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**SECOND EVALUATION OF THE CARCINOGENIC POTENTIAL OF
IMAZALIL**

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**CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS**

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EXECUTIVE SUMMARY

The carcinogenic potential of imazalil was first evaluated by the Health Effects Division (HED) Carcinogenicity Peer Review Committee (CPRC) on August 24, 1994. At that time, the CPRC concluded that imazalil should be classified as a Group C - possible human carcinogen - and recommended that for the purpose of risk characterization, a low dose extrapolation model (Q1*) be applied to the animal data for the quantification of human risk. This conclusion was based on statistically significant pairwise increases in liver adenomas and combined adenomas/carcinomas observed in male Swiss albino mice. An increase in liver carcinomas, while not statistically significant by pairwise comparison with the controls, nevertheless was considered by the CPRC to be biologically significant. In female mice, there were no statistically significant pairwise increases in liver tumors, but there were statistically significant positive trends for liver adenomas and combined adenomas/carcinomas. The CPRC felt that the tumorigenic response in females was supportive of that seen in males. Available information on several structural analogs of imazalil (triazole fungicides), which also induce liver tumors in mice, provided additional support for the classification. The CPRC recommended that for the purpose of risk characterization, the quantification (Q1*) of human risk be based on the total liver tumors (combined adenomas/carcinomas) in the male mouse. In a carcinogenicity study in Wistar rats, there was no apparent increase in any tumors. The CPRC, however, determined that the highest dietary concentration tested in this study (400 ppm) was not adequate for assessing the carcinogenic potential of imazalil in the rat. The Committee recommended that a new 2-year chronic feeding/carcinogenicity study in rats using higher dose levels be performed unless the registrant could properly justify the dose levels which were used in this study. Imazalil did not appear to have mutagenic activity. A data gap, however, was identified by the CPRC and additional mutagenicity testing was required.

At the present meeting of the Cancer Assessment Review Committee (CARC) on July 22, 1998, additional data and information pertaining to the evaluation of the carcinogenic potential of imazalil were evaluated. Regarding the carcinogenicity study in mice, a Pathology Working Group (PWG) report, which presented a re-evaluation of the pre-neoplastic and neoplastic lesions observed in the livers of the male and female mice in this study, was considered. A revised qualitative risk assessment of the liver neoplasms in this study, based on the PWG re-evaluation, was also considered. Although recognizing that fewer liver carcinomas were diagnosed in the imazalil-treated male mice in the PWG report compared to the original report, the CARC, nevertheless, considered the differences between the original reading and the re-evaluation of the liver slides for both males and females to be relatively small and of insufficient magnitude to alter its previous conclusions with respect to the neoplastic findings in this study. In particular, the CARC placed considerable importance on the statistically significant pairwise increase in combined adenomas/carcinomas observed in the male mice at the two highest dose levels in the PWG re-evaluation of the liver slides. Regarding new historical control data on a single study also included in the PWG report, the CARC felt that the results from only one study were of limited usefulness. For purposes of comparison with the imazalil treated animals, the CARC continued to place most importance on the concurrent controls in the study.

Regarding the carcinogenicity study in Wistar rats, the registrant attempted to justify the dose levels used in this study by submitting two new 90-day subchronic feeding (dose range finding and mechanistic toxicity) studies in rats. In one study, Wistar rats were given imazalil in the diet at dietary concentrations up to 800 ppm. In the other study, Wistar (Hannover substrain) rats were given imazalil in the diet at dietary concentrations up to 3200 ppm. Additionally, the registrant initiated a new 2-year chronic feeding/carcinogenicity study in Wistar (Hannover substrain) rats at dietary concentrations up to 2400 ppm. A 6-month interim report on this study was also submitted by the registrant. The in-life phase of this new 2-year study is scheduled to be completed in October, 1998. The registrant has also assessed the dose levels used in the previously submitted carcinogenicity study in Wistar rats in accordance with the April 1996 EPA Proposed Guidelines for Carcinogen Risk Assessment (Proposed Guidelines). The registrant believes that these Proposed Guidelines supercede the 1986 Guidelines for Carcinogen Risk Assessment (1986 Cancer Guidelines) and redefine the criteria for an acceptable high dose level for use in carcinogenicity studies. The registrant believes the highest dietary concentration tested in this study (400 ppm) meets the new high dose requirements in the Proposed Guidelines and has requested a waiver from the requirement for the new (ongoing) 2-year chronic feeding/carcinogenicity study in rats (imposed by the CPRC in 1994).

The CARC considered the two new 90-day subchronic feeding (dose range finding and mechanistic toxicity) studies in rats and the 6-month interim report on the 2-year combined chronic feeding/carcinogenicity study in rats. Regarding body weights and body weight gains in these studies, the CARC concluded that poor palatability of the food (particularly at dietary concentrations ≥ 800 ppm) frequently led to food wastage and/or decreased food consumption which severely compromised the body weight and body weight gain data in these studies. In the 6-month interim report on the ongoing combined study in rats, however, it was determined that food efficiency was not affected at dietary concentrations as high as 2400 ppm. The CARC also recognized that imazalil is a mixed type of liver microsomal enzyme inducer, which results in typical signs of enzyme induction such as increased absolute liver weights, increased liver/body weight ratios, hypertrophy of hepatocytes, increased microsomal protein content, increased cytochrome P450 content, and increased activities of liver glucuronosyltransferase and various monooxygenase isoenzymes. The CARC generally does not consider induction of the liver microsomal enzyme system, in the absence of other more serious signs of toxicity, to be sufficient evidence of adequate dosing in carcinogenicity studies. Other signs of toxicity in these studies were also considered by the CARC to be insufficient evidence of adequate dosing. Noting the apparent lack of significant toxicity at 6 months and also a lack of life threatening toxicity at 17 months in the ongoing 2-year combined chronic feeding/carcinogenicity in rats at dietary concentrations up to 2400 ppm, the CARC determined that this study should be continued to completion, including full pathological evaluation of all surviving animals at termination of the study. The CARC recommended that the registrant's request for a waiver of this study be denied.

Regarding the data gap for mutagenicity testing identified by the CPRC on August 24, 1994, the registrant submitted an acceptable in vivo/in vitro unscheduled DNA synthesis (UDS) assay with mouse liver accompanied by a cell proliferation (S phase) assay as recommended by the CPRC and also two additional mutagenicity assays. The available mutagenicity studies now fulfill the *minimum requirements* for the current mutagenicity testing guidelines. Overall, the available

mutagenicity data indicate that imazalil does not induce mutagenic activity. There was, however, clear evidence of cellular proliferation (mitogenic activity) in the in vivo cell proliferation (S phase) assay conducted in mouse liver.

