



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

OFFICE OF
PREVENTION PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

DATE: February 9, 2000

SUBJECT: **Imazalil:** The HED Toxicology Chapter for the Reregistration Eligibility Decision Document (RED)
PC Code 111901, Case 816389

FROM: Abdallah Khasawinah, Ph.D. Toxicologist and Risk Assessor
Reregistration Branch 4
Health Effects Division (7509C)

THRU: Sanjivani Diwan, Ph.D. Senior Toxicologist
Reregistration Branch 4
Health Effects Division (7509C)
and
Susan V. Hummel, Ph.D. Branch Senior Scientist
Reregistration Branch 4
Health Effects Division (7509C)

TO: Dean Monos/ Betty Shackleford, PM #53
Reregistration Section
Special Review and Reregistration Division (7508W)

TASK ID: DP Code D258087, Submission S548748

Attached is the Toxicology Chapter for Imazalil, to support the Reregistration Eligibility Decision (RED).

TABLE OF CONTENTS

1.0	HAZARD CHARACTERIZATION	2
2.0	REQUIREMENTS	4
3.0	DATA GAP(S)	5
4.0	HAZARD ASSESSMENT	5
4.1	Acute Toxicity	5
4.2	Subchronic Toxicity	6
4.3	Prenatal Developmental Toxicity	9
4.4	Reproductive Toxicity	11
4.5	Chronic Toxicity	12
4.6	Carcinogenicity	13
4.7	Mutagenicity	17
4.8	Neurotoxicity	18
4.9	Metabolism	19
4.10	Special/Other Studies	21
5.0	HAZARD ENDPOINT SELECTION	24
5.1	See Section 9.2 for Endpoint selection table.	24
5.2	Dermal Absorption	24
5.3	Classification of Carcinogenic Potential	24
6.0	FQPA CONSIDERATIONS	25
6.1	Special Sensitivity to Infants and Children	25
6.2	Recommendation for a Developmental Neurotoxicity Study	26
7.0	OTHER ISSUES	26
8.0	REFERENCES	27
9.0	APPENDICES	34
9.1	Toxicity Profile Summary Tables	35
9.1.1	Acute Toxicity Table	35
9.1.2	Subchronic, Chronic and Other Toxicity Table	35
9.2	Summary of Toxicological Dose and Endpoints	40

1.0 HAZARD CHARACTERIZATION

The toxicological data base for Imazalil is partly adequate for hazard characterization. Data gaps exist for an acute, subchronic and developmental neurotoxicity studies in rats. The available studies are listed in Table 1.

Imazalil (1-[2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl]-1H-imidazole) is an imidazole fungicide intended for post harvest control of mildews on citrus fruits as well as barley and wheat seed treatment. It is also used in chicken hatcheries. Imazalil is known as Enilconazole in veterinary medicine. Imazalil inhibits cytochrome P-450 dependent biosynthesis of ergosterol, an essential component of the cell membrane of fungi.

Imazalil exists as a base or as its sulfate salt. Most of the toxicology studies were conducted on the Imazalil base. However, few studies were conducted on the sulfate salt. Acute toxicities of the base and the sulfate salt are comparable which lead to the conclusion that the Imazalil base and its sulfate salt are similar in their toxicological properties.

Imazalil is placed in Category II, III, and IV for oral, dermal and inhalation toxicity, respectively. It is highly irritating to rabbit eye (Category I), but is not a skin irritant (Category IV) or a dermal sensitizer. Imazalil (EC formulation) is readily absorbed by the rat skin with a 41 % absorption of the applied dose within 10 hours of application

The primary target organ for Imazalil toxicity in animals is the liver. Enlarged livers were seen in rabbits after 6 days of dermal application at 250 mg/kg/day (MRID 42085201), increased liver weights and liver to body weight ratios, increased centrilobular swollen hepatocytes and increased vacuolization in hepatocytes after one month of dietary treatment at 32.1 mg/kg/day in rats (MRID 43965704), and similar histopathologic effects in mice (at 38.6 mg/kg/day in the diet (MRID 43222601). In a chronic dietary rat study, there was an increased incidence of intra cytoplasmic inclusion bodies of hepatocytes, increased severity of hepatocyte vacuolization as well as bile duct proliferation at 15.5 mg/kg/day (MRID 47026101). Liver histopathologic lesions were also seen in a 23-month study in mice at 28.0 mg/kg/day (42972001). Increased liver vacuolization was also seen in male rats in a 2-generation reproduction study at 80 mg/kg/day (MRID 42570701 & 42949402). Increased liver weights were seen in dogs treated for one year at 20 mg/kg/day (41328802). The absolute and relative weight of thyroid glands was increased in male rats fed Imazalil for two years at ≥ 65.8 mg/kg/day (MRID 44858001). Microscopic changes were also seen in the affected thyroids.

The data submitted to the Agency as well as those from the published literature do not demonstrate increased sensitivity of rats, mice, or rabbits from *in utero* exposure to Imazalil. The developmental effects in fetuses occurred at or above doses that caused maternal toxicity. There appears to be increased susceptibility of the neonates to Imazalil postnatally. In the 2-generation reproduction study, an increased susceptibility of the pups to Imazalil was reported. The pup survival rate was adversely affected by Imazalil treatment from birth to post natal day 4

in the F2 generation. The HIARC determined that pup deaths resulted from an increased susceptibility to Imazalil from the milk intake during lactation.

Carcinogenicity studies in rodents indicate that Imazalil was carcinogenic to male Swiss albino mice and Wistar rats, based on significant increase in liver adenomas and combined adenomas/carcinomas. In rats there was also increased incidence of combined thyroid follicular cell adenomas/carcinomas. The HED CPRC (1994) and CARC (1998) classified Imazalil a Group C-carcinogen and recommended a linear low dose approach (Q_1^*) for quantification of human cancer risk. The CARC (1999) reclassified imazalil under the July 1999 Draft Guidelines for Carcinogenic Assessment in the category "**Likely to be carcinogenic in humans**". The Committee reaffirmed its earlier decision by recommending a linear low-dose (Q_1^*) extrapolation for quantification of human cancer risk. This extrapolation is supported by the lack of confirmation of the mode of action. The most potent unit risk, $Q_1^*(\text{mg/kg/day})^{-1}$ for imazalil based on male mouse liver adenoma and/or carcinoma combined tumor rates is 6.2×10^{-2} in human equivalents (HED Doc 013842).

Imazalil was non mutagenic both *in vivo* and *in vitro* mutagenicity assays.

2.0 REQUIREMENTS

The requirements (CFR 158.690) for IMAZALIL are in Table 1. Use of the new guideline numbers does not imply that the new (1998) guideline protocols were used.

Table 1. DATA REQUIREMENTS FOR IMAZALIL

Test	Technical	
	Required	Satisfied
870.1100 Acute Oral Toxicity	yes	yes
870.1200 Acute Dermal Toxicity	yes	yes
870.1300 Acute Inhalation Toxicity	yes	yes
870.2400 Primary Eye Irritation	yes	yes
870.2500 Primary Dermal Irritation	yes	yes
870.2600 Dermal Sensitization	yes	yes
870.3100 Oral Subchronic (Rodent)	yes	yes
870.3150 Oral Subchronic (Non-Rodent)	yes	yes*
870.3200 21-Day Dermal	yes	yes
870.3250 90-Day Dermal	no	-
870.3465 90-Day Inhalation	no	-
870.3700a Developmental Toxicity (Rodent)	yes	yes
870.3700b Developmental Toxicity(Non-rodent)	no	
870.3800 Reproduction	yes	yes
870.4100a Chronic Toxicity (Rodent)	yes	yes
870.4100b Chronic Toxicity (Non-rodent)	yes	yes
870.4200a Oncogenicity (Rat)	yes	yes
870.4200b Oncogenicity (Mouse)	yes	yes
870.4300 Chronic/Oncogenicity	yes	yes
870.5100 Mutagenicity—Gene Mutation - bacterial	yes	yes
870.5300 Mutagenicity—Gene Mutation - mammalian	yes	yes
870.5xxx Mutagenicity—Structural Chromosomal Aberrations	yes	yes
870.5xxx Mutagenicity—Other Genotoxic Effects	yes	yes
870.6100a Acute Delayed Neurotox. (Hen)	no	-
870.6100b 90-Day Neurotoxicity Hen)	no	-
870.6200a Acute Neurotox. Screening Battery (Rat)	yes	no
870.6200b 90 Day Neuro. Screening Battery (Rat)	yes	no
870.6300 Develop. Neuro	yes	no
870.7485 General Metabolism	yes	yes
870.7600 Dermal Penetration	yes	yes
Special Studies for Ocular Effects	no	-
Acute Oral (Rat)	no	-
Subchronic Oral (Rat)	no	-
Six-month Oral (Dog)	no	-

* The subchronic study is satisfied by the chronic study in dogs.

3.0 DATA GAP(S)

The toxicological data base for Imazalil is partly adequate for hazard characterization. Data gaps exist for an acute, subchronic and developmental neurotoxicity studies in rats. Based on neurobehavioral effects seen in pups in a published study (Tanaka, 1995) and discussed later, the HIARC determined that a Developmental Neurotoxicity study in rats is required for imazalil (HED Doc. # 013778). HIARC also determined that an Acute Neurotoxicity and Subchronic Neurotoxicity studies in adult rats are required based on the positive effects seen in the Tanaka study and for comparison to DNT results. HIARC also noted that the Tanaka study provides very useful information, but it does not meet the requirement for a DNT study.

4.0 HAZARD ASSESSMENT

4.1 Acute Toxicity

Adequacy of data base for acute toxicity: The data base for acute toxicity is considered complete. No additional studies are required at this time.

Table 2: ACUTE TOXICITY DATA ON IMAZALIL

Guideline Number and Study	MRID #	RESULT	CATEGORY
870.1100 Acute Oral Toxicity - Rat	00031596	LD50=227 - 343 mg/kg	II
870.1200 Acute Dermal Toxicity - Rabbit	41606104 44107213	LD50 ≥ 2 g/kg LD50 ≥ 2 g/kg	III
870.1300 Acute Inhalation Toxicity - Rat	41889802	LC50 ≥ 2.0 mg/L LC50=2.43mg/L	IV
870.2400 Acute Eye Irritation - Rabbit	41606105	Irritating	I
870.2500 Acute Dermal Irritation - Rabbit	41328801 44107216	Non-irritating Non-irritating	IV IV
870.2600 Skin Sensitization Guinea Pig	41718701 40271701	Non-sensitizer Non-sensitizer	NA NA

6

Imazalil is placed in Category II, III, and IV for oral, dermal and inhalation toxicity, respectively. It is highly irritating to rabbit eye (Category I), but is not a skin irritant (Category IV) or a dermal sensitizer. Imazalil (EC formulation) is readily absorbed by the rat skin with a 41 % absorption of the applied dose within 10 hours of application

The acute toxicity data on the Imazalil Technical is summarized in Table 2.

