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RPA 201772 Developmental Study (83-3b)

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DATA EVALUATION RECORD

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

DP BARCODE: D224202
P.C. CODE: 123000
MRID NO.: 43904808
SUBMISSION CODE: S501233
TOX. CHEM. NO.: [New Chemical]

TEST MATERIAL (PURITY): RPA 201772 (99.6%)

SYNONYMS: Isoxaflutole

CITATION: Reader, S.C.J. (1995) RPA 201772 (Active Ingredient). Study of Embryo-Foetal Toxicity in the Rabbit by Oral (Gavage) Administration. Pharmaco LSR Toxicology Services Worldwide, Suffolk, England; Report No. 95/RHA551/1076; November 8, 1995. MRID NUMBER: 43904808. (Unpublished)

SPONSOR: Rhône-Poulenc Agriculture Ltd., Essex, England

EXECUTIVE SUMMARY:

In a developmental toxicity study (MRID# 43904808), RPA 201772 (99.6 a.i.) was administered to twenty-five female New Zealand White Rabbits by gavage at dose levels of 0, 5, 20, or 100 mg/kg/day from gestational days 6-19, inclusive.

Maternal toxicity, observed at 100 mg/kg/day, was manifested as increased incidence of clinical signs (little diet eaten and few feces) and decreased body weight gain (23%) and food consumption (≥15%) during the dosing period. **The maternal LOEL is 100 mg/kg/day, based on increased incidence of clinical signs, decreased body weight gains and food consumption. The maternal NOEL is 20 mg/kg/day.**

Developmental toxicity, observed at 5 mg/kg/day consisted of increased incidence of 27th pre-sacral vertebrae. Additional findings noted at 20 and 100 mg/kg/day were manifested as increased number of postimplantation loss and late resorptions, as well as growth retardations in the form of generalized reduction in skeletal ossification, and increased incidence of 13 pairs of ribs. At 100 mg/kg/day, an increased incidence of fetuses with incisors not erupted was also observed. **The LOEL for developmental toxicity is 5 mg/kg/day, based on increased incidence of fetuses with 27th pre-sacral vertebrae. The developmental NOEL is <5 mg/kg/day.**

RPA 201772 Developmental Study (83-3b)

This study is classified as **acceptable** and **satisfies** the guideline requirement for a developmental toxicity study (83-3b) in rabbits.

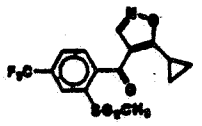
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material: RPA 201772

Chemical Name: 5-Cyclopropyl-4-(2-methylsulfonyl-4-trifluoromethylbenzoyl) isoxazole; isoxaflutole
Description: Beige powder (documented as: fine white cohesive powder, at Pharmaco LSR)
Batch number: JYG708
Lot number: 40 ADM 93
Purity: 99.6%
Stability of compound: Demonstrated in earlier study (#93/RHA518/1193)
Storage Conditions: At room temperature protected from light
CAS NO.: 141112-29-0
Structure:



2. Vehicle Control: 1% w/v Methylcellulose in purified water

3. Test Animals: Species: Rabbit
Strain: New Zealand White
Age and Weight at initiation: 18 to 26 weeks and 3.30 to 4.74 kg, respectively
Source: Froxfield SPF Rabbits Ltd, Hampshire, England
Housing: Individually in cages
Diet - Animals were fed commercial laboratory animal diet STANRAB(P)SQC (Special Diets Services Ltd.,Essex, England) and tap water *ad libitum*
Environmental Conditions: Temperature: 18°C (range: 15-23°C)
Relative Humidity: 55% (range: 40%-70%)
Air changes: at least 12 per hour
Photoperiod: 14-hour light and 10-hour dark cycle
Acclimation period: One week
Males used: Untreated, sexually mature males of proven fertility of same strain

B. PROCEDURES AND STUDY DESIGN:

1. In life dates - start: July 11, 1995
end: August 16, 1995

2. Mating: Following one week of acclimation, females were mated with males of the same strain and source. Following mating, each female was injected intravenously with 25 i.u. of luteinizing hormone. The day of mating was designated Day 0 of gestation.

3. Animal Assignment Animals were assigned to four groups in a sequential manner to ensure even distribution amongst the groups as shown in Table 1.

TABLE 1. Animal Assignment

Test Group	Dose Level (mg/kg/day)	Number Assigned per Group
Control	0	25
Low dose	5	25
Mid dose	20	25
High dose	100	25

3. Dose Selection Rationale: The dose levels were selected based upon the results of preliminary toxicity studies by the sponsor; no details were provided.

