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497AB3

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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SEP 16 1993

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Imazalil Sulphate: review of a developmental toxicity study in rabbits.

Tox.Chem No.: 497AB
MRID No.: 42593601
DP Barcode: D186539
Submission No.: S433281
PC Code: 111901

From: John C. Redden, Toxicologist
Section 3
Toxicology Branch 1
Health Effects Division (H7509C)

8/27/93

To: Kathleen Depukat, PM Team No. 52
Accelerated Reregistration Branch
Special Review and Reregistration Division (H7508W)

Thru: Karen L. Hamernik, Ph.D.
Section Head Section 3
Toxicology Branch 1
Health Effects Division (H7509C)

8/27/93 KB 9/14/93

ACTION:

Review and evaluate MRID No. 42593601 developmental toxicity study in rabbits.

CONCLUSIONS:

A developmental toxicity study was conducted in which Albino rabbits were administered imazalil sulphate daily via gavage at dose levels of 0, 5, 10, or 20 mg/kg/day on gestational days 6-18, inclusively. Maternal toxicity was observed at 10, and 20 mg/kg/day as evidenced by significantly decreased body weight gain and increased mortality (at 20 mg/kg/day). Thus, based on these results, the NOEL for maternal toxicity is 5 mg/kg/day; the LOEL was 10 mg/kg/day (the equivocal weight gain effect at 5 mg/kg/day will not be used to set an LOEL).

Developmental/maternal toxicity, observed at 10 and 20 mg/kg/day, was manifested as an increased incidence of resorptions. Consequently, the NOEL for developmental/maternal toxicity was 5

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mg/kg/day; the LOEL was 10 mg/kg/day.

The study is classified as Core **Supplementary** data, which is potentially upgradable. This study does not meet the minimum requirements set forth under Guideline Series 83-3 for a developmental toxicity study in rabbits, owing to several design and reporting deficiencies listed in the Reviewer's Discussion

FINAL

DATA EVALUATION REPORT

Imazalil Sulphate

Study Type: Developmental Toxicity

Prepared for:

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
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Patricia Bittner, M.S.

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Sharon Segal, Ph.D.

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Contract Number: 68D10075
Work Assignment Number: 2-87
Clement Number: 222
Project Officer: Caroline C. Gordon

EPA Reviewer: Myron Ottley, Ph.D.
Review Section I, Toxicology Branch I/HED

Signature: M Ottley
Date: 6/14/93

EPA Section Head: Marion Copley, D.V.M.
Review Section IV, Toxicology Branch I/HED

Signature: Marion Copley
Date: 8/4/93

DATA EVALUATION REPORT

STUDY TYPE: Developmental Toxicity; Guideline Series 83-3
Rabbit

EPA IDENTIFICATION NUMBERS

PC Code: 111901

Tox Chem. No.: 497AB

MRID No.: 425936-01

TEST MATERIAL: Imazalil sulphate (R27180)

SYNONYM: None reported

SPONSOR: Janssen Pharmaceutica N.V., Beerse, Belgium

STUDY NUMBER: 2615

TESTING FACILITY: Janssen Research Foundation, Beerse, Belgium

TITLE OF REPORT: Oral (gavage) embryotoxicity and teratogenicity study (Segment II) with imazalil sulphate (R 27180) in Albino rabbits.

AUTHORS: P. Dirkx and H. Van Cauteren

STUDY COMPLETION DATE: March 27, 1992

CONCLUSIONS: A developmental toxicity study was conducted in which Albino rabbits were administered imazalil sulphate daily via gavage at dose levels of 0, 5, 10, or 20 mg/kg/day on gestational days (GDs) 6-18, inclusively. Maternal toxicity was observed at 10, and 20 mg/kg/day as evidenced by significantly decreased body weight gain and increased mortality (at 20 mg/kg/day). Thus, based on these results, the NOEL for maternal toxicity ~~is 5 mg/kg/day~~; the LOEL was 10 mg/kg/day (The equivocal weight gain effect at 5 mg/kg/day will not be used to set an LOEL)
Maternal
Developmental toxicity, observed at 10 and 20 mg/kg/day, was manifested as an increased incidence of resorptions. Consequently, the NOEL for developmental/maternal toxicity was 5 mg/kg/day; the LOEL was 10 mg/kg/day.

CLASSIFICATION: Core Supplementary Data - potentially upgradable. This study does not meet the minimum requirements set forth under Guideline Series 83-3 for a developmental toxicity study in rabbits, owing to several study design and reporting deficiencies listed in the Reviewer's Discussion.

