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

MAY 2 4 1993

MEMORANDUM:

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Subject: EPA ID No.: 111901; Imazalil - 6(a)(2) Data on a Study of Reproduction in the Rat/2337 (83-4).
Tox. Chem. No.: 497AB.
Shaughnessy No.: 111901.
Cas Registry No.: 35554-44-0.
Submission No.: S432102.
DP Barcode: D185810.
Case No.: 816389.
MRID No.: 425707-01.

From: David G Anderson, PhD. David G Anderson 5/13/93
Toxicologist, Section 3
Toxicology Branch-1
Health Effects Division (H7509C)

To: Kathy Depukat PM 51
Special Review Branch
Special Review and
Registration Division (H7508C) KD 5/17/93

Thru: Karen Hamernik, PhD. K. Hamernik 5/17/93
Acting Section Head, Section 3
Toxicology Branch-1
Health Effects Division (H7509C)

The registrant has submitted a 2-generation study on reproduction in the rat (83-4) with imazalil {1-[2-((2,4-dichlorophenyl)-2-propenyloxy-ethyl]-1H-imidazole} under 6(a)(2) Data.

P Dirkx and Herman Van Cauteren. R23979 - Imazalil: 2-Generation Reproduction Study with 1 litter per Generation in Wistar Rats, conducted for Jenssen Pharmaceutica, William Goodwine, Agent at Department of Toxicology, Janssen Research Foundation, 2340 Breese, Belgium, study date-October 26, 1992, study No. 2337 (MRID# 425707-01).

The 6(a)(2) data demonstrates potential reproductive effects on pup survival at lower dose levels of < 5 mg/kg/day (LDT) than existing data on reproduction with a NOEL of 40 mg/kg/day, however, the lowest NOEL for chronic studies is unchanged from a NOEL of 1.25 mg/kg/day in dogs. The appropriate toxicity study and NOEL for acute or chronic worker exposure risk assessment has not changed. The 6(a)(2) data was graded supplementary.

Conclusions on the Two-generation Study on Reproduction (The submitted 6(a)(2) Data : Imazalil was administered in the diet to a non-inbred strain of 24 Wistar rats per sex at approximately 0, 5, 20 or 80 mg/kg/day for 60 days prior to mating, through mating, gestation and lactation (females only). The F1 generation was administered the same dietary concentrations for similar periods. Mating was approximately 1 male to 3 females in the second generation rather than 1 male to 1 female. Twenty-four F1 females were mated at the lower dose level, but only 14 matings were conducted for the mating producing the F2 pups at the 80 mg/kg/day dose level.

Study starting date was 1/31/91 and completion date was 9/30/91.

Parental toxicity:
NOEL: 20 mg/kg/day.
LEL: 80 mg/kg/day (HDT) for P0 male body weight gain decrease (90% of controls, $p \leq 0.05$) and body weight decrease (95% of control, $p \leq 0.05$) and probably P0 and F1 female body weight decrement during gestation (76% to 80% of controls) and lactation (94% to 92% of controls, $p \leq 0.05$ and $p \leq 0.0.01$). Food scattering (wastage) by females at the HDT negates food efficiency calculations. Increased liver vacuolation occurred in P0 males (11/24 vs. 0/24 in controls, mean score 0.5, $p \leq 0.05$) and possibly in F1 males (1/7 vs. 0/20 in controls, mean score 0.14, $p \geq 0.05$).

Parental reproductive toxicity:
NOEL: 20 mg/kg/day
LEL: 80 mg/kg/day for increased duration of gestation for the P0 and F1 females.

Offspring toxicity:
NOEL: < 5 mg/kg/day
LEL: 5 mg/kg/day (LDT) for survival of the F1 pups during lactation. Pup survival was affected for the F2 pups during lactation at 5 mg/kg/day and 80 mg/kg/day, but not at 20 mg/kg/day. At the 80 mg/kg/day dose level, the number of live pups born was decreased and the number of dead pups was increased for the F1 and F2 pups, which were statistically significant. Resorptions were nominally increased and implantations were statistically significantly decreased in the second generation only at 80 mg/kg/day.

Core classification: Supplementary. The study is not acceptable for a guideline (83-4) study for effects on reproduction in the rat. Another study may be necessary unless the sponsor adequately responds to the request for additional information and to the questions about the study conduct.

1. Historical control data on the non-inbred strain of Wistar rats used is needed on ring-tail in pups, on the pup survival and body weight to weaning. The data may include data after 1991, but no more than 5 years before 1991.

2. The temperature, humidity and lighting in each animal room used through out the P0 and F1 generations, the groups housed in separate rooms (animals and groups identified with the room used) and the number of animal rooms used for the study must be submitted.

3. The rationale for not mating some selected F1 males for the F1 generation. How were the males selected for the mating trials? This is especially important because the fertility of males dosed in utero were not adequately studied since some F1 offspring selected as parents were not selected for breeding and some of the selected males were bred more than once. The rationale and an explanation of the selection method must be submitted.

4. Please supply the body weight data, food consumption data (if available), clinical observational data and summary tables for the F1 males throughout dosing until sacrificed. It is recognized that the data in the highest dose group may not be meaningful because of food wastage.

5. Please submit the analyses for homogeneity and stability of the test material in the dietary preparations used. Please indicate the dates that each dietary preparation was administered to the animals and analytical concentration data for that dietary preparation.

