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Review Section IV, Toxicology Branch I (7509C)
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Review Section IV, Toxicology Branch I (7509C)

012115

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Study - Rabbit; OPPTS 870.3700 [§83-3b

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

TEST MATERIAL (PURITY): ICIA5504

SYNONYMS: Azoxystrobin

CITATION: Moxon, M.E. 1994. ICIA5504: Developmental Toxicity Study in the Rabbit. Zeneca Central Toxicology Laboratory. Report No. CTL/P/4012 Nov. 11, 1994. MRID 43678143. Unpublished.

SPONSOR: Zeneca Inc., Wilmington, DE

EXECUTIVE SUMMARY:

In a developmental toxicity study (MRID 43678143) ICIA5504, 96.2% a.i. was administered to 20 New Zealand White rabbits/dose by gavage in corn oil at 2 mg/kg bw at dose levels of 0, 7.5, 20, or 50 mg/kg/day from days eight through 20 of gestation.

At 20 mg/kg/d and higher, decreased body weight gain (< 5%) was observed, as well as negligible food consumption in some animals (not fully quantified due to excessive food wastage), which led to their sacrifice in moribund condition. **The maternal LOEL and NOEL for this study could not be determined.**

In the conceptus, increased fused sternebrae (3rd and 4th, and/or 4th and 5th) was observed in the high dose group, along with open eye and cleft palate (litter). **The developmental LOEL and NOEL for this study could not be determined.**

The developmental toxicity study in the rabbit is classified **unacceptable** and does not satisfy the guideline requirement for a developmental toxicity study (OPPTS 870.3700; §83-3 b) in the rabbit. The study was compromised due to excessive food wastage, maternal death and other unidentified factors. A NOEL/LOEL could not be established. The RfD committee concurred with these conclusions.

The results are not appropriate for toxicity risk assessments

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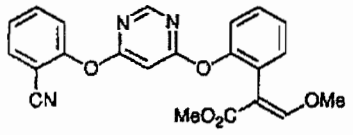
since in subsequent studies (MRIDs 44058702, 44058703, 44058705, 44073201, 44073202) the Submitter has shown that corn oil, at the dose volume it was used here, is toxic to the dams, and also enhances the toxicity of ICIA5504. A subsequent developmental toxicity study (MRID 44058701) showed that when the dose volume of corn oil is 1 ml/kg, the above toxic effects are not seen in dams or fetuses.

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
   Purity: 96.2 % a.i.
   CAS #: None



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

3. Test animals: Species: rabbit
   Strain: New Zealand white
   Age at mating: not specified
   Weight at mating: not specified
   Source: Interfauna UK Ltd., Huntingdon, Cambridgeshire, UK
   Housing: individually in mobile rabbit units
   Diet: SDS Standard Rabbit Diet ad libitum
   Water: filter-sterilized tap ad libitum (initial supply in bowl in cage until satisfactory food intake observed)
   Environmental conditions:
   - Temperature: 59 - 66°F
   - Humidity: 40 - 70%
   - Air changes: 25 - 30/hr
   - Photoperiod: 12 hrs dark/12 hrs light

   Acclimation period (P): Animals arrived on gestation days (gd) two or three, and dosing began on gd 8.

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 testing laboratory on (gd) two or three.

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

TABLE 1 Animal Assignment

Test Group	Dose (mg/kg/day)	Number of Females
Control	0.0	20
Low (LDT)	7.5	20
Mid (MDT)	20.0	20
High (HDT)	50.0	20

4. Dose selection rationale: Not specified

5. Dosage preparation and analysis ICIA5504 was administered in corn oil and the concentrations was adjusted to give a constant volume of 2 ml/kg bodyweight at each dose level. Fresh aliquots were prepared each day, and stored at room temperature.

   Test substance formulations were prepared daily by mixing appropriate amounts of test substance with corn oil and were stored at room temperature. Homogeneity, stability and concentration analyses were performed, with the following results:

   Results - Homogeneity Analysis: within 5% of means.

   Stability Analysis: confirmed at room temperature for 29 days.

   Concentration Analysis: within 3% of nominal.

   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 eight through 20, in a volume of 2 ml/kg of body weight/day. Dosing was adjusted daily according to body weight.