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A. MATERIALS

Test Compound

Purity:	98.2-100% (w/w)
Description:	Off-white to beige powder
Batch number:	ZR027180PUA631
Contaminants:	Not reported
Storage:	Room temperature in closed containers
Receipt date:	Not reported

Vehicle: Deionized water

Test Animals

Species:	Rabbit
Strain:	Albino (non-inbred)
Source:	Buyens, Lichtaart, Belgium
Age:	Not reported
Weight:	2881-3866 g on GD 0

B. STUDY DESIGN

This study was designed to assess the potential of imazalil sulphate to cause developmental toxicity in Albino rabbits when administered daily via gavage from GDs 6 through 18, inclusively.

Mating: Following at least 1 week of acclimation, females were artificially inseminated. Semen was collected from 9 males using an artificial vagina, pooled and prepared to yield 40 million sperm/mL. Three hours before insemination, females were injected with chorionic gonadotropin (50 I.U.). The day of insemination was designated GD 0.

Animal husbandry: Huybrechts pelleted rabbit food and tap water were available ad libitum. No data was reported on the temperature, relative humidity, or photoperiod of the animal room; there were approximately 18 air changes/hour.

Group arrangement: No data were provided regarding method of assignment. Females were assigned to study groups as follows.

Test Group	Dose Level (mg/kg/day)	Number Assigned per Group
Control	0	15
Low-dose	5	15
Mid-dose	10	15
High-dose	20	15

Dose administered: Doses were administered daily via gavage from GD 6 through 18 in a volume of 0.2 mL/kg of body weight. Solutions were prepared using the appropriate amounts of test material and deionized water. Information on the frequency of preparation of dosing solutions was not provided. Control animals were dosed with deionized water. No information was provided regarding which body weight data were used to calculate doses. Concentration and stability of the test material were determined during the study.

Dose rationale: Doses were selected to replicate those chosen for a previous study (Exp. No. 2373), in which 5 and 10 mg/kg/day demonstrated no adverse effects, while 20 mg/kg/day was maternally toxic. Due to a low pregnancy rate among these animals, the study was repeated. No other data regarding this study were provided.

Observations: The animals were observed at least once daily for mortality, moribundity, and clinical signs. Body weight data were recorded on GDs 0, 6, 19, and 28; food consumption was determined on GDs 6, 19, and 28. On GD 28, females were euthanized by decapitation. Necropsy was performed on all females and examination included the following:

- Gross pathology observations
- Gravid uterine weights
- Number of corpora lutea
- Number of implantation sites
- Number of resorptions, and live and dead fetuses

All live fetuses were examined for the following:

- Individual fetal weight and sex
- External anomalies (including dead fetuses) using photographic documentation
- Visceral anomalies using photographic documentation
- Skeletal anomalies using Alizarin s red staining and radiographic documentation

Statistical analysis: The following methods were used:

- Clinical signs, mortality, and pregnancy rate--Chi-square test
- Maternal body weight and weight change, food consumption, gravid uterine weight, corpora lutea, implantations, litter size, fetal anomalies, sex ratio, fetal body weight, and number of live, dead, and resorbed fetuses--Mann-Whitney's U test

Compliance

- A signed Statement of No Data Confidentiality Claim, dated December 2, 1992, was provided.
- A signed Statement of Compliance with EPA GLPs, dated March 27, 1992, was provided.
- A signed Quality Assurance Statement, dated March 27, 1992, was provided.

C. RESULTS

Test Material Analysis

Purity of the test compound ranged from 98% to 100% (w/w). Concentration analyses conducted on dosing solutions ranged from 98% to 101% of target. Stability of the test compound in the vehicle after a 3-week period revealed concentrations from 100% to 102% of nominal values.

Maternal Toxicity

Mortality: Compound-related mortality was observed in 8/15 animals at 20 mg/kg/day. Since individual pathology data were not provided, this could not be verified by the reviewers. The study authors noted that emphysema and/or pneumonia was present in the majority of these animals. Other findings in these animals included weight loss (5/8) and gastrointestinal (GI) changes (2/8) including watery contents or hemorrhagic inflammation. One animal (10 mg/kg/day) that died on GD 18 was noted with focal pneumonia.

Abortion: No abortions were reported.

Clinical observations: Compound-related effects were observed at 10 and 20 mg/kg/day (data not shown). Rough hair coat was noted in animals of the mid- (1/15) and high-dose groups (2/15). Other findings among treated animals included respiratory difficulty at 10 mg/kg/day (2/15) and 20 mg/kg/day (2/15); and thin appearance at 5 mg/kg/day (1/15), 10 mg/kg/day (2/15), and 20 mg/kg/day (3/15). None of these effects were observed among control animals.

