TOXR 007865

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

APR 20 1990

MEMORANDUM

SUBJECT: EPA ID# 41811-2 Review of Studies (DERs) on Imazalil. Current Status of the Toxicity Data Base
MRID Nos.: 410266-01 thru -04

TOX Chem No. 498AB
Project No. 9-1109
Record No. 241875

FROM: Henry Spencer, Ph.D.
Review Section II
Toxicology Branch I
Health Effects Division (H7509C)

TO: Larry Schnaubelt, Team 21
Fungicide Branch
Registration Division (H7505C)

THRU: Marion Copley, D.V.M., Section Head
Review Section II
Toxicology Branch I
Health Effects Division (H7509C)

Action: Requested review of 4 studies submitted by Janssen Pharmaceutica N.V. Beerse, (Belgium) in support of Imazalil

Conclusions:

1. The mutagenicity studies (1) Ames, (2) Micronucleus test were acceptable and (reviewed in a previous action), (3) The In Vitro Cytogenetics study is upgraded to acceptable and does not indicate mutagenic effects. They satisfy the guideline requirement for mutagenicity at this time.

2. The 3-generation Reproduction Study demonstrated no effects in areas of either maternal or developmental toxicity and is therefore considered core-supplementary. The NOEL is greater than or equal to 800 ppm (HDT) and the LEL is greater than 800 ppm (HDT). This area is considered a data gap.

3. The rat teratology study is considered core-minimum with a developmental NOEL = 40 mg/kg; LEL = 80 mg/kg based on a decrease in fetal weights. Maternal toxicity NOEL = 40 mg/kg (LDT) as there was a dose-related decrease in food consumption at all levels.

4. The 90 day mouse subchronic range finding study allowed the agency to consider a 600 ppm dose level as probably adequate to test as the (HDT) for Imazalil in a mouse carcinogenicity study.

Note : The present reviewer's opinion concerning the mouse onco-test doses is discussed later in this memorandum.

5. Additional considerations are listed as well as the following studies.

Studies Submitted Here:

A Mutagenicity

1. Ames. Reverse Mutation test, Experiment #1999.
2. In Vitro Chromosome Aberration Assay on Human Lymphocytes, Study # SCK 86/02D/R23977.
3. Micronucleus test in mice, Experiment #1911.

Note: These studies were reviewed in TOX Branch Document #006818, dated August 5, 1988 by I. Mauer.

B. A rat teratology - Study No. 2003/88/05.

Note: This study has been reviewed by J. Hauswirth on May 9, 1989 and a copy of review is attached.

C. A reevaluation of the rat 3-generation reproduction study with Imazalil. - Study No. 736.

Note: This study has been independently re-reviewed by D.G.Anderson on October 3, 1989 and a copy of the review is attached.

D. Company Response to TOX Branch evaluation of a mutagenicity study - supplement to X9 1988.

Note: This response has been addressed by I. Mauer in a memo dated May 4, 1989 and a copy of this response is attached.

E. The desk copy of a recent 30-day mouse study used to select doses to be used in an oncogennicity study.

Note: This study report dated May 1, 1989 has been sent to Susan Lewis, PM #21, Registration Division and a copy of this report is attached.

Results of the Submissions Reviewed:

A. Mutagenicity

1. Ames test - acceptable
2. In Vitro Cytogenetics - upgraded to acceptable
(addressed by registrant)

3. Micronucleus test - acceptable

B. Teratology in the rat - core, minimum

Maternal toxicity: NOEL=less than 40 mg/kg (LDT) - decreased food consumption

Developmental: NOEL = 40 mg/kg. LEL = 80 mg/kg - decrease in feta. weights.

C. 3-generation reproduction in rats. (rereview of study)

NOEL 800 ppm (HDT)
LEL 800 ppm (HDT) study demonstrated
DQ effects; core classified is supplementary "not likely to be upgraded."

D. Desk copy of the 90 day mouse subchronic range finding study.

Registrant proposed a high dose of 600 ppm for the proposed mouse oncogenicity study due to large weight losses in both sexes at 800 ppm as well as vacuolar degeneration of the liver, decreased albumin, phospholipids and total bilirubin in both sexes noted at 400 ppm.

Agency response: The 600 ppm dose level appeared reasonable from the data as presented. In addition, "care must be taken in the study to be able to determine if a palatability problem exists."

Note: This report was not core-evaluated, therefore it is listed as supplemental information.

This reviewer has perviewed the mouse range finding study and considers the 600 ppm HDT level to be used in the mouse carcinogenicity as probably too low. The data submitted suggest that toxicity was minimal at 800 ppm, and the more

likely dosage to be used should have been the 800 ppm in the diet. Additionally, this reviewer would have used 400 and 200 ppm as the dose levels to complete the study. These comments are based on the current concept of the MTD usage in carcinogenic testing by the National Toxicology Program (NTP), (modified).

Additional items to be considered in this memorandum are:

1. Preliminary results of List B F.I.F.R.A. evaluations.

2. Position of major studies previously supporting a JMPR document on Imazalil.

3. Need of additional data (historical controls).

4. Condition of PP.5F3250, 6G3308, 4F3096, and 7G3530.

Imazalil, a List B chemical, underwent a review for completeness of its toxicological data base under F.I.F.R.A. '88. It was confirmed by the Division administration that chapters written for JMPR do not constitute DERS for the chemical. Therefore, these additional studies are being reviewed to produce official HED DERS.

a. The eighteen month rat oral toxicity study.
Report No. V84.140/220555, May 1984.

b. The chronic dog study.
Report No. 370, 4/12/1977.

c. The carcinogenicity study in mice.

d. Metabolism of Imazalil.

These toxicity data are presently being reviewed.

In order to reduce the time needed to complete the evaluations, historical control data for the strain of rat used in the oncogenicity study is requested to be submitted. The data are to be from only the testing laboratory which produced the rat oncogenicity study. Control studies reported are to include a time period three years on each side of the duration of the rat oncogenicity study.

The information for each control study reported must include the following tumors:

Male: leydig cell tumors
Female: uterus adenocarcinoma and epidermoid carcinoma

a) time period of study, i.e., 1981 - June.
b) report by sex, M, F.
c) tumor types, (see above).
d) numbers at risk (reduce denominator by those dying before the first of any tumor, or 52 weeks [whichever occurs first]).
e) report the number of tumors found.
f) report the range in the total # studies.
g) report the range % in the total # studies.

