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TOXIC SUBSTANCES

**HED DOC. No.**

**DATE:** **JUNE 29, 1999**

**MEMORANDUM**

**SUBJECT:** *IMAZALIL:* - Report of the Hazard Identification Assessment Review Committee.

**FROM:** Abdallah Khasawinah, Toxicologist for AK.
Reregistration Branch 24
Health Effects Division (7509C)

**THROUGH:** Pauline Wagner, Co-Chair for PW
And
Jess Rowland, Co-Chair
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

**TO:** Susan Hummel, Branch Senior Scientist
And
Sanjivani Diwan, Senior Toxicologist
Registration Action Branch 2
Health Effects Division (7509C)

**PC Code:111901**

On June 15 and 22, 1999, the Health Effects Division's Hazard Identification Assessment Review Committee (HIARC) reevaluated the toxicology data base of Imazalil and selected toxicology endpoints for acute dietary, chronic dietary, dermal and inhalation exposure risk assessments, and addressed the potential enhanced sensitivity of infants and children from exposure to Imazalil as required by the Food Quality Protection Act (FQPA) of 1996. The Committee's conclusions are presented in this report.

## Committee Members in Attendance

Members present on June 15, 1999 were Pauline Wagner, Jess Rowland, Yiannakis Ioannou, Sue Makris, Nancy McCarroll, P.V. Shah, Virginia Dobozy, Nicole Paquette, Tina Levine, and Dave Andersen. Members in absentia were Kathy Raffaele,, and Bill Burnam. Also in attendance were Ray Kent, Sanjivani Diwan, Suhair Shallal and David Hrdy of Reregistration Branch 4 and, Seyed Tadayan of Chemistry and Exposure Branch. Data were presented at this and subsequent meetings by Abdallah Khasawinah of Reregistration Branch 4.

Data Presentation &
Report Preparation: _____

Abdallah Khasawinah
Toxicologist

## I. INTRODUCTION

Imazalil (1-[2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl]-1H-imidazole) fungicide intended for post harvest control of mildews on citrus fruits and as ba treatment. It is also used in chicken hatcheries. Imazalil inhibits cytochrom biosynthesis of ergosterol, an essential component of the cell membrane of fu

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

**THIS REPORT SUPERSEDES ANY PREVIOUS RFD AND TES COMMI**

## II. HAZARD IDENTIFICATION

**1. ACUTE DIETARY (Acute RfD)–Females 13+**

**Study Selected:** Developmental Study–Rabbit **Guideline #:83-3b**

**MRID No.:** 42593601

**Executive Summary:** In a developmental toxicity study (MRID 4: sulphate (98.2-100% purity) was administered to 15 female New rabbits/dose by gavage at dose levels of 0, 5, 10 or 20 mg/kg/day from d gestation.

Maternal toxicity was observed at 10 and 20 mg/kg/day as evidence decreased body weight gain (54% and 95%, respectively, during GD difficulty, and increased mortality (8/15 at 20 mg/kg/day). Food significantly decreased at the mid and high doses (18% and 23%, respect 18).

**The maternal LOAEL is 10 mg/kg/day, based on decreased body wei consumption. The maternal NOAEL is 5 mg/kg/day.**

Developmental toxicity was manifested by increased number of reso subsequent decreases in numbers of live fetuses/litter at 10 and 20 mg/ external visceral malformations or variations were reported.

**The developmental LOAEL is 10 mg/kg/day, based on increased decreased number of fetuses per litter. The developmental NOAEL**

This developmental toxicity study in the rabbit is classified **Acceptabl** guideline requirements for a developmental toxicity study **(83-3b)** in ral

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 opthalmological 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 signs of vomiting and soft stools; depressed body weight gains, increased alkaline phosphatase activity and increased liver weights. The NOAEL is 2.5 mg/kg/day.**

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

**Dose and Endpoint for establishing Chronic RfD:** 2.5 mg/kg/day based on clinical toxicity of vomiting and soft stools; depressed body weight gains, increased alkaline phosphatase activity and increased liver weights at 20 mg/kg.