The CARC, at the present meeting, reviewed the mode of action data on Swiss albino mice previously submitted by the registrant and considered at the first peer review meeting on August 24, 1994. It was recalled that imazalil is a mixed type of liver microsomal enzyme inducer, which results in typical signs of enzyme induction in studies in mice (and rats). The present meeting also considered the results in the in vivo/in vitro unscheduled DNA synthesis (UDS) assay and replicative DNA synthesis (RDS) assay in primary mouse hepatocytes. In this study, there was no genotoxic response, but there was clear evidence of increased cellular proliferation (mitogenicity) in the mouse liver cells following treatment with imazalil. Overall, imazalil was determined to be non-genotoxic in a battery of acceptable mutagenicity studies, but mitogenic (causing cellular proliferation) in mouse liver cells. The CARC is aware that tumorigenic findings in the livers of mice are oftentimes associated with non-genotoxic, mitogenic compounds that induce the liver microsomal enzyme system. The CARC, however, does not believe liver enzyme induction to be sufficiently well correlated to tumor induction to be considered as a mode of action for liver tumorigenicity. The CARC is also aware of the current scientific controversy regarding the potential relationship of such findings, particularly mitogenicity, to the induction of liver tumors in mice. At this time, the CARC does not consider this potential relationship to be sufficiently well established to conclude that such findings clearly indicate a mode of action leading to hepatocellular tumors in the livers of mice. Hence, at this time, the CARC does not consider the available mechanistic data on imazalil to be convincing evidence for a mode of action for the carcinogenicity of this compound.

Imazalil is structurally related to the triazole fungicides, at least seven of which have also been shown to induce hepatocellular adenomas, carcinomas, or both in male and/or female mice.

The CARC concluded there was no compelling reason to deviate from the conclusion reached by the CPRC on August 24, 1994 when the CPRC classified imazalil as a Group C Carcinogen (possible human carcinogen). The CARC also determined that the available mechanistic data do not provide a definitive mode of action with respect to the hepatocellular tumors observed in imazalil-treated mice. It was concluded that more data/information will be necessary before a definitive mode of action can be delineated. Since a definitive mode of action for the carcinogenicity of imazalil in mice has not been established, the CARC also concluded there was no compelling reason to deviate from the conclusion of the CPRC on August 24, 1994 that for the purpose of risk characterization, a linear low dose extrapolation model be applied to the animal data for quantification of human risk (Q1*). The CARC also re-affirmed the prior conclusion by the CPRC that the quantification of human risk be based on the total liver tumors (combined adenomas/carcinomas) in the male mouse and recommended that the previously calculated Q1* continue to be used to characterize the carcinogenic risk to humans.

I. INTRODUCTION

On July 22, 1998, the HED Cancer Assessment Review Committee (CARC) met to consider the potential carcinogenicity of imazalil. The carcinogenic potential of imazalil had been previously evaluated by the HED Carcinogenicity Peer Review Committee (CPRC) on August 24, 1994. The material available for review consisted of DERs and other data summaries supplied by Edwin Budd, tables and statistical analyses prepared by Lori Brunzman, and copies of selected documents submitted by the registrant (Janssen Pharmaceutica N.V.). The material included DERs for two 90-day subchronic feeding (dose-range finding and mechanistic toxicity) studies in rats, a 6-month interim report on a 2-year chronic feeding/carcinogenicity study in rats, and three mutagenicity studies. The material also included a Pathology Working Group (PWG) report on a re-evaluation of the male and female liver slides in a previously submitted 23-month carcinogenicity study in mice, a qualitative risk assessment on the results of the PWG report, the report of the first carcinogenicity peer review meeting on imazalil on August 24, 1994 (1), four documents submitted by the registrant (2-5), and a summary of the carcinogenic and mutagenic findings and current EPA classification of ten triazole fungicides considered to be structurally related to imazalil (prepared by Edwin Budd). The material reviewed is attached to the file copy of this report.

II. BACKGROUND INFORMATION

Imazalil (enilconazole) is an imidazole fungicide registered for post-harvest use on bananas and citrus and as a seed treatment for wheat and barley. It also has tolerances for residues in/on cottonseed and for secondary residues in meat and milk (40 CFR 180.413). Imazalil inhibits the cytochrome P450 14-alpha demethylation of sterols and thereby prevents the formation of essential cell wall components of fungi. It is structurally related to the triazole fungicides, including triadimefon, triadimenol, uniconazole, tebuconazole, propiconazole, cyproconazole, hexaconazole, etaconazole, bitertanol and azaconazole. Imazalil contains 2 nitrogen atoms in an imadazole ring, however, whereas the triazole ring contains 3 nitrogen atoms.

At the first evaluation of imazalil (see memo dated December 27, 1994), the CPRC concluded that imazalil should be classified as a Group C - possible human carcinogen - and recommended that for the purpose of risk characterization, a low dose extrapolation model be applied to the animal data for the quantification of human risk (Q1*). This conclusion was based on statistically significant pairwise increases in liver adenomas and combined adenomas/carcinomas observed in male mice in a 1993 23-month carcinogenicity study in Swiss albino mice (MRID 42972001). Statistically significant positive trends for liver adenomas, carcinomas and combined adenomas/carcinomas were also observed in the male mice. The increase in carcinomas, while not statistically significant by pairwise comparison with the controls, nevertheless was considered by the CPRC to be biologically significant (since carcinomas contributed almost equally to the total tumorigenic response and there was an apparent progression of benign to malignant tumors). Further, the incidence of carcinomas exceeded that of historical controls submitted by the registrant. In female mice, there were no statistically significant pairwise increases in liver tumors, but there were statistically significant positive trends for liver adenomas and combined adenomas/carcinomas. The CPRC felt that the tumorigenic response in females was supportive of that seen in males. It was noted that tumors

in this mouse study appeared at dietary concentrations which were not particularly high (200 and 600 ppm). Available information on certain structural analogs of imazalil (etaconazole, uniconazole, cyproconazole, tebuconazole), which also induce liver tumors in mice, provided additional (and important) support for the classification. The CPRC recommended that for the purpose of risk characterization, the quantification of human risk (Q1*) should be based on the total liver tumors (combined adenomas/carcinomas) in the male mouse. In a 1985 30-month carcinogenicity study on Wistar rats (MRID 00162413) and an associated 1984 18-month chronic feeding study on Wistar rats (MRID 00162412), there was no apparent increase in any tumors. The CPRC, however, determined that the highest dietary concentration tested in the carcinogenicity study (400 ppm) was not adequate for assessing the carcinogenic potential of imazalil in the rat. The committee recommended that a new 2-year chronic feeding/carcinogenicity study in rats using higher dose levels be performed unless the registrant could properly justify the dose levels which were used in this study. Imazalil did not appear to have mutagenic activity. A data gap, however, was identified by the CPRC and additional mutagenicity testing was required. The committee recommended that an in vivo/in vitro unscheduled DNA synthesis (UDS) study with liver accompanied with a cell proliferation (S phase) study be submitted.

