1. In life dates

Start: August 27-29, 1991; end: November, 1991 (date not stated)

2. Animal assignment

Animals were assigned by random number (by litter and within litters) to the test groups in Table 1.

42

TABLE 1: Study design

Test Group	Conc. in Diet (ppm)	Dose to Animals (mg/kg/day)	Number of Males	Number of Females
Control	0	0	12	12
Low	200	21.4 20.4 (males) 22.4 (females)	12	12
Mid	2000	217.0 211.0 (males) 223.0 (females)	12	12
High	4000[a]	446.2 443.8 (males) 448.6 (females)	12	12

Data taken from pages 16 and 21 and Appendix I (p. 85), MRID 43678135.
[a] Group originally administered 6000 ppm for 15 days; treatment withdrawn for 5 days followed by dietary administration of 4000 ppm for remainder of study.

3. Diet preparation and analysis

Diet was prepared prior to initiation of the experiment by mixing appropriate amounts of test substance (w/w, corrected for purity) with 1 or 2 kg of CT1 diet. These premixes were added to 29 or 28 kg of diet and mixed in a blender. The mixture was divided into glass feeder jars which were placed in color-coded trays and stored in a freezer. Homogeneity was tested by taking three samples from the low (200 ppm) and high (4000 ppm) concentration levels of the last batch prepared. Chemical stability (determined in an earlier study) of samples taken on day 0 was tested on days 0, 23, and 64. Throughout the study, samples of treated food from all dose levels including controls were analyzed for concentration.

Results -
Homogeneity Analysis: 200 ppm: range 208-230 ppm; 4000 ppm: range 4232-4641 ppm; -0.8 to +1.4% deviation of mean concentration from overall mean concentration.

Stability Analysis: 95.6-101.4% of initial (day 0) concentration.

Concentration Analysis: 200 and 2000 ppm: 92-109% of nominal concentration; 4000 ppm: 105.8-111.4% of nominal concentration.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

4. Statistics

Body weights were compared by analysis of covariance on initial body weight, separately for males and females. Weekly food consumption and food utilization were compared for several periods by analysis of variance separately for males and females. Hematology and clinical blood and urine chemistry were compared by analysis of variance for males and females combined except for urine protein which was considered separately for males and females. Organ weights were considered by analysis of variance and analysis of covariance on final body weight, separately for males and females. All analyses were carried out using the GLM procedure in SAS. Differences from control were provided by the difference between each treatment group least square mean and the control group least square mean. Differences from control were tested statistically by comparing each treatment group least square mean with the control group least square mean using a two-sided Student's t-test, based on the error mean square in the analysis.

C. METHODS

1. Observations

Animals were inspected daily for changes in clinical condition and behavior. Detailed examinations were conducted once weekly.

2. Body weight

Animals were weighed immediately before feeding the experimental diet and then weekly on the same day of the week as the initial weighing.

3. Food consumption and compound intake

Food consumption for each animal was determined and mean daily diet consumption was calculated as g/rat/day for each group on a weekly basis. Food "utilization" or efficiency was calculated by the author as (g body weight gain/g food consumed) × 100 for weeks 1-4, 5-8, 9-13, and 1-13. Compound intake (mg/kg/day) values, based on nominal concentrations, were calculated on a weekly basis and as time-weighted averages from the group mean food consumption and group mean body weight gain data.

4. Ophthalmoscopic examination

Eyes of the control and 4000 ppm group were examined during the week prior to sacrifice.

5. Blood was collected by cardiac puncture at sacrifice for hematology and clinical analysis from all animals. It was not stated that the animals were fasted. The CHECKED (X) parameters were examined.

a. Hematology

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
X	Blood clotting measurements*		
X	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		
X	(Kaolin-cephalin time)		

* Required for subchronic studies based on Subdivision F Guidelines

b. Clinical chemistry

	ELECTROLYTES		OTHER
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*
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (ALK)	X	Total serum protein (TP)*
	Cholinesterase (ChE)	X	Triglycerides
X	Creatine phosphokinase		Serum protein electrophores
	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		

* Required for subchronic studies based on Subdivision F Guidelines

6. Urinalysis*

Urine was collected from fasted animals during the week prior to termination. The CHECKED (X) parameters were examined.

