2-8-00

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

OFFICE OF
PREVENTION PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Imazalil: Review and Update of Toxicology Studies to Support Re-registration Eligibility Decision
P.C. Code: 111901 Case: 816389

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TASK ID: DP Code: D262725 Submission: S548748

Registrant: Janssen Pharmaceutica, Belgium

Action Requested: Update of Data Evaluation Records (DER's) for preparation of the Toxicology Chapter of the Re-registration Eligibility Decision Document

Agency's Action:

HED has prepared/or updated the executive summaries of the Data Evaluation Records (DER's) on the subject studies in light of the new guidelines and classification systems. **The updated executive summaries are attached.**

1. Subchronic Oral toxicity Study - Rat

Executive Summary: In a 6-month toxicity study (Accession No. 00162411) Imazalil base technical (98.1% purity) was administered to groups of SPF Wistar rats (10/sex/dose) in the diet at dose levels of 0, 25, 100 or 400 ppm for a period of 6 months. None of the animals died during the treatment. No dose related clinical signs of toxicity were observed. Food consumption was comparable in all groups. The group mean body weights of the males of the 400 ppm group were slightly but not statistically significantly lower than those of the control group throughout the study.

Hematological determinations at study termination did not reveal compound related effects. Clinical chemistry parameters measured at sacrifice time were not affected in the male groups. But in females there was a significant increase ($p<0.01$) in lactate-dehydrogenase activity at the 400 ppm level. Significant increase ($p<0.01$) in glucose levels were reported in females administered the test material at all levels. No dose-response relationships were noticed.

An increase in the relative weight of the kidney in male rats ($p<0.01$) and in the absolute and relative weights of the kidneys and liver in female rats ($p<0.01$) were observed in the 400 ppm group. An increase in absolute weight of the thymus and the relative weight of the lungs ($p<0.05$) were observed in female rats at the 400 ppm level. The weight changes were not accompanied with enzymatic, morphological or histopathological changes.

The **LOAEL** for systemic toxicity is 400 ppm (20 mg/kg/day, using standard conversion factor), based on increase in relative liver weights (females) at this dose. This finding along with histopathological changes were noted at 400 ppm in a chronic/oncogenicity study in rats (MRID No. 47026101). The **NOAEL** is 100 ppm (5mg/kg/day).

This 6-month toxicity study in rats is **acceptable** and satisfies the guideline requirement for a sub-chronic oral study [870.3100 (82-1b)] in rats.

2. Subchronic Oral toxicity Study - Mouse

Executive Summary: In a 3-month oral mechanistic toxicity study (MRID 43222601, 43292402) designed to study effects on the liver, Imazalil Base (96.9%) was administered in the diet to 25 male and 25 female Swiss mice for 3 months at nominal dosage levels of 0, 50, 200 or 600 ppm (approximate doses of 0, 9.5, 38.6 or 115 mg/kg/day for males and 0, 11.3, 45.6 or 138 mg/kg/day for females, as adjusted for actual achieved concentrations of about 83% of nominal). Additional groups of 15 mice/sex/group were also given Imazalil base in the diet at 0, 50, 200 or 600 ppm, but were sacrificed at 1 month. The following parameters were evaluated: clinical signs, body weights, food consumption, clinical chemistries (3 liver enzymes only), gross necropsy, organ-,weights, histopathology (gall bladder and liver-only), electron microscopy of liver, liver microsomal protein and cytochrome P450 content, liver enzymatic activities of 7 P450 isoenzymes, liver testosterone metabolism and serum concentrations of Imazalil.

No treatment-related effects on mortality, clinical signs, body weights, body weight gains or food

consumption were observed. At 200 ppm, the following treatment-related effects were observed: increased incidence of dark liver at gross necropsy in males, increased incidence and severity of "centrilobular clearer aspect" and of large and/or small vacuoles in the hepatocytes of males and females, increased liver microsomal protein in males and females, and increased microsomal cytochrome P450 content in males and females. At 600 ppm, the following treatment-related effects were observed: increased incidence of dark livers at gross necropsy in males and females, increased absolute liver weights and relative liver/body weight ratios in males and females, increased incidence and severity of "centrilobular clearer aspect" and of large and/or small vacuoles in the hepatocytes of males and females, increased individual cell necrosis in hepatocytes of males, increased diffuse swelling of hepatocytes in females, increased liver microsomal protein in males and females, and increased microsomal cytochrome P450 content in males and females. Electron microscopy revealed increased numbers of lipid droplets in hepatocytes, which corresponded with the increased vacuolization observed by light microscopy, and a morphologically changed rough endoplasmic reticulum (RER) in the hepatocytes of 600 ppm males and females. Regarding liver enzymatic activities of 7 P450 isoenzymes., dosing with Imazalil at 200 ppm and 600 ppm significantly induced certain enzymatic activities but also had an inhibitory effect on other metabolic activities. At 600 ppm, the total activity of testosterone hydroxylases was increased in both males and females. Low levels of Imazalil were detected in the serum of some males and females at 600 ppm only.