4.2 Subchronic Toxicity

Adequacy of data base for subchronic toxicity: The data base for subchronic toxicity is considered complete. No additional studies are required at this time.

870.3100 90-Day Oral Toxicity - Rat

In a 90-day feeding study in rats, technical grade Imazalil base was administered in the diet to groups of 20 male and 20 female SPF Wistar rats at dose levels of 0, 200, 400 or 800 ppm. Ten animals/sex/dose group were sacrificed and examined at 1 month (interim sacrifice) and 10 animals/sex/dose group at 3 months (terminal sacrifice). For the rats sacrificed at 1 month, the mean intake of test material was 0, 20.7, 42.0 and 82.2 mg/kg/day for males and 0, 22.3, 44.6 and 90.1 mg/kg/day for females for the 0 (control), 200, 400 and 800-ppm groups respectively. For the rats sacrificed at 3 months, the mean intake of test material was 0, 15.8, 32.1 and 63.9 mg/kg/day for males and 0, 18.7, 37.9 and 76.4 mg/kg/day for females for the 0 (control), 200, 400 and 800-ppm groups respectively. No treatment-related effects on mortality or clinical signs were observed. It is likely that most, if not all, of the effects on the liver observed in this study were due to stimulation of the liver microsomal enzyme system as demonstrated in Part 3 of this study. **The LOAEL this study is 400 ppm (32.1 and 37.9 mg/kg/day in males and females respectively) and is based on increased absolute liver weights (9-15%) and liver/body weight ratios (9-15%) in males and females at 1 month, possibly increased absolute adrenal weights (15-23%) and adrenal/body weight ratios (15-27%) in females at 3 months, increased centrilobular swollen hepatocytes in males at 1 month, increased vacuolization in hepatocytes in females at 1 month and slightly swollen cortical cells in the adrenals of one 400 ppm and two 800 ppm female rats observed at the 3 month terminal sacrifice. The NOAEL in this study is 200 ppm (15.8, and 18.7 mg/kg/day in males and females respectively) (Acceptable/Nonguideline; MRID 43965704).**

In another 90-day feeding study in rats, technical grade Imazalil base was administered in the diet to groups of 10 male and 10 female Wistar (Hannover substrain) rats at dose levels of 0, 800, 1600, 2400 or 3200 ppm. The dose levels estimated by the study authors were: 0, 64.4, 129, 181 or 252 mg/kg/day in males and 0, 78.7, 150, 236 or 333 mg/kg/day in females. Livers and macroscopic lesions were examined for all animals in all treatment groups, but no other organs or tissues were examined histologically. Electron microscopy of liver sections was also conducted. No treatment-related effects on mortality or clinical signs were observed. Compared to control

7

values, dose-related decreased body weights and body weight gains were observed in treated animals of both sexes throughout the study at all dose levels. At 13-weeks, decreased body weights of 19% in males and 11% in females and decreased body weight gains of 26% in males and 19% in females were recorded in the high dose group. Actual food consumption measurements were severely compromised due to food wastage. Hematological parameters were not affected. Only minor changes were noted in clinical chemistry parameters. Microsomal protein content was statistically significantly increased in livers from male rats treated with 1600 and 2400 ppm Imazalil and in livers from female rats treated with 3200 ppm Imazalil. Hepatic cytochrome P-450 content was statistically significantly increased in males at all dose levels of Imazalil, but increases were not statistically significant in females. UDP-glucuronosyltransferase was statistically significantly increased in female rats, but not in male rats at 2400 and 3200 ppm. Several isoenzyme activities, including N-ethylmorphine – demethylase, 7-ethoxyresorufin O-deethylase, 7-pentoxyresorufin O-dealkylase and 7-ethoxycoumarin O-deethylase activities, were significantly induced in livers of both male and female rats at all dose levels tested. Further, an increase of aniline hydroxylase activity was observed in female rat livers at all dose levels. Imazalil was a mixed type of of microsomal enzyme inducer at all dose levels tested. Urinalyses were unremarkable. Gross necropsies at 3 months revealed increased incidences of dark colored livers and more pronounced lobulation in the liver, particularly in males at 2400 and 3200 ppm and in females at all dose levels. Relative liver/body weight ratios were increased in males at all dose levels. Histopathological examination revealed treatment-related mild hepatocellular hypertrophy in nearly all treated animals at all dose levels. Mild "fatty vacuolization" was observed in males at doses of 1600 ppm and higher and in females at all dose levels. The amount of stainable hepatocytic lipid increased in a dose-related manner in both sexes. **The LOAEL in this study is 800 ppm (grossly estimated by the study authors to be 64.4 and 78.7 mg/kg/day in males and females respectively and by the standard 0.05 conversion factor to be 40 mg/kg/day in both males and females),** and is based on possibly decreased body weights and body weight gains in males and females, possibly decreased triglyceride and phospholipid in males, dark and more pronounced lobulation of livers in females, possibly increased relative liver/body weight ratios in males, mild hepatocellular-hypertrophy in males and females, and mild fatty vacuolation, in the livers of females. NOAEL is < 800 ppm (64.4 and 78.7 mg/kg/day in males and females, respectively).

This study is classified as **Acceptable/non-guideline** It does not satisfy the requirement for a 90-day oral toxicity study in a rodent species since an insufficient number of organs/tissues were examined histologically. Livers and macroscopic lesions were examined for all animals in all treatment groups, but no other organs or tissues were histologically examined. This study may be upgraded if the full set of required organs/tissues is histologically examined (MRID 43965705).

870.3100 90-Day Oral Toxicity - Mouse

In a 3-month oral mechanistic toxicity study, Imazalil base (96.9%) was administered in the diet to groups of Swiss mice (25/sex/dose) 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. 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 enzyme 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 LOAEL 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 NOAEL in this study is 50 ppm (9.5 and 11.3 mg/kg/day in males and females respectively). (Acceptable/Nonguideline; MRID 43222601, 43292402)

870.3150 90-Day Oral Toxicity - Dog

This requirement was satisfied by the chronic study in dogs discussed later.

870.3200 21/28-Day Dermal Toxicity - Rat

In a 21-day dermal toxicity study, groups of 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. There were no treatment related effects attributed to imazalil exposure. A

LOAEL for systemic toxicity was not established in this main study. The doses in this study were based on a range finding study conducted 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 results of these two studies the **NOAEL** of Imazalil is 160 mg/kg/day and the **LOAEL** is 250 mg/kg/day based on changes in the liver. (Acceptable/Guiceline; MRID 42085201).

870.3465 90-Day Inhalation – Rat

This study is not required for Imazalil.

4.3 Prenatal Developmental Toxicity

Adequacy of data base for Prenatal Developmental Toxicity: The data base for prenatal developmental toxicity is considered complete. No additional studies are required at this time.

In developmental studies, there was no evidence of increased susceptibility of rat, rabbit, or mouse fetuses to *in utero* exposure in developmental studies. The effects observed in these species occurred at or above maternally toxic doses (maternal LOAEL/NOAEL in Rat: 40/<40 mg/kg/day vs developmental 80/40 mg/kg/day ; Mice: maternal 40/10 mg/kg/day vs developmental 120/80 mg/kg/day; Rabbit: maternal and developmental 10/5 mg/kg/day, respectively).

870.3700a Prenatal Developmental Toxicity Study - Rat

In a developmental toxicity study, 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. **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 effects reported in the high dose group included 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. The developmental toxicity **LOAEL** is 80 mg/kg/day, based on decreased mean fetal weights. The developmental toxicity **NOAEL** is 40 mg/kg/day.

(Acceptable/guideline; MRID 41026603)

870.3700b Prenatal Developmental Toxicity Study - Rabbit

In a developmental toxicity study, 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**. (Acceptable/guideline, MRID 42593601).

870.3700b Prenatal Developmental Toxicity Study - Mouse

In a developmental toxicity study, imazalil sulphate (99.5% a.i.) was administered by gavage at 0, 10, 40, 80, or 120 mg/kg/day to pregnant mice (30 females/dose) on gestation days (GD) 6-16. Dams were sacrificed on GD 19. At 40 mg/kg, maternal toxicity was characterized by decreased body weight gains during-treatment (\downarrow 13%, GD 6-17) and reduced corrected body weight gains (\downarrow 23%, $p < 0.05$). The decrease in body weight gain continued during the post-treatment period. At 80 mg/kg, maternal toxicity was manifested by the following: death of four dams during treatment (GDs 8-17); reduced mean body weights on GDs 17 and 19 (\downarrow 10-11%, $p < 0.01$); reduced mean body weight gains (\downarrow 22%, days 6-17, $p < 0.05$); decreased gravid uterine weights (\downarrow 18%, not statistically significant); reduced corrected body weight gains (\downarrow 20%, $p < 0.05$); and reduced food consumption (\downarrow 10-15%, GDs 6-18, $p < 0.05$ or 0.01). At 120 mg/kg, maternal toxicity was characterized by the following: death of ten dams during the administration period (days 8-17, $p < 0.05$); clinical signs of toxicity such as, tremors (1 female), prostration and hypothermia (3 females each), convulsions (4 females), excitability (5 females), and piloerection (6 females, $p < 0.05$); reduced mean body weights on days 17 and 19 (\downarrow 25-27%, $p < 0.001$); decreased gravid uterine weights (\downarrow 48%, $p < 0.001$); reduced mean body weight gains (\downarrow 42-59%, days 6-19, $p < 0.05$ or 0.001); reduced corrected body weight gains (\downarrow 54%, $p < 0.001$); reduced food consumption (\downarrow 19-21%, days 6-18, $p < 0.01$ or 0.001). **The maternal toxicity LOAEL is 40 mg/kg/day, based on reduced body weight gains, and reduced corrected body weight**

gains. The maternal toxicity NOAEL is 10 mg/kg/day. The developmental toxicity observed at 120 mg/kg/day was manifested by increased number of resorptions ($p < 0.05$), resorptions/dam ($p < 0.05$), and postimplantation loss resulting in reduced litter size. These findings were noted in an earlier range finding study. The developmental toxicity **LOAEL** is 120 mg/kg/day, based on increased resorptions. The developmental toxicity **NOAEL** is 80 mg/kg/day. (Acceptable/Guideline, MRID 44578201)

In another developmental toxicity study in mice, imazalil sulphate (98.2% a.i.) was administered by gavage at 0, 10, 40, 80, or 120 mg/kg/day to pregnant mice (30 females/dose) on gestation days (GD) 6-16. Dams were sacrificed on GD 19. The maternal toxicity **LOAEL** is 40 mg/kg/day, based on mortality, slightly decreased food consumption and uterus weight. The maternal toxicity **NOAEL** is 10 mg/kg/day. These effects were more prominent at the higher doses in addition to reduced body weight and body weight gain. There was slight developmental toxicity observed at 40 mg/kg/day manifested by increased resorptions, and reduced litter size. The developmental toxicity **LOAEL** is 40 mg/kg/day, based on increased resorptions. The developmental toxicity **NOAEL** is 10 mg/kg/day. (Acceptable/Guideline, MRID 44567802).