X		X	
X	Appearance	X	Glucose
X	Volume	X	Ketones
X	Specific gravity		Bilirubin
X	pH	X	Blood
X	Sediment (microscopic)		Nitrate
X	Protein	X	Urobilinogen

*Not required for subchronic studies

7. Sacrifice and pathology

All animals were sacrificed (by exsanguination using cardiac puncture) on schedule and subjected to gross pathological examination. The CHECKED (X) tissues were collected for histological examination. All tissues from the control and 4000 ppm groups were examined except for the oral and nasal cavities. Gross lesions were examined microscopically. Lungs from the 200 and 2000 ppm groups were also examined. The (XX) organs, in addition, were weighed.

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

* Required for subchronic studies based on Subdivision F Guidelines
+ Organ weight required in subchronic and chronic studies.
T = required only when toxicity or target organ

II. RESULTS

A. OBSERVATIONS

1. Toxicity

Animals of both sexes in the 2000 and 4000 ppm treatment groups had distended abdomens with 8/12 and 12/12 males and 2/12 and 12/12 females affected, respectively. At termination, 11/12 males and 10/12 females in the 4000 ppm treatment group appeared small in size compared with the control and 200 ppm treatment groups.

2. Mortality

All animals survived to termination of the study (>90 days).

B. BODY WEIGHT AND WEIGHT GAIN

Body weights of male and female rats for selected weeks are listed in Table 2. The growth of males and females receiving 6000 ppm was markedly reduced during the first two weeks of the study. Some recovery occurred during the five days when the control diet was administered followed by the change to 4000 ppm for the remainder of the study. Final body weights were reduced in males and females by 32 and 18%, respectively, in the high-dose group compared with controls ($p \leq 0.01$). Final body weights of males and females receiving 2000 ppm in the diet were reduced by 18 and 11%, respectively, compared with controls ($p \leq 0.01$). There were no differences in body weights for males or females between the 200 ppm and respective control groups.

TABLE 2: Mean body weights (g) of male and female rats administered ICIA5504 in the diet for 13 Weeks

Week of Study[a]	Males				Females			
	0	200	2000	4000[b]	0	200	2000	4000[b]
1	75.2	77.8	75.2	76.9	74.6	70.5	71.7	71.1
5	276.8	279.4	222.8**	161.3**	185.6	183.4	168.6*	140.9**
9	382.5	381.0	315.2**	254.0**	229.2	227.1	206.5**	182.6**
13	445.8	445.5	362.3**	304.2**	247.4	251.0	225.3**	205.8**
14	451.3	452.5	369.5**	309.0**	255.8	253.8	226.9**	209.8**

Data taken from Table 5, pp. 39-40, MRID 43678135.
[a]Week 1 corresponds to initial weights on day 0; by week 14, rats had been treated for 13 weeks.
[b]Reduced from 6000 ppm during first 15 days of study followed by 5 days of control diet; 4000 ppm diet commenced on day 21.
*Significantly different from control, $p \leq 0.05$.

**Significantly different from control, p ≤ 0.01.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

Food consumption was significantly lower for males and females in the 4000 ppm groups (p ≤ 0.01 for most weeks); food consumption was reduced by ~40% during the first two weeks of the study, when the high dose group received 6000 ppm, but remained ~20% below that of controls during the 4000 ppm treatment. Food consumption was significantly lower for males in the 2000 ppm group during weeks 5-7 and 10-13 (p ≤ 0.05) and weeks 1-4, 8 and 9 (p ≤ 0.01) and females in the 2000 ppm group during weeks 2, 4, 5, 12 (p ≤ 0.05) and 1, 10, 11 and 13 (p ≤ 0.01). Food consumption was reduced by an average of 10% in the 2000 ppm groups by week 13. Food consumption in the 200 ppm group was similar to that of controls.

2. Compound consumption

Time-weighted average doses for males receiving 200, 2000 or 4000 ppm in the diet were 20.4, 211.0 and 443.8 mg/kg/day. Respective time-weighted averages for females were 22.4, 223.0 and 448.6 mg/kg/day. Overall time-weighted averages were 21.4, 217 and 446.2 mg/kg/day (Table 1).

3. Food efficiency

Food efficiency was significantly reduced in males receiving 2000 or 4000 ppm during weeks 1-4 and for the overall period, weeks 1-13 (p ≤ 0.01). Food efficiency was reduced in females receiving 4000 ppm during weeks 1-4 only (p ≤ 0.05) (Table 3).