The **LOEL** in this study is 200 ppm (38.6 and 45.6 mg/kg/day in males and females respectively) and is based on increased incidence and severity of histopathologic effects, increased microsomal protein and increased microsomal cytochrome P450 content in the livers of both males and females. The **NOEL** in this study is 50 ppm (9.5 and 11.3 mg/kg/day in males and females, respectively).

This study is classified **acceptable/non-guideline.**

3. Subcronic Dermal Toxicity - Rabbits

Executive Summary: In a 21-day dermal toxicity study (MRID 42085201), groups of Albino New Zealand White Rabbits (5/sex/group) received dermal application of Imazalil technical grade (98.1% purity) dissolved in sesame oil, 6 hours a day, 5 days a week, for 21 days at doses of 0, 10, 40 or 160 mg/kg to a shaved area on each animal's back.

No mortalities or behavioral changes were reported. Red stippling of the skin was reported in all groups with higher incidence and more severity in the control group. This was attributed to the sesame oil vehicle and not the test substance. Skin irritation was minimal and was classified as "barely perceptible". Very slight erythema (Draize score 1) and slight scaling was reported in the 10 and 40 mg/kg/day groups. At the 160 mg/kg/day dose, erythema was very slight to well defined (Draize score 1-2) and slight to moderate scaling. There was statistically significant decrease (possibly treatment related) in urinary parameters of creatine levels ($p \leq 0.01$), specific

gravity ($p \leq 0.01$),and urobilinogen levels ($p \leq 0.05$) in the high dose males. In females only the urobilinogen level was decreased ($p \leq 0.05$) in the mid dose group.

A **LOAEL** for systemic toxicity was not established in this study. The doses in this study were based on a range finding study at dose levels of 0, 63, 250 and 1000 mg/kg/day for 6 days. The two highest doses produced significant fissuring, scaling and swollen livers. Based on the combined results of the two studies, the **LOAEL** was 250 mg/kg/day based on liver changes and the **NOAEL** was 160 mg/kg/day.

This 21-day dermal study was initially classified as **unacceptable** due to numerous questions raised by the HED reviewer regarding the methodology, dose selection and deficient data. The registrant subsequently provided satisfactory responses to all these questions and the study was upgraded to **Acceptable** (HED document number 011313) to satisfy the guideline requirement for a 21-day dermal study study [870.3200 (82-2)] in the rabbit.

4. Lifespan Oral Carcinogenicity Study - Rats

Executive Summary: The combined results of an 18-month chronic study and a 30-month chronic/carcinogenicity study are presented in this review. In the 30 month carcinogenicity study, MRID No.47026101, Imazalil technical (98.1%) was administered as a 50% mixture with 50% of equal parts of aerosil (25%) and cornstarch (25%)in the diet to Cpb: Wu Wistar rats, 50/sex/dose at dietary levels of 0, 25, 100, or 400 ppm. Approximate doses were 1.0, 3.7, and 15.5 mg/kg/day for males and 1.2, 4.7, and 20.0 mg/kg/day for females, respectively. In the 18-month study (Accession No. 00162412) 20 rats/sex/dose were used under the same dosing regimen. A previous 6-month study (Accession No. 00162411) used the same dose levels.

Minor losses in body weight gains in females (-3.4%) were seen at 400 ppm after 78 weeks in the 30 month study while a -17% body weight gain loss was noted in the 18 month study. Slight increases in liver weights (+5.4% after 18 months) and (+10.7% after 30 months) were noted in males at the same dose. At 18 months among 400 ppm group males, there was increased incidence of intracytoplasmic inclusion bodies in hepatocytes (5/20 vs 0/20 in controls) and an increase in severity of hepatocyte vacuolization as well as bile duct proliferation. An increased incidence of focal hepatocellular vacuolation was noted in males (6/17 vs 2/17 in controls) at 400 ppm that survived to 30 months. No other treatment effects were reported. Increased incidence of Leydig cell tumors in the testes was noted at 30 months in all dosed groups of males (3/50, 4/50 and 4/47 at low, mid and high dose groups, respectively compared to 1/50 for the controls). The supplemental histopathological information (MRID 41558501) on the historical incidence of Leydig cell testicular tumors dismissed an association with the administration of the test chemical based on the lack of both statistically significant increased incidence and the lack of a dose related increase in tumors. An unusual epidermoid carcinoma of the uterus in female rats (1/50) was reported at the low and high doses only.

This study was evaluated by the HED Cancer Peer Review Committee (CPRC) in 1994. CPRC

found that in this study when Imazalil was administered in the diet to Wistar rats there was no apparent increase in any tumors, however the CPRC determined that the highest dose tested was not adequate enough for assessing the carcinogenic potential of Imazalil in the rat. Another rat study at higher doses was recommended.

Based on the combined results of the two studies, a minimal **LOAEL** could be established at 400 ppm (15.5 and 20.0 mg/kg/day in males and females, respectively) with a **NOAEL** established at 100 ppm (4.7 mg/kg/day in females) based on the liver effects and slight body weight gain reductions (3.7 mg/kg/day in males)

Because Imazalil in this rat study was not tested at a high enough dose to evaluate its carcinogenicity potential, this study is **Unacceptable** for GL -870.4300 (83-5).