**Uncertainty Factor:** 100 was applied to account for both interspecies extrapolation and intra-species variability.

**RfD:** $\frac{2.5 \text{ mg/kg}}{100 \text{ (UF)}} = 0.025 \text{ mg/kg}$

**Comments about Study/Endpoint/Uncertainty Factor(s):** The results of the 18-month feeding study in rats the LOAELs and NOAELs were comparable (LOAEL = 15.9 and 20.3 mg/kg/day and NOAEL = 3.7 and 4.7 mg/kg/day in males and females, respectively; Accession No. 00162412) and the 23-month carcinogenicity in mice ( LOAEL =15.9 and 20.3 mg/kg/day and NOAEL for systemic toxicity = 6.76 mg/kg/day; MRID No. 42972001). Both studies revealed effect on the liver (microscopic changes) and support the findings in the critical study.

## C. OCCUPATIONAL / RESIDENTIAL EXPOSURE—DERMAL

### 1. DERMAL ABSORPTION

**Study Selected:** Dermal Absorption of $^{14}C$- Imazalil in Rats **Guideline #:** §85-3

**MRID No.:** 42913401

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

Blood 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 $mg/cm^2$, blood concentration peaked at 1-2 hours. At 0.4 $mg/cm^2$ it peaked at 1 hour and remained steady for three hours before it declined. At 4.0 $mg/cm^2$ 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 $mg/cm^2$, respectively.

The study was classified **Acceptable** and satisfies the guideline requirement 85-3 for a dermal absorption study.

**Dermal Absorption Factor:** 41% over a 10 hour exposure

**Comments About Proposed Absorption Factor:** A 10 hour exposure period and low level of exposure were assumed in selecting the above factor.

**1. SHORT-TERM DERMAL (1 - 7 days)**

**Study Selected:** 21-Day Dermal Toxicity--Rabbits **Guideline #:** 82-2

**MRID No.:** 42085201

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

No mortalities or behavioral changes were reported. Red stippling of the skin was reported in all groups with higher incidence and more severity in the control group. This was attributed to the sesame oil vehicle and not the test substance. Skin irritation was minimal and was classified as "barely perceptible". Very slight erythema (Draize score 1) and slight scaling was reported at the 10 and 40 mg/kg/day groups. At the 160 mg/kg/day dose, erythema was very slight to well defined (Draize score 1-2) and slight to moderate scaling.

Body weights, body weight gains and food consumption were comparable to controls.

Hematology parameters were comparable in all groups except for a statistically significant dose-related decrease ($p \leq 0.05$) in white blood cell (WBC) count in the high dose males (16.2, 13.4, 11.4, and 10.4 x $10^3$ cells/$mm^3$, at 0, 10, 40, and 160 mg/kg/day, respectively). These values within the normal range.

There were no treatment related gross and microscopic pathological findings.

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 is160 mg/kg/day and the **LOAEL** is 250 mg/kg/day based on changes in the liver.

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

**Dose and Endpoint Selected for Risk Assessment:** 160 mg/kg/day based on liver changes at 250 mg/kg/day

**Uncertainty Factor:** 100

**Comments about Study/Endpoint:** The route and duration of exposure in this study is appropriate for risk assessment.

**Risk Assessment for Short-Term Dermal Exposure is Required**

**2. INTERMEDIATE-TERM DERMAL (1-Week to Several Months)**

**Study Selected: 90-Day Subchronic Study in Rats** **Guideline #: 82-1**

**MRID No.:** 43965704

**Executive Summary:** In a 90-day feeding study in rats (MRID 43965704), 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. The following parameters were evaluated: mortality, clinical signs, body weights, food consumption, hematology, clinical chemistries, urinalyses, gross necropsy and organ weights. In addition, for all animals sacrificed at 1 month, the following organs/tissues

were histologic ally examined: liver, thymus, thyroid (with parathyroid) and all gross-lesions. For all animals sacrificed at 3 months, the following organs/tissues were histologic ally examined: liver, kidneys and all gross lesions. For females only, the adrenals were also examined at 3 months (10/dose group). No other tissues Were histologically examined.