4.4 Reproductive Toxicity

Adequacy of data base for Reproductive Toxicity: The data base for reproductive toxicity is considered complete. No additional studies are required at this time.

Qualitative evidence of increased susceptibility was found following pre-/postnatal exposure to Imazalil in the 2-generation study in rats described below. In this study, the offspring toxicity (pup mortality from birth to day 4) was seen in the presence of minimum maternal toxicity (decreases in body weight/body weight gain and increased liver vacuolation) at the same dose.

870.3800 Reproduction and Fertility Effects - Rat

In a 2-generation reproduction study, imazalil ($\geq 95.0\%$) 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). Only one litter per generation was produced. The parental toxicity **LOAEL** is 80 mg/kg/day based on body weight and body weight gain decreases and increased liver vacuolation in males. The parental toxicity **NOAEL** is 20 mg/kg/day. The reproductive toxicity **LOAEL** is 80 mg/kg/day and the **NOAEL** is 20 mg/kg/day based on the increased duration of gestation for the P0 and F1 females. **Offspring toxicity** consisted of 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 high 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**, even though all responses were not adequate, for a guideline (83-4) study for effects on reproduction in the rat. (MRID# 42570701 & 42949402).

However in the 2-generation reproduction study discussed above, an increased susceptibility of the pups to Imazalil was observed. The parental systemic toxicity **NOAEL/LOAEL** was 20/80 mg/kg/day, respectively. The offspring toxicity **NOAEL/LOAEL** was also 20/80 mg/kg/day, respectively. However, the pup survival rate was adversely affected by the Imazalil in the F2 generation from birth to post natal date 4. The data in the study did not indicate when pup deaths occurred. In the absence of such data, it was assumed that pups were dying as a result of increased susceptibility to Imazalil from the milk intake during lactation. Further more though the study was upgraded to acceptable, it had a number of unanswered deficiencies minimizing confidence in it. These included poor formulation of the test material into the diet, incomplete homogeneity and stability analytical data, deficient historical control data, incomplete characterization of environmental conditions of the study, incomplete body weight data for the F1 males. The HIARC had uncertainty about the accuracy of the dose levels administered.

4.5 Chronic Toxicity

Adequacy of data base for chronic toxicity: The data base for chronic toxicity is considered complete. No additional studies are required at this time. The chronic toxicity of Imazalil was investigated in rats, mice and dogs. The primary target organ of toxicity was the liver and thyroids. In rats there was an increased incidence of intracytoplasmic inclusion bodies of hepatocytes, increased severity of hepatocyte vacuolization as well as bile duct proliferation at 15.5 mg/kg/day dose. Imazalil caused increased thyroid weight (absolute and liver) in male rats at 65.8 mg/kg/day as well as microscopic changes. Liver histopathologic lesions were also seen in a 23 month study in mice at 28.0 mg/kg/day. Increased liver weights were seen in dogs treated for one year with Imazalil at 20 mg/kg/day. At this dose, Imazalil also caused vomiting and soft

stools; depressed body weight gains and increased alkaline phosphatase activity in dogs.

870.4100a (870.4300) Chronic Toxicity – Rat

This is discussed in the carcinogenicity study section.

870.4100b Chronic Toxicity - Dog

In a chronic toxicity study, imazalil base technical ($\geq 97.2\%$ 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 were reported only in the high dose 20 mg/kg/day group. These were increased vomiting, salivation, wasting of food and soft stools. 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. Food consumption was not recorded. There were no abnormal ophthalmological findings. Electrocardiograms and heart rates were within normal range in all treatments. 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. (Acceptable Guideline; MRID 41328802)

4.6 Carcinogenicity

Adequacy of data base for Carcinogenicity: The data base for carcinogenicity is considered complete. No additional studies are required at this time.

The carcinogenic potential of Imazalil was investigated in mice and rats. CPRC (1994) and CARC (1998) concluded that administration of Imazalil in the diet to Swiss albino mice resulted in statistically significant increases in liver adenomas and adenomas/carcinomas in male 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. Information from structural analogs of Imazalil (etaconazole, uniconazole, cyproconazole;

tebuconazole) which also induce tumors at the same site (liver) in mice, provided additional support to classify Imazalil as a Group C carcinogen.

In male rats, Imazalil caused increases in hepatocellular adenomas and combined liver adenomas/carcinomas along with an increased incidence of combined thyroid follicular cell adenomas/carcinomas.

Based on the findings in mice and rats, CARC (1999) classified Imazalil in the category "**likely to be carcinogenic to humans**".

870.4200a Carcinogenicity Study - rat

The combined results of an 18-month chronic study and a 30-month chronic/carcinogenicity study in rats are presented in this review. In the 30 month carcinogenicity study, 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 treated under the same dosing regimen. A previous 6-month study (Accession No. 00162411) was also conducted using the same dose levels. Losses in body weight gains were reported in females (-17 % in the 18-month study and -3.4% after 78 weeks in the 30 month study) at the 400 ppm dose level. 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 intra cytoplasmic 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 related 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** and determined that the highest dose tested was not adequate enough for assessing the carcinogenic potential of imazalil in the rat. Therefore the study was **Unacceptable** for carcinogenic evaluation. 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) based on the liver effects and slight body weight gain reductions with a **NOAEL** established at 100 ppm (4.7 mg/kg/day in females and 3.7 mg/kg/day in males). (**Unacceptable**, MRID 47026101)

15

In another chronic toxicity/oncogenicity study, imazalil ($\geq 97.4\%$ a.i.) was administered in the diet to groups of 50 male and 50 female Hannover substrain (SPF) Wistar-derived rats at concentrations of 0, 50, 200, 1200, or 2400 ppm (equivalent to 0.0, 2.7, 10.8, 65.8, and 134.8 mg/kg/day for males and 0.0, 3.6, 14.6, 85.2, and 168.8 mg/kg/day for females) for two years. No treatment-related effects were observed on survival, clinical signs of toxicity, or the eyes of rats receiving any dose of the test material. In addition, no significant treatment-related effects were observed at 50 or 200 ppm test material for any parameter. Within one week, the body weight and body weight gain of male and female rats in the 1200 ppm and 2400 ppm groups were significantly decreased. The decrease in body weight of male rats remained consistent through the remainder of the study while that of females continued to decline. The food consumption and efficiency of male rats in the 1200 and 2400 ppm groups following the first 26 weeks of the study were not significantly affected, but were decreased for females throughout the study. Male and female rats in the 1200 ppm and 2400 ppm groups had slightly increased RBCs and slightly decreased mean corpuscular volume (MCVs) and mean corpuscular hemoglobin (MCHs) that reached statistical significance at most measurement intervals. The differences, however, were within 6% of controls and the historical control limits for rats of this age, sex, and species. No significant treatment-related effects of biological relevance were found for most clinical chemistry parameters. The absolute liver weight of male rats in the 2400 ppm group was increased while it was decreased in female rats. The associated relative liver weights of male and female rats in the 1200 and 2400 ppm groups were significantly increased 9-26%. In addition, the absolute and relative thyroid weights of male but not female rats in the 1200 and 2400 ppm groups were increased. The effect of treatment on the liver (males and females) and thyroid (males only) were confirmed microscopically, but had distinct sex-related etiologies. The incidence of clear cell and basophilic foci was equivocal while eosinophilic foci were significantly increased for male rats in the 2400 ppm group. In female rats of the 2400 ppm group, the incidences of clear cell and basophilic foci were significantly decreased but the incidence of eosinophilic foci was unaffected. Also, the incidence of hepatocyte fatty vacuolation was increased only in male rats of the 1200 ppm and 2400 ppm groups while the incidence of pigmentation was increased only in females of the 200, 1200, and 2400 ppm groups. In addition, the location of hepatocellular hypertrophy was distinctly different. Female rats in the 1200 and 2400 ppm groups had significant increases in centriacinar and periacinar hypertrophy while male rats only had centriacinar hypertrophy. Finally, the incidence of thyroid follicular cell hyperplasia was increased only in male rats of the 1200 and 2400 ppm groups. The LOAEL for male and female rats was 1200 ppm (65.8 and 85.2 mg/kg/day, respectively) with a corresponding NOAEL of 200 ppm (10.8 mg/kg/day for males, 14.6 mg/kg/day for females). These are based on the effects found on body weight, weight gain, and the macro- and microscopic effects noted in the liver of all rats and the thyroid of male rats.