5. Chronic Oncogenicity Study - Mouse

Executive Summary: In a 23-month carcinogenicity study (MRID 42972001), Imazalil base (96.9% pure) was administered in the diet to 50 male and 50 female Swiss mice for 100-101 weeks at nominal levels of 0, 50, 200, or 600 ppm (approximate doses of 0, 6.76, 28.0, or 88 mg/kg/day for males and 0, 8.29, 34.8, or 110 mg/kg/day for females, as adjusted for actual achieved concentrations of about 83.7% of nominal).

At 600 ppm, body weight (93% of control) and body weight gains (83% of control) in males were significantly decreased ($p \leq 0.001$) over the duration of the study. In females these parameters were decreased but not significantly (97% and 88% of control, respectively). Also at this dose, there was a significantly increased incidence of pigmentation in the sinusoidal cells of the liver in males (20/50 vs 10/50 for controls), focal cellular changes in the pancreas in males (6/49 vs 0/50 in controls, $p<0.05$) and females (5/50 vs 2/50 in controls), increased absolute (+18%, $p<0.05$) and relative (+24%, $p<0.01$) liver weight in males. Also at the 600 ppm dose there were liver effects in females (large vacuoles 5/50 vs 0/50 in controls; parenchymal cellular swelling 4/50 vs 0/50 in controls; and large vacuoles/vacuolization 9/50 vs 1/50 in control). Absolute liver weight (10%) and relative liver weight (14%) in females were increased, but the increases were not statistically significant. At 200 ppm, males-had a significant increase in the incidence of focal cellular changes (10/50 vs 2/50 for controls, $p<0.05$), large vacuoles (8/50 vs 1/50 for controls, $p<0.05$), and swollen sinusoidal cells (37/50 vs 24/50 for controls, $p<0.05$) in the liver. The **LOAEL** for systemic toxicity is 200 ppm (28.0 mg/kg/day) based on the histopathological changes observed in the livers of males. The **NOAEL** is 50 ppm (6.76 mg/kg/day). The **LOAEL** for females is 600 ppm (110 mg/kg/day) based on focal cellullar changes in the pancreas, liver effects and increased absolute and relative liver weight. The **NOAEL** in females is 200 ppm (34.8 mg/kg/day)

The incidence of hepatocytic neoplasms was increased in males in the 200 and 600 ppm groups (50% in both groups versus 26% in controls, $p<0.05$) and in females at 600 ppm (22% versus 8% in controls, $p<0.05$). Of the hepatocytic neoplasms, the incidences for hepatic neoplastic

nodules were increased in males in the 200 and 600 ppm groups (46% at 200 ppm and 34% at 600 ppm versus 16% in controls). Trends for increases in total hepatocytic neoplasms and neoplastic nodules were observed in both males and females. A possible increase in the incidence of hepatocytic carcinomas was observed in males at 600 ppm (22% versus 10% in controls). **A statistical increase ($p<0.05$) in the incidence of vaginal metaplasia (22/48 vs 9/44) was observed.** The dose levels used in this study (0, 50, 200, and 600 ppm in the diet) were previously agreed to by Toxicology Branch I based upon decreased body weight gain seen in males (-25%) and females (-30%) at 800 ppm in the 90-day range finding study in mice.

Supplementary information regarding diet preparation and compound analysis and stability submitted by the registrant (HED Document No. 011162) was reviewed and was found acceptable.

This study is now classified **Acceptable** for carcinogenicity and satisfies the **guideline requirement** for a carcinogenicity study in mouse (Guideline 83-2(b)).

The study was evaluated along with the weight of evidence by the HED Carcinogenicity Peer Review Committee (CPRC; August 24, 1994). CPRC concluded that administration of Imazalil in the diet to CD-1 mice resulted in statistically significant increases in liver adenomas and adenomas/carcinomas in male Swiss albino mice, with a positive trend for adenomas, carcinomas and combined adenomas/carcinomas. It was also noted that tumors in the mouse appeared at a dose which was not particularly high.

6. Chronic Oral Toxicity Study - Dogs

Executive Summary: In a chronic toxicity study (MRID 41328802), Imazalil base technical (≥97.2% purity) was administered by capsule to beagle dogs (4/sex/dose) at dose levels of 0, 1.25, 2.5 or 20 mg/kg/day for a period of 12 months.

All dogs survived the 12 month treatment. Clinical symptoms (increased vomiting, salivation, wasting of food and soft stools)were reported only in the high dose 20 mg/kg/day group. Males were more affected than females. Mean body weights were slightly depressed (12.4% in males and 9.4% in females) at 52 weeks. Body weight gains were significantly decreased (33% of the controls) in this group in both sexes, particularly during the first half of the study.
Serum alkaline phosphatase was markedly increased at 20 mg/kg/day(at least double the control values). Hematological changes were reported to be insignificant or in a non dose related manner. The test material did not appear to affect the urinary parameters.

Liver weights and liver to body weight ratios were significantly increased in a dose related manner in males (2.5 & 20 mg/kg/day) but were not accompanied by histologic changes. The increase in liver to body weight ratio is probably related to the decreased body weight. The increase in liver weight and liver to body weight ratio in the 20 mg/kg/day males was 16% and 30%, respectively. The significant increase in the 2.5 mg/kg/ group was attributed to one dog.