Since the CPRC meeting of August 24, 1994, the registrant has submitted additional data and information pertaining to the evaluation of the carcinogenic potential of imazalil. Regarding the 1993 carcinogenicity study in Swiss albino mice (MRID 42972001), a Pathology Working Group (PWG) was convened by the registrant in 1995 to re-evaluate the pre-neoplastic and neoplastic lesions observed in the livers of the male and female mice in this study. The PWG also evaluated liver slides from male and female control mice in a comparable carcinogenicity study to serve as historical control data. Having determined that the PWG re-evaluation was conducted in accordance with PR Notice 94-5, a revised qualitative risk assessment of the liver neoplasms in this study based on the PWG re-evaluation has been prepared.

Regarding the 1985 carcinogenicity study in Wistar rats (MRID 00162413) and the associated 1984 chronic feeding study in Wistar rats (MRID 00162412), the registrant has attempted to justify the dose levels used in the carcinogenicity study by submitting two new (1996) 90-day subchronic feeding (dose range finding and mechanistic toxicity) studies in rats. In one study (MRID 43965704), Wistar rats were given imazalil in the diet at dietary concentrations of 0, 200, 400 or 800 ppm. In the other study (MRID 43965705), Wistar (Hannover substrain) rats were given imazalil in the diet at dietary concentrations of 0, 800, 1600, 2400 or 3200 ppm. Additionally, the registrant has also now initiated a new 2-year chronic feeding/carcinogenicity study in Wistar (Hannover substrain) rats at dietary concentrations of 0, 50, 200, 1200 or 2400 ppm. A 6-month interim report on this study (MRID 44319901) has been submitted to the Agency. The in-life phase of this new 2-year study is scheduled to be completed in October, 1998.

In recent submissions to the Agency, the registrant has assessed the dose levels used in the previously submitted 1985 carcinogenicity study in Wistar rats (MRID 00162413) in accordance with the April 1996 EPA Proposed Guidelines for Carcinogen Risk Assessment (Proposed Guidelines). The registrant believes that these Proposed Guidelines supercede the 1986 Guidelines for Carcinogen Risk Assessment (1986 Cancer Guidelines) and redefine the criteria

for an acceptable high dose level for use in carcinogenicity studies (see page 50 of the Proposed Guidelines). Accordingly, the registrant has stated that "This assessment indicates that the highest dose, i.e., 400 ppm, tested in the previous chronic toxicity/oncogenicity study in rats meets the new high dose requirements of the Proposed Guidelines. Therefore, Janssen [the registrant] requests that EPA revisit this study in regards to its adequacy in fulfilling the rat oncogenicity requirement and grant a waiver from the conduct of a new chronic toxicity/oncogenicity study in rats with Imazalil." (quoted from page 2 of "Waiver Request From Conducting an Additional Oncogenicity Study in Rats with Imazalil", prepared by Vincent J. Piccirillo (NPC, Incorporated, Sterling, Virginia), submitted by Janssen Pharmaceutica N.V., December 16, 1996). Regarding the data gap for mutagenicity testing identified by the CPRC on August 24, 1994, the registrant has submitted an acceptable in vivo/in vitro unscheduled DNA synthesis (UDS) assay with mouse liver accompanied by a cell proliferation (S phase) assay as recommended by the CPRC and also two additional mutagenicity assays. The available mutagenicity studies now fulfill the minimum requirements for the current mutagenicity testing guidelines.

III. Evaluation of Carcinogenicity Evidence

A. Brief Synopsis of Previously Evaluated Selected Studies in Mice and Rats

1. 23-Month Carcinogenicity Study in Swiss Albino Mice

Reference: Department of Toxicology, Janssen Research Foundation, Experiment No. 2194, October 13, 1993, MRID 42972001.

a. Experimental Design

In a 23-month carcinogenicity study, technical grade imazalil base was administered in the diet to groups of 50 male and 50 female Swiss albino mice at dietary concentrations of 0 (control), 50, 200 or 600 ppm (0, 6.8, 28.0 or 88 mg/kg b.w./day in males and 0, 8.3, 34.8 or 110 mg/kg b.w./day in females).

b. Results

The hepatocellular tumor rates and the qualitative risk assessment based on these rates are presented in Tables 1 and 2.

(i) In male mice, a statistically significant pairwise increased incidence of hepatocellular adenomas was observed in the 200 ($p < 0.05$) and 600 ppm ($p < 0.05$) dietary concentration groups and a statistically significant pairwise increased incidence of hepatocellular combined adenomas/carcinomas was observed in the 600 ($p < 0.01$) ppm dietary concentration group. Statistically significant positive trends for adenomas ($p < 0.01$), carcinomas ($P < 0.05$) and combined adenomas/carcinomas ($p < 0.01$) were also observed in the male mice. The increase in carcinomas in the male mice at 600 ppm, while not statistically significant by pairwise comparison with the controls, was considered by the

CPRC to be biologically significant (since carcinomas contributed almost equally to the total tumorigenic response and there was an apparent progression of benign to malignant tumors). Furthermore, the incidence of carcinomas at 600 ppm exceeded that of historical controls for male mice submitted by the registrant. The CPRC noted that liver tumors in this mouse study appeared at dietary concentrations which were not particularly high (200 and 600 ppm).

(ii) In female mice, there were no statistically significant pairwise increases in hepatocellular tumors, but there were statistically significant positive trends for hepatocellular adenomas ($p < 0.05$) and combined adenomas/carcinomas ($p < 0.05$). The CPRC felt that the tumorigenic response in females was supportive of that seen in males, even though driven mainly by adenomas.

(iii) There were no pertinent historical control data for hepatocellular adenomas in this study because of differences in nomenclature used by the testing laboratory in previously conducted historical control studies and the Agency. Regarding historical control data for hepatocellular carcinomas, the available data were considered to be of only limited usefulness either because of differences in the durations of the historical control studies or because the duration of the historical control studies were not specified by the registrant. Only the concurrent control group was considered by the CPRC to be totally appropriate with respect to the nomenclature used for classifying tumors and the length of the study (23 months).