Body weight: Compound-related effects on body weight and body weight gain were observed at 5, 10, and 20 mg/kg/day. A summary of maternal body weight gain data (calculated and analyzed by the reviewers) for selected intervals is presented in Table 1.

A slight decrease (5%) in body weight was noted at 10 mg/kg/day on GD 19; a significant ($p<0.05$, 7%) decrease was noted at 20 mg/kg/day (data not shown). Dose-dependent decreases in body weight gain were noted during the dosing period for the 5-, 10- and 20-mg/kg/day dose groups (18, 54, and 95%, respectively); these decreases were significant only for the 10- and 20-mg/kg/day groups. Increases in body weight gain were noted in the 5-, 10-, and 20-mg/kg/day groups postdosing (110, 90, and

232%, respectively); the increase at 20 mg/kg/day was significant ($p<0.05$). The decrease at 5 mg/kg/dy is considered equivocal. MC 8/26/97

Food consumption: Compound-related effects on food consumption were noted at 10 and 20 mg/kg/day. A summary of food consumption data is presented in Table 2. A significant ($p<0.05$) decrease in food consumption was noted at 10 mg/kg/day (18%) and 20 mg/kg/day (23%) during GDs 6-18. The significant decrease noted during GDs 0-5 (10 mg/kg/day) was considered incidental.

Gross pathology observations: Individual/summary data for gross pathology observations were not reported.

Cesarean section observations: Compound-related effects were observed at 10 and 20 mg/kg/day. A summary of cesarean section data is presented in Table 3. There was an increase in the mean number of resorptions/doe at 10 and 20 mg/kg/day. Three out of seven rabbits (20 mg/kg/day) surviving to GD 28 had their entire litters resorbed, and another litter had 5/6 embryos resorbed. Although the increase in resorptions at 10 mg/kg/day was within the historical control limits, it is believed to be a compound-related effect in this study due to the pattern of dose dependency. Due to the increased numbers of resorptions at 10 and 20 mg/kg/day, the percentage of postimplantation loss was increased and the numbers of live fetuses/doe was subsequently decreased; however, it is difficult to fully assess the effects seen at 20 mg/kg/day since there were only 4 live litters available for evaluation. The significant change in the sex ratio noted at 5 mg/kg/day was considered incidental.

Developmental Toxicity

Compound-related skeletal anomalies were observed at 20 mg/kg/day. Incidences of skeletal malformations and visceral and selected skeletal variations are presented in Table 4.

External examinations: No external malformations or variations were observed.

Visceral examinations: No visceral malformations were observed. Visceral variations, occurring as single events, consisted of bleeding artery umbilicalis (0 mg/kg/day) and blood in the abdomen (5 mg/kg/day).

Skeletal examinations: Skeletal malformations consisted of dysplasia of thoracic vertebra(e) (0 and 5 mg/kg/day) and bifurcated ribs (0 and 5 mg/kg/day).

Variations occurring in a non-dose-related manner were noted in the ribs and sternum. Significant increases ($p<0.05$) in the fetal incidences of unilateral 13th rib and missing sternum bones were noted at 20 mg/kg/day; however, there were only 4 live litters to evaluate. There was no increase in litter incidence of these variations noted at other dose levels. The significant decrease ($p<0.05$) in rudimentary sternum bones (20 mg/kg/day) was considered incidental.

D. REVIEWERS' DISCUSSION/CONCLUSIONS

Acceptance Criteria

The reviewers have completed an Acceptance Criteria check list (Attachment I) to be included with the evaluation of the study. Criteria which were not satisfied included: (2) insufficient numbers of pregnant animals at 5 and 20 mg/kg/day, (10) necropsy results on individual animals not provided, (11) lack of temporal quality for resorption data (early/late), (15) individual data not provided for skeletal examinations in such a way that the total number of fetuses and litters affected could be determined.

Test Material Analyses

Analyses of concentrations of the test material in the vehicle were within ±2% of the nominal values. The compound was stable in the vehicle for 3 weeks.

Maternal Toxicity

Although the effect (Body wt gain depression) at 5mg/kg/day was equivocal. Compound-related maternal toxicity was observed at all dose levels. It was manifested as increased mortality (20 mg/kg/day); decreased body weight gain (10, and 20 mg/kg) during GDs 6-18; and decreased food consumption (10 and 20 mg/kg/day) during GDs 6-18. Based on these results, the NOEL = 5 mg/Kg/day ; the LOEL for maternal toxicity was 10 mg/kg/day.