Liver weight changes in females were insignificant.

The **LOAEL** is 20 mg/kg/day, based on clinical toxicity of vomiting and soft stools; depressed body weight gains, increased alkaline phosphatase activity and increased liver weights. The **NOAEL** is 2.5 mg/kg/day.

This chronic toxicity study in the dog is **Acceptable** and satisfies the guideline requirement for a chronic oral study [870.4100 (83-1b)) in dogs.

7. Prenatal Developmental Study - Mouse

Executive Summary: In a developmental toxicity study (Accession No. 000258128), imazalil (purity not specified) was administered to 24 female cobs mice/dose by oral gavage in Arabic gum solutions at dose levels of 0, 2.5, 10 or 40 mg/kg/day from days 6 through 16 of gestation.

No maternal toxicity was observed at any level. There were no treatment related mortalities. Body weight gains and food consumption were comparable to controls. Pregnancy was comparable in all groups.

There was no fetal toxicity either. All measured parameters (number of live fetuses per litter, number of dead fetuses per litter, litter size, number of resorptions, number of implantations, weight of living pups) were similar in all groups. No fetal abnormalities related to treatment were reported. The maternal systemic and Fetotoxic NOAEL is >40 mg/kg/day, the highest dose tested.

This developmental toxicity study in the mouse is classified as **Unacceptable** and **not upgradable.** The highest dose tested did not produce any evidence of maternal or fetal toxicity. No information on the test material purity was provided.

8. Prenatal Developmental Study - Rat

Executive Summary: In a developmental toxicity study (MRID 41026603), imazalil sulphate (99.9% purity) was administered to 24 Sprague-Dawley rats/dose by oral gavage in aqueous solutions at dose levels of 0, 40, 80 or 120 mg/kg/day from days 6 through 16 of gestation.

Maternal toxicity was observed at all dose levels as evidenced by significantly decreased mean food consumption (9.8%, 17.1% and 18.7%, for the low, mid and high doses, respectively) during the dosing period. Mean body weights on gestation day 17 were significantly decreased for the mid (3.5%) and high (4.6%) dose groups compared to the control group. The mean body weight gain for the low dose group and the control group was comparable. For the high dose group, mean body weight on gestation day 22 (6.2%) and mean corrected body weight gain (19%) for gestation days 1-22 were significantly decreased compared to the control group. **The maternal toxicity LOAEL is 40 mg/kg/day, based on decreased mean food consumption.**

The maternal toxicity NOAEL is <40 mg/kg/day (LDT).

Developmental toxicity was manifested by a dose-related significant decrease in mean fetal weights in the mid (7.1%) and high (17.9%) dose groups compared to the controls. Other toxic effects were reported in the high dose group. These were: a significantly decreased mean litter size (11.2 vs 13.9 for the control), a significantly decreased number of live fetuses/litter (11.1 vs 13.8 for the control group), a significantly increased number of resorbed fetuses/litter (3.7 vs 0.4 for the control group). An increase in the number of fetuses (but not litters) with rudimentary extra ribs(6/247 vs 0/333 for the control group) was noted in the high dose group. This was found to be litter effect (See page 26) and therefore was not considered to be treatment related. The litter incidence for rudimentary extra ribs was 2/22 for the high dose group and 0/24 for the control group. No other treatment related effects were reported. **The developmental toxicity LOAEL is 80 mg/kg/day, based on decreased mean fetal weights. The developmental toxicity NOAEL is 40 mg/kg/day.**

This developmental toxicity study in the rat is classified as **Acceptable** and satisfies the guideline requirement for a developmental toxicity study [870.3700 (83-3)] in rats.

9. Prenatal Developmental Study - Rabbit

Executive Summary: In a developmental toxicity study (MRID 42593601), imazalil sulphate (98.2-100% purity) was administered to 15 female New Zealand Albino rabbits/dose by gavage at dose levels of 0, 5, 10 or 20 mg/kg/day from days 6 through 18 of gestation.

Maternal toxicity was observed at 10 and 20 mg/kg/day as evidenced by significantly decreased body weight gain (54% and 95%, respectively, during GD 6-18), respiratory difficulty, increased resorptions and increased mortality (8/15 at 20 mg/kg/day). Food consumption was significantly decreased at the mid and high doses (18% and 23%, respectively during GD 6-18). The maternal **LOAEL** is 10 mg/kg/day, based on decreased body weight gain and food consumption and increased resorptions and mortality. The maternal **NAOEL** is 5 mg/kg/day.

Developmental toxicity was manifested by increased number of resorptions/litter, with subsequent decreases in numbers of live fetuses/litter at 10 and 20 mg/kg/day doses. No external visceral malformations or variations were reported. **The developmental LOAEL is 10 mg/kg/day, based on increased resorptions and decreased number of fetuses per litter. The developmental NOAEL is 5 mg/kg/day.**

The developmental toxicity study in the rabbit was initially classified **supplementary** due to numerous deficiencies. These issues were subsequently resolved by additional information from the sponsor (HED document no, 011239). The study was upgraded to **Acceptable** and satisfies the guideline requirement for a developmental toxicity study [OPPTS 870.3700; §83-3b)] in rabbits.