TABLE 3: Food efficiency of male and female rats administered ICIA5504 in the diet for 13 weeks

Week of Study	Males Treatment Group (ppm)				Females Treatment Group (ppm)			
	0	200	2000	4000	0	200	2000	4000
1-4	28.75	28.35	25.44**	20.27**	19.81	20.34	19.42	18.24*
5-8	12.22	11.58	12.33	14.36	7.30	7.18	6.85	8.60
9-13	6.63	6.79	5.96	6.77	3.68	3.75	3.07	4.47
1-13	14.46	14.19	13.13**	12.37**	9.63	9.76	9.04	9.39

Data taken from Table 7, pp. 43 and 44, MRID 43678135.
*Significantly different from control, p ≤ 0.05.
**Significantly different from control, p ≤ 0.01.

D. OPHTHALMOSCOPIC EXAMINATION

Findings in the treatment groups were similar to those in the control groups.

E. BLOOD WORK

1. Hematology

There were minimal but statistically significant reductions in hemoglobin, mean cell volume and mean cell hemoglobin in females receiving 4000 ppm ($p \leq 0.05$ or 0.01) (Table 4). Reductions in mean cell volume ($p \leq 0.05$) and mean cell hemoglobin ($p \leq 0.01$) also occurred in females receiving 2000 ppm. These reductions did not occur in males at either dose. White cell counts were increased in both sexes receiving 4000 ppm ($p \leq 0.01$) and this increase was accompanied, in most cases, by increases in neutrophil, lymphocyte and monocyte counts. Platelet counts were decreased in both sexes receiving 4000 ppm ($p \leq 0.01$). The reduction in MCV of females receiving 200 ppm was also statistically significant although the percent change was small and not toxicologically significant.

TABLE 4: Clinical hematology parameters for male and female rats fed ICIA5504 for 13 weeks

Parameter	Males Treatment Group (ppm)				Females Treatment Group (ppm)[a]			
	0	200	2000	4000	0	200	2000	4000
Hemoglobin (g/dL)	14.8	14.8	14.8	14.3	15.2	15.0	14.9	14.5*
MCV (fL)	50.7	51.3	51.2	50.9	53.8	52.4*	52.0*	51.6**
MCH (pg)	17.0	16.9	16.8	17.0	18.2	18.0	17.7**	17.3**
White cell count (10^9/L)	5.27	5.22	5.91	7.33**	4.57	4.38	5.58	7.17**
Neutrophils (10^9/L)	0.97	0.88	1.36	2.08**	0.74	0.91	1.70**	2.07**
Lymphocytes (10^9/L)	4.03	4.13	4.37	4.88**	3.58	3.28	3.73	4.77**
Monocytes (10^9/L)	0.117	0.092	0.092	0.26**	0.117	0.075	0.075	0.200
Platelets (10^9/L)	760	807	807	650**	777	775	753	666**

Data taken from Table 9, pp. 46-49, MRID 43678135.
MCV = Mean cell volume.
MCH = Mean cell hemoglobin.
*Significantly different from control, $p \leq 0.05$.
**Significantly different from control, $p \leq 0.01$.

2. Clinical chemistry

The author excluded two males in the 4000 ppm group from the analyses because of extremely high plasma bilirubin, cholesterol, and triglyceride concentrations, as well as high activities of most plasma enzymes. For the remaining animals in both the 2000 and 4000 ppm groups, there were decreases in triglycerides for both sexes and in cholesterol concentrations for males (all $p \leq 0.01$) (Table 5). Decreases in alanine transaminase and aspartate transaminase activities and an increase in gamma glutamyl transferase activity occurred in both sexes in the 4000 ppm treatment groups ($p \leq 0.01$). Decreases in alkaline phosphatase and creatine kinase activities occurred in males only ($p \leq 0.01$). There was a decrease in plasma glucose concentration in females receiving 2000 or 4000 ppm. In the 2000 ppm group, changes were less marked and/or occurred in only one sex. For electrolyte concentrations, there were increases in phosphate and potassium in both sexes receiving 4000 ppm ($p \leq 0.01$) and an increase in calcium in females receiving 4000 ppm ($p \leq 0.01$). There were no effects in rats receiving 200 ppm.