No treatment-related effects on mortality or clinical-signs were observed. Ophthalmological examinations were negative. Although slight decreases in body weight (3-6%)and body weight gains (6-11%) were observed in 800 ppm males throughout the study, these decreases were not considered to be toxicologically significant because slight decreases in food consumption (1-8%) were also observed-in the same animals throughout the study. It is likely that the decreased body weights were due to decreased food consumption caused by poor palatability of the food. Except for a significant decrease in food consumption in 800 ppm females during week 1 no other treatment-related changes in body weights, body weight gains or food consumption were observed in male rats at 200 or 400 ppm or in female rats at any dose level. No treatment-related effects on hematological parameters, clinical chemistries, urinalyses or gross necropsies were observed in the male or female rats. Treatment-related increased absolute liver weights (9-15%) and liver/body weight ratios (9-15%) were observed in the male and female rats at 400 and 800 ppm at the 1 month interim sacrifice, but not at the 3 month terminal sacrifice. An equivocal increase in liver weights in the male and female rats was also observed at 200 ppm at 1 month. Possibly treatment-related increases in adrenal weights(15-23%) and adrenal/body Weight ratios (15-27%) were noted in female rats at 400 and 800 ppm at 3 months. A treatment-related increased incidence of centrilobular swollen hepatocytes in male rats at 400 and 800 ppm and of vacuolization in the hepatocytes of female rats at 400 and 800 ppm was observed at 1 month, but not at 3 months. 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. Slightly swollen cortical cells in the adrenals of one 400 ppm and two 800 ppm female rats observed at the 3 month terminal sacrifice were considered to be possibly related to treatment with the test material.

**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 and liver/body weight ratios in males and females at 1 month, possibly increased absolute adrenal weights and adrenal/body weight ratios in females at 3 months, increased centrilobular swollen hepatocytes in males at 1 month, and increased vacuolization in hepatocytes in females at 1 month. **The NOAEL in this study is 200 ppm (15.8,and 18.7 mg/kg/day in** males and females respectively).

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

**Dose and Endpoint Selected for Risk Assessment:** 15.8 mg/kg/day based on liver changes at 32.1 mg/kg/day. Since an oral NOAEL was selected, a dermal absorption factor of 41% is used for this risk assessment.

**Comments about Study/Endpoint:** the duration of this study is appropriate for this exposure period of concern (i.e., 7 days to several months) with clear histopathologic effects

on the liver at one month of exposure.

**Risk Assessment for Intermediate-term Dermal Exposure is Required**

**4. LONG-TERM DERMAL (Several Months to Lifetime)**

**Study Selected:** 12-month chronic oral study in dogs **Guideline #:** §83-1b

**MRID No.:** 41328802.

**Executive Summary:** See - 3. Chronic Dietary (Reference Dose RfD) section

**Dose and Endpoint for Risk Assessment:** 2.5 mg/kg/day based on clinical signs of vomiting and soft stools; depressed body weight gains, increased alkaline phosphatase activity and increased liver weights at 20 mg/kg. Since an oral NOAEL was selected, a dermal absorption factor of 41% is used for this risk assessment.

**Comments about Study/Endpoint:** The dose and endpoint are relevant because the chronic dosing regimen simulates the exposure period of concern (several months to life-time).