TUMOR TYPE	STUDY DATE		INCIDENCE M	INCIDENCE F	N=8-RANGE	
	June	'80-				
	"	'82				
Leydig cell tumors	"	"	4/47	-	0-4	0%-8.5%
Uterus-adenocarcinoma	"	"	-	2/50		
Epidermoid-carcinoma	"	"	-	5/50		

The support is incomplete at this memo/date for the added tolerances from

4F3096 Post harvest use on pome fruits at 7ppm and apple pomace at 30 ppm.

and 7G3530 on melons, citrus, sweetcorn and milk, meat [liver]

Only the PP#5F3250 was supportable because additional residues would not exceed existing tolerances in milk and meat.

Summary of existing data gaps:

1.	Multi-generation reproduction study in rats.	*Required without further evaluation.
2.	Oncogenicity mouse	-in process.
3.	Chronic feeding-rat	-being reviewed.
4.	Oncogenicity-rat	-being reviewed.
5.	Teratology Study in rabbits (second species)	*Required without further evaluation.
6.	Metabolism study	-being reviewed.
7.	21 day dermal toxicity study in rats or rabbits.	*required without further evaluation.

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Reviewed by: Judith W. Hauswirth, Ph.D., Chief
Toxicology Branch I - IRS

5/9/89

DATA EVALUATION REPORT

STUDY TYPE: Teratology - rat (83-3) TOX. CHEM. NO.: 497AB

MRID NO.: 410266-03

TEST MATERIAL: imazalil sulfate

SYNONYMS: R 27180

STUDY NUMBER: 2003/88-05

TESTING FACILITY: Research Department, Laboratoires Janssen, France

TITLE OF REPORT: Embryotoxicity and Teratogenicity Study in Sprague-Dawley Rats

AUTHOR(S): J M Gillardin and H Van Cauteren

REPORT ISSUED:* July 5, 1988

CONCLUSION:

Maternal NOEL = not determined, <40mg/kg based upon a significant decrease in food consumption from days 6-16 of gestation. In addition, at 80 and 120 mg/kg there was a decrease in body weight at day 17 of gestation when compared to the controls and of body weight gain at the highest dose tested.

Developmental NOEL = 40 mg/kg; LEL = 80 mg/kg based upon a significant decrease in fetal weights. In addition, at 120 mg/kg there was a decrease in litter size and the number of live fetuses, an increase in the number of resorbed fetuses and an increase in the number of fetuses with rudimentary extra ribs.

Core Grade: Minimum

A. MATERIALS:

1. Test Compound: Imazalil sulfate (R 27180); off-white to beige powder; Melting range 130.0°-131.8°C; Technical material, 99.9% pure.

2. Animals: Species: rat; Strain: Sprague-Dawley (CFA.SD); Age: -3 months; Weight: 227-312 g; Source: IFFA CREDO. Animals were housed individually in a temperature controlled room and were given free access to food (pelleted diet, A04C, U.A.R.) and water.

B. STUDY DESIGN:

1. Animal assignment: 96 pregant rats were randomly assigned to four groups, 24 per group.

2. Mating was considered successful if sperm were found in the vaginal smear. The day mating was confirmed was designated as day 1 of gestation.

3. Treatement: Females were treated on days 6 to 16 of gestation. Dosage levels were 0, 40, 80 or 120 mg/kg and the material was given by gavage in an aqueous solution. Test article stability was determined prior to initiation of the study and a sample was to be retained for a period of five years.

4. Statistics: The statistical procedures used in analyzing the numerical data are attached in Appendix I.

5. A signed and dated quality assurance statement was attached to the report.

C. METHODS AND RESULTS:

1. Observations: The rats were observed at least once daily for clinical signs of toxicity and mortality.

Only one female died (#34), in the 40 mg/kg group, by unknown cause. This animals was not pregnant. The only clinical sign noted was food wastage in one animal (#41 of the 40 mg/kg group).

2. Body weight: Animals were weighed on days 1, 6, 17 and 22 of gestation.

The following body weight and weight gain data were extracted from table 1 (pg. R7) of the report.

Mean Body Weight and Weight Gain Data of Dams

Dose Level (mg/kg)	Body Weight (g) Day 1	6	17	22	Body Wt. Gain[1]
0	252.0	276.8	340.7	413.4	52.9
40	245.9	270.1	323.5*	399.4	52.8
80	250.8	274.6	328.8*	406.4	44.3
120	252.4	278.0	325.0**	387.9**	42.8*

Body weight data were collected on 24 controls, 20 low dose, 23 mid dose, and 22 high dose dams.
[1] Body weight gain after subtracting out the weight of the gravid uterus.
* $p<0.05$ ** $p<0.01$

Body weights were statistically significantly lower than controls for all treated groups at day 17 of gestation although not in a dose-related manner. Body weights were statistically significantly lower than controls for the high dose only on day 22 of gestation. Overall body weight gain, day 1 through 22 of gestation (less uterine weight), was statistically significantly

less for the high dose group only.

3. Food consumption: Food consumption was recorded on days and 22.

The following food consumption data were extracted directly from the report (pg. R2).

Food Consumption of Dams

Dose Level (mg/kg)	Mean Food Consumption (g) Day 1-5	Day 6-16	Day 17-21
0	156.3±2.9	335.3±5.0	161.2±2.2
40	156.5±3.4	302.6±6.7***	159.4±3.4
80	152.1±3.3	278.0±5.2***	153.7±4.3
120	157.9±3.5	272.7±6.3***	150.1±4.4

Food consumption was averaged from 24 dams of the control group, 19 of the low dose group, 23 of the mid dose group, and 22 of the high dose group.
***$p<0.001$.

Food consumption was statistically significantly reduced in all treatment groups from day 6-16 of gestation and in a dose-related manner.

4. Sacrifice and necropsy examination: Dams were sacrificed on day 22 of gestation by decapitation. No findings upon necropsy were noted for any dams.

5. Reproductive effects: According to the report:

> The dams are examined for number of live and dead fetuses, presence of empty implantation sites and embryos undergoing resorption....In case a reduced litter size (0-3 fetuses) is notes, the Salewski technique is performed in order to discriminate between resorption and pseudopregnancy.

There were 24, 20, 23 and 22 pregnant females in the control, 40, 80 and 120 mg/kg groups, respectively. Litter size and number of live fetuses were statistically significantly decreased from controls at the high dose, and the number of resorbed fetuses was signficantly increased at this dose level (see following table extracted from table on pg K3 of the report).