(iv) The dose levels used in this study were considered adequate by the CPRC for assessing the carcinogenic potential of imazalil in mice.

(v) The following treatment-related non-neoplastic effects were observed in this study: decreased body weights at 600 ppm over the duration of the study in males (5-7%) and in females (3-5%); decreased body weight gains at 600 ppm in males (about 18%) and at 200 and 600 ppm in females (6-13%); increased absolute (and relative) liver weights at 600 ppm in males (18%) and at 600 ppm in females (10%); increased incidences of histopathologic lesions in the livers of males (focal cellular changes and hyperplasia at 200 and 600 ppm, and large vacuoles and pigmentation of sinusoidal cells at 600 ppm). No treatment-related non-neoplastic histopathological changes were observed in the livers of females.

NOTE – The liver slides for all male and female mice in this study were later re-evaluated by a Pathology Working Group (PWG) and reported in a subsequent submission to the Agency in 1997. The results of this re-evaluation are presented under “Evaluation of New Carcinogenicity Evidence” and in Tables 3 and 4 of this document.

TABLE 1. IMAZALIL - CHARLES RIVER SPF SWISS ALBINO MOUSE STUDY

Male Hepatocellular Tumor Rates^a and Exact Trend Test and Fisher's Exact Test Results (p values)

ORIGINAL READING OF LIVER SLIDES (1993)

	<u>Dietary Concentration (ppm)</u>			
	0	50	200	600
Adenomas (%)	5/50 (10)	2/47 (4)	14 ^a /50 (28)	13/48 (27)
p =	0.003**	0.244 ^b	0.020*	0.027*
Carcinomas (%)	5/50 (10)	6/47 (13)	6/50 (12)	11 ^b /48 (23)
p =	0.033*	0.456	0.500	0.072
Combined (%)	10/50 (20)	8/47 (17)	17 ^c /50 (34)	22 ^d /48 (46)
p =	0.001**	0.455 ^b	0.088	0.006**

^aNumber of tumor bearing animals/Number of animals examined, excluding those that died before week 53.

^bNegative change from control.

^aFirst adenoma observed at week 55, dose 200 ppm.

^bFirst carcinoma observed at week 72, dose 600 ppm.

^cThree animals in the 200 ppm dose group had both an adenoma and a carcinoma.

^dTwo animals in the 600 ppm dose group had both an adenoma and a carcinoma.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then p < 0.05. If **, then p < 0.01.

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TABLE 2. IMAZALIL - CHARLES RIVER SPF SWISS ALBINO MOUSE STUDY

Female Hepatocellular Tumor Rates^a and Exact Trend Test and Fisher's Exact Test Results (p values)

ORIGINAL READING OF LIVER SLIDES (1993)

	<u>Dietary Concentration (ppm)</u>			
	0	50	200	600
Adenomas (%)	3/45 (7)	3 ^a /48 (6)	0/45 (0)	7/47 (15)
p =	0.035*	0.630 ^a	0.121 ^a	0.176
Carcinomas (%)	0/45 (0)	2/48 (4)	2/45 (4)	3 ^b /47 (6)
p =	0.103	0.264	0.247	0.129
Combined (%)	3/45 (7)	5/48 (10)	2/45 (4)	9 ^c /47 (19)
p =	0.027*	0.394	0.500 ^a	0.070

^aNumber of tumor bearing animals/Number of animals examined, excluding those that died before week 53.

^aNegative change from control.

^aFirst adenoma observed at week 83, dose 50 ppm.

^bFirst carcinoma observed at week 88, dose 600 ppm.

^cOne animal in the 600 ppm dose group had both an adenoma and a carcinoma.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then p < 0.05. If **, then p < 0.01.

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2. 3-Month Oral Mechanistic Toxicity Study in Swiss Albino Mice With 1-Month Interim Sacrifice (in 4 Parts)

Reference: Janssen Research Foundation, Experiment No. 3140, Reports No. R 23979/FK1600 and R 23979/FK1682, February 18, 1994, February 11, 1994 and December 9, 1993, MRID 43222601 (Part 1), 43202402 (Part 2), 43202402 (Part 3), and 43202402 (Part 4).

a. Experimental Design

In a 3-month oral mechanistic toxicity study designed to study effects on the liver, technical grade imazalil base was administered in the diet to groups of 25 male and 25 female Swiss albino mice at nominal dietary concentrations of 0 (control), 50, 200 or 600 ppm (0, 9.5, 38.6 or 115 mg/kg b.w./day in males and 0, 11.3, 45.6 or 138 mg/kg b.w./day in 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 after 1 month. The following parameters were evaluated: mortality, clinical signs, body weights, body weight gains, food consumption, clinical chemistries (three liver enzymes only), gross necropsy, organ weights, and histopathology (liver and gall bladder only). In additional special studies, the following parameters were also evaluated: liver content of microsomal protein and cytochrome P450, liver enzymatic activities of seven P450 isoenzymes, liver testosterone hydroxylase activity, electron microscopy of liver sections, and concentrations of imazalil in serum.

b. Results

(i) 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, and increased incidence and severity of "centrilobular clearer aspect" and of large and/or small vacuoles in the hepatocytes of males and females. At 600 ppm, the following treatment-related effects were observed: increased alkaline phosphatase in males, 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, and increased diffuse swelling of hepatocytes in females.

(ii) Increased microsomal protein and increased microsomal cytochrome P450 content were observed in the livers of males and females at 200 and 600 ppm.

(iii) Regarding liver enzymatic activities of seven P450 isoenzymes, the results indicated a mixed type of induction at 200 and 600 ppm. Dosing with imazalil significantly

induced some enzymatic activities but also had an inhibitory effect on other enzyme

activities.

(iv) The total activity of testosterone hydroxylases was increased in both males and females at 600 ppm.

(v) 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 in the hepatocytes of males and females at 600 ppm.

(vi) Low levels of imazalil were detected in the serum of only some males and females and only at 600 ppm.