**Risk Assessment for Long-Term Dermal Exposure is Required**

**5. INHALATION EXPOSURE**

**a) Acute Inhalation Exposure (<1 day)**

An acute inhalation risk assessment is not required because Imazalil is classified as Toxicity Category IV for inhalation ($LC_{50}$ = 2.43 mg/L in males and females; MRID 44107214). Imazalil also has a very low vapor pressure (0.0093 mPa 25°C) to be of concern for acute inhalation exposure.

**b) Short Term Inhalation Exposure (1-7 days)**

**Study Selected:** Developmental Study–Rabbit **Guideline #:83-3b**

**MRID No.:** 42593601

**Executive Summary:** See II-1 above

**Dose and Endpoint Selected for Risk Assessment:** 5 mg/kg/day with oral equivalent to be used

**Comments about Study/Endpoint:** The duration of exposure in the selected study simulates short term intermittent inhalation exposure

**Risk Assessment for Short Term Inhalation Exposure is Required.**

**c) Intermediate and Long Term Inhalation Exposure ( 1-3 months and more than three months)**

**Study Selected:** 12-month chronic oral study in dogs **Guideline #:** §83-1b

**MRID No.:** 41328802.

**Executive Summary:** See II-3 above

**Dose and Endpoint Selected for Risk Assessment:** 2.5 mg/kg/day with oral equivalent to be used

**Comments about Study/Endpoint:** See II-3 above

Since the doses identified for inhalation risk assessment are from oral studies route-to-route extrapolation should be as follows:

Step I: The inhalation exposure component (i.e. $\mu$g a.i./day) using a 100% absorption rate (default value) and an application rate should be converted to an **equivalent oral dose** (mg/kg/day)

Step II: The dermal exposure component (i.e. mg/kg/day) using a 41% dermal absorption factor and an application rate should be converted to an **equivalent oral dose**. This dose should then be combined with the converted oral dose in Step I.

Step III: The combined dose from Step II should then be compared to oral NOAEL of 2.5 mg/kg/day to create the MOE's for Intermediate- and Long-Term exposures.

NOTE: The dermal and inhalation MOE's can not be combined for Short Term since a dermal NOAEL was selected for the Short Term scenarios.

**Risk Assessment for Inhalation Exposure is Required.**

**Margin of Exposure (MOE):** An MOE of 100 is adequate for occupational exposure. There are no residential uses. Residential risk assessment is not required.

**Recommendation for Aggregate (Food, Water, Dermal, Inhalation) Exposure Risk Assessments.** Since there are no residential uses, aggregate exposure risk assessments for food and water only is required.



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## IV. CARCINOGENICITY SCREEN:

### 1. Combined Chronic Toxicity/Carcinogenicity Study in Rats

**Guideline #:** 83-5

**MRID No.:** 47026101

**Executive Summary**: 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, MRID No. 47026101, Imazalil technical (98.1%) was administered as a 50% mixture with 50% of equal parts of aerosil (25%) and cornstarch (25%)in the diet to Cpb: Wu Wistar rats, 50/sex/dose at dietary levels of 0, 25, 100, or 400 ppm. Approximate doses were 1.0, 3.7, and 15.5 mg/kg/day for males and 1.2, 4.7, and 20.0 mg/kg/day for females, respectively. In the 18-month study (Accession No. 00162412) 20 rats/sex/dose were 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.

**Discussion of Tumor Data.** An increased incidence of Leydig cell tumors in the testes was noted at 30 months in all dosed groups of males (3/50, 4/50 and 4/47 at low, mid and high dose groups, respectively, compared to 1/50 for the controls). The supplemental histopathological information (MRID 41558501) on the historical incidence of Leydig cell testicular tumors dismissed an association with the administration of the test chemical based on the lack of both statistically significant increased incidence and the lack of a dose related increase in tumors. An unusual epidermoid carcinoma of the uterus in female rats (1/50) was reported at the low and high doses only.