Dose Level (mg/kg)	Litter Size	Fetuses Live	Fetuses Dead	Fetuses Resorbed
0	13.9	13.8	0.0	0.4
40	12.1	12.0	0.1	0.5
80	13.0	13.0	0.0	2.0
120	11.2**	11.1**	0.1	3.7***

**p<0.01; ***p<0.001

The number of corpora lutea per litter were similar for all groups.

6. Fetal effects: Live fetuses were individually weighed. All live and dead fetuses were examined for external anomalies. Radiological examination was made of all fetuses. One half of the fetuses were examined for visceral findings and one half for skeletal findings.

Fetal body weights were statistically significantly reduced when compared to the controls for the mid and high dose groups. Fetal body weight data can be found summarized in the following table taken from the report (pg. R4).

Fetal Body Weights

Dose Level (mg/kg)	Fetal Weights (g)
0	5.6±0.1
40	5.5±0.1
80	5.2±0.1*
120	4.6±0.1***

*p<0.05; ***p<0.001

The most commonly occurring skeletal findings were split vertebrae center and rudimentary extra ribs. The fetal incidence of these findings was 8/333, 10/241, 10/299, and 11/247 for split vertebrae center and 0/333, 3/241, 2/299, and 6/247 for rudimentary extra ribs for the control, low, mid and high dose, respectively. The litter incidence for rudimentary extra ribs was 0, 3, 2, and 2 for the control, low, mid, and high dose groups, respectively.

D. DISCUSSION:

No clinical signs of toxicity were reported in this study for the dams. There was one death in the 40 mg/kg group, which was unexplained, and not considered to be due to treatment. Body weights were statistically decreased when compared to the control group for all treatment groups on gestation day 17; however, body weight gain during gestation was only statistically

decreased at the highest dose tested. This reviewer does not consider the effect on body weight at the low dose to be treatment related since 1) the effect is not dose-related at the low dose, 2) body weights for this group were lower than the control group on gestation day 1, 3) the percentage decrease is approximately 5%, and 4) body weight gain during the gestation period was the same for this group and the control group. The effect at the mid dose is considered to be treatment related since it is dose-related and there is a decrease in body weight gain overall during the gestation period at this dose level, although not statistically significant.

Food consumption was statistically significantly reduced in the treatment groups in a dose-related manner from day 6-16 of gestation. This effect is considered to be treatment related even at the low dose since it was a 10% decrement.

At the highest dose tested, there was a statistically significant decrease in litter size and the number of live fetuses and an increase in the number of resorbed fetuses. At the mid and high dose, there was a dose-related and statistically significant decrease in fetal weights. All of the above effects are considered to be treatment related.

There were no treatment related visceral findings. There was an increase in the number of fetuses but not litters with rudimentary extra ribs at the highest dose tested. This finding is considered to be equivocal. Historical control data would aid in the evaluation of this effect as to whether it is or is not treatment related.

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

PESTICIDES AND

Subject: Re-evaluation of the 3-Generation Study of Imazalil on Reproduction in the Rat (Study no, 736), and the Addendum, and Responses to the Sponsor.

Tox. Chem. No: 497AB.
Project No.: 9-1100.
Record No.:

To: Henry Spencer, PhD.
Section 2, Toxicology Branch 1 (IRS)
Health Effects Division (H7509C)

From: David G Anderson, PhD David G Anderson 10/4/89
Section 2, Toxicology Branch 1 (IRS)
Health Effects Division (H7509C)

Thru: Marion Copley, DVM
Chief Section 2, Toxicology Branch 1 (IRS)
Health Effects Division (H7509C)

and

Karl Baetcke, Chief
Toxicology Branch 1 (IRS)
Health Effects Division (H7509C)

CONCLUSIONS: This memorandum covers the portion of Bill Goodwine's letter concerning the study on reproduction, study number 736, accession no. 097233, and the addendum, MRID no. 410266-04. The study in question has been re-reviewed, and the study was given a core classification of supplementary with a low probability that it can be upgraded to minimum.

A. BACKGROUND:

Under cover of a letter, dated May 30, 1989, to Edward (Sic) Budd (Ewin Budd), Acting Chief, Toxicology Branch 1 (IRS); Bill Goodwine, Manger, Plant Protection Division of Janssen Pharmaceutica supplied additional information requested in a memorandum on the toxicity data base for Imazalil to Lois Rossi,

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Product Manger #21, Registration Division, OPP from Judith W Hauswirth, then Acting Chief Toxicology Branch I (IRS . Hea.th Effects Division, OPP, dated 10/7/88. In this letter deficiencies were designated in 2 teratogenicity studies 556 and 597, 2 studies on reproduction 616 and 736, 1 24-month rat study 667 and 1 carcogenicity study in mice 666. This memorandum covers the portion of Bill Goodwine's letter concerning the study on reproduction, study number 736, accession no. 097233, and the addendum, MRID no. 410266-04.

B. RESPONSE TO THE SPONSOR'S COMMENTS:

The sponsor has commented on the study deficiencies noted in Judith Hauwirth's memorandum. The study in question is the final Three-Generation Study in Wistar Rats, Study No. 736, dated 3/15/78, Accession No. 097233 and the addendum MRID No. 410266-01 and 410266-04.

A summary of the sponsor's comment is presented first and immediately following is the Agency's reply.

Sponsor's Comment:

It is acknowledged that dosing prior to mating did not occur in the study no. 736. However, if the initial generation is considered the P0 generation, then the parents were exposed in utero, during lactation, and through early adulthood through mating. This should be a sufficient duration of exposure.

The Agency's Response:

Guideline 83-4 states that dosing should continue for 8 weeks prior to mating. However, the dosing period is not at issue here. In this study, 736 only the 1st generation had a mating ratio of 1 male to 2 females, which is minimally acceptable. Subsequent generations had a mating ratio 1 male to 3 females. Subtle effects on male fertility are difficult at best to detect when the number of sampling units is 10. If the sampling unit decreases below 10 as it must have in subsequent generations, the sensitivity of the study to male fertility effects is unacceptable.

In addition, the study was so poorly reported that the number of males mated in subsequent generations cannot be determined. The number of females mated in the first mating differed from the number of females mated for the second mating after the first generation. How was the selection of females for the second mating made, and was it from among females which successfully produced litters in the first mating? Were the males the same for the first and second matings in generations after the first. Mating pairs were not identified. There was no opportunity to detect any parental toxicity or other effects because the animals were not meaningfully evaluated even with regard to

body weight. The mating pairs in no generation could be determined.

Sponsor's Comment:

The dose levels of 0, 5, 20, and 80 mg/100g feed used were evaluated in several other studies. The 80 mg/100g feed was slightly toxic to rats of unspecified sex, and 20 mg/100g feed was borderline toxic.

