9-26-96

E5504 **Developmental Study OPPTS 870.3700 (§83-3(a))**

EPA Reviewer: Myron S. Ottley, Ph.D. MSOttley, Date 9/26/96
Review Section IV, Toxicology Branch I (7509C)
EPA Secondary Reviewer: Marion P. Copley, D.V.M., D.A.B.T. MCopley, Date 9/26/96
Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Study - Rat; OPPTS 870.3700 [§83-3a]

DP BARCODE: D218319 SUBMISSION CODE: S489692
P.C. CODE: 128810 TOX. CHEM. NO.: None

TEST MATERIAL (PURITY): E5504 (95.2%)

SYNONYMS: Azoxystrobin

CITATION: Moxon, M.E. 1994. (ICIA5504) E5504: Developmental Toxicity Study in the Rat. Zeneca Central Toxicology Laboratory. Report No. CTL/P/3633 Nov. 11, 1994. MRID 43678142. Unpublished.

SPONSOR: Zeneca Inc., Wilmington, DE

EXECUTIVE SUMMARY:

In a developmental toxicity study (MRID 43678142) E5504, 95.2% a.i. was administered to 24 Wistar-derived rats/dose by gavage at dose levels of 0, 25, 100 or 300 mg/kg/day from days seven through 16 of gestation.

At 300 mg/kg/d maternal lethality caused the discontinuance of dosing at that level. At 100 mg/kg/d, minimally reduced body weights (< 2%) were observed ($p < 0.05$), although body weight gain and food consumption were not affected. Clinical signs included diarrhea (42%), urinary incontinence (17%) and salivation (71%). At 25 mg/kg/d salivation was observed in 29% of animals. **The maternal LOEL is 25 mg/kg/day, based on increased salivation. The maternal NOEL is not established.**

In the conceptus, no significant adverse developmental effects were observed. **The developmental LOEL is >100 mg/kg/day. The developmental NOEL is 100 mg/kg/day.**

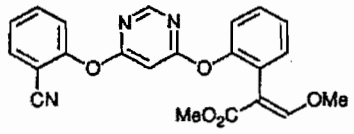
Due to maternal toxicity at the high dose level, this study must be considered a two dose study, which makes it deficient. However, since valid NOEL and LOEL were obtained from the data, the developmental toxicity study in the rat is classified acceptable and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3 a) in the rat.

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 #: P32/A1016/34 CTL Ref # Y06654/004
   Purity: 95.2 % a.i.
   CAS #: None



2. Vehicle: corn oil
   Description: None provided
   CTL Ref #: Y00790/004
   Purity: 100% a.i.

3. Test animals: Species: rat
   Strain: Alpk:APfSD (Wistar-derived)
   Age at mating: 12 weeks
   Weight at mating: 176 - 271 g
   Source: Zeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK
   Housing: individually in suspended rat cages
   Diet: CT1 rat diet ad libitum
   Water: automatic tap ad libitum
   Environmental conditions:
   - Temperature: 66 - 73°F
   - Humidity: 45 - 65%
   - Air changes: 25 - 30/hr
   - Photoperiod: 12 hrs dark/12 hrs light

   Acclimation period (P): Animals arrived on gestation day (gd) one, and dosing began on gd 6.

B. PROCEDURES AND STUDY DESIGN

1. In life dates - not specified

2. Mating: method not specified. Animals were mated at the supplier, and arrived at the testing laboratory on gestation day (gd) one.

3. Animal Assignment: Animals were assigned to dose groups as indicated in Table 1. Assignment was random.

2 656

TABLE 1 Animal Assignment

Test Group	Dose (mg/kg/day)	Number of Females
Control	0	24
Low (LDT)	25	24
Mid (MDT)	100	24
High (HDT)	300	24

Animals were received over an 8-day period and three animals/group were place on study on each of these days.

4. Dose selection rationale: Not specified

5. Dosage preparation and analysis: ICIA5504 was administered in corn oil and the concentrations were adjusted to give a constant volume of 1 ml/100g bodyweight at each dose level. Fresh aliquots were prepared each day, and stored at room temperature.

   Test substance formulations were prepared and divided into daily aliquots at the start of the study by mixing appropriate amounts of test substance with corn oil. They were stored at room temperature. Concentration was verified at the start of dosing; homogeneity of the formulations was evaluated by analyzing it at the start, middle and end of subdividing it into aliquots.

   Results - Homogeneity Analysis: within 3% of nominal values.

   Stability Analysis: within 3% of nominal values.

   Concentration Analysis: within 4% of nominal values.

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

6. Dosage administration: All doses were administered once daily by gavage, on gestation days seven through 16, in a volume of 1 ml/100g of body weight/day. Dosing was adjusted daily according to body weight and performed on the morning of each day.

C. OBSERVATIONS

1. Maternal Observations and Evaluations - The animals were

3 666

checked daily for mortality, clinical signs and behavioral changes. Body weights were recorded on gestation days 1, 4, 7 - 16, 19 and 22. Food consumption was determined at 3-day intervals through day 22. Dams were sacrificed on day 22 of gestation by halothane overexposure. Examinations at sacrifice consisted of: uterine weight, number and positions of implantations; corpora lutea in each ovary; individual fetal weights, percent preimplantation loss, percent post-implantation loss, early and late intra-uterine deaths.