Based on HED's assessment (Brunsman, 1999), rats had significant differences in the pair-wise comparison of the 2400 ppm dose group with the controls for hepatocellular adenomas, and combined adenomas/carcinomas, both at $p < 0.05$. The incidence of adenomas exceeded the historical control range (adenomas: 6%-10%; carcinomas: 0%-4%) and the increase in the

combined incidence was driven by the adenomas. There were also significant increasing trends for hepatocellular adenomas and combined adenomas/carcinomas, both at $p < 0.01$. In addition there were significant differences in the pair-wise comparisons of the 1200 and 2400 ppm dose groups with the controls, for combined thyroid follicular cell adenomas/carcinomas, at $p < 0.05$. The incidence of these tumors at 1200 and 2400 ppm exceeded the range for the historical controls (adenomas: 6%-16%; mean 11.5%; carcinomas: 0-4%; mean 2% and combined: 6%-16%; mean 12.5%). The increased incidence of thyroid adenomas at 50 and 200 ppm was not considered by the CARC to be biologically significant because of lack of dose response. There was also a significant ($p < 0.05$) increasing trend for the combined thyroid follicular adenomas/carcinomas. There were no compound related increases in tumors in female rats. (Acceptable /guideline; MRID 44858001)

870.4200b Carcinogenicity (feeding) - Mouse

In a 23-month carcinogenicity study, 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, 7, 28, or 88 mg/kg/day for males and 0, 8, 35, 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 cellular 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). (Acceptable guideline; MRID 42972001)

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

17

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 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 Swiss albino mice resulted in statistically significant increases in liver adenomas and adenomas/carcinomas in male mice, with a positive trend for adenomas, carcinomas and combined adenomas/carcinomas. The increase in carcinomas, while not statistically significant by pair-wise comparison with controls, was considered by the CPRC to be biologically significant (carcinomas contributed equally to the total response and there was an apparent progression of benign to malignant tumors). Furthermore the incidence of carcinomas exceeded that of the historical controls submitted by the registrant. In female mice there was only a statistically significant positive trend for liver adenomas and combined adenomas/carcinomas, but the CPRC considered that the tumor response in females was supportive of that seen in males, even though driven mainly by the adenomas. It was also noted that tumors in the mouse appeared at a dose which was not particularly high. Information from structural analogs of Imazalil (etaconazole, uniconazole, cyproconazole, tebuconazole) which also induce tumors at the same site (liver) in mice, provided additional support to classify Imazalil as a Group C carcinogen.

4.7 Mutagenicity

Adequacy of data base for Mutagenicity: The data base for Mutagenicity is considered adequate based on pre 1991 and 1991 mutagenicity guidelines.

Imazalil was non mutagenic in both *in vivo* and *in vitro* assays.

Gene Mutation

<p>GLN 870.5100, MRID 40729301 Acceptable, pre 1991 guidelines</p>	<p>5-500 µg/plate, 3 plates/dose. Salmonella typhimurium strains ,imazalil (dissolved in dimehtylsulfoxide). Imazalil was negative up to cytotoxic doses (250-500 ug/plate ±S9)</p>
--	---

Cytogenetics

18

<p>GLN 870.5375 , MRID 40729302 HED 006818) Acceptable, pre 1991 guidelines</p>	<p>In an in vitro human lymphocytes chromosome aberration study, imazalil did not result in any increased chromosomal aberrations at concentrations ranging from 23 to 909 µg/ml.</p>
---	---

Other Genotoxicity

<p>GLN 870.5300, MRID 43735003 Acceptable, pre 1991 guideline</p>	<p>In an in vitro cytogenetic study in mammalian cells (Chinese hamster V79 cells), imazalil, at doses ranging from 10 to 100 µg/ml, was not mutagenic both with and without activation</p>
---	---

<p>GLN 870.5395 , MRID 40729303, HED Doc. 006818 Acceptable. Pre 1991 guideline</p>	<p>In a mutagenicity Micronucleus Test in mice, oral administration of imazalil (0, 20, 80 or 320 mg/kg) did not result in any increase in micronucleated polychromatic erythrocytes</p>
<p>GLN 870.5395 , MRID 00031599, HED Doc. 000057 Acceptable. Pre 1991 guideline</p>	<p>In a mutagenicity Micronucleus Test in rats, imazalil did not result in any increase in micronucleated polychromatic erythrocytes in any of the intraperitoneally doses tested (0, 20, 40 or 160 mg/kg</p>

<p>GLN 870.5500, MRID 43965702 Acceptable. 1991 guideline</p>	<p>In an in vivo/in vitro unscheduled DNA synthesis assays in mice administered single oral doses of imazalil of 125 or 250 mg/kg, imazalil was negative for genotoxicity but positive for cellular proliferation when tested at overtly toxic (250/ mg/kg; mortality 29/54) and cytotoxic doses</p>
<p>GLN 870.5500, MRID 43780201 Acceptable pre 1991 guideline</p>	<p>In an unscheduled DNA synthesis (UDS) assay in rat hepatocytes imazalil at concentrations ranging from 0.09 to 9.0 µg/ml did not cause increased UDS</p>

4.8 Neurotoxicity

Adequacy of data base for Neurotoxicity: Imazalil is not an organophosphate pesticide. Therefore, delayed neurotoxicity testing in hen is not required. However, HIARC determined that acute neurotoxicity testing is required for imazalil.

870.6100 Delayed Neurotoxicity Study - Hen

This is not required for Imazalil.

870.6200 Acute Neurotoxicity Screening Battery

This is a data gap for Imazalil.

870.6200 Subchronic Neurotoxicity Screening Battery

19

This is a data gap for Imazalil.

870.6300 Developmental Neurotoxicity Study

This is a data gap for Imazalil.

4.9 Metabolism

Adequacy of data base for metabolism: The data base for metabolism is considered to be complete. No additional studies are required at this time.

¹⁴C-imazalil was rapidly absorbed, distributed, almost completely metabolized and eliminated in rats following single oral or multiple oral doses and single i.v. dosing. 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. The similar excretion and metabolites pattern following oral and i.v. dosing indicated that imazalil was efficiently absorbed. Biliary excretion was suggested by the high degree of fecal elimination in the i.v. dosed group. ¹⁴C tissue residues after 4 days were ≈ 1% of the AD irrespective of the dosing regimen. 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. Metabolites were characterized and identified. 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.

870.7485 Metabolism - Rat

In a metabolism study (MRID 42012003), the absorption, distribution, metabolism and excretion of Imazalil labeled with ¹⁴C 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 ¹⁴C-labeled imazalil on day 15.

¹⁴C-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. The similar excretion and metabolites pattern following oral and i.v. dosing indicated that imazalil was efficiently absorbed. Biliary excretion was suggested by the high degree of fecal elimination

in the i.v. dosed group. Peak blood levels were not measured.

¹⁴C tissue residues after 4 days were \approx 1% of the AD irrespective of the dosing regimen. Tissue residues were proportional to the AD and were not identified. 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. Ten metabolites or metabolite fractions (representing about 30% of the AD) were chosen for mass balance determination on the basis of their abundance or their co-elution with reference compounds. In both sexes of all dosing groups two metabolites referred to as metabolites 3 and 4 were the most abundant representing 50% of the urine radioactivity (females in the single low oral and i.v. groups had higher levels than in the corresponding males). Two metabolites 8 and 10 were tentatively identified by co-chromatography with reference compounds as (\pm)1-[2-(2,4-dichlorophenyl)-2-[(2,3-dihydroxypropyl)oxy]ethyl]-2,5-imidazolinedione (R61000) and (\pm)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. No major dose-, route-, or sex-related differences in the metabolite patterns determined by HPLC were observed. 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 (\pm)-alpha-(2,4-dichlorophenyl)-H-imidazole-1-ethanol, respectively. No attempt was made to analyze glucuronic acid or sulphate conjugates in the feces.

Urinary metabolites 3 and 4 were further identified (HED Doc 011313; MRID 43016803). Metabolite 3 consisted of 2 metabolite subgroups, 3A and 3B. 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).

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. (Acceptable/Guideline , MRID 42012003, HED Doc 010485; MRID 43016803, HED Doc 011313)

870.7600 Dermal Absorption - Rat

In a dermal absorption study, young adult male Wistar rats (4/dose/exposure duration) received applications of ^{14}C - Imazalil EC formulation on a 12 cm² shaven dorso-lumbar area at 0.004, 0.04, 0.4 or 4.0 mg/cm² 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 collected from the orbital plexus. Samples analyzed for radioactivity were skin wash, skin of application site, blood, carcass, urine, feces, and cage wash.

Blood concentration of ^{14}C - Imazalil radioactivity increased with increasing dose but the pattern with time changed with the dose. At doses of 0.004 and 0.4 mg/cm², blood concentration peaked at 1-2 hours. At 0.4 mg/cm² it peaked at 1 hour and remained steady for three hours before it declined. At 4.0 mg/cm² it peaked at 10 hours of exposure and remained steady until sacrifice (24 hours). 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/cm², respectively (Acceptable/Guideline, MRID 42913401)

4.10 Special/Other Studies (Published Literature)

a. Acute Toxicity

Male ddY mice gavaged with 10 mg/kg Imazalil daily for three consecutive days exhibited enhanced cytochrome P450-catalysed ethoxyresorufin *O*-deethylase and pentoxyresorufin *O*-deethylase (PROD) activities in microsomes from small intestinal mucosa and liver, indicating the induction of cytochrome P450 subfamilies CYP1A and CYP2B (Muto et al., 1997). Immunochemical analysis also demonstrated enhanced expression of CYP2B, CYP2C and CYP3A subfamilies in both tissues. Imazalil was a potent inhibitor of cytochrome P450-dependent monooxygenase activities in microsomes from both tissues.

Male F344/DuCrj rats given single doses of 170, 255, or 340 mg/kg Imazalil by stomach tube exhibited dose-dependent decreases, statistically significant at the two higher doses ($p < 0.05$), in serum activity of aspartate aminotransferase 24 hours later (Nakagawa and Tayama, 1997). The highest dose level significantly ($p < 0.05$) decreased concentrations of serum lipids, cholesterol, triglycerides, and phospholipids.

In an in vitro study, Imazalil was added to rat hepatocyte suspensions at a concentration of 0.75mM. Within a three hour period acute cell death, accompanied by depletion of intracellular levels of glutathione and protein thiols, and a rapid depletion of cellular ATP were noted

(Nakagawa and Moore, 1995). Cell death was also accompanied by accumulation of intracellular malondialdehyde, indicating initiation of lipid peroxidation.