10. Reproduction Study - Rat

Executive Summary: In a 2-generation reproduction study (MRID 42570701), imazalil (≥95.0%) 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 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 each level except for the high dose level where 14 females were mated only to produce the F2 generation. Only one litter per generation was produced.

Parental toxicity was seen at the highest dose level. A statistically significant increase in dystocia was seen in the P0 dams (3/24 in controls vs 6/24, $p \leq 0.05$) and F1 dams (1/24 in controls vs 8/14, $p \leq 0.05$) associated with the end of the gestation. A red vaginal discharge (incidence frequency not reported in the DER) was seen in the P0 dams during lactation. Food wastage occurred at the high dose in the P0 females from week 2 prior to mating, during gestation and lactation and in F1 females during pregnancy and lactation which negates food efficiency calculations. Body weight and body weight gains were decreased (95% of controls) in P0 males. Body weight decreases occurred in P0 and F1 females during gestation (76-80% of controls, $p \leq 0.05$) and lactation (94% of controls, $p \leq 0.01$). 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. $p \geq 0.05$). The parental toxicity **LOAEL** is 80 mg/kg/day based on body weight and body weight gain decreases and increased liver vacuolation in males in addition to the other toxic symptoms described above. The parental toxicity **NOAEL** is 20 mg/kg/day.

Parental Reproductive Toxicity (a treatment related effect on the length of gestation, significantly increased by approximately 1 day in both P0 and F1 females) was noted at the highest dose. Based on the increased duration of gestation for the P0 and F1 females, the reproductive toxicity **LOAEL** is 80 mg/kg/day and the **NOAEL** is 20 mg/kg/day.

Offspring Toxicity. 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 the highest dose. The number of dead pups at birth were also statistically ($p \leq 0.05$ to $p \leq 0.0001$) increased at the high dose in both generations. A nominal trend (statistical analysis was not conducted for trend) for decreased implantation sites in both generations was observed. The decreased number of implantation sites was statistically significant ($p \leq 0.05$) in the F2 females at the high dose. Survival during lactation was significantly ($p \leq 0.05$ to $p \leq 0.0001$) reduced at all dose levels in the F1 pups and at the low dose and high dose levels in the F2 pups. Additional information provided by the registrant in response to several questions by the EPA reviewers of the study clarified the pup survival issue. A review of these responses (HED document no. 011019) considered the apparent decreased F1 pup survival at all dose levels as not real because there was a strong litter effect in the data and the registrant based the statistical

analysis on the fetus. Additional statistical analysis on pup mortality by litter was significant only at the HDT. The offspring toxicity **LOAEL** is 80 mg/kg/day based on pup mortality from birth to day 4 and the **NOAEL** is 20 mg/kg/day.

This reproductive study in rats was initially classified **supplementary** (HED document no.010278) due to several questions by the reviewers regarding historical control data, environmental conditions, mating rational, additional data on F1 males, homogeneity and stability of the test material in the diet and data clarifications. The registrant's responses were found acceptable (HED document no.011019) and the study was upgraded to **Acceptable** for a guideline [OPPTS 870.3800;(83-4)] study in the rat.

11. Reproduction Study - Rat

Executive Summary: In a 3-generation reproduction study (Accession No. 097233 and amendment MRID 41026604) imazalil (98.8%) was administered in the diet to 10 male and 20 female Wistar rats per dose at approximately 0, 50, 200 or 800 ppm at the time of mating and continuously there after. The mating ratio was 1:2 for the F0 and 1:3 for the subsequent generations. Two litters per generation were produced. During the first mating, only 40% of the high dose females delivered viable litters. However, this finding was considered incidental since 100% of the dams of this dose group produced a normal number of litters from the second mating.

No treatment related effects were noted in the parental as well as developmental parameters measured. These included body weight of dams, food consumption, fertility, number of litters, litter size, pup viability, pup body weight or malformations, with the exception of the results of the first mating in the first generation at the high dose.

LOAEL for parental (systemic and reproductive) as well as developmental toxicity is > 800 ppm based on lack of effect in parameters measured. The **NOAEL** is ≥800 ppm.

The original review of the study (HED # 000065) classified this study as core minimum (Acceptable). Reevaluation of the study (HED # 007865) determined the study contained many deficiencies and was downgraded to **supplementary**. Therefrore this study is classified **Unacceptable** for a reproduction guideline study [OPPTS 870.3800;(83-4)].

12. Bacterial Reverse Mutation Assay

Executive Summary: In a bacterial reverse mutation Ames test (MRID 40729301), Salmonella strains were exposed to seven concentrations of Imazalil (dissolved in dimethylsulfoxide) ranging from 5-500 µg/plate (3 plates/dose) both in the presence and absence of a rat metabolic activation system.

Imazalil up to toxic concentrations of 250-500 µg/plate was found to be negative for increasing

reversion to his$^+$ under the conditions of this test.

This bacterial reverse mutation study is classified as **Acceptable** and satisfies the guideline requirement [870.5100(84-2)].