4. Dosage Preparation, Analysis and Administration: All doses were in a volume of 5 ml/kg of body weight/day prepared fresh daily, as suspensions in 1% aqueous methylcellulose, during the dosing period. Dosing was based on the daily body weight during gestation day 6 through 19. An appropriate quantity of vehicle was gradually added to a pre-weighed quantity of RPA 201772 and the mixture was ground to form a paste. The paste was then diluted with the remaining vehicle and the resulting suspension was mixed with a mixer for two minutes at low speed. The suspension was then thoroughly hand stirred followed by four minutes on a magnetic stirrer before dosing. The four samples of each test concentration, taken on the first and the last day of treatment, and on three occasions during the treatment period were analyzed to determine the concentration of the test material in the vehicle. The purity of the test compound was analyzed by HPLC chromatography.

Results - The concentration analysis for the samples for the three dose groups conducted before and after dosing indicated values within ±5% of nominal concentration (95%-98%) except on

one occassion. Homogeneity analyses conducted on the test samples revealed values ranging between 95% to 99% (S.D.= 1.6) and within ±6% of target, thus showing that the test compound was homogeneously distributed in the dosing suspension. The recovery of RPA 201772 from spiked samples ranged between 97% and 105%.

C. OBSERVATIONS:

1. Maternal Observations and Evaluations - The animals were weighed and checked for any for overt signs of toxicity daily during the study period. Food consumption was recorded for the following periods: GD 1-5, 6-12, 13-19, 20-23, and 24-28. Dams were sacrificed on day 29 of gestation. Examinations at sacrifice consisted of:

- Gross pathology observations for evidence of adverse reaction to treatment and specimens of abnormal tissues were retained
- Individual placental weights
- Number of corpora lutea
- Number of implantation sites; the uteri from apparently nonpregnant animals were stained using the Salewski (1964) technique to detect the implantation sites
- Numbers of resorptions (early and late) and live and dead fetuses
- Number and distribution of fetuses in each uterine horn

2. Fetal Evaluations - The fetuses were examined in the following manner:

- Individual fetal weight and sex
- External anomalies
- Visceral anomalies by dissecting the neck and the thoracic as well as abdominal cavities of all fetuses from each litter
- Skeletal anomalies for all fetuses using the method of Dawson and staining with Alizarin
- Head anomalies (approximately one-third of the fetal heads) following fixation in Bouin's solution

D. DATA ANALYSIS

1. Statistical Analysis: The following methods were used:

- Maternal body weight and body weight change, food consumption, fetal and placental weights and litter size--One-way analysis of variance and/or t-test

- Corpora lutea, implantations, early and late resorptions, and pre- and post-implantation loss--Mann-Whitney U-test

2. Historical Control data: Historical control data were provided to allow comparison with concurrent controls.

II. RESULTS

B. MATERNAL TOXICITY

1. Mortality - No compound-related mortalities were noted. The incidental findings included death of one control and one mid-dose (20 mg/kg/day) female on GD 10 and 19, respectively. Necropsy of these animals revealed that these deaths were incidental. These animals suffered body weight loss and had thymic hemorrhage, few or no signs of implantations, frothy material in trachea and/or congested lungs. The mid-dose female also had hemorrhagic stomach. At 100 mg/kg/day, one female that aborted on GD 26 of gestation was sacrificed; gross examination at necropsy revealed pale liver and enlarged gall bladder with multiple pale areas on the surface. The cause of death was considered to be unrelated to treatment.

2. Clinical observations - Compound-related clinical signs observed at 100 mg/kg/day included increased number of does with little diet consumed and few feces in the cages (6/19 and 17/19 females, respectively), beginning from GD 6 through 19 (Table 2).

3. Body weight - Body weight gain data are summarized in Table 3. Compound-related decreases in body weight and weight gain were observed at 100 mg/kg/day. At this dose level, significant decreases in body weight gain were observed for GD 6-20 (86%), and GD 0-20 (23%). During GD 20-28, a compensatory increase (120%) was noted for this dose group. Maternal body weight (data not shown) was slightly (4-5%) but significantly ($p \leq 0.001$) decreased during GD 6-20 of treatment period and remained lower (4-6%) from GD 20 through 28 compared to controls during the post-treatment period. The body weights at 5 and 20 mg/kg/day were unaffected.