6. Please clarify the Tables 12 through 19, page 000060 through 000067. There is a discrepancy in the designed sex between Table 20 and the text at the top of the page for Tables 12 through 19. Animal numbers 1 through 114 refer to female animals whereas the Tables 12 through 19 indicate the animal numbers refer to male animals. Also the mean body weight gain within Table 10, page 000058 indicates female animal numbers when these numbers are referred to as male animal numbers outside Table 10 and in the text, page 000024. Please clarify these tables and any other discrepancies occurring.

7. There appears to discrepancies in histological mean scores between Tables 56, 57, 58, 59 and 60 and the Tables on individual animals data in Tables A 8.1 through A 8.36, page 000532 to 000567. Perhaps the apparent discrepancy would be explained if the sponsor would please explain the method used to compute the mean histological scores in Tables 56, 57, 58, 59 and 60.

8. Other information and data may be requested depending on the response to the requirements in 1 to 6.

Memo for 6(a)(2) data/Repro/Imazalil/2337/D185810/425707-01/ B:\IMAZAL49.7AB\CMREPRAT.293/DANDERSON/2/19/93(Edited 5/5/93&5/13/93)*.

Primary reviewer: David G Anderson, PhD. David G Anderson 5/5/93
Section 3, Tox. Branch 1 (H7509C).
Secondary reviewer: Karen Hamernik, PhD. K. Hamernik 5/17/93
Section 3, Tox. Branch 1 (H7509C).

DATA EVALUATION REPORT

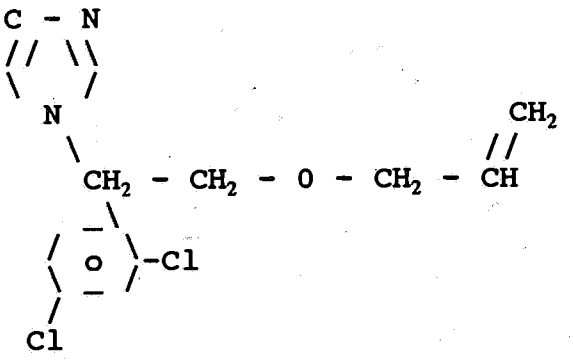
STUDY TYPE: Reproduction/Rat/(83-4)/Imazalil/2337.

CAS REG. No.: 35554-44-0.
MRID No.: 425707-01.
PC CODE: 111901.
DP BARCODE: D185810.
TOX. CHEM. No.: 429AB.
SUBMISSION No.: S432102.

TEST MATERIAL: Imazalil, technical, (Deccozil™).

SYNONYMS: R23979; 1-[2-((2,4-dichlorophenyl)-2-propenyloxy)-ethyl]-1H-imidazole.

STRUCTURE:



SPONSOR: Janssen Pharmaceutica, William Goodwine, Company Agent.

TESTING FACILITY: Janssen Foundation, Department of Toxicology, 2340 Beerse, Belgium.

STUDY NO.: 2337.

REPORT TITLE: 2-Generation Reproduction Study with 1 Litter Per Generation in Wistar Rats.

AUTHOR(S): Paula Dirkx and Herman Van Cauteren.

REPORT ISSUED: October 26, 1992.

CONCLUSIONS: Imazalil was administered in the diet to a non-inbred strain of 24 Wistar rats per sex at approximately 0, 5, 20 or 80 mg/kg/day for 60 days prior to mating, through mating, gestation and lactation (females only). The F1 generation was administered the same dietary concentrations for similar periods. Mating was approximately 1 male to 3 females in the second generation rather than 1 male to 1 female. Twenty-four F1 females were mated at the lower dose level, but only 14 matings were conducted for the mating producing the F2 pups at the 80 mg/kg/day dose level.
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Parental reproductive toxicity:
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NOEL: < 5 mg/kg/day
LEL: 5 mg/kg/day (LDT) for survival of the F1 pups during lactation. Pup survival was affected for the F2 pups during lactation at 5 mg/kg/day and 80 mg/kg/day, but not at 20 mg/kg/day. At the 80 mg/kg/day dose level, the number of live pups born was decreased and the number of dead pups was increased for the F1 and F2 pups, which were statistically significant. Resorptions were nominally increased and implantations were statistically significantly decreased in the second generation only at 80 mg/kg/day.

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2. The temperature, humidity and lighting in each animal room used through out the P0 and F1 generations, the groups housed in separate rooms (animals and groups identified with the room used) and the number of animal rooms used for the study must be submitted.

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5. Please submit the analyses for homogeneity and stability of the test material in the dietary preparations used. Please indicate the dates that each dietary preparation was administered to the animals and analytical concentration data for that dietary preparation.

6. Please clarify the Tables 12 through 19, page 000060 through 000067. There is a discrepancy in the designed sex between Table 20 and the text at the top of the page for Tables 12 through 19. Animal numbers 1 through 114 refer to female animals whereas the Tables 12 through 19 indicate the animal numbers refer to male animals. Also the mean body weight gain within Table 10, page 000058 indicates female animal numbers when these numbers are referred to as male animal numbers outside Table 10 and in the text, page 000024. Please clarify these tables and any other discrepancies occurring.

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8. Other information and data may be requested depending on the response to the requirements in 1 to 6.

A. MATERIALS:

1. Test compound 1: R 23979 (Imazalil), technical, Description: slightly yellow crystalline solid, sometimes an oily liquid; Batch # ZR023979 G3A231; Purity- ≥ 95.0% a.i..

2. Test animals: Species: Rats, Strain: Wistar, Male age: 60 days, (8.6 weeks), Female age: 2-3 months. Weight (range in means of the groups selected for study at study week 1): Males - 247.9 - 253.3 g, Females - 160.5 - 165.6, Source: Janssen Foundation non-inbred colony. Animals were acclimatized for 13 days and abnormal animals were removed.