Agency's Response:

The Agency can not determine whether or not this study demonstrated toxicity, and since test material concentration in the feed was not analyzed, the Agency has no way to verify whether or not the animals were treated with Imazalil.

The failure of the study on reproduction to demonstrate any toxicity is part of the reason the study is unacceptable as reported. There was no opportunity to evaluate whether or not the female rat demonstrates more or less toxicity during pregnancy. It could be suggested that any study not demonstrating toxicity at 2 dose levels, known to be toxic and differing by a factor of 4, is a study which is indeed poorly reported.

Sponsor's Comment:

More detailed individual data on parental and pup parameters are enclosed in the addendum.

Agency's Response:

The response is insufficient.

The data submitted contains added information on individual dam body weights at day 1, 7, 14 and 21 of gestation, and pup data. However, the data is not summarized either in the original submitted report or in the addendum. The sponsor needs to summarize the body weights of the dams during gestation and lactation and resubmit it. Summaries and individual weekly body weights of dams and food consumption and efficiency on all appropriate generations from the initiation of dosing to termination should be submitted as well. Summaries and individual data on weekly body weights, food consumption and efficiency of parental males from the initial dosing to termination should be submitted. Similarly, the pup data missing from the summaries in the originally submitted study needs to be added, i.e., pup weights at day 4, and day 7, and variability data for day 7. Individual animal data is needed to support summary data, but it can not be substituted for it.

c. RE-REVIEW OF THE STUDY ON REPRODUCTION (716):

The abstract of the study no. 736 is copied below.

ABSTRACT:

Wistar rats were fed a diet containing 0, 50, 200, or 800 ppm of Imazalil, technical for 3-generations of rat reproduction. Dosing was initiated at the time of mating the F0 generation [males (10) about 350 g and females (20) about 180-220 g and about 3-4 months old] to produce the F1a and F1b pups of the F1 generation. The mating ratio was male:females = 1:2 for the F0 generation and 1:3 for subsequent generations. Two litters per generation were produced. Approximately 33 to 44 dams were selected from the F1b litters for the first mating of the F1 generation and producing the F2a and inexplicably 23 to 31 were selected for the second mating of the F1 generation producing the F2b. Approximately 32 to 36 dams were selected from the F2b litters for the first mating of the F2 generation and producing the F3a and inexplicably 20 to 29 were selected for the second mating of the F2 generation producing the F3b. These differences in numbers of dams selected or why were never explained. The F3b litters were not allowed to deliver but were taken on day 22 of gestation for teratological evaluation.

No dose or treatment related effects were noted in the body weight of dams, food consumption of dams, on fertility, number of litters, litter size, pup viability, pup body weight or malformations, except in the results of the first mating in the first generation at the HDT. The number of dams pregnant in this group was reported to be 40%, and it was speculated that some of the dams had probably cannibalized their litters. However, the only verification of pregnancy was a normal mean body weight gain during the third week of gestation. The dams of this group produced a normal number of litters from the second mating, therefore, the low number of pregnancies produced by the first mating was possibly incidental, but this can not be determined. In addition, the effects seen in this group are consistent with some type of significant trauma to these animals. This episode also demonstrates the possiblility of GLP failure. Untraumatized female rats seldom cannibalize normal litters.

The study contained many deficiencies, and while the probability of effects on fertility from Imazalil consumption is low, a study on reproduction is designed for screening for various parameters impacting on reproduction. The possible effects on these other parameters can not be determined from the data presented. There was no indication that the study was conducted under the GLP. No test chemical stability data was reported. No analyses were reported of the test material in the diets administered. Weekly body weights of males and females were not reported, and summaries of body weight gains of females were reported only for the third week of gestation for the 3 generations. Food consumption was reported only for the same periods and for lactation. No necropsy data were reported for the parents of any generation. Pup necropsies were conducted on the second litters of the third generation only. The matings

producing the second litters had insufficient number of males. Specific data about the identity of mated males and females were absent.

E. SUMMARY:

Doses administered: 0, 50, 200, or 800 ppm in the diet to Wistar rats.

NOEL: > 800 ppm.
LEL: > 800 ppm for effects on offspring, reproduction, and other systemic effects. Study demonstrated no effects.
Core classification: Supplementary because additional information must be submitted. It is doubtful that the study can be upgraded.

F. NEEDED INFORMATION AND RESPONSES:

1. A GLP statement must be signed and submitted if possible.

2. Stability studies of the test material in the diet must be submitted.

3. Analyses of the concentration of the test material in the diet must be submitted.

4. Since the substance tested was synsysized in the testing laboratory, it may have a different range of contaminants than the MUP. What is the diference in types and concentration of contaminants between the two.

5. Weekly body weights and food consumption must be submitted on all parental animals.

6. Summary observational data, and observational data on individual animals during dosing should be submitted.

7. The identity of male and female mating pairs should be submitted.

8. An explanation of the change in numbers of females used for mating between the first and second matings and among the generations should be submitted.

9. An explanation for the use of a ratio of male:females = 1:3 after the first generation should be submitted.

10. Summary data should be submitted for pups weights at birth,

day 4, day 7, day 14, and day 21 postnatally, and viability on these same days. Summary data was supplied only for pups at birth, day 14, and day 21 of lactation.

11. Histological data must be submitted on the vagina, uterus, ovaries, epididymides, seminal vesicles, prostate, testes, and any target organs from appropriate animals used for mating.

DER for a 3-generation/rat/Imazalil/497AB/B:3-GENRAT/D Anderson/6/23/89.

007865

Primary reviewer: David G Anderson, PhD. David G Anderson 6-3-89
Section 2, Tox. Branch 1 (IRS) (H7509C).
Secondary reviewer: Marion P Copley DVM. Marion Copley
Section 2, Tox. Branch 1 (IRS) (H7509C).

DATA EVALUATION REPORT

STUDY TYPE: Re-Review and Amendment/3-Gen-Reproduction(83-4)/Study No 736/Rat.

TOX. CHEM. No.: 497AB

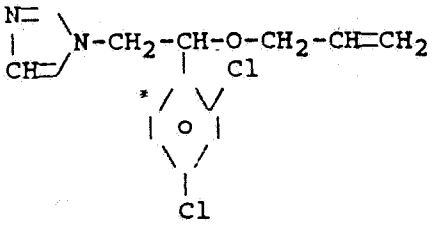
ACCESSION No.: 097233 for original 3-gen study. (TB Doc # 000065)

MRID No.: 410266-04 for amendment to Accession No. 097233, the original study.