TABLE 2. Clinical Signs During Treatment Period[a]

Clinical signs	Dose Groups (mg/kg/day)			
	0	5	20	100
No. of animals with signs/No. Examined				
Little diet eaten	1/19	0/21	1/23	6/19
Few feces	6/19	5/21	5/23	17/19

[a]Data were extracted from Study No. 95/1076, Table 2, page 26; includes females that survived to Day 29 of gestation

TABLE 3. Mean Body Weight Gain (kg)[a]

Dose in mg/kg/day (# does/group)	Prior to Dosing Period (GD 0-6)	Dosing Period (GD 6-20)	Post-Dosing Period (GD 20-28)	Gestation Period (GD 0-28)
0 (19)	0.10 ± 0.07	0.22 ± 0.13	0.10 ± 0.17	0.44 ± 0.20
5 (21)	0.11 ± 0.09	0.27 ± 0.10	0.17 ± 0.21	0.57 ± 0.26
20 (23)	0.14 ± 0.10	0.24 ± 0.17	0.23 ± 0.09	0.61 ± 0.15
100 (19)	0.09 ± 0.09	0.03 ± 0.13c	0.22 ± 0.17c	0.34 ± 0.16c

[a]Data were extracted from Study No. 95/1076, Table 3, page 27 and Appendix 4, pages 79-86; calculated by the Reviewer

[b]Gravid uterine weight was not provided and, therefore, the corrected body weight change could not be calculated.

[c]Body weight gain decreased during GD 6-20 and 0-20 (86% and 23%, respectively) and increased during GD 20-28 (120%) compared to controls; no statistical analyses were conducted.

4. Food consumption - Food consumption data are summarized in Table 4. A compound-related significant decrease (15-24%, $p < 0.05$) in food consumption was observed at 100 mg/kg/day, during GD 6-19. A compensatory increase (17-32%); $p<0.01$) in food consumption was noted during post-treatment, on GD 20-28. No adverse effects on food consumption were noted at lower dose levels.

5. Gross Pathology - No compound-related gross pathology findings were noted.

6. Cesarean section Data - Data are summarized in Table 5. Compound-related effects were observed at 100 mg/kg/day.

   The number of corpora lutea and implantation were unaffected by treatment. At 100 mg/kg/day, the mean number of late resorptions was slightly higher than that of concurrent and historical controls (1.5 versus 0.7 resorptions/litter in concurrent control; historical control range: 0.4-1.3). This resulted in an increased post-implantation loss (21% versus 11% in control) and reduced the mean number of viable pups (8.3 versus 9.8/litter). In addition, mean fetal weight was slightly but nonsignificantly lower (3%) compared to controls.

B. DEVELOPMENTAL TOXICITY

Incidences of fetal anomalies with selected external, visceral, and skeletal anomalies are presented in Tables 6 and 7. No compound-related anomalies were observed during external and visceral examinations of fetuses. Compound-related effects were observed during skeletal examination at all treatment levels. These were manifested as reduction in skeletal ossification (20 and 100 mg/kg/day) and increased incidence of fetuses with 13 pairs of ribs (20 and 100 mg/kg/day) and 27 presacral vertebrae (5, 20 and 100 mg/kg/day) as well as fetuses with incisors not erupted (100 mg/kg/day). Incidences of these anomalies, on a litter basis, were higher than the concurrent control values and in some cases exceeded the range for historical controls. When analyzed on a litter basis, the overall number of fetuses and litters with external, visceral, and skeletal anomalies was unaffected. The compound-related anomalies are discussed below.

1. External Examination - The incidental finding noted was occurrence of small fetuses (Table 6) in all dose groups. Additional findings noted at 20 mg/kg/day included three fetuses in two litters with flexure of the forelimb and one fetus from a separate litter having a thread-like tail with constriction followed by a swollen tip; no bone was apparent.

TABLE 4. Mean Food Consumption (g/animal/day)[a]

Study Period in Days	Dose Groups (mg/kg/day)			
	0	5	20	100
Pre-treatment				
0-5	166	180	165	167
Treatment				
6-12	157	167	168	134* (-15)
13-19	123	152* (+24)	149* (+21)	94* (-24)
Post-treatment				
20-23	147	162	166	173 (+18)
24-28	100	132* (+32)	148**(+48)	132*(+32)

[a]Data were extracted from Study No. 95/1076, Table 4, p. 28
[b]Value in parenthesis indicates percent increase or decrease over controls
*Significantly different from control ($p<0.05$)
**Significantly different from control ($p<0.01$))

TABLE 5. Cesarean Section Observations[a]