3. Environment: The animals were maintained in an air conditioned room with positive pressure. The temperature, humidity and lighting for the animal rooms were not given.
During mating animals were cohoused 1:1 = male:female in wire mesh cages. Premating and post mating caging was not stated. Mating for the 2nd generation was stated to be 1 male:3

females (page 000023 of the submitted report), but the number of F1 males mated did not confirm this latter ratio.

B. STUDY DESIGN:

1. Animal Assignment - Animals were assigned randomly to the test groups, including pup selection for the F1. However, the mating of the F1 males did not appear to be conducted by random selection. The day vaginal sperm was detected was designated day 1 of gestation. Morning smears were taken after an evening of cohabitation. Litter mates were not mated. The number of animals assigned to controls, nominal dose levels and assigned animal numbers are presented in Table A.

2. Study Purpose and Protocol - The study is designed to provide information on the effect of imazalil on male and female fertility. The dose was administered to 9 week old male rats for 60 days prior to mating and during mating and to 2-3 month old female rats for 60 days prior to mating, during mating, gestation and lactation. F1 pups selected for parenting the F2 were dosed from birth (page 000029 of the submitted report) to end of mating for males and to the end of lactation for females (page 000030 of the submitted report).

Animals were co-housed for up to 3 weeks. Only 1 litter per generation was produced. Only 14 females were cohoused with males to produce the F2 pups at the HDT. Presumably the lower number of matings in the F1 at the 80 mg/kg/day dose level was due to the poor pup survival during the lactation of the P0 females for the F1 pups. The dose levels, the number of animals per dose level and animal numbers for the P0 and F1 groups are presented in Table A.

3. Diet preparation - The dosages administered are given in mg/kg/day, however the study dosages are comparable to a study conducted at a constant ppm. The diet was prepared such it would deliver an average of ≈5, ≈20 and ≈80 mg/kg/day to the rats consuming the diet during the premating period. The concentration in the diet was 0.05, 0.20 and 0.80 mg/g and was not adjusted for increased food consumption during gestation and lactation. Therefore the relative dose administered to lactating dams is similar to a study conducted at constant ppm (About 2-3 times higher during lactation than for the premating period).

The dosage of the test material was calculated from the food consumption and concentration of the test material in the feed (Table B). The food consumption data for the 80 mg/kg/day dose group and during the latter part of lactation for the 20 mg/kg/day groups are not accurate because of food wastage observed at this dose level (See the section on clinical observations). Data are presented for P0 males and females for the premating period and P0 females and F1 females during gestation (Table B). Data were collected for test material consumption for the lactation of the P0 and F1 females, but

Table A. Test groups and the number and identification number of animals co-housed from each generation.

Dose, (nominal dose) level (mg/kg/day)	Mean calculated dose level during the premating period. (mg/kg/day)	Number of P0 co-housed to produce the F1		Identity # of the P0		Identity # of the F1	
		Males	Females	Male	Female	Males	Females
0.0 Control	0.0	24	24	201-224	1-24	5-65[a]	601-624
5 LDT	5	24	24	231-254	31-54	101-163[b]	631-654
20 MDT	20	24	24	261-284	61-84	201-251[c]	661-684
80 HDT	80	24	24	291-314	91-114	301-324[d]	691-704

[a] Includes 19 different F1 males for breeding; 5 were bred twice.
[b] Includes 18 different F1 males for breeding; 6 were bred twice.
[c] Includes 15 different F1 males for breeding; 5 were bred twice; 1 was bred 5 times.
[d] Includes 14 different F1 males for breeding; 4 were bred twice; 1 was bred 3 times.

Table B. The calculated dosages from the food consumption data and dietary concentration data during the premating period for P0 males and females and during gestation for the P0 and F1 females. The food wastage noted in the 80 mg/kg/day dose group may cause some error in the calculated HDT. However, this would not affect the two lower dose groups.

Period of study	5 mg/kg/day dosage group (mg/kg/day)	20 mg/kg/day dosage group (mg/kg/day) [a]	80 mg/kg/day dosage group (mg/kg/day) [b]
Premating for the first generation			
P0 Males	4.2	17.6	70.5
P0 Females	5.0	21.5	104.3
First generation gestation (females)			
P0 Females	4.0	16.3	86.8
Second generation gestation (females)			
F1 Females	4.3	18.6	87.8

[a] Food wastage was noted toward the end of lactation for the F2.
[b] Food wastage was noted 2 weeks prior to mating, gestation and lactation in females. In males, food wastage was noted 3 weeks prior to mating.

summary means were not presented. Data were not collected on body weight or food consumption for the F1 males.

The diet was prepared from a premix of the test material, and stored at room temperature until used. If a vehicle was used in the preparation, it was unidentified. The frequency of diet preparation was not stated, but may have been at 6 to 8 weeks intervals. Samples of the diet were collected and analyzed approximately every 6 to 8 weeks during the study. It was stated that the test material was stable in the diet until used, but the only data presented to verify the statement were the analyses at 6 to 8 week intervals. The results of 6 analyses of the diets used during the study were all within acceptable variation from nominal (91% to 109% of nominal, only two values were outside this range, 114% and 118%). Since the frequency of dietary preparation was not stated, it is not possible to verify the concentration of the test material in each batch prepared. Only one analysis on each of the dose levels (9/19/91 for the diet prepared on 8/29/91) was conducted during the period of the F1 gestation and lactation for the F2 pups (approximately 7/29/91 to 9/27/91).