TABLE 5: Clinical chemistry parameters for male and female rats fed ICIA5504 for 13 weeks

Parameter	Males Treatment Group (ppm)				Females Treatment Group (ppm)			
	0	200	2000	4000	0	200	2000	4000
Triglycerides (mg%)	102.5	100.0	64.7**	34.9**	64.3	68.0	47.8**	34.7**
Cholesterol (mg%)	95.8	93.5	79.8**	71.3**	88.8	90.3	88.0	97.5*
Alkaline phosphatase (IU/L)	192	197	138**	127**	113	111	92	104
SGOT (IU/L)	63.0	61.2	54.8	45.7**	53.7	51.7	36.7**	34.0**
SGPT (IU/L)	58.3	57.6	43.1**	37.2**	60.5	57.7	51.0	43.8**
Creatine kinase (IU/L)	151	153	102*	78**	124	110	106	78
GGT (IU\L)	0.3	0.7	0.5	2.8**	0.1	0.8	0.7	3.6**
Glucose (mg%)	167	166	166	152	196	194	170*	164**
Phosphate (mg%)	6.81	7.16	7.42*	8.42**	6.89	7.23	7.93**	9.25**
Potassium (mEq/L)	3.88	4.22	4.09	4.47**	3.84	3.92	4.32*	4.48**
Calcium (mg%)	10.9	10.8	10.8	11.3	10.8	11.3	10.9	11.7**

Data taken from Table 10, pp. 50-53, MRID 43678135.
SGOT = alanine transaminase.
SGPT = aspartate transaminase.
GGT = gamma-glutamyl transferase.
*Significantly different from control, $p \leq 0.05$.
**Significantly different from control, $p \leq 0.01$.

F. URINALYSIS

The total urinary protein of males receiving 4000 ppm was reduced. Blood was present in the urine of control males and a number of animals of both sexes in the 2000 and 4000 ppm treatment groups.

G. SACRIFICE AND PATHOLOGY

1. Organ weight

Differences in absolute organ weights between controls and the two higher dose groups generally reflected differences in final body weights (Table 6). However, there was an increase in liver weight relative to body weight for both sexes in the 2000 and 4000 ppm treatment groups (no statistics), particularly for the two males with high liver enzymes noted in the clinical chemistry discussion (Study report Volume 2, Appendix 9, p. 255). Liver weight adjusted for body weight was significantly increased for both sexes in the 2000 and 4000 ppm treatment groups ($p \leq 0.01$). There was a decrease in absolute kidney weights (significant for males at the two higher doses, $p \leq 0.01$) but relative kidney weights and kidney weights adjusted for body weight were increased for females at all dose levels and males receiving 4000 ppm ($p \leq 0.05$ or 0.01).

TABLE 6: Selected absolute, relative and adjusted organ weights of male and female rats fed ICIA5504 for 13 weeks

Parameter	Males Treatment Group (ppm)				Females Treatment Group (ppm)			
	0	200	2000	4000	0	200	2000	4000
Absolute organ weights (g)								
Liver	19.2	19.1	17.9	17.3	10.0	9.8	10.2	10.9*
Kidneys	3.14	3.14	2.71**	2.50**	1.82	1.88	1.80	1.70*
Adrenals	0.063	0.068	0.065	0.067	0.081	0.076	0.070**	0.065**
Brain	1.99	2.01	1.89**	1.82**	1.83	1.83	1.80*	1.73**
Organ/body weight (%)								
Liver	4.2	4.2	4.8	5.6	3.9	3.9	4.5	5.2
Kidney	0.69	0.69	0.74	0.81	0.71	0.74	0.79	0.81
Organ weight adjusted for body weight (g)								
Liver	15.9	15.7	19.5**	22.5**	9.3	9.2	10.6**	11.9**
Kidney	2.72	2.72	2.91	3.15**	1.68	1.76*	1.86**	1.89**

Data taken from Table 14, pp. 62-66, MRID 43678135.
*Significantly different from control, $p \leq 0.05$.
**Significantly different from control, $p \leq 0.01$.

2. Gross pathology

One male in the 4000 ppm treatment group had an enlarged pale liver, enlarged hepatic lymph node and distended extrahepatic bile duct.