A 43-year old female veterinary technician developed an acute eczematous contact dermatitis of the hand and forearm two weeks after being treated for a dermatophytic skin infection with Imaverol® (a 0.2% solution of enilconazole) (Van Hecke and De Vos, 1983). Patch tests resulted in strong positive reactions to eniconazole and Imaverol®, and positive reactions to Econazole, Isoconazole, and Miconazole, other β -substituted 1-phenylethyl imidazoles used as antimycotics.

b. Subchronic/Chronic Effects

A 51-year old woman was treated for a chronic fungal infection of the nose, palate, and sinuses with Imazalil for six months (Stiller and Stevens 1986). Imazalil was initially applied topically twice daily, in lubricating jelly or polyethylene glycol-400, in increasing concentrations, up to 5 g/100 ml, when a burning sensation was noted. Local irrigation in saline was also given up to 4 times daily up to a dose of 0.4g/100ml, the maximum tolerated concentration. Oral administration of Imazalil dissolved in ethanol was initiated with 50 mg once daily, and gradually increased to 1200 mg once daily. At doses of ≥ 800 mg, nausea lasting several hours was noted, which prevented further elevation of the dose. A total of 170g of Imazalil had been given when treatment was stopped after six months. No apparent changes in clinical chemistry parameters were noted.

c. Carcinogenicity

Imazalil was found to be a tumor promoter in a rapid bioassay based on the induction of glutathione S-transferase placental (GST-P) form positive foci in rat liver. Rats were given a 200 mg/kg injection of diethylnitrosamine (DEN), followed two weeks later by a diet containing 1000 ppm Imazalil (for six weeks) and partial hepatectomy at week three. Numbers and areas of GST-P positive foci per unit area of liver were increased significantly ($p < 0.001$) in the group treated with DEN and then fed Imazalil compared with the group treated with DEN only (Hasegawa and Ito, 1992).

d. Developmental Neurotoxicity

In a published study on the reproductive and neurobehavioral effects of Imazalil (Tanaka 1995), Imazalil (99%) was administered to Crj:CD-1 mice (10/sex/group) at dietary doses of 0, 0.012, 0.024, or 0.048% (0, 120, 240, or 480 ppm) from 5 weeks of age of the F_0 generation to 9 weeks of age in the F_1 generation. Selected reproductive parameters (litter size, litter weight, and sex ratio) and neurobehavioral parameters (surface righting reflex, negative geotaxis, cliff avoidance, swimming behavior, olfactory orientation) were measured in the F_1 generation. Exploratory behavior (number of movements, total distance; number of vertical activities, vertical time,

number of turnings, average distance, average speed and number of defecations) was examined in the F₀ and F₁ generation offspring. **In the F₀ generation**, Exploratory behavior (number of movements, movement time, total distance and number of turnings) at 8 weeks of age was significantly increased in males of the high dose group. Number of vertical activities was significantly increased in the mid dose group, and number of defecations was increased in the low dose group. Females were not affected. Average body weights were not affected by treatment during the preconception, gestation and lactation periods. **In the F₁ generation**, there were no significant adverse effects observed in litter size, litter weight and sex ratio at birth. The average body weight of offspring during the early lactation period was significantly decreased in the high and mid dose groups of both males and females. With regard to neurobehavioral effects, surface righting reflex in all treated females, in the high dose male offspring group on post natal day (PND) 4 and in the mid dose group on PND 7 was significantly affected in a dose related manner. Swimming behavior of head angle in the high dose males and females at PND 4 was significantly affected in a dose related manner. Other neurobehavioral parameters were not affected. The number of turnings (exploratory behavior) in female offspring was significantly increased in the mid dose group, the other groups showed an increase compared to controls. Other exploratory behavior parameters were not affected in males or females. There were some significant effects on multiple water T-maze performance in females, but not in males. By week 8 there were no effects exploratory behavior in either sex. These results suggest that neurobehavior can be adversely affected in (mice) exposed during development to Imazalil in their diet.

In another published study (Mason *et al* 1987), it was found that Imazalil and other Imidazole antimycotics are selective inhibitors of steroid aromatization and progesterone hydroxylation with imazalil being a potent inhibitor of progesterone 21-hydroxylase.

5.0 HAZARD ENDPOINT SELECTION

On June 15 and 22, 1999, the Health Effect Division's (HED) Hazard Identification Assessment Review Committee (HIARC) reviewed the toxicology database for imazalil and selected doses and toxicology endpoints for risk uncertainty factors and margins of exposures for dietary and non-dietary risk assessments assessment, based solely on **animal toxicity studies**. **The June HIARC report supersedes all other reports (RfD, TES, HIARC, etc) for imazalil.** (HED Doc. No. 013539). Table 3 provides a summary of Toxicology Endpoint Selection.

5.1 See Section 9.2 for Endpoint selection table.

5.2 Dermal Absorption

Dermal Absorption Factor: 41 %

The committee determined (June 15, 1999) that a absorption factor of 41% based on a dermal absorption study (MRID 42913401).

The dermal absorption factor is required for intermediate and long term dermal risk assessment since oral doses were selected for these exposure periods. Dermal absorption factor is not required for short term dermal exposure risk assessment since a dermal dose from a 21-day dermal toxicity study was selected for this time period.

5.3 Classification of Carcinogenic Potential

5.3.1 The HED carcinogenicity Peer Review Committee (CPRC) met on August 24, 1994 and classified Imazalil as a group C (possible human carcinogen) and recommended a linear approach for quantification of risk (see memo dated December 22, 1994). This classification was based on results of a 1993 mouse carcinogenicity study discussed above. **The HED Cancer Assessment Review Committee (CARC)** reevaluated the carcinogenic potential of Imazalil on September 4, 1998 (HED document no. 012870) in light of the new data submitted by the registrant on the above mouse study, mechanistic data in 90-day rat feeding studies, 6-month interim report on an ongoing 2-year rat combined chronic/carcinogenicity feeding study and new mutagenicity studies. The CARC 1998 report reaffirmed the original conclusions of the 1994 CPRC report.

On October 27, 1999, the CARC met to evaluate the new rat chronic/carcinogenicity study discussed above. The CARC concluded that Imazalil was carcinogenic to male rats because: 1) There was significant increase by pair-wise comparison of the 2400 ppm group with the controls for hepatocellular adenoma and combined adenomas/carcinomas. The incidence of adenomas was within the range for the historical controls and the combined incidence was driven by the adenomas. 2) There were also significant increasing trends for hepatocellular adenomas and combined adenomas/carcinomas. and 3) There was significant increase by pair-wise comparisons of the 1200 and 2400 ppm groups with the controls for thyroid follicular cell combined adenomas/carcinomas. There was also a significant increasing trend for thyroid follicular cell combined adenomas/carcinomas. No increase in liver and thyroid tumors was noted in females. The dosing at the highest dose in both sexes was considered to be adequate and not excessive based on decrease in body weight gains and non-neoplastic changes in the liver in both sexes and thyroid in males. The CARC considered the liver and thyroid tumors in males to be treatment-related.

5.3.2 Classification of Carcinogenic Potential

Under the Draft Guidelines for Carcinogen Risk Assessment (July, 1999), Imazalil is classified in the category "**Likely to be carcinogenic in humans**". The CARC's decision was based on the following:

1. There was increase in hepatocellular adenomas and combined liver adenomas/carcinomas in **male** Swiss albino mice and Wistar rats. In male rats, there was also increased incidence of combined thyroid follicular cell adenomas/carcinomas.
2. Imazalil was **non mutagenic** in *in vitro* and *in vivo* mutagenicity assays. Imazalil is structurally related to triazole compounds which have been shown to be both hepatocarcinogens in mice.

5.3.3 Quantification of Carcinogenic Potential

The Committee recommended a linear low-dose (Q_1^*) extrapolation approach for the quantification of human cancer risk based on the most potent liver tumors in mice. This approach is supported by the lack of confirmation of the mode of action. The most potent unit risk, Q_1^* (mg/kg/day)⁻¹ of those calculated for imazalil is that for male mouse liver adenoma and/or carcinoma combined tumor rates at 6.11×10^{-2} in human equivalents (HED Doc 013842: Lori Brunsman memo to Abdallah Khasawinah, October 27, 1999). The CARC's 1999 evaluation supersedes earlier evaluations.

6.0 FQPA CONSIDERATIONS

6.1 Special sensitivity to Infants and Children

The FQPA Safety Factor Committee met on September 20, 1999 to evaluate hazard and exposure data for imazalil and recommend application of the FQPA Safety Factor (as required by Food Quality Protection Act of August 3, 1996), to ensure the protection of infants and children from exposure to imazalil. The Committee recommended that the FQPA safety factor for protection of infants and children (as required by FQPA) should be retained at 10x when assessing chronic dietary exposure and reduced to 3x when assessing acute dietary exposure to this pesticide (HED Doc. # 013762). The rationale for the retention of the 10x factor was:

- ▶ the toxicology database for imazalil is incomplete (acute, subchronic, and developmental neurotoxicity studies are required);
- ▶ there is qualitative evidence of increased susceptibility following pre-/postnatal exposure to imazalil in the 2-generation reproduction study in rats (developmental toxicity was seen in the presence of minimal maternal toxicity at the same dose); and
- ▶ there is concern for neurobehavioral effects in offspring following prenatal exposure to imazalil which were reported in a published literature study conducted in mice (Tanaka 1995).

6.2 Recommendation for a Developmental Neurotoxicity Study (DNT)

Based on the positive neurobehavioral effects seen in mice offspring in the Tanaka study described above, the HIARC determined that a DNT study in rats should be required for imazalil. HIARC also determined that Acute Neurotoxicity and Subchronic Neurotoxicity studies in adult rats should be required based on the positive effects seen in the Tanaka study and for comparison to DNT results. HIARC also noted that the experimental design of the Tanaka study differs from the developmental neurotoxicity study and therefore, the DNT is required to assess the potential effects of this chemical in the developing fetuses.

In regard to structural activity similarities to other related fungicides, it was pointed out that imazalil is an imidazole ring based chemical. All other -conazole agricultural fungicides are triazole compounds. Imazalil is more structurally related to some human therapeutic antimycotic agents with an imidazole ring structure. However, there were no data available on the developmental effects of these agents. Therefore, HIARC determined that the results of the Tanaka study provided sufficient concern to require the DNT.