Parameter	Dose Level (mg/kg/day) 0	5	20	100
No. animals assigned	25	25	25	25
No. animals mated	25	25	25	25
No. animals pregnant	20	21	23	20
Pregnancy rate (%)	100	100	100	100
Maternal wastage				
No. died/nonpregnant	0	0	0	0
No. died/pregnant	1	0	1	0
No. nonpregnant	5	4	1	5
No. aborted	0	0	0	1
No. premature delivery	0	0	0	0
Total corpora lutea	247 (19)	280 (21)	291 (23)	236 (19)[c]
Corpora lutea/dam	13.0 ± 3.1[b]	13.3 ± 2.2	12.7 ± 2.3	12.4 ± 2.3
Total implantations	210	247	248	201
Implantations/dam	11.1 ± 3.2	11.8 ± 2.8	10.8 ± 3.8	10.6 ± 3.1
Total live fetuses	186	210	209	159
Live fetuses/dam	9.8 ± 2.7	10.0 ± 2.4	9.0 ± 3.3	8.3 ± 2.7
Total resorptions	24	37	39	42
Early	10	13	21	13
Late	14	24	18	29
Resorptions/dam	1.3 ± 1.1	1.8 ± 1.3	1.7 ± 1.3	2.2 ± 1.5
Total dead fetuses	0	0	0	0
Dead fetuses/dam	0	0	0	0
Fetal weight/litter (g)	38.9 ± 1.6	40.0 ± 1.8	40.8 ± 1.6	37.9 ± 1.5
Preimplantation loss (%)	16.3	12.7	16.2	15.9
Postimplantation loss (%)	11.4	15.0	15.7	20.9
Sex ratio (% male)	54	51	46	48

[a]Data were extracted from Study No. 954/1076, Tables 1, 6 and 7, pages 25, 31 and 32 and Appendix 7, pages 100-107
[b]Mean ± S.D.
[c]One dam aborted on GD 26; data excluded from analysis

2. Visceral Examination - There were no compound-related visceral anomalies observed. Incidental findings noted in one or more dose groups consisted of grossly abnormal fetus, anomalies of liver (e.g. pale areas, lobular pattern, cyst on the liver lobe), gall bladder (e.g. reduced, rudimentary or hemorrhagic; agenesis), bilobed renal cavitation and distended urinary bladder.

TABLE 6. Fetal External and Visceral Examination[a]

Findings[b]	Dose Level (mg/kg/day) 0	5	20	100
External Anomalies				
No. fetuses (litters) examined	186 (19)	210 (21)	209 (23)	159 (19)
Small fetus (<32 g)	38 (10)	28 (13)	38 (11)	31 (8)
Total No. fetuses (litters) with external malformations[c]	38 (10)	28 (13)	42 (12)	31 (8)
No. fetuses/litter with external malformations[c]	3.8	2.2	3.5	3.8
Visceral Anomalies				
No. fetuses (litters) examined	186 (19)	210 (21)	209 (23)	159 (19)
Total No. fetuses (litters) with visceral malformations[c]	16 (9)	24 (9)	16 (9)	7 (6)
No. fetuses/litter with visceral malformations[c]	1.7	2.6	1.7	1.1

[a]Data were extracted from Study No. 95/1076, Table 8 , pages 33-35 and Appendix 7, pages 98-107
[b]More than one type of anomaly may be found in one fetus.
[c]Calculated by the Reviewer

3. Skeletal Examination - Skeletal anomalies noted at 20 and 100 mg/kg/day consisted of generalized reduction in the degree of skeletal ossification, possibly associated with reduced fetal growth, increased incidences of fetuses with 13 pairs of ribs, rudimentary first ribs (at 100 mg/kg/day only), reduced or lack of ossification of heads of limb long-bones, metacarpals, phalangeal and pubic bones and 27 pre-sacral vertebrae. The incidences of these findings exceeded that of historical controls (see Attachment for historical control data). At 5 mg/kg day, the incidence of fetuses with 27 pre-sacral vertebrae was higher than that of concurrent and historical controls (% fetal incidence (number of litters): 34.8% (19) versus 16.7 (12) in controls; historical control range: 11.6%-34.2%; see report page 40); although this finding was not associated with effects on the number of ribs, when analysed on a litter basis, the incidence increased in a dose-related manner at all three dose levels (3.8, 5.0 and 7.6 at 5, 20, and 100 mg/kg/day versus 2.5 in controls). Therefore, this finding was considered to be compound-related (Table 7).

   Additional findings noted at 100 mg/kg/day included increased incidence of medium anterior fontanelle, incomplete ossification of hyoid body, additional 6th sternebra and incompletely ossified centrales, reduced ossification of 1st rib or ribs and incomplete ossification of pubic bones. Incidences of these anomalies were higher than that of concurrent controls.