3. Microscopic pathology

a) Non-neoplastic - Slight to moderate proliferation of the intrahepatic bile ducts/ductiles and oval cells was observed in two males in the 4000 ppm treatment group. Cholangitis of the extrahepatic bile duct, inflammatory cell infiltrate of the pancreas, active hepatocellular hyperplasia and a reactive hepatic lymph node were also observed in one of the two males. There was a reduction in renal tubular basophilia in males receiving 4000 ppm. Histopathological lesions were not observed in any other tissues or organs.

b) Neoplastic - Neoplastic lesions were not observed.

III. DISCUSSION

A. DISCUSSION

Male and female Wistar-derived (Alpk:APfSD) rats were administered ICIA5504 in the diet at concentrations of 0, 200, 2000 or 4000 ppm (high-dose groups initially received 6000 ppm for 15 days) for 13 weeks in a Specific Pathogen Free unit. As a result of effective failure of the groups receiving 6000 ppm to grow during the first two weeks of the study, these groups were fed the control diet for 5 days followed by a lowered concentration of 4000 ppm for the remainder of the study. Diet and water were provided *ad libitum*. Animals were inspected daily for changes in clinical condition and behavior and weighed weekly. Hematological and clinical chemistry parameters were measured at termination of the study.

All animals survived to termination of the study without evident toxic signs except for distended abdomens and small body size of both sexes receiving the 2000 and 4000 ppm concentrations. The lower final body weights of these mid- and high-dose animals compared with the control groups were statistically significant (32 and 18% lower for males and females, respectively, in the 4000 ppm treatment groups and 18 and 11% lower for males and females, respectively, in the 2000 ppm treatment groups) and reflected reduced food consumption. Food efficiency was reduced only during the first four weeks of the study, primarily for males, thus indicating that the effect on body weights was a result of reduced food

15A

consumption (due to the probable unpalatability of the food) and was not a toxic response.

Some trends in the hematology and clinical chemistry findings were observed. In the 4000 ppm treatment groups, effects on hematology (reduced hemoglobin in females and increased white cell counts and platelets in both sexes) and clinical biochemistry (reduced plasma enzyme levels and reduced cholesterol, glucose and triglycerides) were observed. Similar, but lesser effects were present in the 2000 ppm treatment group. According to the author, the reduced nutritional status and reduced liver activity was evidenced by the small size of the animals, their distended abdomens, and clinical chemistry findings of reduced cholesterol, glucose and triglycerides and reduced plasma enzymes. The reviewer agrees with the author's statement concerning the effects of reduced nutritional status.

At necropsy, absolute liver weights of treated groups were not significantly different from control groups except for a slight increase in absolute weight for females in the high-dose group. Absolute kidney weights for males in the mid- and high-dose groups and females in the high-dose group were reduced compared to controls. However, liver weights adjusted for body weights in the mid- and high-dose groups of both sexes were significantly increased as were kidney weights for males in the high-dose group and for females in all dose groups. The increased adjusted organ weights are likely due to the lower final body weights of these animals. For 10/12 males and 12/12 females in the 4000 ppm treatment group there were no microscopic findings in the liver and kidneys, respectively (the reduction in renal tubular basophilia in males receiving 4000 ppm may have been treatment related, but its significance is unknown). The lack of histopathological correlates indicates that the organ weight changes were not toxicologically significant. In the absence of histopathological change in other organs, significant differences from controls in other absolute organ weights are also not toxicologically significant.

Two males in the 4000 ppm treatment group showed clear signs of liver toxicity with marked elevations in total bilirubin, cholesterol and triglycerides and alkaline phosphatase, alanine transaminase and aspartate transaminase activities. These two animals were excluded from the statistical analyses for these parameters as reductions in these values were observed in the remaining males in this group. The increase in liver weight relative to body weight was greatest for these two males compared with the remainder of this test group. The effect on the liver of these two animals was observed

microscopically as proliferation of the intrahepatic bile duct/ductiles and oval cells. Hepatocellular hyperplasia and an enlarged hepatic lymph node was observed in one of the two. Although there was no supporting histopathology in the other animals, relative and adjusted liver weights were generally increased in a dose-response manner reflecting the normal response of the liver to chemical treatment and exaggerated by the reduced nutritional status of these animals. Results from the chronic study with rats (MRID 43678139) confirm that the bile duct is the target tissue of this chemical, with secondary effects on the liver due to bile duct blockage. Based on decreased weight gain, clinical observations of distended abdomens and reduced body size, and clinical pathology findings attributable to reduced nutritional status in both sexes, the LOEL is 2000 ppm. Based on the absence of effects, the NOEL is 200 ppm.