4. Animals received food and water *ad libitum* - Food was prepared by Janssen Pharmaceutica N.V. and contained no medications. The food was sterilized by 2.5 megarad of gamma radiation at the Belgium National Institute of Nuclear Energy (Fleurs). The water used was a government regulated system.

5. Statistics - The following procedures were utilized in analyzing the numerical data. The Chi-square test for pairwise comparison with control according to Siegel (two-tailed, Yates' correction for continuity) was used as the statistical method for:

clinical observations
mortality
copulation, fertility and gestation rate
survival rate

The Mann-Whitney U test for pairwise comparison with control according to Siegle (two-tailed, correction for ties) was used as the statistical method for:

body weight
food consumption
cohabitation-mating interval
duration of gestation
live, dead and resorbed fetuses - mean litter size -
implantations
anomalies
histopathology

Siegle S., Non-parametric Statistics, McGraw-Hill, NY, 1956.

6. The quality assurance statement was signed on 10/26/92 by P. Lenaerts of the Quality Assurance Unit.

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6

C. METHODS AND RESULTS FOR P0 AND F1 GENERATIONS: (Numbered tables were copied from the submitted report. Lettered tables were constructed from data in the submitted report.

1. Observations - Animals were inspected daily for signs of toxicity and mortality. However, no observational data were presented for F1 males after weaning.

Results - Toxicity - Test material related food wastage occurred in females of the 80 mg/kg/day dose level from week 2 prior to mating, during gestation and lactation. A slight increase ($p \geq 0.05$) in the incidence of male food wastage occurred during the last 3 weeks prior to mating. Increased piloerection also occurred in these females during pregnancy and lactation. In the second generation this food wastage was seen at the 80 mg/kg/day dose level during pregnancy and lactation. Possible treatment related food wastage was seen toward the later part of lactation for the F2 at 20 mg/kg/day dose level. A statistically significant increase in dystocia (3/24 in controls versus 6/24*, $p \leq 0.05$ at the HDT) was seen in the P0 dams and in the F1 dams (1/24 in controls versus 8/14***, $p \leq 0.001$, at the HDT) associated with the end of gestation at the 80 mg/kg/day dose level.

A red vaginal discharge was seen in P0 dams during lactation at the HDT.

Pups were observed 8 and 12 hours after birth and at post natal day (pnd) 4, 14 and 21. F1 pups with "bad condition" (otherwise undefined) were observed more frequently at the 80 mg/kg/day dose level (8/15)[1] than in controls (3/22) and F2 pups with "bad condition" were observed only at the 5 mg/kg/day dose level (3/22) and the 80 mg/kg/day dose level (2/13). The pups with "bad condition" were probably related to the decreased pup survival.

There also was a decreasing incidence of F1 pups with ring-tail with an increasing dose of imazalil (Table C). Ring-tail was observed in F2 litters at the 5 mg/kg/day dose level only (Table C). Ring-tail has no infectious component and Harkness and Wagner[2] indicate that ring-tail in pups is caused by < 20% humidity. The apparent negative correlation of the ring-tail observation in pups (Table C) with the dose related decrease in F1 pup survival and the equivocal dose relationship in F2 pup survival (Table G) may have a common origin and indicate that there is a contributing environmental component, if the ring-tail referred to by Harkness and Wagner is the same as the ring-tail referred to in the study report.

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[1] (# of litters with" bad condition"/# of litters in group)

[2] Harkness, John E and Joseph E Wagner in The Biology and Medicine of Rabbits and Rodents, 1977 edition, Lea Febiger, Philadelphia, PA, pp 119, 133 and 142.

Table C. Incidence of ring tail in pups from the P0 dams and F1 dams.

Group	Control	5 (mg/kg/day)	20 (mg/kg/day)	80 (mg/kg/day)
Incidence in F1 litters of ring-tail. Litters affected (pup incidence).				
# of litters	22	22	21	15
Ring-tail and tail necrosis	8 (45)	6 (23)	2 (5)	1 (2)
Incidence in F2 litters of ring-tail. Litters affected (pups incidence)				
# of litters	22	20	20	13
Ring-tail and tail necrosis	0	1 (5)	0	0

Mortality (Survival) - Among adult animals, no mortality occurred among males. Two females died or were sacrificed moribund during lactation at the 80 mg/kg/day dose level in each of the P0 and F1 females. The report authors did not consider these deaths to be test material related.

2. Body Weight and Food Consumption for P0 and F1 Adults and Pups - The premating body weight and food consumption was determined weekly for P0 males and females and F1 females; these parameters were determined through gestation and lactation of the P0 females and F1 females. No body weight data were presented for the F1 males except as pups prior to weaning.

a. Results, body weight P0 and F1 Adults - Body weight gains for P0 males were statistically significantly decreased during the premating period to 90% of controls, $p \leq 0.05$ (Table D), and body weights were statistically significantly decreased toward the latter half of the premating period at the 80 mg/kg/day dose level. Body weight gains for P0 females during the premating period were nominally decreased to 89% of controls, $p \geq 0.05$ (Table D), at 80 mg/kg/day and body weights were nominally decreased for the premating period. P0 and F1 female body weight gain decrement was statistically significantly decreased during gestation and lactation.

Table D. Mean body weight change in males and females of the P0 and F1 generations for periods indicated.