TEST MATERIAL: Imazalil R 23979, Technical.

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

STRUCTURE:



SPONSOR: Janssen Pharmaceutica, 40 Kingsbridge Road, Piscataway, NJ 08854.

TESTING FACILITY: Janssen Pharmaceutica, Research Laboratories, 2340 Breesem Belgium.

STUDY NO.: 736

REPORT TITLE: Oral Three-Generation Study in Wistar Rats.

AUTHOR(S): Anonymous

REPORT ISSUED: 15/3/78

CONCLUSIONS:
Doses administered: 0, 50, 200, or 800 ppm in the diet to Wistar rats.

NOEL: > 800 ppm.
LEL: > 800 ppm for effects on offspring, reproduction, and other systemic effects. Study demonstrated no effects.

Re-Review and Amendment/3-Gen-Reproduction(83-4)/Study No 736/Rat.

Core classification: Supplementary because additional information must be submitted. It is doubtful that the study can be upgraded.

A. MATERIALS:

1. Test compound: Imazalil R 23979, synthesized by the testing laboratory, Description yellow-brown viscous oily liquid, Batch # R.E.11.108. = C03/1, Purity 98.8% by assay, 101.2% by GC.

2. Test animals: Species: Rat, Strain: Wistar (bred in house strain), Age: 3-4 months, Weight: males 350 g, females 180-220 g, Source: Jenssen Laboratories.

3. Environmental: Animals housed in wire cages in air conditioned rooms. Temperature: 21-23 degrees C, humidity: 50-60%, light:dark = 12:12.

B. STUDY DESIGN:

1. Animal Assignment - Animals were assigned randomly to groups. The ratio of male:females = 1:2 for the 1st generation only. For subsequent generations the ratio of male:females = 1:3. The exact number of males/group used for mating in the F1 and F2 generations was not specified.

Test group	Dose in diet ppm	1st Gen. Female	2nd Gen. Female	3rd Gen. Female
1st mating				
1. Cont.	0.0	20	33	32
2. Low (LDT)	50	20	42	32
3. Mid (MDT)	200	20	44	33
4. High(HDT)	800	20	43	36
2nd mating				
1. Cont.	0.0	18	23	27
2. Low (LDT)	50	20	31	29
3. Mid (MDT)	200	20	30	26
4. High(HDT)	800	20	29	20

Note: The reason for these variations in numbers of animals mated must be clarified.

2. Diet preparation - diet was prepared weekly, and stored at room temperature. The analyses for stability and concentration of test material in samples of treated food were NOT reported.

Results - None.

3. Animals received food (Coppen's rat diet) and tap water ad libitum.

4. Statistics - Statistical methods were not used on the study data, and the data presented did not need statistical analysis.

5. Quality assurance was NOT INCLUDED.

6. Study conduct - Pups were not reduced at day 4 after birth. Day 0 was the day of birth. The first litter was discarded, and the breeders producing the next generation were selected from the second litter. Malformations noted at birth were subjected to radiological examination.

F0 generation:
- 10 males: 20 females/group
- Dosing initiated at mating, and continuous there after.
- Mated at 3-4 months of age (180-220 g, females), (350 g, males)
- First litter (F1a) was weighed and counted at day 1 and discarded.
- Second litter (F1b) was weighed, counted, and survival each determined at day 1, day 4, day 14, and day 21 of lactation.

F1 generation:
- Selected from and immediately after weaning the F1b pups.
- Dosing in the diet initiated immediately after weaning.
- Mated at sexual maturity (3 months) (brother sister matings avoided). Mating ratio = 1 male:3 females.
- First litter (F2a) was weighed and counted at day 1 and discarded.
- Second litter (F2b) was weighed, counted, and survival each determined at day 1, day 4, day 14, and day 21 of lactation.

F2 generation:
- Selected from and immediately after weaning the F2b pups
- Dosing in the diet was initiated immediately after weaning.
- Mated at sexual maturity (3 months) (brother sister matings avoided). Mating ratio = 1 male:3 females.
- First litter (F3a) was weighed and counted at day 1 and discarded.
- Second litter (F3b) was taken by caesarean section for developmental toxicity studies at gestational day 22. Numbers of viable fetuses, dead fetuses, resorptions, and implantation sites counted, and fetuses examined for soft tissue and skeletal malformations.

C. METHODS AND RESULTS:

1. Observations - Animals were inspected daily for signs of toxicity and mortality.

Results - Toxicity - [No summary or individual observational data was reported. Mortality data is presented in Table A below. No dose related or treatment related mortality was noted, and no

information was presented on the cause of the 2 deaths in the HDT.

Table A.

Mortality among females.

Dose group (mg/100g feed)	Dose in diet (ppm)	Number dead females/total in test 1st Gen. Female	2nd Gen. Female	3rd Gen. Female
1st litter				
1. Cont.	0.0	0/20	0/33	0/32
2. 5 (LDT)	50	0/20	0/42	0/32
3. 20 (MDT)	200	0/20	0/44	0/35
4. 80 (HDT)	800	0/20	1/43	0/36
2nd litter				
1. Cont.	0.0	0/18	0/23	0/27
2. 5 (LDT)	50	0/20	0/31	0/29
3. 20 (MDT)	200	0/20	0/30	0/26
4. 80 (HDT)	800	0/20	1/29	0/20

2. Body Weight - [Weekly body weights were not determined as required by 83-2 Guidelines from EPA. Male body weights were not reported, except for pup body weights during lactation. Dams were weighed 1st, 7th, and 21st day of presumed pregnancy. However, the only summary body weight changes reported were for the period of the third week of pregnancy. The individual body weights of dams were reported for day 1, 7, and 21 of presumed pregnancy.

Results - No dose or treatment related changes in body weight gain of dams during the third week of pregnancy occurred.

3. food consumption and compound intake - Consumption was determined during the third week of presumed pregnancy for the Fa and Fb litters of their respective generations, and during lactation for the Fb litters of their respective generations.

Results - Food consumption - No dose or treatment related effects occurred in food consumption during the third week of gestation for all 3 generations.

Food efficiency - Efficiency was not determined because no weight changes or food consumption changes were noted.

Compound intake - Not reported.

4. Organ Weights and Pathology - No organ weights were determined on adults or pups.

Results - None.

5. Adult Reproductive Parameters - The (a) percent pregnancy, (b) litter size, (c) duration of gestation, (d) percent live pups, and (e) pup weights at birth, day 14 and 21 of lactation, and (f) percent survival at day 21 post delivery were determined and summary data reported.