C. OBSERVATIONS

1. Maternal Observations and Evaluations - The animals were checked for mortality or clinical signs at least twice daily. Body weights were recorded upon arrival on gestation days 4, 8 - 20, 23, 26, and 30. Food consumption data were obtained on gestation days 8, 11, 14, 17, 20, 23 and 26. Dams were sacrificed on day 30 of gestation. 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.

2. Fetal Evaluations - The fetuses were first examined externally including oral cavity. An internal examination 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:

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

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

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

II. **RESULTS**

A. MATERNAL TOXICITY

1. Mortality and Clinical Observations: The occurrence of intercurrent deaths was 2, 4, 3, and 7 in the control, 7.5, 20 and 50 mg/kg/d groups, respectively, most killed by investigators after excessive morbity was observed

usually between gd 12 and 20. The following clinical observations were reported: blood on tray, sporadic in 2, 1, 2, 6 animals in the control, 7.5, 20 and 50 mg/kg/d groups, respectively; general coat staining in 2, 2, 3, 5 animals in the control through high-dose groups, respectively; diarrhea in 2, 4, 7, 7 animals in the control through high-dose groups, respectively. While these occurrences were observed in a dose-related manner, their toxicological significance is uncertain.

2. Body Weight - Body weight data are summarized in Fig. 1 (from study report) and in Table 2, and as follows: Maternal body weights were similar to controls throughout the conduct of the study in all treatment groups, with the exception of the high-dose group during gd 9 (-3%), 10 (-3%), and 13 (-5%). The table also shows that body weight gain was lower than controls in the mid and high dose groups, although not in a dose-related manner. The mid dose group was more severely affected, and for a longer period.

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

Interval	Dose in mg/kg/day (# of Dams)			
	Control (N)	7.5 (N)	20 (N)	50 (N)
Pretreatment: Days 4 - 8	97(20)	61(19)	114(18)	90(19)
Treatment: Days 9 - 20	118(18)	107(17)	-93(17)	29(15)
Posttreatment: Days 23- 30	211(18)	246(15)	150(16)	238(12)

a Data extracted by reviewerfrom report no. CTL/P/4012 and table no. 7B (p 48, 49)

3. Food Consumption (Table 3) - Food consumption data were inconclusive due to food wastage and other factors. Some animals showed very little food consumption. Some of these became moribund and had to be sacrificed intercurrently (see Mortality section). The number of animals showing negligible food consumption during the dosing period were 5, 6, 8 and 9 in the control, low, mid and high dose groups, respectively.

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TABLE 3 Mean Maternal Food Consumption (g)[a] (includes intercurrent deaths

Interval	Dose in g/animal (# of Dams)			
	Control (N)	7.5 (N)	20 (N)	50 (N)
Pretreatment:				
Days 4 - 8	146±44(17)	146±37(17)	176±36(11)	159±47(15)
Treatment:				
Days 8 - 11	24±25(19)	38±41(17)	27±34(18)	15±20(18)
Days 11 - 14	53±48(19)	64±55(18)	38±41(18)	39±41(17)
Days 14 - 17	54±52(19)	75±53(15)	26±25(16)	53±46(15)
Days 17 - 20	68±51(17)	80±48(14)	38±38(15)	64±54(11)
Post-treatment:				
Days 20 - 23	118±41(15)	134±46(13)	124±50(13)	134±52(08)
Days 23 - 26	149±51(15)	171±39(15)	168±31(13)	172±59(11)
Days 26 - 30	152±32(16)	159±31(11)	139±35(12)	159±44(09)

a Data extracted by reviewer from report no. CTL/P/4012 and table no. 8B (p 51)

4. Gross Pathology - No dose-related adverse effects were noted during necropsy, either in animals that died during the study, or in animals sacrificed at the end of the study.

5. Cesarean Section Data - As summarized in Table 4, no dose-related adverse observations were made at the time of test animal sacrifice.