Dosage group (mg/kg/day)	Mean premating P0 male body weight change (g)	Mean premating P0 female body weight change (g)	Mean premating F1 male body weight change (g)	Mean premating F1 female body weight change (g)
0.0, Control	273.4	142.0	No data submitted	No summary data submitted
5, LDT	270.7	136.8	No data submitted	No summary data submitted
20, MDT	265.0	135.5	No data submitted	No summary data submitted
80, HDT	246.5*	125.8	No data submitted	No summary data submitted
	Mean body weight change in P0 dams from gd 1 to gd 22		Mean body weight change in F1 dams from gd 1 to gd 22	
0.0, Control	156.1		161.9	
5, LDT	163.4		156.5	
20, MDT	150.0		151.4	
80, HDT	119.4*		128.8***	
	Delivery	Mean P0 body weight during lactation. Lactational day 4	Lactational day 14	Lactational day 21
0.0, Control	379.1	385.7	393.8	372.2
5, LDT	374.7	377.6	390.7	371.3
20, MDT	368.7	377.0	387.5	375.5
80, HDT	338.9**	349.3**	350.8***	349.5*
	Delivery	Mean F1 body weight during lactation. Lactational day 4	Lactational day 14	Lactational day 21
0.0, Control	353.5	356.7	367.8	354.2
5, LDT	343.8	348.6	357.9	348.7
20, MDT	337.2	341.8	351.6	347.1
80, HDT	308.7***	307.4***	319.3***	321.0**

Statistically significance: * = $p \leq 0.05$, ** = $p \leq 0.01$, ** = $p \leq 0.001$.

b. Results, food consumption for P0 and F1 Adults - Food consumption in males remained comparable to control consumption at all dose levels (Data not shown). Therefore the body weight gain decrement and the decreased body weights at the 80 mg/kg/day dose level were considered test compound related. Data were not presented for the F1 males.

Increased food consumption occurred at the HDT in P0 and F1 females (Table E). The statistically significantly increased food consumption at the HDT is not meaningful because of the observation that food wastage occurred at the HDT in P0 and F1 adult females during the P0 premating period and the P0 and F1 females during the gestational and lactational periods. The food wastage by P0 and F1 females obscures the meaning of body weight gain decrement seen in females during gestation and lactation.

Table E. Food consumption data for the P0 and F1 females during gestation and lactation for the F1 and F2 pups. Food wastage was noted at 20 mg/kg/day during the latter part of lactation and during gestation and lactation for the F1 and F2 pups. Food consumption data may be in error during these periods.

Dosage group	Mean food consumption (g)/day during gestation First generation	Second generation		
0.0, Control	32.3	30.0		
5, LDT	31.7	32.1		
20 MDT	31.8	32.8		
80 HDT	38.4	37.3**		
Dosage group	Mean food consumed (g)/day by P0 females lactating for the F1 pups. Day 0-3	Day 4-13	Day 14-20	Day 0-20
0.0, Control	41.8	61.7	75.8	62.6
5, LDT	33.9	57.2	74.1	58.4
20 MDT	44.6	56.5	71.4	59.1
80 HDT	54.2	72.3	79.3	72.4
Dosage group	Mean food consumption/day by P0 females lactating for the F2 pups. Day 0-3	Day 4-13	Day 14-20	Day 0-20
0.0, Control	40.4	66.5	81.4	66.7
5, LDT	41.0	65.5	81.5	66.2
20 MDT	49.6	67.2	83.8	69.4
80 HDT	50.6	54.7*	73.8	60.2

Statistically significance: * = $p \leq 0.05$, ** = $p \leq 0.01$.

c. Results on Pup weights for the F1 and F2 during Lactation - Pup weights were recorded individually and as litter means to the nearest 0.1 g at birth (day 0), and at postnatal day (pnd) 4, 7, 14, and 21 (weaning). The data is presented in Table 23 and 40 for the first and second generations, respectively. The number of pups per litter were not reduced to 8 per litter at pnd 4.

No trends or statistically significant pup weight reductions at birth or during lactation compared with control values occurred at any dose level (Table 23 and 40) in the F1 or F2 generation litters. It is noted that equivocal body weight decrement occurred in adult females at the same dose level (HDT) causing compound related death of 2 adult females during lactation.

4. Reproductive Parameters - The reproductive performance and other parameters are summarized in Table 23 and 40 and reproduced in the Appendix from the submitted report. The body weight and food consumption during pregnancy and lactation, litter and pup weights in Table 23 and 40 have already been discussed. Table 23 summarizes data for the first generation and Table 40 summarizes data for the second generation.

The following data, related to the reproductive potential of adult males and/or females, were collected: copulation, fertility and gestation rates, estrous cycles on adult females, length of gestation, incidence of decreased corpora lutea and the number of implantation sites. The copulation, fertility and gestation rate are used instead of mating, fertility and gestation indexes as recommended by EPA, however, the respective meaning is equally valid.

Reproductive performance was reported as:

- copulation rate = [(# females with vaginal sperm)/(# males housed)],
- fertility rate = [# females pregnant)/(# females with vaginal sperm),
- and gestation rate = [(#females delivery)/(# pregnant females)].
- Gestation length = gestation length to the nearest day of parturition. Animals failing to deliver by day 26 were sacrificed.

The definitions of the above rates were not defined by the submitter, however, the definitions could be anticipated from the individual animal data used. The above definitions were defined by the reviewer from the individual animal data (page 000072-000080 for the first generation and page 000091-00097 for the second generation in the submitted report).

The litter data were also calculated differently than usual. Litters sizes were not reduced on lactational day 4 to 8 pups per litter.

- Survival rates = [(# surviving pups on day 4, 14 or 21]/(total # live pups born)] X 100.
- The mean litter size = [(# live plus # dead pups)/(# pregnant females)].