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

3. 30-Month Carcinogenicity Study in Wistar Rats and Associated 18-Month Chronic Feeding Study in Wistar Rats

Reference: Netherlands Organization for Applied Scientific Research: Division for Nutrition and Food Research, Study No. B82-0555 and Study No. 419, November 1985 and May 1984, MRID 00162413 and 00162412.

a. Experimental Design

In the 30-month carcinogenicity study, technical grade imazalil was administered in the diet to groups of 50 male and 50 female Cpb:Wu Wistar rats at dietary concentrations of 0, 25, 100 or 400 ppm (0, 1.0, 3.6 or 15.0 mg/kg b.w./day in males and 0, 1.2, 4.7 or 19.7 mg/kg b.w./day in females). In the 18-month chronic feeding study, technical grade imazalil was administered in the diet to groups of 20 male and 20 female Cpb:Wu Wistar rats at dietary concentrations of 0, 25, 100 or 400 ppm (0, 1.0, 3.7 or 15.5 mg/kg b.w./day in males and 0, 1.2, 4.9 or 20.0 mg/kg b.w./day in females). Both studies were initiated at the same time in the same testing laboratory using the same batch of test material using the same strain of rats from the same supplier.

b. Results

(i) A treatment-related increase in neoplasms was not observed in either the 30-month or 18-month study. Although increased incidences of testicular Leydig cell tumors, uterine adenocarcinomas, and uterine epidermoid carcinomas (rare tumor type) were observed in the 30-month carcinogenicity study, the CPSC considered these incidences to be within the historical control range and not related to treatment with the imazalil.

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(ii) The following non-neoplastic effects were observed in the 18-month study:

- Slightly decreased body weights in females at 400 ppm during the first 52 weeks of the study (254 gm at 400 ppm vs 278 gm for controls at 52 weeks). This decrease was considered by the Toxicology reviewer to be due to poor palatability of the food.
- Slightly increased absolute liver weights in males at 400 ppm (+5.4%).
- Slightly increased absolute kidney weights in females at 100 and 400 ppm.
- Increased multivacuolar hepatocytes in males at 400 ppm (9/20 at 400 ppm vs 3/20 for controls).

(iii) The following non-neoplastic effects were observed in the 30-month study:

- Slight reductions in body weight gain in females at 400 ppm after 78 weeks.
- Slightly increased absolute liver weights in males at 400 ppm (+10.7%).
- Slightly increased focal vacuolation in hepatocytes in males at 400 ppm (6/17 at 400 ppm vs 2/17 for controls).

(iv) The CPRC, at the first meeting on August 24, 1994, did not consider the dietary concentration of 400 ppm in the 30-month study to be high enough to adequately evaluate the carcinogenic potential of imazalil since only minor signs of toxicity were observed in either the 30-month or 18-month study. Accordingly, the CPRC recommended that a new 2-year chronic feeding/carcinogenicity study in rats using higher dose levels be performed unless the registrant could properly justify the dose levels which were used in this study.

B. Evaluation of New Carcinogenicity Evidence

1. Pathology Working Group (PWG) Supplemental Report on 23-Month Carcinogenicity Study in Swiss Albino Mice.

Reference: Butler, W.H. and Vandenberghe, J. (1997). Summary of Histopathological Evaluation and Pathology Working Group Review, Janssen Study No. 2194, Mouse Carcinogenicity Study on Imazalil. Janssen Research Foundation, March 25, 1997, MRID 44269601.

a. Background

A Pathology Working Group (PWG) was convened by the registrant in 1995 to re-evaluate the neoplastic lesions observed in the livers of the male and female mice in this study. The PWG also evaluated liver slides from male and female control mice in a previously conducted comparable study to serve as historical control data. Having

determined that the PWG re-evaluation was conducted in accordance with PR Notice 94-5, a revised qualitative risk assessment based on the PWG re-evaluation of the liver neoplasms in this study has been prepared.

b. Revised Qualitative Risk Assessment

The results of the re-evaluation of the mouse liver slides as presented in the PWG report and the qualitative risk assessment based on these results are presented in Tables 3 and 4. (*Memorandum*:Lori Brunsman to Edwin Budd, dated June 11, 1998.)

(i) The statistical evaluation of mortality indicated no significant incremental changes with increasing doses of imazalil in male or female mice.

(ii) In male mice, a statistically significant pairwise increase in adenomas was observed at 200 ppm (19/50, $p < 0.05$) compared to controls (8/50). An increase in adenomas at 600 ppm (14/48, $p = 0.093$) was not statistically significant by pairwise comparison to controls. A statistically significant pairwise increase in combined adenomas/carcinomas was observed at 200 ppm (22/50, $p < 0.05$) and at 600 ppm (22/48, $p < 0.05$) compared to controls (13/50). Statistically significant positive trends were also observed for adenomas ($p < 0.05$) and for combined adenomas/carcinomas ($p < 0.01$). For carcinomas, neither a statistically significant pairwise increase nor a statistically significant positive trend was noted.

(iii) In female mice, an increase in adenomas at 600 ppm (9/47, $p = 0.133$) was not statistically significant by pairwise comparison to controls (4/45). Similarly, an increase in combined adenomas/carcinomas at 600 ppm (10/47, $p = 0.086$) was not statistically significant by pairwise comparison to controls (4/45). Statistically significant positive trends were observed, however, for adenomas ($p < 0.05$) and for combined adenomas/carcinomas ($p < 0.05$). For carcinomas, neither a statistically significant pairwise increase nor a statistically significant positive trend was noted.

c. Historical Control Data

Liver slides for control male and female mice from a comparable historical control study (expt. no. 2166) were reviewed by the PWG in the same manner as the liver slides from the imazalil mouse study. This historical control study (expt. No. 2166) was conducted at the same facility, using Swiss albino mice from the same supplier and for a similar duration. For males, the incidence of hepatocellular adenomas was 5/50 (10%) and of hepatocellular carcinomas was 8/50 (16%). For females, the incidence of hepatocellular adenomas was 3/50 (6%) and of hepatocellular carcinomas was 1/50 (2%).

2. Evaluation of PWG Report by CARC

Although recognizing that fewer hepatocellular carcinomas were diagnosed in the livers of imazalil-treated male mice in the PWG report (1997) compared to the original report (1993), the CARC, nevertheless, considered the differences between the original reading and the re-evaluation of the liver slides for both males and females to be relatively small and of insufficient magnitude to alter its previous conclusions with respect to the neoplastic findings in this study. In particular, the CARC placed considerable importance on the statistically significant ($p < 0.05$) pairwise increase in combined adenomas/carcinomas observed at both 200 and 600 ppm in the PWG re-evaluation of the liver slides. It was noted that in the original reading, a statistically significant ($p < 0.01$) pairwise increase in combined adenomas/carcinomas was observed only at 600 ppm. Regarding the new historical control data included in the PWG report, the CARC felt that the results from only one study were of limited usefulness. For purposes of comparison with the imazalil treated animals, the CARC continued to place most importance on the concurrent controls in the study.