7. **OTHER ISSUES:** None

8.0 REFERENCES

- 00031596 Niemegeers, C.J.E. (1979) Comparative Acute Oral Toxicity Studies of the Different Salts of Imazalil in Rats. (Unpublished study received Mar 7, 1980 under 43813-1; prepared by Janssen Pharmaceutica, submitted by Janssen R & D, Inc., New Brunswick, N.J.; CDL:099285-B)
- 00031599 Vanparys, P.; Marsboom, R.; Lenaerts, P. (1979) Micronucleus Test in Rats: Experiment No. 916 (79.12.18). (Unpublished study received Mar 7, 1980 under 43813-1; prepared by Janssen Pharmaceutica, submitted by Janssen R & D, Inc., New Brunswick, N.J.; CDL:099285-E)
- 00152439 Appleman, L.; Woutersen, R. (1983) Acute Inhalation Toxicity Study of an Imazalil Containing Smoke, Developed by a Smoke-Generator, in Rats: Report No. V 83.308/230831: Project No. B83-0831. Un-published report prepared by Netherlands Organization for Applied Scientific Research. 20 p.
- 40729301 Vanparys, P.; Marsboom, R. (1988) Ames Reverse Mutation Test with Salmonella typhimurium: [Imazalil]: Project ID: X7-1988. Unpublished study prepared by Janssen Pharmaceutica N.V. 35 p.
- 40729302 Leonard, A.; Deknudt, G. (1986) Report on the in vitro Chromosome Aberration Assay on Human Lymphocytes (Study and Protocol No. SCK 86/02D/R23979): [Imazalil]: Project ID: X9 1988. Unpublished study prepared by S.C.K./C.E.N. 53 p.
- 40729303 Vanparys, P.; Marsboom, R. (1988) Micronucleus Test in Mice: [Imazalil]: Project ID: X8-1988. Unpublished study prepared by Janssen Pharmaceutica N.V. 45 p.
- 41026603 Gillardin, J.; Sanz, G.; Van Cauteren, H.; et al. (1989) Embryotoxicity and Teratogenicity Study in Sprague-Dawley Rats (Segment II): Proj. ID V1 1989. Unpublished study prepared by Janssen-Le Brun. 129 p.
- 41328801 Teuns, G.; Marsboom, R. (1987) Primary Dermal Irritation Study in Rabbits: Lab Project Number: 1864/(87/06/22). Unpublished study prepared by Janssen Pharmaceutica N.V. 22 p.
- 41328802 Verstraeten, A.; Teuns, G.; Vandenberghe, J. et al. (1989) Chronic Toxicity Study in Beagle Dogs (Imazalil Base): Lab Project Number: 1899. Unpublished study prepared by Janssen Research Foundation. 600 p.

- 41606104 Teuns, G.; Marsboom, R. (1990) Imazalil: R 23979 (Technical Grade): Acute Dermal Toxicity Study in New Zealand White Rabbits: Final Report: Lab Project No.: 2344. Unpublished study prepared by Janssen Research Foundation. 29 p.
- 41606105 Teuns, G.; Marsboom, R. (1990) Imazalil: R 23979 Technical Grade: Primary Eye Irritation Study in New Zealand White Rabbits: Final Report: Lab Project No.: 2253. Unpublished study prepared by Janssen Research Foundation. 22 p.
- 41718701 Teuns, G.; Coussement, W.; Van Cauteren, H. et al. (1990) Imazalil R 23979: Dermal Sensitization Study According to the Magnusson Guinea-Pig Maximization Test: Lab Project Number: 2417. Unpublished study prepared by Janssen Research Foundation, Dept. of Tox. 40 p.
- 41889802 Reuzel, P.G.J.; De Smet, M. (1990) Addenda to MRID 00152439 (Phase 3 Summary - MRID 92072006) Acute Inhalation toxicity Study of an Imazalil Containing Smoke, Developed by a Smoke Generator, in Rats: Report No. V 83.308/230831
- 42012003 Mannens, G., Van Leemput, L. And Heykants, J. 1991. General Metabolism of Imazalil in the Rat. Study number R23979/FK1116. Dept. of Drug Metabolism and Pharmacokinetics, Janssen Research Foundation, 2340 Beerse, Belgium. June 13, 1991. Unpublished.
- 42085201 Teuns, G.; VanDenberghe, J.; Lampo, A.; et al. (1991) Subchronic Toxicity Study in New Zealand White Rabbits: Lab Project Number: 2418. Unpublished study prepared by Janssen Research Foundation. 206 p.
- 42570701 Dirx, P. (1992) 2-generation Reproduction Study with 1 Litter Per Generation in Wistar Rats: R 23979-Imazalil: Final Report: Lab Project Number: 2337. Unpublished study prepared by Janssen Research Foundation. 567 p.
- 42593601 Dirx, P.; Van Cauteren, H. (1992) Embryotoxicity and Teratogenicity Study in Albino Rabbits: R27180 Imazalil Sulphate: Final Report: Lab Project Number: 2615. Unpublished study prepared by Janssen Research Foundation. 132 p.
- 42913401 van Beijsterveldt, L.; Leemput, L.V.; Heykants, J. 1993. Dermal absorption of ¹⁴C- Imazalil in male rats after topical application of its Fungaflor 500 EC formulation. Study number R23979/FK1326. Janssen Research Foundation, 2340 Beerse, Belgium. February 1, 1993. Unpublished.

- 42949402 Dirx, P.; Van Cauteren, H. (1992) 2-Generation Reproduction Study with 1 Litter per Generation in Wistar Rats: Supplement to: (Janssen Response to EPA Deficiency Letter of June 4, 1993): Lab Project Number: 2337. Unpublished study prepared by Janssen Pharmaceutica. 38 p.
- 42972001 Verstraeten, A. (1993) Carcinogenicity Study in Swiss Mice: Imazalil Base: Final Report: Nonclinical Laboratory Study: Lab Project Number: 2194: R 23979. Unpublished study prepared by Janssen Research Foundation. 1004 p.
- 43016802 Janssen (1993) Supplement to MRID 42085201: Repeated Dose Dermal Toxicity Study in New Zealand White Rabbits (21 Days)--R23979 Imazalil: Lab Project Number: 2418. Unpublished study. 52 p.
- 43154201 Janssen Research Foundation. (1994) Embryotoxicity and Teratogenicity Study in Albino Rabbits: Imazalil: Supplement to MRID 42593601: Lab Project Number: 2615. Unpublished study. 34 p.
- 43202401 Van Duen, K.; Vandenberghe, J.; Hess, R.; et al. (1994) Safety Assessment of Imazalil (Enilconazole)-Relevance of Liver Tumor Formation in Mice: Lab Project Number: PPD-4. Unpublished study prepared by Janssen Research Foundation, Dept. of Toxicology. 8 p.
- 43202401 Van Duen, K.; Vandenberghe, J.; Hess, R.; et al. (1994) Safety Assessment of Imazalil (Enilconazole)-Relevance of Liver Tumor Formation in Mice: Lab Project Number: PPD-4. Unpublished study prepared by Janssen Research Foundation, Dept. of Toxicology. 8 p.
- 43222601 Van Deun, K. (1994) Three-Month Oral Mechanistic Toxicity Study with One Month Interim Sacrifice in SPF Albino Swiss Mice: Imazalil: R 23979: Final Report: Lab Project Number: R 23979: 3140. Unpublished study prepared by Janssen Research Foundation. 433 p.
- 43242401 Verstraeten, A. (1993) Carcinogenicity Study in Swiss Mice: Imazalil Base: Supplement: Lab Project Number: 2194. Unpublished study prepared by Janssen Research Foundation. 34 p.
- 43242402 Deun, K. (1994) Three-Month Oral Mechanistic Toxicity Study with One Month Interim Sacrifice in SPF Albino Swiss Mice: Imazalil: Supplement: Lab Project No.: 3140. Unpublished study prepared by Janssen Research Foundation. 16 p.
- 43735001 Hess, R. (1995) Imazalil (Enilconazole): Relevance of Liver Tumor Formation in

30

Mice: Supplement to MRID 43202401: Lab Project Number: PPD-4:
R23979/FK1600: 3140. Unpublished study prepared by Janssen Research
Foundation. 9 p.

- 43735002 Butler, W. (1995) Carcinogenicity Study in Swiss Mice: Imazalil Base: Supplement No. 2: (Pathology Working Group Review): Lab Project Number: 2194. Unpublished study prepared by Janssen Research Foundation. 18 p.
- 43735003 Gompel, J. (1995) In Vitro Mammalian Gene Mutation Assay: Imazalil: Final Report: Lab Project Number: R023979: 3470. Unpublished study prepared by Janssen Research Foundation. 31 p.
- 43780201 Fautz, R. (1990) Unscheduled DNA Synthesis in Primary Hepatocytes of Male Rats in vitro With Imazalil: Lab Project Numbers: 920: 192600. Unpublished study prepared by CCR Cytotest Cell Research GmbH & Co. KG. 39 p.
- 43965702 Clare, C. (1996) Imazalil: Measurement of Unscheduled DNA Synthesis and Replicative DNA Synthesis in Mouse Liver Using an in vivo/in vitro Procedure: Final Report: Lab Project Number: 1073/3-1052: R023979-EXP NO.3666. Unpublished study prepared by Corning Hazleton (Europe). 68 p.
- 43965704 Van Deun, K. (1996) Three-Month Oral Dose Range Finding and Mechanistic Toxicity Study with One Month Interim Sacrifice in SPF Wistar Rats: Final Report: Lab Project Number: R023979: R023979/FK1600: 3514. Unpublished study prepared by Janssen Research Foundation Dept. of Toxicology. 499 p.
- 43965705 Van Deun, K. (1996) 3-Month Dose Range Finding And Mechanistic Toxicity Study in SPF Wistar Rats: Final Report: Lab Project Number: R023979: 773A: 773B. Unpublished study prepared by Janssen Research Foundation Dept. of Toxicology. 396 p.
- 44107212 Dreher, D. (1990) Imazalil Technical: Acute Oral Toxicity Test in the Rat: Lab Project Number: 306/1: TX 1894. Unpublished study prepared by SafePharm Labs, Ltd. 26 p.
- 44107213 Dreher, D. (1990) Imazalil Technical: Acute Dermal Toxicity (Limit Test) in the Rat: Lab Project Number: 306/2: TX 1895. Unpublished study prepared by SafePharm Labs, Ltd. 14 p.
- 44107214 Blagden, S. (1990) Imazalil Technical: Acute Inhalation Toxicity Study, Four

- Hour Exposure (Nose Only), in the Rat: Lab Project Number: 306/3: TX 1896.
Unpublished study prepared by SafePharm Labs, Ltd. 43 p.
- 44107215 Glaza, S. (1996) Primary Eye Irritation Study of Magnate (Imazalil) Technical in Rabbits: Final Report: Lab Project Number: CHW 6413-138: TP6189.
Unpublished study prepared by Corning Hazleton, Inc. 31 p.
- 44107217 Dreher, D. (1990) Imazalil Technical: Magnusson & Kligman Maximisation Study in the Guinea Pig: Lab Project Number: 306/6: TX 1899. Unpublished study prepared by SafePharm Labs, Ltd. 25 p.
- 44109915 Glaza, S. (1996) Primary Dermal Irritation Study of Magnate (Imazalil) 50 EC in Rabbits: Final Report: Lab Project Number: CHW 6413-135: TP6187.
Unpublished study prepared by Corning Hazleton Inc. 26 p.
- 44178501 Verstraeten, A. (1996) Carcinogenicity Study in Swiss Mice: Imazalil Base: Supplement No. 3 (Response to the EPA Review of the PWG of the Mouse Carcinogenicity Study on Imazalil): Lab Project Number: 2194: 2166: JPA/004.
Unpublished study prepared by Janssen Research Foundation. 24 p.
- 44269601 Piccirillo, V. (1997) Overall Assessment of Rodent Chronic Toxicity and Oncogenicity Data for Imazalil for Purposes of Carcinogenicity Peer Review: Lab Project No.: 2194M/2166M/3817R. Unpublished study prepared by NPC, Inc. 62p.
- 44567802 Dirx, P. (1992) R 27180: Embryotoxicity and Teratogenicity Study in Mice. Department of Toxicology, Janssen Research Foundation, 2340 Beerse, Belgium. Experiment No. 2712, November 13, 1992. Unpublished.
- 44567803 Gillardin, J.M., Sanz, J. and Marsboom, R. (1987) R 27180: Embryotoxicity and Teratogenicity Study in COBS CD1 Mice. Research Department Laboratories Janssen, 93300 Aubervilliers, France. Experiment No. 787/86-07, January 14, 1987. (Range-Finding Study) Unpublished
- 44578201 Levron, J.C. (1991) Embryotoxicity and Teratogenicity Study in Cobs CD1 Mice. Research Department, Laboratoires Janssen, Aubervilliers, France, Laboratory Project ID. 2374/90-06, June 3, 1991. Unpublished.
- 44858001 Van Deun, K. 1999. Combined oral chronic toxicity/carcinogenicity study with Imazalil in the SPF Wistar rat. Dept. Toxicology, Janssen Research Foundation, 2340 Beerse, Belgium. Laboratory report number, 3817, June 8, 1999. Unpublished.