TABLE 4 Cesarean Section Observations[a]

Observation	Dose (mg/kg/day)			
	0	7.5	20	50
# Animals Assigned (Mated)	18	16	17	13
# Animals Pregnant Pregnancy Rate (%)	100	93.8	88.2	92.3
# Nonpregnant	0	1	2	2
Maternal Wastage # Died # Aborted # Premature Delivery	 2 N.R. N.R.	 4 N.R. N.R.	 3 N.R. N.R.	 7 N.R. N.R.
Corpora Lutea/Dam	11.5	11.7	11.1	11.9
Implantations/Dam	10.11	9.00	9.87	10.00
Total # Litters	18	15	15	12
Live Fetuses/Dam	9.17	8.00	7.87	8.75
Resorptions/Dam Early Late Litters Affected Early Late	 3.0 6.1 4 6	 6.9 3.5 5 3	 7.2 14.2 9 9	 6.8 5.4 5 4
Mean Fetal Weight (g) Mean Litter Weight (g)	37.3 339	42.3** 328	39.1 307	40.1 349
Sex Ratio (% Male)	47.3	48.5	57.8	46.9
Mean Preimplantation Loss (%) Implants affected Litters affected	 12.1 78.0	 23.1 80.0	 10.8 60.0	 16.3 83.3
Mean Postimplantation Loss (%) Implants affected Litters affected	 9.1 55.6	 10.4 46.7	 21.4** 93.3*	 12.2 66.7

a Data extracted from Report No. CTL/P/4012 and Table No. 10
* $p \leq 0.05$
** $p \leq 0.01$

B. DEVELOPMENTAL TOXICITY

1. External/Visceral - In the High-dose group nine fetuses (8.6%) from two litters (16.7%) had open eye, the majority bilateral. One high dose fetus had cleft palate. Other effects, the incidence of which are included in Table 4a), were of low occurrence, were not dose related, and were not associated with treatment.

These effects included internal hydrocephaly, encephalocoele, fenestration in parietal, reduced pulmonary artery, enlarged aorta. Many of these effects occurred in the controls only.

2. Skeletal Examination - Fused sternebrae was noted in the high-dose group. The occurrence was statistically ($p \leq 0.01$) above control levels an involved 12 fetuses from four litters.

TABLE 5a. External/Visceral Examinations[a]

Observations+	Dose (mg/kg/day)			
	0	7.5	20	50
#Fetuses(litters) examined	165(18)	120(15)	118(15)	105(12)
#Fetuses(litters) affected	4(3)	0(0)	2(2)	3(2)
Cleft Palate	0(0)[b]	0(0)	0(0)	5(1)
Open Eye(s)	0(0)	0(0)	0(0)	9(2)

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

TABLE 5b. Skeletal Examinations[a]

Observations+	Dose (mg/kg/day)			
	0	7.5	20	50
#Fetuses(litters) examined	165(18)	120(15)	118(15)	105(12)
#Fetuses(litters) affected	4(3)	0(0)	2(2)	3(2)
Fused Sternebrae	1(1)[b]	1(1)	3(2)	12(4)

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

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS

Dose levels of 20 and 50 mg/kg/d of ICIA5504 were maternally toxic. The principal effects were reduced food intake and body weight gain. Reduced food intake was so marked at the high-dose group that some animals were removed in moribund condition. **The maternal LOEL was therefore 20 mg/kg/d. The NOEL was 7.5 mg/kg/d.**

The high-dose group showed evidence of developmental

toxicity, the principal effects being open eye and sternebral fusion. **The developmental LOEL was 50 mg/kg/d. The NOEL was 20 mg/kg/d.**

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY: After an initial decrease in food consumption, most animals recovered sufficiently to leave body weight gains unaffected, compared with controls. In the mid and high dose groups, however, reduced food intake was marked, compared with controls. Some animals became moribund and had to be removed from the study. The incidence of this was highest in the high dose group.

2. DEVELOPMENTAL TOXICITY: The effects seen on eye opening and sternebral fusions took place at maternally toxic dose levels, making it unclear concerning the primacy of these effects. Since maternal toxicity was observed at lower dose levels, in the absence of developmental effects, the maternal endpoints should be used in determining the NOEL for this study.

The reviewer cannot determine valid LOELs/NOELs from this study. Hence, The results of this study are not appropriate for toxicity risk assessments since in subsequent studies (MRIDs 44058702, 44058703, 44058705, 44073201, 44073202) the Submitter has shown that corn oil, at the dose volume it was used here, is toxic to the dams, and also enhances the toxicity of ICIA5504. A subsequent developmental toxicity study (MRID 44058701) showed that when the dose volume of corn oil is 1 ml/kg, the above toxic effects are not seen in dams or fetuses.

C. STUDY DEFICIENCIES Historical control data would have been beneficial in assessing the toxicological significance of some observations such as eye opening. Due to the absense of clear LOELs/NOELs this study is not acceptable for regualory purposes.