13. *In vitro* Mammalian Chromosome Aberration - Human Lymhocytes

Executive Summary: In an *in vitro* human lymphocytes chromosome aberration study (MRID 40729302), imazalil was incubated in human peripheral blood lymphocytes (from human donors) at 0 (DMSO Solvent), 50, 200, 400 or 800 µg/culture (22.73-909.09 µg/mL), as well as 5 or 10 µg/culture of positive control agent mitomycin for the inactivated system or 500 or 1000 µg/culture of positive control agent cyclophosphamide in the presence of an S9 activation system. Two cultures/treatment/donor were established. Fifty metaphase/donor/treatment/dose were examined and structural chromosome aberrations, simple breaks, complex chromatid exchanges were scored on coded slides by different observers.

There were no increased chromosomal aberrations in any of the imazalil lymphocyte treated culture. According to the study authors imazalil was negative in inducing chromosomal aberrations in human lymphocytes. The study was initially classified as **Unacceptable** due to major reporting deficiencies which were subsequently satisfied by the registrant(HED # 007865 and the study was upgraded to **Acceptable** satisfying the guideline requirement [870.5375 (84-2)] for an in vitro mammalian chromosome aberration assay.

14. Micronucleus Test - Mice

Executive Summary: In a mutagenicity Micronucleus Test in Mice (MRID 40729303), 12 groups of 5 animals/sex each were given the test article (suspended in propylene glycol) at single oral doses of 0, 20, 80, or 320 mg/kg, and groups at each level sacrificed 24, 48 or 72 hours post-dose and a positive control (cyclophosphamide, 40 mg/kg) group sacrificed after 48 hours. The doses were selected after a preliminary toxicity test with imazalail was run at 160, 320, or 640 mg/kg doses. Immediately after sacrifice, femoral bone marrow from each animal was processed for microscopy according to standard procedures.

In the preliminary test, all the 640 mg/kg group animals died. One male of the 320 mg/kg group died after hours. All survivors in both the low and mid dose groups except one animal lost weight (2-3.7 gm) and manifested a dose-related decrease in polychromatic erythrocyres/normochromatic erythrocytes (PCE/NCE) ratios.

In the main study at the high dose 2 females of the 24 hour sampling time, 1 male and 3 females of the 48-hour sampling time and 3 males and one female of the 72-hour sampling time died before their respective sacrifice. All the low and mid dose animals survived the test periods. Body weights of high dose animals were significantly reduced compared to controls ($p < 0.01$) at the 48 and 72 hour sampling time. In the surviving high dose animals hematopoiesis was

depressed ($p<0.01$-0.001) 48 and 72 hours after dosing.

No increase in micronucleated PCEs over solvent control was found in any of dose group sacrificed at any sampling time. A significant increase ($p<0.001$) in micronucleated PCEs was recorded in the positive control group. The study demonstrates that the test material, imazalil, was negative for micronucleus induction in mice treated with acute oral doses up to toxic levels (320 mg/kg) causing clinical and hematopoietic toxicity.

The study is **Acceptable** and satisfies the guideline requirement [970.5395 (84-2)].

15. Micronucleus Test - Rats

Executive Summary: In a micronucleus assay in rats (Accession No. 00031599), four groups of male rats (number/group not specified in DER) were admintered intraperitoneally the test article at 0, 10, 40, or 160 mg/kg at 30 hours and 6 hours prior to the preparation of the bone marrow. A total of 2,500 erythrocytes per animal were screened for the presence of micronuclei. Structural chromosomal aberrations were evaluated by the enumeration of the micronuclei in polychromatic and normo-chromatic erythrocytes. A positive control group of male rats were dosed I.P. with 40 mg/kg cyclophosphamide.

The rate of micronucleated polymorphic cells in the negative control and the three Imazalil treated groups were considered normal spontaneous rates. In the cyclophosphoamide positive control rats there was an increase in the number of micronucleated erythrocytes as a result of chromosome breakages.

It was concluded that the test material Imazalil R 23979, administered intraperitoneally, was not mutagenic at the doses tested.

This study is classified **Acceptable** and satisfies the guideline requirement [870.5395 (84-2)]

16. Dermal absorption - Rats

Executive Summary: In a dermal absorption study (MRID 42913401), young adult male Wistar rats (4/dose/exposure duration) received applications of ^{14}C- Imazalil EC formulation on a 12 cm^2 shaven dorso-lumbar area at 0.004, 0.04, 0.4 or 4.0 mg/cm^2 for durations of 0.5, 1, 2, 4, 10 or 24 hours. Rats were housed individually in stainless steel cages where urine and feces were collected separately. At the end of the exposure period, rats were anesthetized and 3 ml of blood colleted from the orbital plexus. Samples analyzed for radioactivity were skin wash, skin of application site, blood, carcass, urine, feces, and cage wash.

Blood concentation of ^{14}C- Imazalil radioactivity increased with increasing dose. At 10 hours 41%, 25%, 17% and 26% and at 24 hours 47.93, 39.39, 30.92, and 29.23% of the applied doses were absorbed at doses of 0.004, 0.04, 0.4 or 4.0 mg/cm^2, respectively.