Developmental Toxicity

(a) Deaths/resorptions: Increased numbers of resorptions/litter, with subsequent decreases in numbers of live fetuses/litter were noted at 10 and 20 mg/kg.

(b) Altered growth: No compound-related effects were observed.

(c) Developmental anomalies: Increased incidences of fetuses with a unilateral 13th rib and missing sternum bones were noted at 20 mg/kg/day. However, these findings could not be examined on a litter basis.

Based on decreased numbers of live pups and increased numbers of resorptions, the NOEL for developmental toxicity was 5 mg/kg/day; the LOEL was 10 mg/kg/day.

Study/Reporting Deficiencies

The highest dose tested (20 mg/kg/day) was highly toxic to the does, causing a high number of maternal deaths and resorptions in surviving animals. A lower dose should have been selected to allow more animals to survive to study termination, thus providing more maternal and fetal data for evaluation. Doses were selected based on those used in a previous study, however, the results of that study were not provided.

The high maternal mortality should have been predicted by this earlier study, but apparently it was not.

In addition, the following reporting deficiencies were noted:

-- Individual fetal data on skeletal malformations/variations were not reported in such a way that the total number of fetuses/group with malformations/variations could be determined.

-- Body weight data were not recorded as per Guidelines, but were recorded on GDs 0, 6, 19, and 28; this did not affect the interpretation of results.

-- Clinical signs were reported only for GDs 0-5, 6-18, and 19-27 intervals, although it seems that the registrant had data on daily observations.

-- No data were provided regarding which body weight data were used to calculate the dosing concentrations.

-- No data were provided on the age or breed of animals or how they were assigned to treatment groups; temperature, humidity or photoperiod of the animal room were also not provided.

-- Uteri were not stained to detect early resorptions, therefore the number of resorptions may have been artificially low.

E. CORE CLASSIFICATION: Supplementary, this study ~~can~~ may be upgraded (see note below) if reporting deficiencies are resolved. MC 8/4/93

Maternal NOEL - 5 mg/kg/day MC 8/4/93
Maternal LOEL - 10 mg/kg/day
Developmental Toxicity NOEL - 5 mg/kg/day
Developmental Toxicity LOEL - 10 mg/kg/day (Maternal)

F. RISK ASSESSMENT: Not applicable

The lack of 12 litters at the low and high doses; and excessive maternal toxicity (HDT) are serious deficiencies.

However, a new study would not be expected to alter the conclusion that developmental toxicity (- resorptions) occurred at 10 mg/kg/dy and the NOEL is clearly 5 mg/kg/dy. This is a maternally toxic dose.

Although the 7 litters (at the high dose) could compromise the study, at the LDT, 11 litters is only marginally less than the guidelines request

MC 8/4/93

1 10

TABLE 1. Mean Body Weight Gain (g ± S.D.)[a,b]

Dose Group (mg/kg/day)	Prior to Dosing Period (GDs 0–6)[c]		Dosing Period (GDs 6–19)[c]		Post Dosing Period (GDs 19–28)[c]		Entire Gestation Period (GDs 0–28)[c]		Corrected Body Weight Change (GDs 0-28)[d]	
0	151	60	282	90	80	175	513	232	93	74
5	141	72	231	92	168	64	540	89	155	56
10	141	87	130*	194	152	93	422	166	100	61
20	133	113	13*	150	266*	116	412	300	194	115

[a]Data were extracted from study number 2615, Tables 2-5.

[b]Body weight data were recorded on the morning of GDs 0, 6, 19, and 28; weights reflect the intervals shown.

[c]Calculated and statistically analyzed by the reviewers using one-way ANOVA with Schefe's test

*Statistically different from control ($p<0.05$)

[d]Corrected body weight change = (body weight through GD 27 - body weight on GD 0) - gravid uterus weight

TABLE 2. Mean Food Consumption (g ± S.D.)[a,b]

Dose Group (mg/kg/day)	Prior to Dosing Period (GDs 0-5)[c]		Dosing Period (GDs 6-18)[c]		Post Dosing Period (GDs 19-27)[c]	
0	1298	33	2726	117	1651	360
5	1250	27	2635	107	1484	92
10	1160	51*	2227	178*	1495	78
20	1155	84	2096	238*	1748	146

[a]Data were extracted from study number 2615, Tables 2-5.

[b]Food consumption data were only recorded on GDs 6, 19, and 28.