2. Fetal Evaluations - The fetuses were first weighed and examined externally including oral cavity. An internal examination (using magnification) followed which included sex determination and observation for visceral abnormalities. They were then eviscerated and fixed in 70% industrial methylated spirits. After approx. 24 hrs the brain was examined for macroscopic abnormalities and the carcasses stained with Alizarin Red S for skeletal evaluation.

D. DATA ANALYSIS

1. Statistical analyses: performed according to standard techniques.

2. Indices: The following indices were calculated from cesarean section records of animals in the study: Preimplantation loss and post-implantation loss were calculated as follows:

$$\% \text{ preimplantation} = \frac{(\#\text{ corpora lutea} - \#\text{ implantations})}{(\#\text{ corpora lutea})} * 100$$

$$\% \text{ postimplantation} = \frac{(\#\text{ implantations} - \#\text{ live fetuses})}{(\#\text{ implantations})} * 100$$

3. Historical control data: Historical control data were not provided.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations: Of the 24 animals in the high-dose group, three of the first 12 placed on study died following two days of treatment, and a third had to be killed in moribund condition. These findings resulted in the suspension of treatment in all remaining animals in this group. The following treatment-related clinical observations were reported:

300 mg/kg/d (12 dams only): piloerection (4 animals), signs of diarrhea (10 dams), and signs of urinary incontinence (5 dams)
100 mg/kg/d (24 dams): diarrhea (10 dams) and urinary incontinence (4 dams) starting about day 8, slight to moderate salivation (17 dams) between days 9 and 16.
25 mg/kg/d (24 dams): slight to mod. salivation (7 dams) between days 11 and 16 usually lasting only one day.

2. Body Weight - Body weight gain data are summarized in Table 2. Maternal body weights were not measured in the high dose group since that group was removed from the study. Maternal body weights in the LDT were not significantly different from controls. Although statistically significant ($p \leq 0.01$ or $p \leq 0.05$) reductions in body weights were seen in the MDT compared with controls on gd 8, 9, 12, 13, 14, 15, 16, these differences were all less than 2%, and not considered toxicologically significant.

TABLE 2 Maternal Body Weight Gain (g)[a] (includes intercurrent deaths) Pregnant animals only

Interval	Dose Group (mg/kg/d)			
	Control (N)	25 (N)	100 (N)	300 (N)
Pretreatment: Days 1 - 7	31(24)	39(21)	33(24)	N/A
Treatment: Days 8 - 16	46(24)	46(21)	44(24)	N/A
Posttreatment: Days 16- 22	65(24)	68(21)	71(24)	N/A

a Data extracted from report no. CTL/P/3633 and table no. 7 (p 39, 40)

3. Food Consumption - In the MDT, food consumption was sig. reduced ($p \leq 0.01$, up to 23%) during dosing (gd 7-16) compared with controls. Food consumption on gd 17-22 recovered, and was 13% ($p \leq 0.01$) above controls.

In the LDT no toxicologically significant changes were observed in food consumption.

4. Gross Pathology - In HDT animals found dead, red areas and thin walls were observed in the stomach or jejunum. In the MDT, two animals showed hemorrhagic areas in the stomach at terminal necropsy. No other macroscopic findings were made.

5. Cesarean Section Data - As summarized in Table 3, no dose-related adverse effects were observed at the time of test animal sacrifice.

TABLE 3 Cesarean Section Observations[a]

Observation	Dose (mg/kg/day)			
	0	LDT	MDT	HDT
# Animals Assigned (Mated)	24	24	24	24
# Animals Pregnant Pregnancy Rate (%)	100	87.5	100	100
# Nonpregnant	0	3	0	0
Maternal Wastage # Died # Aborted # Premature Delivery	 0 N.R.[b] N.R.	 0 N.R. N.R.	 0 N.R. N.R.	 4## N.R. N.R.
Corpora Lutea/Dam	13.6	13.6	13.3	N/A
Implantations/Dam	11.9	11.0	11.4	N/A
Total # Litters	24	21	24	N/A
Live Fetuses/Dam	11.3	10.7	10.7	N/A
Resorptions/Dam Early Late Litters Affected Early Late	 0.58 0.04 10 1	 0.10 0.00 4 0	 0.46 0.13 5 2	 N/A N/A N/A N/A
Mean Fetal Weight (g) Mean Litter Weight (g)	4.77 52.7	4.86 51.2	4.77 51.4	N/A N/A
Sex Ratio (% Male)	47.3	52.1	55.0*	N/A
Mean Preimplantation Loss (%) Implants affected Litters affected	 12.9 15	 20.1* 15	 14.0 13	 N/A N/A
Mean Postimplantation Loss (%) Implants affected Litters affected	 5.5 10	 4.0 4	 5.5 6	 N/A N/A

a Data extracted from Report No. CTL/P/3633, Tables #5 and #10
b Not reported
* $p \leq 0.05$
** $p \leq 0.01$
HDT discontinued on study due to high mortality (33% of first 12 animals entered on study)

B. DEVELOPMENTAL TOXICITY

1. External/Visceral - As stated earlier, the high-dose group was discontinued due to excessive maternal toxicity. Therefore, no developmental endpoints were evaluated for this group. One LDT fetus had hindlimb edema. This single incidence was not considered treatment related.