TABLE 3. IMAZALIL - CHARLES RIVER SPF SWISS ALBINO MOUSE STUDY

Male Hepatocellular Tumor Rates⁺ and Exact Trend
Test and Fisher's Exact Test Results (p values)

PATHOLOGY WORKING GROUP RE-EVALUATION OF LIVER SLIDES (1997)

	<u>Dietary Concentration (ppm)</u>			
	0	50	200	600
Adenomas (%)	8/50 (16)	4/47 (9)	19 ^a /50 (38)	14/48 (29)
p =	0.020*	0.210 ⁿ	0.012*	0.093
Carcinomas (%)	5/50 (10)	5/47 (11)	3/50 (6)	8 ^b /48 (17)
p =	0.123	0.590	0.358	0.250
Combined (%)	13/50 (26)	9/47 (19)	22/50 (44)	22/48 (46)
p =	0.005**	0.288 ⁿ	0.046*	0.033*

⁺Number of tumor bearing animals/Number of animals examined, excluding those that died before week 53.

ⁿNegative change from control.

^aFirst adenoma observed at week 55, dose 200 ppm.

^bFirst carcinoma observed at week 72, dose 600 ppm.

Note: Significance of trend denoted at control.
 Significance of pair-wise comparison with control denoted at dose
 level. If *, then $p < 0.05$. If **, then $p < 0.01$.

TABLE 4. IMAZALIL - CHARLES RIVER SPF SWISS ALBINO MOUSE STUDY

Female Hepatocellular Tumor Rates[†] and Exact Trend
Test and Fisher's Exact Test Results (p values)

PATHOLOGY WORKING GROUP RE-EVALUATION OF LIVER SLIDES (1997)

	<u>Dietary Concentration (ppm)</u>			
	0	50	200	600
Adenomas (%)	4/45 (9)	5 ^a /48 (10)	0/45 (0)	9/47 (19)
p =	0.036 [*]	0.542	0.058 ^a	0.133
Carcinomas (%)	0/45 (0)	1/48 (2)	2 ^b /45 (4)	1/47 (2)
p =	0.329	0.516	0.247	0.511
Combined (%)	4/45 (9)	6/48 (12)	2/45 (4)	10/47 (21)
p =	0.034 [*]	0.412	0.338 ^a	0.086

[†]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 53.

^aNegative change from control.

^aFirst adenoma observed at week 83, dose 50 ppm.

^bFirst carcinoma observed at week 95, dose 200 ppm.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then p < 0.05. If **, then p < 0.01.

3. Subchronic Feeding (Dose Range Finding and Mechanistic Toxicity) Study in Wistar Rats (MRID 43965704)

Reference: Van Deun, K. (1996). Three-Month Oral Dietary Dose Range Finding and Mechanistic Toxicity Study With One Month Interim Sacrifice in SPF Wistar Rats with Imazalil Base (R023979). Janssen Research Foundation, Department of Toxicology, Beerse, Belgium. Experiment No. 3514. March 13, 1996. MRID 43965704.

Part 1 – 90-Day Feeding Study

a. Experimental Design

In a 90-day feeding study, technical grade imazalil base was administered in the diet to groups of 20 male and 20 female SPF Wistar rats at dietary concentrations 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 b.w./day for males and 0, 22.3, 44.6 and 90.1 mg/kg b.w./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 b.w./day for males and 0, 18.7, 37.9 and 76.4 mg/kg b.w./day for females for the 0 (control), 200, 400 and 800 ppm groups respectively. The following parameters were evaluated: mortality, clinical signs, body weights, body weight gains, 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 histologically examined: liver, thymus, thyroid (with parathyroid) and all gross lesions. For all animals sacrificed at 3 months, the following organs/tissues were histologically examined: liver, kidneys and all gross lesions. For females only, the adrenals were also examined at 3 months (10/dose group). No other organs or tissues were histologically examined.

b. Results

- (i) 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 dietary concentration.
- (ii) No treatment-related effects on hematological parameters, clinical chemistries, urinalyses or gross necropsies were observed in the male or female rats.
- (iii) Treatment-related increased absolute liver weights (9-15%) and liver/body weight ratios (9-19%) 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.

(iv) 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.

(v) The LOEL in this study is 400 ppm (32.1 and 37.9 mg/kg b.w./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 NOEL in this study is 200 ppm (15.8 and 18.7 mg/kg b.w./day in males and females respectively).

Part 2 -- Toxicokinetics

Individual blood samples collected at necropsy from all rats at the interim sacrifice (1 month) and the terminal sacrifice (3 months) were analyzed for plasma concentrations of imazalil. Median plasma concentrations of imazalil in treated male and female rats were generally low, but appeared to be dose-related (increasing for the most part proportionally with increasing concentrations of imazalil in the food). Plasma concentrations in both male and female rats were similar. Plasma concentrations at 3 months were not appreciably different than those at 1 month indicating a steady state plateau in plasma concentrations was reached prior to the 1 month interim sacrifice.

Part 3 -- Induction and/or Inhibition of Hepatic Drug Metabolizing Enzymes

Samples of liver were collected at the 1 month interim sacrifice and at the 3 month terminal sacrifice from 4 rats/sex/dose group and assayed for possible induction and/or inhibition of drug metabolizing enzymes. Microsomal protein content, cytochrome P450 content, UDP-glucuronosyltransferase activity and several monooxygenase isoenzyme activities were assayed by standard procedures. Microsomes of rats pre-treated with specific inducers were used to validate the enzyme assays. "After one month of dosing, the microsomal protein and cytochrome P-450 contents and several enzyme activities were increased, especially the 7-ethoxyresorufin, 7-pentoxyresorufin and 7-ethoxycoumarin O-dealkylase activities in livers of both male and female rats as well as the N-ethylmorphine N-demethylase activity in livers of female rats. The results indicated a mixed type of induction. Prolongation of the dosing period up to three months did not cause any additional induction of the enzyme activities in male rat livers. In livers of female rats, treatment for three months with imazalil resulted in a significant increase of the cytochrome P-450 content and a supplementary induction of the aniline hydroxylase, 7-ethoxyresorufin O-deethylase, lauric acid hydroxylase and UDP-

glucuronosyltransferase activities" (quoted from page 135 of the study report).

4. Subchronic Feeding (Dose Range Finding and Mechanistic Toxicity) Study in Wistar (Hannover substrain) Rats (MRID 43965705)

Reference: Van Deun, K. (1996). Three-Month Oral Dietary Dose Range Finding and Mechanistic Toxicity Study in SPF Wistar Rats with Imazalil Base (R023979). Janssen Research Foundation, Department of Toxicology, Beerse, Belgium. Experiment No. 3672. March 13, 1996. MRID 43965705.