32

- 92072008 Goodwine, W. (1990) Janssen Pharmaceutic Phase 3 Summary of MRID 1026603. Embryotoxicity and Teratogenicity Study in Sprague Dawley Rats (R27180): Report No. 2003 (88-05), Rec #327. Prepared by Laboratories Janssen, Research Dept. 11 p.
- 92072010 Goodwine, W. (1990) Janssen Pharmaceutic Phase 3 Summary of MRID 40729301. Ames Reverse Mutation Test with Salmonella typhimurium: Report No. 1999, March 22, 1988. Prepared by Janssen Pharmaceut. 11 p.
- 92072011 Van Parijs, P. (1990) Janssen Pharmaceutic Phase 3 Summary of MRID 40729302. in vitro Chromosome Aberration Assay on Human Lymphocytes: Report No. SCK EXP-833. Prepared by Janssen Mammalian Gen. LAB. 9 p.
- 92072012 Goodwine, W. (1990) Janssen Pharmaceutic Phase 3 Summary of MRID 40729303. Micronucleus Test in Mice: Report No. 1911, February 29, 1988. Prepared by Dept. of Toxicology Janssen Pharmaceut. 12 p.
- 92072033 Lampo, A. (1990) Janssen Pharmaceutic Phase 3 Summary of MRID 41328802. Chronic Toxicity Study in Beagle Dogs-Imazalil Base (R23979): Report No. 1899, Rec #11. Prepared by Janssen Pharmaceut. 8 p.

PUBLISHED STUDIES

- Hasegawa, R. and N. Ito. 1992. Liver medium-term bioassay in rats for screening of carcinogens and modifying factors in hepatocarcinogenesis. *Fd. Chem. Toxic.* 30: 979-992.
- Mason, JI; Carr, BR; and Murry, BA. 1987. Imidazole Antimycotics: Selective inhibitors of Steroid Aromatization and Progesterone Hydroxylaiton. *Steroids* 50 (1-3): 179-189.
- Muto, N., H. Hirai, T. Tanaka, N. Itoh, and K. Tanaka. 1997. Induction and inhibition of cytochrome P450 isoforms by Imazalil, a food contaminant, in mouse small intestine and liver. *Xenobiotica* 27: 1215-1223.
- Nakagawa, Y., and G. Moore. 1995. Cytotoxic effects of postharvest fungicides, ortho-phenylphenol, thiabendazole and imazalil, on isolated rat hepatocytes. *Life Sci.* 57: 1433-1440.
- Nakagawa, Y., and K. Tayama. 1997. Acute hepatotoxic potential of Imazalil fungicide in rats. *Bull. Envir. Contam. Toxicol.* 58: 402-407.
- Stiller, R., and D. Stevens. 1986. Studies with a plant fungicide, Imazalil, with vapor-phase activity, in the therapy of human alternariosis. *Mycopathologia* 93: 169-172.

33

Tanaka, T. 1995. Reproductive and neurobehavioral effects of Imazalil administered to mice. *Reprod. Toxicol.* 9: 281-288.

Thienpont, D., J. Van Cutsem, H. Van Cauteren, and R. Marsboom. 1981. The biological and toxicological properties of Imazalil. *Arzn. Forsch.* 31: 309-314.

Van Hecke, E. and L. de Vos. 1983. Contact sensitivity to enilconazole. *Contact Derm.* 9:144.

Van Leemput, L., and E. Swysen, R. Woestenborghs, et al. 1989. On the terrestrial toxicity of fungicide Imazalil (enilconazole) to the earthworm species *Eisenia foetida*. *Ecotoxicol. Environ. Saf.* 18: 313-320.

34

9.0 APPENDICES
Tables for Use in Risk Assessment

35

9.1 Toxicity Profile Summary Tables

9.1.1 Acute Toxicity Table - See Section 4.1

9.1.2 Subchronic, Chronic and Other Toxicity Table

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Results
870.3100 90-Day oral toxicity rats	43965704(1996) Acceptable/non-guideline 0, 200, 400, 800 ppm M: 0, 16, 32, 64, mg/kg/d F: 0, 19, 38, 76 mg/kg/d	NOAEL: 200 ppm (15.8 and 18.7 mg/kg/day in ♂ and ♀, respectively). LOAEL: 400 ppm (32.1 and 37.9 mg/kg/day in ♂ and ♀, respectively, based on increased absolute and relative liver weights in ♂ and ♀ at 1 month, possibly increased absolute and relative adrenal weights in females at 3 months, increased vacuolization in hepatocytes in ♀ at 1 month.
870.3100 90-Day oral toxicity rats	43965705(1996) Acceptable/non-guideline 0, 800,1600, 2400, 3200 ppm M: 0, 65, 129, 181, 252 mg/kg/d F: 0, 79, 150, 236, 333 mg/kg/d	NOAEL: <800 ppm (64.4 and 78.7 mg/kg/day in ♂ and ♀ , respectively). LOAEL: 800 ppm (64.4 and 78.7 mg/kg/day in ♂ males and ♀ females, respectively), based on possibly decreased bw and bw gains in ♂ males and ♀, possibly decreased triglyceride and phosholipid in ♂males, dark and more pronounced lobulation of livers in ♀, possibly increased relative liver/bw ratios in ♂, mild hepatocellular hypertrophy in ♂ and ♀, and mild 'fatty vacuolation' in the livers of ♀.
870.3100 90-Day oral toxicity mice	43222601 & 43292402 (1994) Acceptable/nonguideline 0, 50, 200, 600 ppm M: 0, 10, 39, 115 mg/kg/day F: 0, 11, 46, 138 mg/kg/day	NOAEL = 50 ppm (9.5 and 11.3 mg/kg/day in ♂ and ♀, respectively) LOAEL = 200 ppm (38.6 and 45.6 mg/kg/day in ♂ and ♀, respectively) based on increased incidence and severity of histopathologic effects, increased microsomal protein and increased microsomal cytochrome P450 content in the livers of both sexes.
870.3150 90-Day oral toxicity Dog	See one year study	See one year study
870.3200 21-Day dermal toxicity-Rabbit	42085201 (1991) 5/sex at 0, 10, 40 or 160 mg/kg/day 6-day range finding at 0, 63, 250 or 1000 mg/kg/day	NOAEL :160 mg/kg/day LOAEL : 250 mg/kg/day based on significant fissuring, scaling and swollen livers in the range finding study. No systemic toxicity was reported in main 21-day study.

36

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Results
870.3250 90-Day dermal toxicity	NA	NA
870.3465 90-Day inhalation toxicity	NA	NA
870.3700a Prenatal developmental in rodents-rats	41026603 (1988) Imazalil Sulfate technical: 0, 40, 80 or 120 mg/kg/day Acceptable/guideline	Maternal NOAEL = <40 mg/kg/day LOAEL = 40 mg/kg/day based on decreased food consumption Developmental NOAEL = 40 mg/kg/day LOAEL = 80 mg/kg/day based on decreased fetal weight
870.3700a Prenatal developmental in rodents-mouse	41026603 (1991) Imazalil Sulfate technical: 0, 10, 40, 80 or 120 mg/kg/day Acceptable/guideline	Maternal NOAEL = 10 mg/kg/day LOAEL = 40 mg/kg/day based on reduced bodyweight gains and corrected bodyweight gains Developmental NOAEL = 80 mg/kg/day LOAEL = 120 mg/kg/day based on increased resorption, postimplantation loss and reduced litter size
870.3700a Prenatal developmental in rodents-mouse	44567802 (1992) Imazalil Sulfate technical: 0, 10, 40, 80 or 120 mg/kg/day Acceptable/guideline	Maternal NOAEL = 10 mg/kg/day LOAEL = 40 mg/kg/day based on reduced bodyweight gains, corrected bodyweight gains and food consumption Developmental NOAEL = 40 mg/kg/day LOAEL = 80 mg/kg/day based on significant increase of fetuses and litters with extra 14 th pair of ribs
870.3700b Prenatal developmental in nonrodents - rabbit	42593601 (1992) Imazalil Sulfate technical: 0, 5, 10 or 20 mg/kg/day Acceptable/guideline	Maternal NOAEL = 5 mg/kg/day LOAEL = 10 mg/kg/day based on decreased body weight and food consumption and increased mortality Developmental NOAEL = 5 mg/kg/day LOAEL = 10 mg/kg/day based on increased resorption and decreased number of fetuses.
870.3800 Reproduction and fertility effects 2-generation, rat	42570701 & 42949402 (1992) 0, 5, 20, 80 mg/kg/day Acceptable/guideline	Parental/Systemic NOAEL = 20 mg/kg/day LOAEL = 80 mg/kg/day based on decreased body weight gain (♂ & ♀) and increased liver vacuolation (♂). Reproductive NOAEL = 20 mg/kg/day LOAEL = 80 mg/kg/day based on increased duration of gestation. Offspring NOAEL = 20 mg/kg/day LOAEL = 80 mg/kg/day based on increased pup mortality from birth to day 4.