This study was evaluated by the **HED Cancer Peer Review Committee (CPRC)** in 1994. CPRC found no apparent increase in any tumors, however the CPRC determined that the highest dose tested was not adequate enough for assessing the carcinogenic potential of Imazalil in the rat. **Another rat study at higher doses was recommended.**

Based on the combined results of the two studies, a minimal **LOAEL** could be established at 400 ppm (15.5 and 20.0 mg/kg/day in males and females, respectively) 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)

**Adequacy of the Dose Levels Tested** : Imazalil was not tested at adequate doses.

**2. Carcinogenicity Study in Mice**

**Guideline #:** 83 - 2

**MRID No.:** 42972001

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

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

**Discussion of Tumor Data.** The incidence of hepatocytic neoplasms was increased in males in the 200 and 600 ppm groups (50% in both groups versus 26% in controls, $p<0.05$) and in females at 600 ppm (22% versus 8% in controls, $p<0.05$). Of the hepatocytic neoplasms, the incidences for hepatic neoplastic nodules were increased in males in the 200 and 600 ppm groups (46% at 200 ppm and 34% at 600 ppm versus 16% in controls). Trends for increases in total hepatocytic neoplasms and neoplastic nodules were observed in both males and females. A possible increase in the incidence of hepatocytic carcinomas was observed in males at 600 ppm (22% versus 10% in controls). **A statistical increase ($p<0.05$) in the incidence of vaginal metaplasia (22/48 vs 9/44) was observed.** The dose levels used in

this study (0, 50, 200, and 600 ppm in the diet) were previously agreed to by Toxicology Branch I based upon decreased body weight gain seen in males (-25%) and females (-30%) at 800 ppm in the 90-day range finding study in mice.

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

The study was evaluated by the HED Carcinogenicity Peer Review Committee (CPRC; August 24, 1994). CPRC concluded that administration of Imazalil in the diet to CD-1 mice resulted in statistically significant increases in liver adenomas and adenomas/carcinomas in male Swiss albino mice, with a positive trend for adenomas, carcinomas and combined adenomas/carcinomas. The increase in carcinomas, while not statistically significant by pairwise 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 felt 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. In a subsequent HED memo (Bernice Fisher to Henry Spencer, march 7, 1995), the $Q_1^*$ was estimated to be $6.20 \times 10^{-2}$ $(mg/kg/day)^{-1}$.

The HED Cancer Assessment Review Committee (CARC) reevaluated the carcinogenic potential of Imazalil on September 4, 1998 (HED document no. 012870) considering new data submitted by the registrant on the above mouse study, mechanistic data in 90-day rat feeding studies, progress reports on an ongoing 2-year rat combined onco/chronic feeding study and new mutagenicity studies. The CARC 1998 report reaffirmed the original conclusions of the 1994 CPRC report.

**Cancer Classification and Basis:**

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.

Mutagenicity studies were all negative.

**The registrant subsequently submitted the following:**

- Two new 90-day subchronic feeding (dose range finding and mechanistic toxicity) studies in rats.

- Pathology Working Group Report re-evaluating the pre-neoplastic and neoplastic lesions observed in the livers of the male and female mice in the mouse carcinogenicity study.
- 6-month interim report on a new 2-year combined chronic feeding/carcinogenicity study in rats.
- Two new mutagenicity assays in addition to an acceptable *in vivo/in vitro* unscheduled DNA synthesis study with liver accompanied by a cell proliferation (S-phase) assay.

The HED Cancer Assessment Review Committee (CARC) met on July 22, 1998 to consider the new data and issued its report on September 4, 1998 reaffirming the earlier classification of Imazalil as a Group C carcinogen.