The study was classified **Acceptable** and satisfies the guideline requirement [(870.7600 (85-3)] for a dermal absorption study.

17. Metabolism Study - Rat

Executive Summary: In a metabolism study (MRID 42012003), the absorption, distribution, metabolism and excretion of Imazalil labeled with ^{14}C at the 2-ethyl position was investigated in groups of Wistar rats administered a single intravenous (i.v.) dose of 1.25 mg/kg, a single oral gavage dose of 1.25 mg/kg or 20 mg/kg, or 14-day repeated oral doses of 1.25 mg/kg unlabeled imazalil followed by a single dose of 1.25 mg/kg ^{14}C-labeled imazalil on day 15.

^{14}C-imazalil was rapidly absorbed, distributed, almost completely metabolized and eliminated in rats under all dosing regimens. Recovered radioactivity in urine and feces was 83.9-93.9% after 24 hours and 86.6-98.3% of the administered dose (AD)after 96 hours. Female rats eliminated more of AD in the urine (55-60%) than in the feces; the difference was smaller in the males. Peak blood levels were not measured. ^{14}C tissue residues after 4 days were $\approx$1% of the AD irrespective of the dosing regimen. The largest residues were in the liver followed by lungs and kidneys and adrenal gland.

Parent compound was not detected in the urine. Less than 1% was detected in fecal extracts. Over 25 metabolites were detected in the 0-24 hour urine pools by radio HPLC. Metabolite patterns were quantitatively and qualitatively similar for both sexes and all dosage groups. Two metabolites identified as 3 and 4 were the most abundant representing 50% of the urine radioactivity. Metabolite 3 consisted of 2 metabolite subgroups, 3A and 3B (HED no. 011313 of MRID 43016803). Metabolite 3A was determined to be carboxylic acid form as 3-[1-(2,4-dichlorophenyl)-2-(1H-imidazol-1-yl)ethoxy]-2-hydroxypropanoic acid. Metabolite 3B was composed of 2 HPLC fractions MS 6 and MS 7, is an alanine conjugate of a carboxylic acid metabolite of metabolite 3A or metabolite 4. Metabolite 4 is composed of 2 HPLC fractions: MS 8 and MS 9, both carboxylic acid forms and equivalent to MS 5 (probably a diastereomer of metabolite 3A). Two metabolites 8 and 10 were tentatively identified by co-chromatography with reference compounds as (±)1-[2-(2,4-dichlorophenyl)-2-[(2,3-dihydroxypropyl)oxy)ethyl]-2,5-imidazolidinedione (R61000) and (±)1-[2-(2,4-dichlorophenyl)-2-[(2,3-dihydroxypropyl)oxy)ethyl]-1H-imidazole (R42243). Glucuronic acid and sulfate conjugates were not detected following incubation of urine samples with the proper enzymes. In the 24-48 hour urine less than 0.1% of the AD was found in any metabolite fraction.

Methanol extractable material accounted for 62% of the radioactivity in the feces from male rats and 70% for female rats. Over 25 metabolites were detected; less polar metabolites were more abundant in feces than in urine. Unchanged imazalil (0.6-1.0% of the AD) was found in feces from both sexes. Metabolites 8,9 and 10 were the most abundant accounting for a maximum of 3.4, 3.0 and 4.7% of the AD, respectively. These were tentatively identified as R61000, R42243 and R14821 (±)-alpha-(2,4-dichlorophenyl)-H-imidazole-1-ethanol , respectively.

A metabolic pathway was proposed based on the initial epoxidation of the allyl ring, followed by epoxide hydrolysis, imidazole oxidation, imidazole ring scission, N-dealkylation and oxidative dealkylation. A second metabolic pathway with O-dealkylation as the first step followed by imidazole oxidation, imidazole ring scission, and N-dealkylation.

The study was classified **Acceptable** and satisfies the guideline requirement for a metabolism study [870.7485 (85-1)] in the rat.

18. Acute Oral Toxicity

Executive Summary: In an acute oral toxicity study (Accession No. 00031596) inbred Wistar rats 10/sex/dose were administered imazalil base or one of its technical salts orally by gavage at 160, 320 or 640 mg/kg as an aqueous suspension at a volume of 1 ml/100 gm body weight. The rats were observed at regular intervals for 14 days for signs of toxicity and mortality.

The LD_{50} of compounds tested were (Toxicity Category II):

Imazalil base = 343 (262-448) mg/kg (M), 227 (174-297) mg/kg (F)
Imazalil sulfate = 355 (272-464) mg/kg (M), 309 (237-404) mg/kg (F)
Imazalil nitrate = 343 (262-448) mg/kg (M), 288 (221-297) mg/kg (F)
Imazalil acetate = 371 (284-485) mg/kg (M), 309 (237-404) mg/kg (F)

Study classified **Acceptable** for guideline OPPTS 870.1100 [§81-1].

19. Primary Eye Irritation

Executive Summary: In a primary eye irritation study [Accession No. 00031603], 0.1 ml of Imazalil technical grade, 97.1 % a.i., was instilled into the lower conjunctival sac of one eye of each of six rabbits. The opposite eye of each rabbit remained untreated and served as the control. Animals then were observed for 10 days. The DER does not specify the method of scoring.