Part 1 – 90-Day Feeding Study

a. Experimental Design

In a 90-day feeding study, technical grade imazalil base was administered in the diet to groups of 10 male and 10 female Wistar (Hannover substrain) rats at dietary concentrations of 0, 800, 1600, 2400 or 3200 ppm. Due to food wastage, most likely resulting from poor palatability of the food, it was not possible to accurately calculate the dose levels in units of mg/kg b.w./day. Nevertheless, the following dose levels were estimated by the study authors: 0, 64.4, 129, 181 or 252 mg/kg b.w./day in males and 0, 78.7, 150, 236 or 333 mg/kg b.w./day in females. It should also be noted that application of the standard conversion factor of 0.05 for older rats (A. J. Lehman, 1959) results in dose levels of 0, 40, 80, 120 or 160 mg/kg b.w./day for both males and females. The following parameters were evaluated: mortality, clinical signs, body weights, body weight gains, food consumption, hematology, clinical chemistries, urinalyses, gross necropsy and organ weights. Livers and macroscopic lesions were examined for all animals in all treatment groups, but no other organs or tissues were histologically examined. Electron microscopy of liver sections was also conducted.

b. Results

(i) No treatment-related effects on mortality or clinical signs were observed. Compared to control 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 for 3200 ppm animals, 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. There was considerable uncertainty, however, regarding how much of these body weight decreases may have been due to direct effects of the test material and how much may have been due to poor palatability of the food. Considerable dose-related food

wastage noted during the study was not directly measured and, therefore, measurements of actual food consumption were severely compromised.

(ii) Hematological parameters were not affected. The following minor changes in clinical chemistries observed at 3 months were possibly related to the test material: decreased triglyceride in males at all treatment levels and in females at 3200 ppm, decreased phospholipid in males at all treatment levels, decreased blood urea nitrogen in males at 1600, 2400 and 3200 ppm, and decreased albumin in females at 2400 and 3200 ppm. Urinalyses were unremarkable.

(iii) 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.

(iv) Histopathological examination revealed treatment-related hepatocellular hypertrophy in nearly all treated animals at all dose levels. This hypertrophy was mild and there was essentially no difference between treatment groups in incidence or severity. Mild "fatty vacuolization" was observed in males at doses of 1600 ppm and higher and in females at all dietary concentrations. The amount of stainable hepatocytic lipid increased in a dose-related manner in both sexes. It is likely that many 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.

(v) The LOEL in this study is 800 ppm (grossly estimated by the study authors to be 64.4 and 78.7 mg/kg b.w./day in males and females respectively and by the standard Lehman conversion factor of 0.05 to be 40 mg/kg b.w./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. No NOEL was demonstrated in this study in either males or females since treatment-related effects were observed at all dose levels tested: NOEL < 800 ppm; estimated by the study authors to be 64.4 and 78.7 mg/kg b.w./day in males and females respectively and by the standard 0.05 conversion factor to be 40 mg/kg b.w./day in both males and females.

Part 2 -- Toxicokinetics

Individual blood samples were collected at necropsy from all rats (9 or 10/sex/dose group) and levels of imazalil in the plasma were quantitatively determined. Mean plasma levels of imazalil in treated male and female rats appeared to increase with increasing concentrations of imazalil in the feed in a roughly proportional (dose-related) manner. Large standard deviations, indicating considerable variation in individual values, were observed in female rats at dietary concentrations of 1600 and 3200 ppm. Plasma levels in

female rats were somewhat higher than in male rats probably reflecting the higher intake of test material (in units of mg/kg b.w./day) by the female rats compared to the male rats.

Part 3 -- Induction and/or Inhibition of Hepatic Drug Metabolizing Enzymes

Samples of liver were collected at necropsy from 4 rats/sex/dose group and assayed for possible induction and/or inhibition of drug metabolizing enzymes. Microsomal protein content, cytochrome P450 content, UDP-glucuronosyltransferase activity and several monooxygenase isoenzyme activities were assayed by standard procedures. Microsomes of rats pre-treated with specific inducers were used to validate the enzyme assays. 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 P450 content was statistically significantly

increased in males at all dose levels of imazalil, but increases were not statistically significant in females. UDP-glucuronosyltransferase activity was statistically significantly increased in female rats, but not in male rats at 2400 and 3200 ppm. Several isoenzyme activities, including N-ethylmorphine N-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 demonstrated to act as a mixed type of of microsomal enzyme inducer at all dose levels tested.

5. Two-year Combined Chronic Feeding/Carcinogenicity Study in Wistar (Hannover substrain) Rats (6-Month Interim Report)

Reference: Van Deun, K. (1997). Combined Oral Chronic Toxicity/ Carcinogenicity Study (Exp. No. 3817) with Imazalil (R023979) in SPF Wistar Rats. Interim Report (6 Months). Janssen Research Foundation, Department of Toxicology, Beerse, Belgium. Experiment No. 3817. LMD No. N125353/1. June 27, 1997. MRID 44319901.

a. Experimental Design

In a 24-month combined chronic feeding/carcinogenicity study, technical grade imazalil base was administered in the diet to groups of 50 male and 50 female Wistar (Hannover substrain) rats at dietary concentrations of 0, 50, 200, 1200 or 2400 ppm (0, 3.2, 12.8, 78 or 159 mg/kg b.w./day in males and 0, 4.1, 16.3, 96 or 189 mg/kg b.w./day in females for the first 6 months of the study). The following parameters were evaluated during the first 6 months of the study: mortality, clinical signs, pre-treatment ophthalmological examinations, body weights, body weight gains, food consumption, hematology, clinical chemistries and urinalyses. Gross necropsies, organ weights and histopathology were not performed since there was no interim sacrifice at the 6 month time point in the study.

b. Results

(i) No treatment-related effects on mortality were observed and other than food wastage, no treatment-related clinical signs of toxicity were noted. Dose-related food wastage, most likely due to poor palatability of the food, was frequently observed at 2400 ppm in males and females and at 1200 and 2400 ppm in females. Ophthalmological examinations were performed prior to initiation of the study, but were not conducted during the first 6 months of the study. No treatment-related effects on absolute body weights or body weight gains were observed in male or female rats at 50 or 200 ppm during the first 6 months of the study. At 1200 and 2400 ppm, however, dose-related decreases in absolute body weights and body weight gains were consistently observed in both male and female rats during the entire first 6 months of the study. Mean body weights were decreased approximately 5% in both males and females at 1200 ppm and were decreased 13% in males and 9% in females at 2400 ppm. Mean body weight gains were decreased approximately 10% in both males and females at 1200 ppm and approximately 20% in both males and females at 2400 ppm. It was established, however, that these decreases in absolute body weight and body weight gain were not due to a direct (toxic) effect of the test material, but rather to decreased food consumption which was consistently observed in both male and female rats at these same dose levels during

the entire first 6 months of the study. Food efficiency was not affected. The decreased food consumption was most likely due to poor palatability of the food.