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3. **Mutagenicity** – The following table presents the mutagenicity findings for IMAZALIL BASE, TECHNICAL GRADE:

| GL # | MRID | Study Type | Results and Classification |
|---|---|---|---|
| 84-2 | 40729301 | Ames Assay<br>5-500 µg/plate<br>March 22, 1988 | Negative in Salmonella strains up to toxic concentrations of 250-500 µg/plate with or without S-9 activation. **Acceptable/guideline** |
| 84-2 | 40729302 | In vitro mammalian chromosomal Aberration<br>0, 50, 200, 400 or 800 µg per culture<br>October 21, 1996 | Negative with or without metabolic activation.<br>**Acceptable/guideline** |
| 84-2 | 40729303 | In vivo micronucleus test - mice; 0, 20, 80 or 320 mg/kg | Negative for induction of micronuclei in bone marrow cells at all levels tested.<br>**Acceptable/guideline** |
| 84-2 | 43780201 | Unscheduled DNA synthesis in primary rat hepatocytes<br>August 20, 1990 | Negative for inducing Unscheduled DNA Synthesis.<br>**Acceptable/guideline** |
| 84-2 | 00031599 | Micronucleus Test: Rats<br>0, 10, 40 or 160 mg/kg, i.p.<br>Janssen<br>December 1979 | Not mutagenic<br>**Acceptable/guideline** |
| 84-2 | 43735003 | In vitro mammalian cell gene mutation: Chinese hamster lung V79 cells<br>April 27, 1995 | negative for gene mutations at all doses 10-100 µg/mL in the presence or absence of enzyme activation<br>**Acceptable/guideline** |
| 84-2 | 43965702 | In vivo/in vitro Unscheduled DNA synthesis in primary mouse hepatocytes<br>February 1996 | Negative for Unscheduled DNA synthesis (genotoxicity) at the single oral doses 125 or 250 mg/kg<br>Positive for cellular proliferation (Replicative DNA synthesis) at overtly and cytotoxic doses<br>**Acceptable/guideline** |

## V. FQPA CONSIDERATIONS

### 1. Adequacy of the Data Base

No neurotoxicity studies were available. Developmental toxicity studies in rat, mice, and rabbits and a reproductive toxicity study in rats with Imazalil have been submitted and were classified as acceptable.

2. **Neurotoxicity Data**

No neurotoxicity studies were available. Neurotoxic signs were noted at lethal doses in maternal animals prior to death in mouse and rabbit developmental studies. The literature search revealed limited data regarding neurobehavioral effects in offspring.

3. **Developmental & Reproductive Toxicity**

(i) **Developmental Toxicity:** There is no evidence for 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).

(ii) **Reproductive Toxicity:** In a 2-generation reproduction study (MRID 42570701), imazalil (≥95.0%) was administered in the diet to a non-inbred strain of 24 Wistar rats per sex at approximately 0, 5, 20 or 80 mg/kg/day for 60 days prior to mating, through mating and lactation (females only). 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** appeared as 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.

(iii) Neurobehavioral Toxicity. In a published study on the reproductive and neurobehavioral effects (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 equivalent to 19, 39 and 79 mg/kg/day in males and 26, 45 and 102 mg/kg/day in females during the preconception period) from 5 weeks of age of the $F_0$ generation to 9 weeks of age in the $F_1$ generation. Chemical intake during the lactation period increased to 65, 153 and 262 mg/kg/day at the dietary levels of 120, 240, 1nd 480 ppm, respectively. 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 $F_0$ and $F_1$ generation. Data were subjected to appropriate statistical analysis. **In the $F_0$ 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 $F_1$ 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 neurobehavioral effects can occur in mice exposed prenatally to Imazalil in their

diet.

(iv) **Other Reproductive Effects.** 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.