*Significantly different from control ($p<0.05$)

TABLE 3. Cesarean Section Observations[a]

Parameter	Dose Level (mg/kg/day) 0		5		10		20	
No. animals assigned	15		15		15		15	
No. animals pregnant	15		11		14		13	
Pregnancy rate (%)	100		73		93		87	
Maternal wastage								
No. died/nonpregnant	0		0		0		2	
No. died/pregnant	0		0		1		6	
No. nonpregnant	0		4		1		2	
No. aborted	0		0		0		0	
No. examined at necropsy[b]	15		11		13		7	
Gravid uterine weight (g)	420		384		322		218	
Litters w/live fetuses	15		11		13		4	
Total corpora lutea[c]	134		95		119		54	
Corpora lutea/doe[d]	8.9	0.7	8.6	0.5	9.2	0.6	7.7	0.9
Total implantations[c]	113		75		84		51	
Implantations/doe[d]	7.5	0.6	6.8	0.8	6.5	0.8	7.3	0.9
Total live fetuses[b]	96		68		65		25	
Live fetuses/doe[d]	6.4	0.8	6.2	0.9	5.0	0.8	3.6	1.6
Total resorptions[b]	10		7		19		26	
Resorptions/doe[d]	0.7	0.3	0.6	0.3	1.5	0.6	3.7	1.6
Total dead fetuses[c]	7		0		0		0	
Dead fetuses/doe[d]	0.5	0.4	0		0		0	
Mean fetal weight (g)[d]	41.3	1.5	42.4	1.2	45.2	2.2	39.0	1.5
Preimplantation loss (%)[c]	16		21		29		6	
Postimplantation loss (%)[c]	15		9		23		51	
Sex ratio (% male)	47		35*		54		60	

[a]Data were extracted from study number 2615, Tables 1-5.

[b]Data excludes does that either died on study or were not pregnant; all mean calculations are based on the number of does examined at necropsy.

[c]Calculated (but not statistically analyzed) by the reviewers

[d]Mean S.D.

*Significantly different from control ($p<0.05$)

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TABLE 4. Incidences of Fetal Abnormalities[a]

Findings[b]	Dose Level (mg/kg/day) 0	5	10	20
No. fetuses (litters) examined	96 (15)	68 (11)	65 (13)	25 (4)
Visceral Variations				
Bleeding artery umbilicalis (bladder)	1	0	0	0
Blood in abdomen	0	1	0	0
No. of fetuses (litters) with any visceral variations	1	1	0	0
Skeletal Malformations				
Dysplasia of thoracic vertebra(e)	1	1	0	0
Bifurcated rib(s)	1	2 (1)	0	0
No. of fetuses (litters) with any skeletal malformation(s)	1 (1)	2 (1)	0	0
Skeletal Variations				
One rudimentary 13th rib (unilateral)	12 (7)	10 (6)	10 (6)	3 (3)
One 13th rib (unilateral)	4 (2)	6 (3)	5 (3)	6* (3)
13th pair of ribs	17 (9)	13 (7)	18 (8)	9 (3)
Rudimentary sternum bone(s)	18 (8)	19 (9)	19 (9)	11* (4)
Sternum bone(s) : missing	4 (4)	15 (4)	3 (3)	6* (3)

[a]Data were extracted from study no. 2615, Tables 7-11.

[b]More than one type of anomalies may be found in one fetus; total number of fetuses with anomalies could not be determined since individual data were not provided

*Significantly different from control ($p<0.05$)

ATTACHMENT I

83-3 Teratology Studies

ACCEPTANCE CRITERIA

Does your study meet the following acceptance criteria?

1.	YES	Technical form of the active ingredient tested.
2.	NO	At least 20 pregnant animals/dose group for mice, rats, or hamsters are available. At least 12 pregnant animals/dose group for rabbits are available.
3.	YES	At the high dose, overt maternal effects such as slight weight loss are reported (or a limit dose is given, 1,000 mg/kg).
4.*	YES	At the low dose, no developmental toxicity is reported.
5.	YES	Dosing duration is at least during the period of major organogenesis, but may extend up to one day prior to term.
6.*	YES	Analysis for test material stability, homogeneity, and concentration in dosing medium.
7.	YES	Individual daily observations.
8.	YES	Individual body weights.
9.	YES	Individual food consumption.
10.	Y/N	Necropsy on all animals.
11.	YES	Individual uterine examination, including numbers of fetal deaths, early and late resorptions, and viable fetuses per sex.
12.	YES	All ovaries examined to determine number of corpora lutea.
13.	YES	Individual litter weights and/or individual fetal weights/sex/litter.
14.	YES	Individual fetal external examination.
15.	Y/N	Individual fetal skeletal examination for 1/3 to 1/2 of each litter for rodents and all for rabbits.
16.	YES	Individual fetal soft tissue examination.

Criteria marked with an * are supplemental, may not be required for every study.

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