• # of implantations = # of implantation scars present at necropsy. The number includes resorptions, live births and dead births. The number of resorptions were calculated by the reviewer from the number of implantations and the number of live and dead births.

a. Results on Copulation, fertility and gestational rates - The copulation, fertility ~~and gestational~~ rates were not affected at any dose level. The gestation rate was nominally affected at the 80 mg/kg/day dose level for the P0 females (0.71 at the HDT and 0.96 in controls), but not for the F1 females. The nominally decreased gestation rate in P0 females is consistent with the decreased number of live and dead pups born to the P0 females. Summary information of the results of the mating trials for the P0 and F1 generation are presented in Tables 23 and 40, reproduced in the Appendix from the submitted report.

The reproductive parameters discussed above are less definitive when applied to the F1 mating because mating was approximately 1 male to 1 female to 1 male to 5 females (Table A). It would appear that the purpose of the second mating for the second generation was to study the dams and the pups produced rather than any potential effects on male fertility of in utero dosed males. Thus, any potential effects initiated in utero on male fertility are not adequately studied. In addition, the rationale for the selection of the animals for mating and the rationale for animals selected for multiple matings are not adequately described.

b. Results on the Length of Gestation - A treatment related effect on the length of gestation was statistically significantly increased by approximately 1 day in both P0 and F1 females (Tables 23 and 40), but only at 80 mg/kg/day.

c. Results on Average Number of Implantation sites, Pups at Birth and Pup Viability (Tables 23 and 40; copies from the submitted report in the Appendix I) -

There was a statistically significant decreased litter size at birth from the dams producing the F1 and F2 litters (54% and 51% of control values for F1 and F2, respectively) at 80 mg/kg/day. Dead pups at birth were also statistically significantly increased at the 80 mg/kg/day dose level in both generations. Total litter size (dead plus live) were not statistically significantly reduced and neither were implantation sites except in the F1 dams. However, there is a nominal trend (statistical analysis was not conducted for trend) for decreased implantation sites in both generations (Table F). The decreased number of implantation sites is statistically significant in the F2 females at the 80 mg/kg/day dose level (Table F, below).

Survival during lactation was statistically significantly reduced at all dose levels in the F1 pups (Table G, below), and could be biologically significantly reduced for the F2 pups at 5 mg/kg/day. The survival data demonstrated a good dose related response in the F1 pups but not in the F2 pups.

The statistically significant survival in the F2 pups at the 5 mg/kg/day dose level and was not considered to be dose related by the study authors. There was an nominal increase in survival at lactational day 4, 14 and 21 at the 20 mg/kg/day dose level in F2 pups. The report stated that the only dose level causing test material related affects on survival was the 80 mg/kg/day dose level.

Table F. The number of live and dead pups at birth and implantations per pregnant P0 and F1 female for the F1 and F2 litters.

Dosage group (mg/kg/day)	Number of F1 pups at birth per pregnant P0 female First generation				
	Live pups	Dead pups	Live + dead pups	Resorptions[a]	# Implantations
0 Control	10.9	0.96	11.9	1.80	13.7
5 LDT	12.6	0.50	13.1	0.90	14.0
20 MDT	11.1	0.24	11.3	1.50	12.8
80 HDT	5.9**	3.48*	9.4	3.10	12.5
	Number of F2 pups at birth per pregnant F1 female Second generation				
0 Control	13.2	0.41	13.6	0.50	14.1
5 LDT	11.5	0.48	12.0	1.50	13.5
20 MDT	11.1	0.48	11.6	0.90	12.5
80 HDT	6.7***	2.38***	9.1***	3.00	12.1*

Statistical significance: * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$.

[a] Resorptions = (# implantations) - (# live + dead pups); no statistical analysis was conducted on the resorption data.

The pup survival was apparently dose related in the first generation, but not in the second generation. The biological significance of the decreased survival in F1 pups at the two lowest dose levels is not easily dismissed as suggested by the report authors, partially because the values were statistically significant with a $p \leq 0.001$.

This difference in the dose relationship of the pup survival data could be due to many factors including disease, possible differences in test material consumption (i.e., lack of homogeneity in the concentration and/or possible errors in the concentration of the test material in the food administered), genetic factors, incidental random variation, or other differences between the two generations.

In addition, the meaning of the observation on ring-tail in pups of the first generation (the decreasing incidence with increasing dose of imazalil), but not in pups of the second

generation, except at the LDT and HDT, needs exploration (See Table C). The finding that all dose levels demonstrating ring-tail pups also show a statistically significant decrease in survival rate (Table G) may indicate that an environmental component is correlated with the decreased pup survival. The decrease in the pup survival rate at 80 mg/kg/day in first and second generations is a compound related effect accepted by the report authors as well as the reviewer. Only the effects at the mid and lowest dose level are in dispute.

Dose analyses and food consumption data indicate no significant difference between the generations. Food consumption if anything was nominally higher at the 20 mg/kg/day dose level in the second generation where no effects on survival were demonstrated. However, data on the analyses of the dietary preparations is not sufficient to determine the concentration of the test material in the feed administered during the gestation and lactation for the F2 pups. In addition, data on the dietary homogeneity was not submitted.

There was an apparent dose related decrease in implantation sites in both generations, which were statistically significant only at the 80 mg/kg/day dose level in the second generation. There was also a general downward trend in the number of corpora lutea generations and statistically significant decreases in corpora lutea of lactation at the 80 mg/kg/day dose level (Table 56 and 57, reproduced in the Appendix from the submitted report) (Also see the section on Microscopic Examination).