2. Skeletal Examination - General reduced ossification was seen in all dose groups, the incidence of which was not statistically different between controls and treatment groups. As illustrated by the MANUS and PES scores in Table 4b. mean scores/litter showed a non-statistically significant increase with dose. There was, however, a statistical increase (6.9% vs 2.6%, $p \leq 0.05$) in the animals with a PES score of 6 at 100 mg/kg/d vs controls.

TABLE 4a. External/Visceral Examinations[a]

Observations+	Dose (mg/kg/day)			
	0	25	100	300
#Fetuses(litters) examined	271(24)	225(21)	259(24)	N/A
#Fetuses(litters) affected	0(0)	1(1)	0(0)	N/A

\+ Some observations may be grouped together
a Data extracted from Report No. CTL/P/4012, Table No. 12.
b Fetal (litter) incidence

TABLE 4b. Skeletal Examinations[a]

Observations+	Dose (mg/kg/day)			
	0	25	100	300
#Fetuses(litters) examined	271(24)[b]	225(21)	259(24)	N/A
Major Skeletal Defects	0(0)	0(0)	0(0)	N/A
MANUS Scores				N/A
Prop. with score 2 (%)	2 (0.7)	0 (0.0)	0 (0.0)	
Prop. with score 3	10 (3.7)	2 (0.9)	4 (1.5)	
Prop. with score 5	172 (63.5)	163*(72.4)	167 64.5)	
Prop. with score 5	85 (31.4)	60 (26.7)	84 (32.4)	
Prop. with score 6	2 (0.7)	0 (0.0)	4 (1.5)	
Mean MANUS Score/litter	4.22±0.47 (24)	4.25±0.36 (21)	4.34±0.38 (24)	N/A

7 206

TABLE 4b. Skeletal Examinations[a]

Observations+	Dose (mg/kg/day)			
	0	25	100	300
PES Scores				N/A
Prop. with score 2	10 (3.7)	0**(0.0)	0**(0.0)	
Prop. with score 3	44 (16.2)	49 (21.8)	33 (12.7)	
Prop. with score 5	210 (77.5)	175 (77.8)	208 (80.3)	
Prop. with score 5	7 (2.6)	1 (0.4)	18* (6.9)	
Prop. with score 6				
Mean PES Score/litter	4.74±0.51 (24)	4.78±0.32 (21)	4.94±0.32 (24)	N/A

\+ Some observations may be grouped together
* $p \leq 0.05$
** $p \leq 0.01$
a Data extracted from Report No. CTL/P/4012, Table No. 13.
b Fetal (litter) incidence

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS

Dose levels of 300 mg/kg/d of E5504 caused maternal lethality and toxicity to the extent that dosing at this level was suspended prematurely, and no assessments of developmental toxicity were made. At 100 mg/kg/d maternal toxicity in the form of reduced body weight gain and food consumption, diarrhea, salivation and urinary incontinence was observed. At 25 mg/kg/d increased salivation was observed. **The maternal LOEL was therefore 25 mg/kg/d. The NOEL was not established.** The salivation observed is considered to be a minor pharmacological effect, and of no toxicological significance.

Developmental toxicity assessments were made at 100 and 25 mg/kg/d only. 100 mg/kg/d administration was associated with generally reduced ossification which was generally not statistically significant, except for an increased ($p \leq 0.05$) PES score at the 100 mg/kg/d dose level (6.9% vs 2.6%). No effects were observed at 25 mg/kg/d. The developmental LOEL was 100 mg/kg/d. The NOEL was 25 mg/kg/d.

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY: The incidence of increased salivation observed was dose-related: 71% at 100 mg/kg/d, 29% at 25 mg/kg/d, 0% in controls. While the authors characterize this as a "minor pharmacological effect . . . of no toxicological significance", it should be regarded as an adverse effect in the regulatory process.

2. DEVELOPMENTAL TOXICITY: Developmental toxicity was not observed at the dose levels tested. The increases in reduced ossification observed were not statistically significant, or were not dose-related, with the exception of an increased number of animals with a PES score of six at the 100 mg/kg/d. Since this increase was not supported by increases in other related endpoints, and since the mean pes score/litter was not statistically different from controls, it is not considered to be of toxicological significance. Therefore, **the developmental LOEL was >100 mg/kg/d. The NOEL was 100 mg/kg/d.**

C. STUDY DEFICIENCIES Due to significant maternal toxicity, dosing at 300 mg/kg/d was discontinued, and no maternal or fetal data were generated at the high dose, making this a two dose study. While the absence of the third treatment level represents a significant deficiency, sufficient data were obtained from the other two dose levels to determine a valid LOEL and a NOEL. No major deficiencies were noted.