Serial sections of fetal heads revealed increased incidence of fetuses (litters) with incisors not erupted at 100 mg/kg/day, suggesting a delay in development (# fetuses (# litters): 20 (12) versus 8 (6) in controls; see report page 43). The incidence of this finding, on a litter basis, was also higher compared to controls (1.6 versus 1.3 in controls). Incidental findings noted included anomalies of incisors, retina and various parts of the brain.

III. DISCUSSION:

A. MATERNAL TOXICITY: Compound-related maternal toxicity was observed at 100 mg/kg/day and was manifested as an increased incidence of does with little diet eaten and few feces in the cages, decreased body weight gain, and food consumption during the dosing period.

   Based on these results, the maternal LOEL was 100 mg/kg/day; the NOEL was 20 mg/kg/day.

B. DEVELOPMENTAL TOXICITY:

1. Deaths/Resorptions: At 100 mg/kg/day, there were compound-related increases in late resorptions and percent post-implantation loss causing reduction in the number of viable pups.

TABLE 7. Fetal Skeletal Examination[a]

Findings[b]	Dose Level (mg/kg/day) 0	5	20	100
No. fetuses (litters) examined	186 (19)	210 (21)	209 (23)	159 (19)
Head				
Medium anterior fontanelle	17 (7)	21 (9)	18 (9)	33 (13)
Incomplete ossification of hyoid body	18 (8)	34 (14)	34 (13)	37 (13)
Sternebrae and Ribs				
Sternebrae				
- Additional bone between 5th and 6th sternebrae	0	3 (2)	6 (4)	8 (6)
Ribs				
- 13/13	53 (14)	79 (17)	128 (22)	119 (19)
(% fetal incidence)[c]	28.5%	37.6%	61.2%	78.8%
- Rudimentary 1st rib or ribs	0	0	0	3 (2)
Vertebrae, Limbs and Girdles				
27th presacral vertebrae	31 (12)	73 (19)	100 (20)	137 (18)
(% fetal incidence)[d]	16.7%	34.8%	47.8%	86.2%
Heads of limb long-bones,				
- unossified	14 (7)	24 (9)	46 (12)	52 (13)
One or both centrale,				
- incompletely ossified	1	3 (2)	12 (5)	6 (5)
- unossified	1	3 (3)	4 (3)	1
Pubic bones,				
- incompletely ossified	3 (2)	3 (3)	6 (3)	12 (7)
- unossified	1	0	2 (2)	1
Metacarpals and/ or phalanges,				
- unossified	8 (7)	16 (6)	35 (9)	17 (10)
- incompletely ossified	12 (8)	14 (7)	32 (11)	21 (11)
Total No. fetuses (litters) with skeletal malformations[e]	94 (18)	121 (21)	159 (23)	112 (19)
No. fetuses/litter with skeletal malformations[e]	5.2	5.8	6.9	5.9

[a]Data were extracted from Study No. 95/1079, Tables 9 and 10, pages 36-42 and Appendix 9, pages 119-127
[b]More than one type of anomaly may be found in one fetus.
[c]Fetal incidence in historical control: 22.7-57.3%
[d]Fetal incidence in historical control: 11.6-34.2%
[e]Calculated by the Reviewer

2. Altered Growth: At 20 and 100 mg/kg/day, a generalized reduction in skeletal ossification was observed. Additionally, at 100 mg/kg/day, there was increase in the number of fetuses with incisors not erupted suggesting a delay in development.

3. Developmental Anomalies: At 20 and 100 mg/kg/day, there was delayed ossification in the generalized skeletal development consisting of increased incidence of fetuses with rudimentary 1st ribs, reduced or lack of ossification of heads of limb long-bones, metacarpals, phalangeal and pubic bones, compared to control values. In addition, there was increase in the incidence of 13 pairs of ribs (20 and 100 mg/kg/day) and 27 pre-sacral vertebrae (in all dose groups) above that of controls. The percentage of fetuses affected was outside the historical control range. Therefore, these anomalies were considered to be compound-related.

Based on the increased incidence of 27th pre-sacral vertebrae, the developmental LOEL and NOEL were 5 and <5 mg/kg/day, respectively.

C. STUDY DEFICIENCIES:

- Gravid uterine weights were not reported; therefore, the corrected body weight change could not be calculated.

- Individual Clinical observations were not provided.

- Since no developmental NOEL was identified in this study, it is not adequate by guideline standards.

However, the above deficiencies do not negatively impact upon the outcome of the study.

ATTACHMENT

Insufficient Review

Page _____ is not included in this copy.

Pages 46 through 47 are not included in this copy.

The material not included contains the following type of information:

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