Other studies, all indicate no effects of any kind below 10 mg/kg/day in the rat, the 2-year dog study yields the lowest NOEL of 1.25 mg/kg/day: (1) a core minimum oncogenicity study in rats (Accession/MRID# 099285 and 245311, HED Doc# 000057, 001337, 004795 and 004853) indicates a systemic NOEL of 10 mg/kg/day, (2) a core minimum 2-year feeding study in dogs (Accession/MRID# 097234, 246010 and 070091, HED Doc.# 00065, 000057 and 001337) indicates a NOEL of 1.25 mg/kg/day for decreased body weight, (3) a core minimum developmental toxicity study in rats (Accession/MRID# 097234, HED Doc.# 000065) on imazalil nitrate indicates a fetotoxic NOEL of 40 mg/kg/day, (4) a core minimum developmental toxicity study in the rat (410266-03, HED Doc.# 007865) with imazalil sulfate indicates a fetal NOEL of 40 mg/kg/day for fetal weight and an effect level for reduced litter size reduced live fetuses at 120 mg/kg/day, (5) a supplementary reproduction study in rats (Exp. 736, 410266-04, HED Doc.# 000065, 004795, 004853 and 007865) indicate a NOEL of > 40 mg/kg/day for various effects.

The reports submitted on the studies on reproduction contained no data on the analysis of the concentration of the test material in the food administered.

Metabolism studies indicate that imazalil is rapidly metabolized and excreted. More than 80% within 48 hours. Therefore the lethality in the pups at birth and during lactation is probably not due to "dumping" accumulated imazalil from the dam into the offspring.

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Table G. Survival rates for the F1 and F2 pups during lactation.

Dosage group (mg/kg/day)	Survival rate = [(# F1 live pups)/ (# live F1 pups at birth)] X 100 First generation		
	Lactational day 4	Lactational day 14	Lactational day 21
0 Control	84.9	79.7	73.3
5 LDT	71.1***	65.3***	63.9*
20 MDT	77.3*	61.4***	60.9**
80 HDT	66.9***	54.9***	54.9***
	Survival rate = [(# live F2 pups)/ (#live F2 pups at birth)] X 100 Second generation		
0 Control	95.2	94.3	91.5
5 LDT	91.3	85.1***	82.6**
20 MDT	97.6	94.9	94.5
80 HDT	75.0***	70.0***	70.0***

* Statistically significant at $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$.

3. Necropsy - A necropsy was conducted on P0 and F1 adults selected for mating. Pups were observed during lactation, but were not necropsied. Only histological observations and grades were reported for accessary male genital glands (prostate and seminal vesicles), epididymides, kidneys, liver, ovaries, testes, uteri, vaginas. The estrus stages for ovaries, uteri and vaginas were reported. The data are presented in Tables 56, 57 and 58 for males and females of the P0 generation and in Table 59 and 60 for males and females of the F1 generation. The number appearing in the tables represents an average grade and number of affected animals (severity slight to low)

c. Microscopic examination - Test material related findings noted were large vacuoles in hepatocytes of males (11/24 vs. 0/24 in controls) in P0 males and possibly in F1 Males (1/7 vs. 0/20 in controls), but the values were statistically significant only for the P0 males at the 80 mg/kg/day dose level. These effects on the hepatocytes were considered to be the only compound related histopathology noted in this study.

A decreased number of corpora lutea of lactation were seen for P0 females which probably is a function of the decreased number of pups during lactation at the 80 mg/kg/day dose level. A decrease in corpora lutea generations and clear aspect of interstitial cells occurred at all dose levels in the F1 females

(Table 60). The study authors attributed this to the stage in weaning of the F2 pups when the dam was killed and not to the compound treatment. The statistically significant increase in ovarian proestrus and metestrus seen at 80 mg/kg/day in the F1 dams was considered to be within normal limits by the study authors. These latter evaluations on the corpora lutea, clear aspects and estrus stage of the ovary appear to be within the normal limits of variation for the results on these types of parameters.

Slightly fewer histological findings of inflammation in the kidneys and liver were noted in the F1 females than in P0 females at the 20 mg/kg/day dose level, but these were not statistically significant and may not have been biologically significant.

D. ABSTRACT AND DISCUSSION:

Imazalil was administered in the diet to 24 an non-inbred strain of Wistar rats per sex from the testing laboratory at approximately 0, 5, 20 or 80 mg/kg/day for 60 days prior to mating, through mating, gestation and lactation (premating). The F1 generation was administered the same dietary concentrations for similar periods. Mating was 1 male to 1 female in the first generation, but inexplicably varied from 1 male to 1 females to approximately 1 male to 5 females in the second generation. Twenty-four F1 females were mated at the lower dose levels, but only 14 matings were conducted for the mating producing the F2 pups at the 80 mg/kg/day dose level; presumably the fewer number of F1 matings at the highest dose level were due to fewer F1 animals caused by the poor survival during the lactation for the F1 pups.

The slight male and female body weight gain decrease at the 80 mg/kg/day dose level was considered to be compound related. In males, food consumption was comparable with control values and indicated that the body weight gain decrement was not due to reduced food consumption. Body weight data for F1 males were not reported. For P0 and F1 females, the body weights were statistically significantly less than control values during part of the premating period, gestation and lactation. In addition, food scattering and consumption was statistically significantly increased at the 80 mg/kg/day dose level during the premating and gestation for the P0 and F1 females and for the lactation period for the P0 females. This statistically significant body weight decrease may have been only partly due to toxicity because food scattering among females at 80 mg/kg/day dose level was observed frequently.