Results - The (a) percent pregnancy is reported in Table B. In the first litter of the first generation at the HDT there were only 40% pregnant females. Since these same dams all produced litters from the second mating, the report stated that the 40% pregnancies from the first mating was believed due to failure of the animal caretaker to note cannibalism among these litters. While this may be true, the study report failed to demonstrate whether or not all these dams were pregnant. In addition, the dams delivering in this same group, delivered on day 24 (Table D), slightly later t[illegible] in any other group.

Table B.

Percent females delivering viable litters.

Dosage group (mg/100 g feed)	Dosage (ppm)	% females pregnant (number mated) 1st gen.	2nd gen.	3rd gen.
1st litter				
0 (Control)	0.0	100 (20)	96.9 (33)	100 (32)
5 (LDT)	50	95 (20)	97.6 (42)	100 (32)
20 (MDT)	200	100 (20)	97.7 (44)	100 (35)
80 (HDT)	800	40 (20)	97.6 (43)	100 (36)
2nd litter				
0	0.0	94.4 (18)	95.7 (23)	88.9 (27)
5	50	100 (20)	96.8 (31)	89.7 (29)
20	200	100 (20)	96.6 (30)	80.8 (26)
80	800	100 (20)	96.6 (29)	85.0 (20)

Results - The (b) litter size was normal in all litters except the first litter in the first generation at the HDT (Table C). In this one dosage group, the litter size was nominally lower, and may have been statistically significantly lower if tested.

Table C.

Re-Review and Amendment/3-Gen-Reproduction(83-4)/Study No 736/Rat.

Mean litter size.

Dosage group (mg/100 g feed)	Dosage (ppm)	Mean size (number dams with litters) 1st gen.	2nd gen.	3rd gen.
1st litter				
0 (Control)	0.0	12.3 (20)	12.1 (31)	11.9 (32)
5 (LDT)	50	11.6 (19)	11.7 (39)	11.9 (32)
20 (MDT)	200	11.2 (20)	11.6 (42)	11.8 (35)
80 (HDT)	800	9.9 (8)	11.3 (42)	11.9 (36)
2nd litter				
0	0.0	11.0 (16)	11.4 (18)	12.0 (24)
5	50	10.1 (18)	12.7 (26)	12.7 (26)
20	200	11.4 (17)	12.1 (26)	13.0 (21)
80	800	11.6 (18)	13.2 (28)	12.8 (17)

Results - The (c) duration of pregnancy is presented in Table D. Although slightly longer gestational periods were noted in association with the first litter of the first generation of the HDT, these results probably are not significant. The testing laboratory believed that a large number of these litters were cannibalized, but were unnoticed by the animal caretaker, and thus these animals were not considered pregnant. If this was true then obviously the duration of gestation could not have been adequately recorded. The duration of gestation requires accurate observation of the end of the parturition process.

Table D.

The duration of gestation.

Dosage group (mg/100 g feed)	Dosage (ppm)	The duration of gestation (days) 1st gen.	2nd gen.	3rd gen.
1st litter				
0 (Control)	0.0	23.2 (20)	23.2 (31)	23.4 (32)
5 (LDT)	50	23.2 (19)	23.5 (41)	24.0 (32)
20 (MDT)	200	23.3 (20)	23.5 (43)	23.7 (35)
80 (HDT)	800	24.0 (8)	23.8 (42)	23.8 (36)
2nd litter				
0	0.0	23.6 (17)	23.4 (22)	*
5	50	23.2 (18)	23.6 (30)	
20	200	23.6 (20)	23.3 (29)	
80	800	23.7 (20)	23.7 (28)	

* No data because these dams were killed on day 22 of gestation, and the fetuses examined for developmental anomalies.

Results - The (d) percent live pups, and (e) pup weights at birth, and (f) percent survival at day 21 post delivery were all normal.

6. Developmental Segment - The F3b fetuses were evaluated for developmental effects. Thus, fetuses were removed from the dams

by caesarean section at day 22 of gestation. The report indicated that 1/3 of the fetuses were examined for soft tissue anomalies and that 2/3 of the fetuses were examined for skeletal anomalies. However, only a terse summary of skeletal malformations (ossification delays not reported) was noted in the report. Fetal weight, and the number of resorption sites were reported.

Results - The developmental toxicity evaluation conducted on the second litter from the third generation demonstrated no dose or treatment related effects. The extent of the evaluation of the developmental effects was not clear. Much of the detail normally presented in developmental toxicity studies in tables was absent, such as ossification delays, and other developmental variations. This portion of the study, if submitted under Guideline 83-3, would be consider supplementary pending the submission of more data.

D. ABSTRACT AND DISCUSSION:

Wistar rats were fed a diet containing 0, 50, 200, or 800 ppm of Imazalil, technical for 3-generations of rat reproduction. Dosing was initiated at the time of mating the F0 generation [males (10) about 350 g and females (20) about 180-220 g and about 3-4 months old] to produce the F1a and F1b pups of the F1 generation. The mating ratio was male:females = 1:2 for the F0 generation and 1:3 for subsequent generations. Two litters per generation were produced. Approximately 33 to 44 dams were selected from the F1b litters for the first mating of the F1 generation and producing the F2a and inexplicably 23 to 31 were selected for the second mating of the F1 generation producing the F2b. Approximately 32 to 36 dams were selected from the F2b litters for the first mating of the F2 generation and producing the F3a and inexplicably 20 to 29 were selected for the second mating of the F2 generation producing the F3b. These differences in numbers of dams selected or why were never explained. The F3b litters were not allowed to deliver but were taken on day 22 of gestation for teratological evaluation.

No dose or treatment related effects were noted in the body weight of dams, food consumption of dams, on fertility, number of litters, litter size, pup viability, pup body weight or malformations, except in the results of the first mating in the first generation at the HDT. The number of dams pregnant in this group was reported to be 40%, and it was speculated that some of the dams had probably cannibalized their litters. However, the only verification of pregnancy was a normal mean body weight gain during the third week of gestation. The dams of this group produced a normal number of litters from the second mating, therefore, the low number of pregnancies produced by the first mating was possibly incidental, but this can not be determined. In addition, the effects seen in this group are consistent with some type of significant trauma to these animals. This episode

also demonstrates the possiblility of GLP failure. Untraumatized female rats seldom cannibalize normal litters.