Corneal opacity (Grade 2) was observed in all 6 animals at 24 hours. The corneal opacity persisted throughout the study (Grade 4) in 4/6 animals and (Grade 3) in 2/6 animals through day 10. Due to corneal opacity no scoring could be done on the iris. Severe conjunctival irritation persisted throughout the study. In this study, Imazalil technical is a **severe eye irritant.** Imazalil technical is placed in **Toxicity Category I** for primary eye irritation based on corneal opacity which was not reversible within the duration of the study of 10 days.

This study is classified as **Acceptable** and satisfies the guideline requirement for a primary eye irritation study OPPTS [870.2400 (§81-4)] in the rabbit.

20. Primary Dermal Irritation

Executive Summary: In a primary skin irritation study (Accession No. 00031604), 0.5 ml of the test material (97.10%) was applied to the intact and abraded skin of six albino rabbits weighing about 4 kg. The sample was introduced under a double gauze layer to an area of skin 1" x 1" square. The patches were then covered and maintained in contact with the skin for 24 hours. After 24 hours the patches were removed and the skin reactions were evaluated.

Primary skin irritation index was 1.75. Very slight to well defined erythema and very slight edema were noted at 24 and 72 hours. Imazalil is placed in toxicity Category III for primary dermal irritation. The study was classified as **Acceptable** [OPPTS 870.2500 (§81-5)].

21. Primary Eye Irritation

Executive Summary: In a primary eye irritation study [MRID 41606105], 0.1 g of Imazalil technical grade, 98.1 % a.i., was administered as a fine dust into the conjunctival sac of the left eye of three young adult female New Zealand White rabbits. Eyes were unwashed for at least 24 hours. Animals then were observed for 21 days. Irritation was scored by the Draize method .

Corneal opacity was observed in all 3 animals (grades 1 &2) through day 14 and persisted in 2 of 3 (grade 1) through day 21. Iritis (grade 1) was observed in all 3 animals by day 3 and persisted in 2 of 3 animals through day 7 and persisted in 1 animal through day 10. Redness was seen in all 3 animals through day 1 and persisted in 1 animal (grade 1) through day 4. Chemosis (grades 1 &2) was seen in all 3 animals by 24 hours, in 2 animals by 48 hours, and persisted in 1 animal (grade 1) through day 10. Discharge (grade 1) was seen in 1 animal from 48 hours through day 4. An average group irritation score of 28.9 was recorded indicating moderate eye irritation. In this study, Imazalil technical is an **eye irritant**. Imazalil technical is **Toxicity Category I** for primary eye irritation based on corneal opacity which was not reversible within 21 days.

This study is classified as **Acceptable** and satisfies the guideline requirement for a primary eye irritation study (81-4) in the rabbit.

22. Acute Dermal Toxicity

Executive Summary: In an acute dermal toxicity study (MRID 41606104), young Albino New Zealand White Rabbits (5/sex/group) received dermal application of Imazalil technical grade, (97.6% purity) at doses of 0 or 2000 mg/kg to at least 10% of the body surface under occlusive patch for 24 hours. Animals then were observed for 14 days.

In the imazalil treated group, 3 males and 3 females showed slight sedation on day 1 after exposure. The sedation was completely gone after day 1. From day 4 through day 13, all treated animals developed slight to moderate scaling. At the end of the study no visible skin changes were seen. All treated animals gained weight comparable to the control animals. At necrosy

slightly thickened skin area or slightly swollen and pale liver with pronounced lobulation were noted in 2 females of the treated group. Dermal LD_{50} Males = >2000 mg/kg (males and females).

Imazalil is **Toxicity Category III** based on the LD_{50} of >2000 mg/kg in males and females. This acute dermal study is classified as **Acceptable** and satisfies the guideline requirement for an acute dermal limit dose study [OPPTS 870.1200 (81-2)] in the rabbit.

23. Dermal Sensitization - Guinea Pigs

Executive Summary: In a dermal sensitization study (MRID 41718701) with imazalil technical grade (98.1% a.i.), adult male Pirbright Guinea pigs using the maximization test of Magnusson and Kligman were tested as follows: 1% imazalil in sesame oil in the intradermal induction phase, 10% imazalil in petrolatum in the epicutaneous induction phase and 5% imazalil in petrolatum in the challenge phase. There were two groups, imazalil-induced and vehicle induced, each consisted of 20 Guinea pigs.

All animals survived the treatment. The first intradermal induction produced slight necrosis at the injection site of all animals in both the vehicle and imazalil groups. The epicutaneous induction produced slight erythema in 13 and 15 animals of the vehicle and imazalil groups, respectively. All vehicle-induced animals were normal within 48-72 hours after the challenge phase. One animal of the imazalil induced animals had slight erythema following the challenge phase. The sensitization rate is 5%. Comparing these results with the results of a positive control study with 2,4-dinitro-1-chlorobenzene, it was concluded that **imazalil technical is a weak dermal sensitizer (grade I)**. This study is classified as **acceptable** and satisfies the guideline requirement for a dermal sensitization study [OPPTS 870.2600 (81-6)] in the Guinea pig.

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