The duration of gestation was affected and the gestation rate may have been affected at the HDT. The number of pregnant P0 females delivering, but not F1 females delivering were nominally decreased at the 80 mg/kg/day dose level (gestation rate = 0.71 versus controls at 0.96 at 80 mg/kg/day and controls, respectively). The duration of gestation was affected in the P0 and F1 females (approximately 1 day longer than controls) at the 80 mg/kg/day dose level. Mating and fertility were unaffected for the P0 males and females and for F1 females. These rates

were not of value for F1 males because of the mating procedure, but no effects were reported.

Microscopic examination indicated compound related adverse effects of the P0 male livers. Hepatocyte vacuolation was significantly increased in males, but only nominally increased in P0 females and F1 males at the 80 mg/kg/day dose level. The liver effect in males was considered to be test compound related. Other histological effects on the ovary at the 80 mg/kg/day dose level, such as corpora lutea of lactation and other ovarian effects were considered to be due to the decreased pup survival or within the normal variation for such effects.

Litter size was reduced at birth in the P0 ($p \geq 0.05$) and F1 ($p \leq 0.05$) generations at the 80 mg/kg/day dose level. Implantations were statistically significantly reduced ($p \leq 0.05$) in the F1 gestation at the 80 mg/kg/day dose level, however, this decrease may have been due to the increased litter size in controls and may not have been compound related.

Pup weights were not affected at birth or during lactation, but pup survival appeared to be decreased during lactation for F1 pups at all dose levels and in F2 pups at the LDT and HDT (survival of mid dose F2 pups was comparable with control values). The lack of a good dose response in the survival of F2 pups at the mid dose level complicates the interpretation. F1 pup survival was statistically significantly decreased at all dose levels at post natal day (pnd) 4, 7, 14 and 21, becoming progressively worse at each period. Each period of determination was dose related except at pnd 4, which was lower than controls ($p \leq 0.05$), but higher than either the 5 or the 80 mg/kg/day dose level. F2 pup survival was statistically significantly lower at 5 and 80 mg/kg/day than control values, but nominally higher at 20 mg/kg/day than controls.

The finding of decreased survival with no decrease in pup weight throughout lactation is unusual. The meaning of these findings is unclear. A disease factor could possibly be responsible for the decrease in survival, however, the factor would not be expected to cause a dose related decrease in pup survival in the absence of a decreased body weight.

The authors of the submitted report stated that the 5 and 20 mg/kg/day dose levels were NOELs. The authors pointed out that the response in the second generation was not dose related. In addition, another study on reproduction in Wistar rats (Experiment 736, Accession # 097233, addendum MRID# 410266-01 and 410266-04) dosed at 0, 5, 20 or 80 mg/kg/day demonstrated no effects on pup survival in the first or second generation. This latter study demonstrated no toxicity and can not be used as supporting evidence of no toxicity to offspring. This latter study was poorly reported and showed no analytical evidence verifying that the animals were dosed.

With the evidence available, the current study is considered to demonstrate no NOEL for pup survival in the first and possibly in the second generation. There was a good dose response in decreased pup survival in the first generation except at pnd 4 that demonstrated a marginal dose relationship at the mid dose. The lack of a dose relationship in the second generation was due to the mid dose, which was inconsistent with the first generation

and the statistically significantly decreased pup survival at the lowest and highest dose tested in the second generation. It would appear that the aberrant dose group may be the mid dose group in the second generation, not the low and mid group in the first generation and the lowest dose group in the second generation.

No sufficient reason could be found for ignoring the dose related and statistically significantly decreased pup survival demonstrated at the lowest and the mid dose levels in the first generation. No sufficient reason could be found for ignoring the dose related and statistically significantly decreased pup survival demonstrated at the lowest dose level in the second generation. (Ordinarily a lack of dose response may be used to ignore data, but in this case, the failure may have an alternative reason.) The lack of effects demonstrated at the mid dose level of the second generation could be due to inhomogeneity of the diet, or other factors. It was noted that dietary analysis was conducted only once for each dosage during the period of gestation and lactation for the F2 pups and it was not reported whether or not all the feed administered to F1 dams during this period was analyzed for homogeneity and concentration of the test material. It is also noted that female rats apparently did not like the taste of the test material (food wastage observations), thus, inhomogeneity in the diet may have allowed the female rats to avoid eating the test material. The sponsor is invited to respond with additional evidence, such as historical control data, potential disease in the animals used, dietary homogeneity data or other data, which may better explain the effects on F1 pup survival at the two lowest dose levels and the apparent effects on F2 pup survival at the lowest dose level and not at the mid dose level. The effects on F1 and F2 pup survival at 80 mg/kg/day is not at issue, since the sponsor accepts these effects at the HDT.

This 2-generation study only allowed a single litter per female per generation, thus additional survival data on other litters was unavailable to confirm a dose response or a lack of a dose response.

In addition, the effect on fertility of imazalil on dosing of males in utero is unknown, although the study demonstrated no effect on this parameter. An effect on the fertility of the F1 males could have been masked by an inappropriate selection of the F1 males for mating. Additional questions about the validity of the study are caused by the failure to mate all the F1 males selected as pups for the F1 generation parents, the failure to provide an adequate explanation and description of the F1 animal selection process and the process of selection of the F1 animals for mating.

F. APPENDIX I: Tables 23, 40, 56,57, 58, 59 and 60 copied from the submitted report and referenced in the DER.

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Imazalil Tox Review

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