The study contained many deficiencies, and while the probability of effects on fertility from Imazalil consumption is low, a study on reproduction is designed for screening for various parameters impacting on reproduction. The possible effects on these other parameters can not be determined from the data presented. There was no indication that the study was conducted under the GLP. No test chemical stability data was reported. No analyses were reported of the test material in the diets administered. Weekly body weights of males and females were not reported, and summaries of body weight gains of females were reported only for the third week of gestation for the 3 generations. Food consumption was reported only for the same periods and for lactation. No necropsy data were reported for the parents of any generation. Pup necropsies were conducted on the second litters of the third generation only. The matings producing the second litters had insufficient number of males. Specific data about the identity of mated males and females were absent.

E. SUMMARY:

Doses administered: 0, 50, 200, or 800 ppm in the diet to Wistar rats.

NOEL: > 800 ppm.
LEL: > 800 ppm for effects on offspring, reproduction, and other systemic effects. Study demonstrated no effects.
Core classification: Supplementary because additional information must be submitted. It is doubtful that the study can be upgraded.

F. NEEDED INFORMATION AND RESPONSES:

1. A GLP statement must be signed and submitted if possible.

2. Stability studies of the test material in the diet must be submitted.

3. Analyses of the concentration of the test material in the diet must be submitted.

4. Since the substance tested was synsysized in the testing laboratory, it may have a different range of contaminants than the MUP. What is the diference in types and concentration of contaminants between the two.

5. Weekly body weights and food consumption must be submitted on all parental animals.

6. Summary observational data, and observational data on individual animals during dosing should be submitted.

7. The identity of male and female mating pairs should be submitted.

8. An explanation of the change in numbers of females used for mating between the first and second matings and among the generations should be submitted.

9. An explanation for the use of a ratio of male:females = 1:3 after the first generation should be submitted.

10. Summary data should be submitted for pups weights at birth, day 4, day 7, day 14, and day 21 postnatally, and viability on these same days. Summary data was supplied only for pups at birth, day 14, and day 21 of lactation.

11. Histological data must be submitted on the vagina, uterus, ovaries, epididymides, seminal vesicles, prostate, testes, and any target organs from appropriate animals used for mating.

DER for a 3-generation/rat/Imazalil/497AB/B:3-GENRAT/D Anderson/6/23/89.

Comments on the Oral Three-Generation Study in Wistar Rats, Study No. 736, dated 3/15/78, Accession No. 097233 and the addendum

The study and addendum (consisting of individual data on dams and pups) was submitted to the Agency in response to a letter on the toxicity data base for Imazalil to Lois Rossi, Product Manger #21, Registration Division, OPP from Judith W Hauswirth, then Acting Chief Toxicology Branch I (IRS), Health Effects Division, OPP, dated 10/7/88. In this letter deficiencies were designated in 2 teratogenicity studies 356 and 597, 2 studies on reproduction 616 and 736, 1 24-month rat study 667 and 1 carcogenicity study in mice 666. The study on reproduction is the subject of this statment.

The study on reproduction, 736, was initially classified core minimum by Carlos Rodriguez, 11/3/78 (Document Number 000065). On re-review of the study, it was clearly supplementary at best, with doubt that it can be upgraded.

However, there are several mitigating circumstances and other data which indicate that another study on reproduction for potential fertility effects may not be necessary. The study demonstrated no probable effects at over 4 times the dose level causing effects on liver histology (Study No. 342. reviewed in Document 000065). In addition, a major deficiency, failure to adequately study male reproductive effects, may be partly overcome by a previous study on male and female fertility, study number 598.

In study number 598, 20 Wistar males/group were dosed at the same dose levels as study number 736 for 60 days prior the mating to 20 females/group. No effects were noted on fertility or pups in this study. The study was considered core minimum. With re-review of study number 598, it may adequately demonstrate that the potential male fertility effects from Imazalil are of no concern.

The probability that undetected frank fertility effects were produced in the study on reproduction is low, however, other more subtle effects could not have been detected from the data presented in study number 736. Thus, since the Agency has requirements for these other possible effects, the study must be repeated or an adequate response must given to requested information about the study.

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: IMAZALIL - Company Response to TB Evaluation of a Mutagenicity Study

TOX Chem No.: 497AB
TB Project No.: 8-0063
(Contingency Fund)

FROM: Irving Mauer, Ph.D., Geneticist
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

TO: Judith W. Hauswirth, Ph.D., Chief
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

Registrant: Janssen Pharmaceutica, Beerse (Belgium)

Request

The registrant has submitted a supplement to the report of the following mutagenicity study,

"in vitro Chromosome Aberration Assay on Human Lymphocytes," (with R 23979, enilconazole, imazalil), Study/Protocol No. SCK 86/02D/R23979, performed by the Mammalian Genetics Lab., Biology Dept., S.C.K./C.E.N., Mol (Belgium); Report dated October 20, 1986. [Project ID: X9 1988; EPA MRID No. 40729302],

in response to deficiencies transmitted in a Toxicology Branch (TB) review dated August 5, 1988.

Background

This study was judged UNACCEPTABLE because of the following deficiencies:

"1. A description of the test article as received, and the purity (% ai) if supplied by the sponsor.

"2. The sex, age, current and/or previous medical status, as well as therapies/medications, of the two sets of donors (for the toxicity test, as well as the main assay).

"3. The procedures used in establishing the cultures, and their treatments (especially the concentrations per mL culture).

"4. Data from the preliminary toxicity test.

"5. Confirmation of protein content and activity of the S9 preparation obtained elsewhere (from Louvain).

"6. The number of cultures per treatment.

"7. The number of slides per concentration or per treatment made, or analyzed."

[Quoted from Memorandum: I. Mauer to L. Rossi, August 5, 1988, with attached DER, TB Document No. 006718.]

Current Submission

The current submission, received as a supplement to the original report of the study, contains the following (additional) information:

1. Purity of test article: 98.3%, certification of chemical analysis is included.

2. All donors were healthy males (ages 22, 24 and 47, 51 for the two aspects of the study), without current or immediately previous therapy or medication.

3. The concentrations ranged from 125 to 5000 ug per culture, or for the 5.5 mL culture volumes: 22.73 to 909.09 ug/mL.

4. Data from the preliminary toxicity test are now provided in the table that follows:

CELLS IN MITOSIS

Treatment (ug/mL)	Donor I -S9	Donor I +S9	Donor II -S9	Donor II +S9
Solvent control (18.18 uL DMSO)	70	78	60	104
R 23979:				
909.09	0	0	0	0
454.55	0	0	0	0
227.27	3	18	2	14
22.73	59	68	66	75

5. The protein content of the S9 (from Louvain) used in this study was 36.92 mg/mL. Further, S9s are routinely tested in Salmonella TA100, TA1535, TA98, and TA97 against the promutagen, 2-aminoanthracene.