(v) **Structure Activity Relationships.** In the structurally related fungicide, **TEBUCONAZOLE** (PC Code 128997), the HED Hazard Identification Review Committee (HIARC) determined that on the basis of comparative NOAELs and LOAELs, there was no indication of increased susceptibility of mice, rats or rabbits *in utero* and /or postnatal exposure (HED Doc. No. 012534). However, HIARC determined that a postnatal developmental neurotoxicity study in rats with this chemical is **required** based on the weight of evidence. In the developmental toxicity studies, there was evidence of alterations to the development of the fetal nervous system in mice (increased malformations of the brain and spinal cord and exencephaly), in rats (anophthalmia) and in rabbits (neural tubule defects characterized as meningocoele and spina bifida and hydrocephalus) at doses 10 fold higher than the developmental toxicity NOAEL. With the exception of Etaconazole and Hexaconazole, other structurally related compounds (Triadimefon, Triademenol, Bitertanol, Uniconazole, Propiconazole, Azaconazole, and Cyproconazole) have been shown to cause developmental toxicity with a LOAEL below the maternal toxicity LOAEL.

**4. Determination of Susceptibility**

The data submitted to the Agency as well as those from the published literature do not demonstrate increased sensitivity of rats, mice, or rabbits to *in utero* exposure to Imazalil. The developmental effects in fetuses occurred at or above doses that caused maternal toxicity. 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. The offspring toxicity NOAEL/LOAEL was also 20/80 mg/kg/day. 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 is assumed that pups are 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.

5. **Determination of the Need for Developmental Neurotoxicity Study**

Although neurotoxicity studies were not conducted, additional neurotoxicity studies including the developmental neurotoxicity study are not required because there is no evidence of neurotoxicity, neuropathology, or abnormalities in the development of the fetal nervous system was seen in the available toxicity studies.

6. **Determination of the FQPA Factor:**

Based on the increased susceptibility of pups seen in the reproduction study in rats, the HIARC recommended that the 10x FQPA safety factor (10X) be retained. The final recommendation will be made by the FQPA Safety Factor Committee.

## VI. HAZARD CHARACTERIZATION

The data base for Imazalil is adequate for hazard characterization. Imazalil has moderate oral (Category II), low dermal (Category III), and inhalation (Category IV) toxicity. It is Toxicity Category IV for primary dermal irritation and Category I for primary eye irritation. It is not a dermal sensitizer. Imazalil (EC formulation) is readily absorbed by the rat skin with a 41 % absorption rate within 10 hours of application

The primary target organ for Imazalil in animals is the liver. Increased liver weights were seen in dogs treated for one year (20 mg/kg/day) , swollen livers in rabbits after dermal treatment (250 mg/kg/day) for short period (6 days), increased liver weights and liver to body weight ratios, increased centrilobular swollen hepatocytes and increased vacuolization in hepatocytes after one month of treatment ( 32.1 mg/kg/day) in rats, and similar histopathologic effects in mice (38.6 mg/kg/day). In chronic 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 were seen at 15.5 mg/kg/day. Liver histopathologic lesions were also seen in mice 23-month study at 28.0 mg/kg/day. Increased liver vacuolization was also seen in male rats in a 2-generation reproduction study at 80 mg/kg/day.

The data submitted to the Agency as well as those from the published literature do not demonstrate increased sensitivity of rats, mice, or rabbits to *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 postnataly. In the 2-generation reproduction study, an increased susceptibility of the pups to Imazalil was reported. The pup survival rate was adversely affected by the Imazalil in the F2 generation from birth to post natal day 4. The HIARC determined that pups' deaths resulted from an increased susceptibility to Imazalil from the milk intake during lactation.

Carcinogenicity studies in mice indicated that Imazalil was carcinogenic to male mice, based on significant increase in liver adenomas and adenomas/carcinomas. The available rat study did not provide evidence of carcinogenicity in male or female rats. The HED CPRC

and CARC classified Imazalil a Group C-carcinogen and recommended a linear low dose approach ($Q_1^*$) for quantization of human risk. The $Q_1^*$ is $6.2 \times 10^{-2}$(mg/kg/day)$^{-1}$. Imazalil was not mutagenic both *in vivo* and *in vitro*.

## VII. DATA GAPS

There are no data gaps. A combined chronic toxicity/carcinogenicity study in rats has been recently submitted by the registrant (June 24, 1999) and is pending the HED review.