37

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Results
870.4100a Chronic toxicity rodents - rats	00162412 (1984) 18-Month study 0, 25, 100 or 400 ppm 47026101(1985) Chronic/Oncogenicity 0, 25, 100 or 400 ppm M: 0, 1, 3.7, 15.5 m/kg/d F: 1.2, 4.7, 20 mg/kg/day Unacceptable: not tested at adequate high dose	NOAEL = 3.7 mg/kg/day LOAEL = 15.5 mg/kg/day based on liver effects: increased incidence of intracytoplasmic inclusion bodies in hepatocytes, increased hepatocyte vacuolization and bile duct proliferation.
870.4100b Chronic toxicity dogs	41328802 (1989) 12-Month Chronic Oral Toxicity (Capsule) - 0, 1.25, 2.5, or 20 mg/kg/day Acceptable/guideline	NOAEL = 2.5 mg/kg/day LOAEL = 20 mg/kg/day, based on decreased body weight gain (♂ & ♀), increased alkaline phosphatase (♂ & ♀), increased liver weight (♂) and clinical symptoms of vomiting and soft stools.
870.4200 Carcinogenicity rats	44858001 (1999) Chronic/Oncogenicity 0, 50, 200, 1200, 2400 ppm M: 0, 2.7, 10.8, 65.8, 134.8 mg/kg/d F: 0, 3.6, 14.6, 85.2, 168.8 mg/kg/d Acceptable/guideline	NOAEL = 10.8 & 14.6 mg/kg/day in ♂ & ♀, respectively LOAEL = 65.8 & 85.2 mg/kg/day in ♂ & ♀, respectively based on reductions in body weight and weight gain and macro and micro-scopic effects in the liver of ♂ & ♀ rats and thyroid of ♂ rats. Positive for liver and thyroid neoplasm in male rats. Classified by HED CARC (1999) as "Likely to be carcinogenic in humans" - with a Q_1^* of 6.11×10^{-2} (mg/kg/day) ⁻¹
870.4300 Carcinogenicity mice	42972001 (1993) 23-Month Carcinogenicity in Mice 0, 50, 200 or 600 ppm M: 0, 6.8, 28, 88 mg/kg/d F: 0, 8.3, 35, 110 mg/kg/d : :	NOAEL = 50 ppm (6.8 mg/kg/day) in ♂; 200 ppm (110 mg/kg/day) in ♀ LOAEL = 200 ppm (28 mg/kg/day) in ♂ and 600 ppm (35 mg/kg/day) in ♀, based on increased liver histopathology Positive for liver neoplasm in male mice. Classified by HED CPRC (1994) and CARC (1998) as a Group C - Possible human carcinogen - with a Q_1^* of 6.11×10^{-2} (mg/kg/day)⁻¹
Gene Mutation 870.5100	40729301(1988) Ames assay 5-500 µg/plate Acceptable/guideline	Negative in Salmonella strains up to toxic concentrations of 250-500 µg/plate with or without S-9 activation.

38

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Results
Cytogenetics 870.5375	40729302 (1986) HED 006818 Acceptable, pre 1991 guidelines	In an in vitro human lymphocytes chromosome aberration study, imazalil did not result in any increased chromosomal aberrations at concentrations ranging from 23 to 909 µg/ml culture
Other Effects 870.5300 870.5395 870.5500 870.5500	43735003 (1988) Acceptable, pre 1991 guideline 00031599 (1979) HED Doc. 000057 Acceptable. Pre 1991 guideline 43965702 (1996) Acceptable. 1991 guideline 43780201(1990) Acceptable pre 1991 guideline	In an in vitro cytogenetic study in mammalian cells (Chinese hamster V79 cells), imazalil, at doses ranging from 10 to 100 µg/ml, was not mutagenic both with and without activation In a mutagenicity Micronucleus Test in rats, imazalil did not result in any increase in micronucleated polychromatic erythrocytes in any of the intraperitoneally doses tested (0, 20, 40 or 160 mg/kg In an in vivo/in vitro unscheduled DNA synthesis assays in mice administered single oral doses of imazalil of 125 or 250 mg/kg, imazalil was negative for genotoxicity but positive for cellular proliferation when tested at overtly toxic (250/ mg/kg; mortality 29/54) and cytotoxic doses In an unscheduled DNA synthesis (UDS) assay in rat hepatocytes imazalil at concentrations ranging from 0.09 to 9.0 µg/ml did not cause increased UDS.
870.6200a Acute neurotoxicity screening battery	DATA GAP	DATA GAP
870.6200b Subchronic neurotoxicity screening battery	DATA GAP	DATA GAP
870.6300 Developmental neurotoxicity	DATA GAP	DATA GAP
870.7485 Metabolism and pharmacokinetics	42012003 (1991) Metabolism C ¹⁴ - Rat Single IV dose 1.25 mg/kg Single oral 1.25 mg/kg Single oral 20 mg/kg 14-day repeated oral 1.25 mg/kg Acceptable/guideline	¹⁴ C- Imazalil rapidly absorbed, distributed, metabolized and excreted in roughly equal amounts in urine and feces within 24 hours. Metabolized to more than 25 metabolites. Major metabolites identified. A metabolic pathway proposed. No significant bioaccumulation in tissues. No significant sex differences observed. No significant differences between dosing regimens.

39

Guideline No./ Study Type	MRID No. (year)/ Classification /Doses	Results
870.7600 Dermal penetration	42913401 (1993) Dermal Absorption-Rats 0.004, 0.04, 0.4 or 4.0 mg/cm ² Acceptable/guideline	Peak blood concentration at 1 hour for the 0.00- 0.4 mg/cm ² ; at 10 hours for the 4.0 mg/cm ² . Percent absorption at 10 hours was 41%, 25%, 17% and 26% and at 24 hours was 48%, 39%, 31% and 29% of the applied doses of 0.004, 0.04, 0.4 or 4.0 mg/cm ²
Special studies	NA	NA

47

9.2 Summary of Toxicological Dose and Endpoints for Imazalil for Use in Human Risk Assessment¹

Exposure Scenario	Dose Used in Risk Assessment, UF	FQPA SF and Endpoint for Risk Assessment	Study and Toxicological Effects
Acute Dietary <u>females 13-50 years of age</u>	NOAEL = 5 mg/kg/day UF = 100 Acute RfD = 0.05 mg/kg/day	FQPA SF = 3X aPAD = $\frac{0.05}{3}$ = 0.017 mg/kg/day	Developmental Study-Rabbits: 42593601 LOAEL = 10 mg/kg/day based on increased resorptions, decreased litter size
Acute Dietary <u>general population</u> Not Applicable	NA	NA	NA
Chronic Dietary <u>all populations</u>	NOAEL= 2.5 mg/kg/day UF = 100 Chronic RfD = 0.025 mg/kg/day	FQPA SF = 10x cPAD = $\frac{0.025}{10}$ = 0.0025 mg/kg/day	12-m chronic study-dogs 41328802 LOAEL = 20 mg/kg/day based on vomiting and soft stools, ↓ bw gain, ↑ alkaline phosphatase activity and ↑ liver weight
Short-Term Dermal (1-7 days) (Occupational/ Residential)	dermal study NOAEL= 160 mg/kg/day dermal absorption rate = 41%	acceptable MOE = 100 (Occupational) acceptable MOE = Residential, NA	21-d dermal toxicity-rabbit, 42085201 LOAEL = 250 mg/kg/day based on skin fissuring and scaling and swollen livers
Intermediate-Term Dermal (1 week - several months) Occupational	oral study NOAEL= 15.8 mg/kg/day dermal absorption rate = 41%	acceptable MOE = 100 (Occupational) acceptable MOE = Residential NA	90-d subchronic study-rats 43965704 LOAEL = 32.1 mg/kg/day based on increased absolute liver weight and liver/bw ratios in ♂ & ♀ at one month and adrenal glands and liver effects
Long-Term Dermal (several months - lifetime) Occupational	oral study NOAEL= 2.5 mg/kg/day dermal absorption rate = 41%	acceptable MOE = 100 Occupational acceptable MOE = Residential, NA	12-m chronic study-dogs 41328802 LOAEL = 20 mg/kg/day based on vomiting and soft stools, ↓ bw gain, ↑ alkaline phosphatase activity and ↑ liver weight
Short-Term Inhalation (1-7 days) Occupational	oral study NOAEL= 5 mg/kg/day inhalation absorption rate = 100%	acceptable MOE = 100 Occupational acceptable MOE = Residential NA	Developmental Study-Rabbits: 42593601 LOAEL = 10 mg/kg/day based on increased resorptions, decreased litter size

41

Exposure Scenario	Dose Used in Risk Assessment, UF	FQPA SF and Endpoint for Risk Assessment	Study and Toxicological Effects
Intermediate-Term Inhalation (1 week - several months) Occupational	oral study NOAEL= 2.5 mg/kg/day (inhalation absorption rate = 100%)	acceptable MOE = 100 (Occupational) acceptable MOE = Residential NA	12-m chronic study-dogs 41328802 LOAEL = 20 mg/kg/day based on vomiting and soft stools, ↓ bw gain, ↑ alkaline phosphatase activity and ↑ liver weight
Long-Term Inhalation (several months - lifetime) Occupational	oral study NOAEL= 2.5 mg/kg/day (inhalation absorption rate = 100%)	acceptable MOE = 100 (Occupational) acceptable MOE = Residential NA	12-m chronic study-dogs 41328802 LOAEL = 20 mg/kg/day based on vomiting and soft stools, ↓ bw gain, ↑ alkaline phosphatase activity and ↑ liver weight
Cancer (oral, dermal, inhalation)	likely carcinogen; CARC 1999, HED# 013885	low-dose extrapolation approach for quantifying the carcinogenic risk $Q1^* = 6.11 \times 10^{-2} \text{ (mg/kg/day)}^{-1}$	an increase (both trend and pair-wise) in combined liver adenomas/carcinomas in male Swiss albino mice and male Wistar rats and an increase in combined thyroid follicular adenomas/carcinomas in Wistar rats

¹ UF = uncertainty factor, FQPA SF = FQPA safety factor, NOAEL = no observed adverse effect level, LOAEL = lowest observed adverse effect level, PAD = population adjusted dose (a = acute, c = chronic) RfD = reference dose, MOE = margin of exposure

42

IMAZALIL/FEBRUARY 2000

RED (Registration Toxicology Chapter)

SignOff Date:	2/9/00
DP Barcode:	D258087
HED DOC Number:	013993
Toxicology Branch:	RRB4

43