B. STUDY DEFICIENCIES

All parameters required for subchronic studies were measured and there were no major deficiencies in the study. However, the reviewer has a few suggestions that might make the data more meaningful. The statistical analysis and toxicological significance of the parameter "organ weight adjusted for body weight" was not fully explained. When it was observed early in the study that food consumption was reduced and the animals failed to grow, a paired feeding study in which animals are administered the same amount of food as the high-dose groups should have been undertaken. The result would be a clear indication of the effects of nutritional status vs. the effects of treatment.

ATTACHMENTS

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

DRAFT
Subdivision O
Guideline Ref. No. 82-1

82-1 Subchronic Feeding in the Rodent and Nonrodent

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?:

1. ✓ Technical form of the active ingredient tested.
2. ✓ At least 10 rodents or 4 nonrodents/sex/group (3 test groups and control group).
3. ✓ Dosing duration daily for 90-days or 5 days/week for 13 weeks.
4. ✓ Doses tested include signs of toxicity at high dose but no lethality in nonrodents or a limit dose if nontoxic (1000 mg/kg).
5. ✓ Doses tested include a NOEL.
6. • ✓ Analysis for test material stability, homogeneity and concentration in dosing medium
7. ✓ Individual daily observations.
8. ✓ Individual body weights.
9. ✓ Individual or cage food consumption.
10. • ✓ Opthalmoscopic examination (at least pretest and at term) control and high dose.
11. ✓ Clinical pathology data of 12 & 13 at termination for rodents, before, monthly or midway and at termination for nonrodents.
12. ✓ Hematology.
    - ✓ Erythrocyte count
    - ✓ Hemoglobin
    - ✓ Hematocrit
    - ✓ Leucocyte count
    - • ✓ Differential count
    - ✓ Platelet count (or clotting measure)
13. ___ Clinical chemistry.
    - ✓ Alkaline phosphatase
    - ✓ Aspartate aminotransferase
    - • ✓ Creatinine ~~kinase~~ (phospho)
    - ___ Lactic dehydrogenase
    - ✓ Glucose
    - ✓ Bilirubin
    - ✓ Cholesterol
    - • ✓ Creatinine
    - ✓ Total Protein
    - ✓ Albumin
    - ✓ Urea (Blood urea nitrogen)
    - ✓ Inorganic phosphate
    - ✓ Calcium
    - • ✓ Potassium
    - ✓ Sodium
    - • ✓ Chloride
14. • ✓ Urinalysis, only when indicated by expected or observed activity. As scheduled in 11.
    - ✓ Blood
    - ✓ Protein
    - ✓ Ketone bodies
    - ✓ Appearance
    - ✓ Glucose
    - ___ Total bilirubin
    - • ✓ Urobilirubin
    - ✓ Sediment
    - ✓ Specific gravity (osmolality)
    - • ✓ Volume
15. ✓ Individual necropsy of all animals.
16. ✓ Histopathology of the following tissues performed on all nonrodents and rodents, all control and high dose animals, all animals that died or were killed on study, all gross lesions on all animals, target organs on all animals and lungs, liver and kidneys on all other animals.

Criteria marked with a • are supplemental and may not be required for every study.

DRAFT
Subdivision O
Guideline Ref. No. 82-1

✓ aorta	✓ jejunum	✓ peripheral nerve
✓ eyes	✓ bone marrow	✓ kidneys†
✓ caecum	✓ liver†	✓ esophagus
✓ colon	✓ lung†	✓ ovaries† (not weighed)
✓ duodenum	✓ lymph nodes	___ oviduct
✓ brain†	✓ stomach	✓ pancreas
✓ skin	✓ mammary gland	✓ rectum
✓ heart† (not weighed)	✓ spleen† (not weighed)	✓ spinal cord (3x)
✓ testes†	✓ musculature	✓ thyroid / parathyroids
✓ pituitary	✓ epididymis	✓ salivary glands
✓ ileum	✓ adrenals†	✓ thymus
✓ trachea	✓ uterus	✓ urinary bladder

† organs to be weighed

Criteria marked with a * are supplemental and may not be required for every study.

Azoxystrobin

Page _____ is not included in this copy.

Pages 21 through 27 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

X FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.