6. Two cultures per treatment per donor were established.

7. Two slides per concentration were analyzed in the toxicity test, four per concentration in the main assay.

TB Conclusions

All the deficiencies noted in the review of the original study have been adequately addressed with this additional information. Hence, the study can now be upgraded to ACCEPTABLE.

CASWELL FILE

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

007865

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Imazalil - Dose Selection for Mouse Oncogenicity Study. Submitted by Janssen Pharmaceutica by Fax on April 7, 1989.

Tox. Chem. No.: 497AB

TO: Susan Lewis
Product Manager #21
Registration Division (H7505C)

FROM: Judith W. Hauswirth, Ph.D., Chief
Toxicology Branch I - IRS
Health Effects Division (H7509C)

Judith W. Hauswirth 4/28/89

THRU: William L. Burnam, Acting Director
Health Effects Division (H7509C)

WLB 5/1/89

The registrant has sent a desk copy of a recently completed 90 day mouse study on imazalil to aid in the dosage selection for an oncogenicity study. Subsequent to this submission, Dr. H. J. van Cauteren of Janssen Pharmaceutica in Belgium called to discuss the study and to set appropriate dosage levels for the oncogenicity study. This telephone conversation took place during the week of March 20, 1989. He then faxed his version of this conversation to this reviewer along with his proposed high dosage level for the mouse study (A copy of the faxed material is attached for information).

Dr. van Cauteren proposed a high dose of 600 ppm for the oncogenicity study. Toxicology Branch I agrees to this dosage level based upon depressed body weight gain seen in males and females at 800 ppm in the 90 day range finding study. The percentage body weight decrement at this dosage level was by Toxicology Branch's calculations 30% in females and 25% in males. The differences in body weight gains were statistically significant at several time points during the 90 day study for females but not for males. Toxicology Branch notes that although the food consumption table in the report indicates that the dosed groups ate more than the control group, the report states that food wastage was a problem in this study. We also note that the mice were housed 2-3 per cage which could have contributed to wastage. The report further states that there could have been a palatability problem with the treated diet. Based upon the values given in the table for food consumption, it is difficult to determine whether this was a problem. We urge that csre be taken in the long term study to determine whether there is a palatability problem at 600 ppm.

Other effects seen at 800 ppm in the range finding study were hepatocellular

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vacuolar degeneration, a decrease in albumin, phospholipids, and total bilirubin in males and females and a decrease in AST in females only.

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DATE : April 7, 1989

FAX 00/1/703 557 ~~0233~~ 3106

ATTENTION : Dr. J.W. HAUSWIRTH - Arlington VA 22202 -U.S.A.

FROM : Dr. H. VAN CAUTEREN - Janssen Pharmaceutica - BELGIUM

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TELEFAX - No.703-5570233
TELEPHONE NUMBER: 703-5577397

TO: Dr. Judith W. HAUSWIRTH - Arlington VA 22202 - U.S.A.

FROM: Dr. H. Van Cauteren - Janssen Pharmaceutica - Belgium

DATE: April 7, 1989

SUBJ.: Imazalil
Dose levels for mouse carcinogenicity study

Dear Dr. Hauswirth,

Referring to correspondence dated March 8, 1989, from Bill Goodwine to you, a desk copy of a subchronic feeding study in mice (Exp.2020, December 2, 1988) was submitted for review and comment.

Subsequently, we spoke by phone and agreed that the finding, with regard to MTD, supported a high dose between 400 and 800 ppm.

Please find below, my dose level suggestions and justification for the 24-month mouse carcinogenicity study.

Protocol
In general, the protocol of this mouse carcinogenicity study will be fully compliant with the EPA guidelines (1984). More specifically, we will meet the criteria of the test procedures with regard to age (5 weeks at start), sex and number of mice (50 males and females/group), clinical observations (daily, weekly), measurements of body weight and of food consumption (weekly, monthly), clinical pathology (at 12 and 18 months and terminally), gross necropsy (including organ weights in terminal animals), and histopathology.

Route of administration and dose level selection
Imazalil will be admixed into the diet at levels of 50, 200 and 600 ppm. These levels have been selected based upon the following:
- Fifty ppm is an appropriate low dose since it is estimated to be a no-toxic effect level (NOEL). This level is in the same order of magnitude as the medium dose of the previously conducted mice carcinogenicity study (25 ppm in the drinking water is approximately equivalent to 50 ppm into the feed assuming mice drink about the double of the dry feed they consume).
- As an intermediate dose, 200 ppm will be used. It is estimated to be at the borderline of toxicity based upon a 3-month dose range finding study (Exp.No.2020) whereby dosing at 200 ppm resulted in slightly decreased aspartate aminotransferase, cholesterol and phospholipid values in the serum of females and in a vacuolar degeneration in the liver of males. This dose level also falls in the same order of magnitude of the high dose of the previously conducted mice carcinogenicity study (100 ppm in the drinking water is approximately equivalent to 200 ppm into the diet).

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- The high dose will be 600 ppm. In a 3-month dose range finding study (Exp.2020), dosing at 400 ppm resulted in toxicity which was characterized by some altered serum parameters (decrease of albumin, total bilirubin and phospholipids in males and decrease of cholesterol, phospholipids, total bilirubin and AST in females) and by histological modifications on the liver (vacuolar degeneration and centrilobular swelling). These effects were also, but more pronounced, present at 800 ppm. In addition, the liver weight was increased and macroscopically showed a swollen and dark aspect at this dose. In females, dosing at 800 ppm also resulted in a lower body weight gain (about 12%). This study indicated that the dose level of 400 and 800 ppm into the diet are toxic with cholesterol and lipid metabolism and the liver as potential targets.
  Since it could not be fully excluded that survival might not be adversely affected at 800 ppm, it was decided to select 600 ppm as the intermediate between 400 and 800 ppm.

Prior to initiating the study, we would like to receive verification, from you, that the subchronic feeding study (Exp.2020) has been reviewed and supports the proposed dose levels. Bill suggested that you might handle this by way of an Internal Memorandum to the Registration Division, with a copy faxed to Janssen, U.S.A. at (201)524-9895, so that we may proceed with our plans to initiate the study later this month. Alternatively, we would be most grateful to receive a letter directly from you on this matter by way of facsimile. We will leave it up to you to follow the best route.

I will ask Bill Goodwine to follow-up this letter within the same week.

Sincerely,

Herman Van Cauteren