## VIII. ACUTE TOXICITY

| Guideline No. | Study Type | MRIDs # | Results | Toxicity Category |
|---|---|---|---|---|
| 81-1 | Acute Oral: Rats | 00031596<br>44107212 | $LD_{50}$ = 343 mg/kg<br>$LD_{50}$ = 480-679 mg/kg | II<br>II |
| 81-2 | Acute Dermal: Rabbits | 41606104<br>44107213 | $LD_{50}$ = >2000 mg/kg<br>$LD_{50}$ = >2000 mg/kg | III<br>III |
| 81-3 | Acute Inhalation: Rats | 44107214 | $LC_{50}$ = 2.43 mg/L | IV |
| 81-4 | Primary Eye Irritation | 41606105 | Irritating | I |
| 81-5 | Primary Skin Irritation | 44107216 | Mild-irritation | IV |
| 81-6 | Dermal Sensitization | 41718701<br>40271701 | Non-sensitizer<br>Non-sensitizer | IV |
| 81-8 | Acute Neurotoxicity | — | | |

References

Tanaka, T. 1995. Reproductive and Neurobehavioral effects of Imazalil Administered to Mice. Toxicology 9 (3): 281-288.

Mason, JI; Carr, BR; and Murry, BA. 1987. Imidazole Antimycotics: Selective inhibitors of Steroid Aromatization and Progesterone Hydroxylation. Steroids 50 (1-3): 179-189.

## VII. SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

| EXPOSURE SCENARIO | DOSE (mg/kg/day) | ENDPOINT | STUDY | MOE* |
|---|---|---|---|---|
| Acute Dietary Females 13-50 General Population: Not Relevant | NOAEL=5<br>UF = 100 | Increased resorption and decreased fetuses | Developmental-Rabbit Study | Not Relevant |
| | **Acute RfD =0.05 mg/kg/day** | | | |
| Chronic Dietary | NOAEL=2.5<br>UF= 100 | Systemic toxicity: vomiting, soft stools, ↓body weight gain, ↑liver weight, ↑alkaline phosphatase | Chronic Toxicity-Dogs | Not Relevant |
| | **Chronic RfD =0.025 mg/kg/day** | | | |
| Dermal Absorption | 41% based on a dermal absorption study in male rats | | | |
| Short-Term (Dermal) | Dermal NOAEL=160 | Skin effects and swollen livers | 21 Day Dermal - Rabbit | 100 |
| Intermediate-Term (Dermal)[a] | Oral NOAEL=15.8 | Severe liver effects | Subchronic Study - Rats | 100 |
| Long-Term (Dermal)[a] (Non-cancer) | Oral NOAEL=2.5 | Systemic toxicity: vomiting, soft stools, ↓body weight gain, ↑liver weight, ↑alkaline phosphatase | Chronic Toxicity-Dogs | 100 |
| Cancer Chronic Dietary** | $Q_1^* = 6.2 \times 10^{-2}$ $(mg/kg/day)^{-1}$ | Hepatocytic neoplasm | Carcinogenicity Study Mice | NA |
| Inhalation (Acute) | Not required: acute inhalation is category IV. Acute exposure not likely | | | |
| Inhalation (Short-term) | Oral NOAEL = 5 | Increased resorption and decreased fetuses | Developmental-Rabbit Study | |
| Inhalation (Intermediate and long term) | Oral NOAEL = 2.5 | Systemic toxicity: vomiting, soft stools, ↓body weight gain, ↑liver weight, ↑alkaline phosphatase | Chronic Toxicity-Dogs | |

[a] = Since an oral value was selected, a 41% dermal absorption factor should be used for route to route extrapolation.

* MOEs are for occupational exposure risk assessments; there are no registered residential uses at the present time.

** For dermal Cancer risk assessment use 41% absorption factor.



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