(ii) Although possible treatment-related effects on a number of hematological, clinical chemistry and urinalyses parameters were noted at the 6 month time point in this study, except for several clinical chemistry parameters which suggested impaired liver function (even possibly at the lowest dietary concentration of 50 ppm), most of these effects were minimal in magnitude and not convincing evidence of toxicity at this time point in the study. Final determinations of treatment-related effects on these parameters should await the results from additional time points (scheduled for 12, 18 and 24 months at termination of the study).

(iii) Based on effects other than clinical chemistries, the tentative LOEL in this study at the 6 month time point is 1200 ppm (78 mg/kg b.w./day in males and 96 mg/kg b.w./day in females) and is based on food wastage in females, decreased absolute body weights and body weight gains in males and females, and decreased food consumption in males and females. These effects may have been due in large part to poor palatability of the food. It is noted, however, that several clinical chemistry parameters suggested possible impairment of liver function at dietary concentrations below 1200 ppm at the 6 month time point. Final determinations of these parameters should await results from additional time points. The tentative NOEL in this study at the 6 month time point, based on effects other than clinical chemistries, is 200 ppm (12.8 mg/kg b.w./day in males and 16.3 mg/kg b.w./day in females).

NOTE – In a recent communication from the registrant (FAX from Bill Goodwine, dated March 26, 1998), EPA was informed of the mortality rate in this study at the 17 month time point (shown on the next page). Clearly, there were very few deaths and no treatment-related effect on mortality at 17 months in this study.

Mortality at 17 Months

	<u>Males</u>		<u>Females</u>
0 ppm	2/50	0 ppm	4/50
50 ppm	4/50	50 ppm	5/50
200 ppm	3/50	200 ppm	2/50
1200 ppm	4/50	1200 ppm	2/50
2400 ppm	1/50	2400 ppm	1/50

C. Evaluation of three New Studies In Rats by CARC

The CARC considered the experimental data in the two 90-day subchronic feeding (dose range finding and mechanistic toxicity) studies in rats (MRID 43965704 and 43965705), the 6-month interim report on the 2-year combined chronic feeding/carcinogenicity study in rats (MRID 44319901) and the FAX dated March 26, 1998. The CARC concluded that poor palatability of the food (particularly at dietary concentrations ≥ 800 ppm) frequently led to food wastage and/or decreased food consumption which resulted in decreased body weights and body weight gains in the imazalil treated animals. Further, in the 6-month interim report on the ongoing combined study in rats, it was determined that food efficiency was not affected at dietary concentrations as high as 2400 ppm. Hence, data for absolute body weights and body weight gains in these studies at dietary concentrations ≥ 800 ppm were of only limited usefulness with respect to evaluating the adequacy of the doses in the previously evaluated 30-month carcinogenicity study in rats (MRID 00162413). The CARC also recognized that imazalil is a mixed type of liver microsomal enzyme inducer, which results in typical signs of enzyme induction such as increased absolute liver weights, increased liver/body weight ratios, hypertrophy of hepatocytes, increased microsomal protein content, increased cytochrome P450 content, and increased activities of liver glucuronosyltransferase and various monooxygenase isoenzymes. The CARC generally does not consider induction of the liver microsomal enzyme system, in the absence of other more serious signs of toxicity, to be sufficient evidence of adequate dosing in carcinogenicity studies. Other signs of toxicity in these studies (e.g. minor changes in clinical chemistries, possibly increased adrenal weights, possible histopathology in the adrenal, and vacuolization of hepatocytes) were also considered by the CARC to be insufficient evidence of adequate dosing.

Noting the apparent lack of significant toxicity at 6 months and also the lack of life threatening toxicity at 17 months in the ongoing 2-year combined chronic feeding/carcinogenicity in rats at dietary concentrations up to 2400 ppm, the CARC determined that this study should be continued to completion, including full pathological evaluation of all surviving animals at termination of the study. The CARC recommended that the registrant's request for a waiver of this study be denied.

IV. ADDITIONAL TOXICOLOGY DATA ON IMAZALIL

A. Mutagenicity

Tabulated below are the results of the mutagenicity studies previously evaluated at the August 24, 1994 CPRC meeting (*Memorandum, 12/27/94*)

Study Type	MRID No.	Results
Salmonella assay	40729301	Negative for increasing reversions at doses into the toxic ranges
<i>In vitro</i> cytogenetics	40729302, 41026602	Negative for inducing chromosomal aberrations in human lymphocytes at concentrations of 125-5000 µg/culture plate and 22-909µg/mL
Micronucleus test	40729303	Negative for induction of micronuclei in bone marrow cells of mice treated up to toxic doses (320 mg/kg b.w.)

At the August 24, 1994, the CPRC required that an outstanding data gap for mutagenicity be filled and recommended that an *in vivo/in vitro* unscheduled DNA synthesis (UDS) study with liver accompanied with a cell proliferation (S phase) study be submitted to satisfy the requirement. These studies; and two additional mutagenicity studies, have been submitted and the results are summarized below:

1. *In vivo/in vitro* unscheduled DNA synthesis (UDS) assay and replicative DNA synthesis (RDS) assay in primary mouse hepatocytes

Reference: Clare, C. (1996). Imazalil: Measurement of Unscheduled DNA Synthesis and Replicative DNA Synthesis in Mouse Liver Using an *in vivo/in vitro* Procedure. Corning Hazleton (Europe), Harrogate, England. Report No. 1073/3-1052. February 1996. MRID 43965702.

Results: There was no indication of a genotoxic response at any dose or sacrifice time. However, a clear dose-related increase in the percentage of cells in S-phase was obtained from hepatocytes harvested 48 hours posttreatment. The response was significant ($p \leq 0.025$) at 250 mg/kg b.w. and represented a 2.8-fold increase in replicative DNA synthesis (5.2% of the cells in S-phase versus 1.88% in the vehicle control group). The data indicate that imazalil was negative for genotoxicity but positive for cellular proliferation (mitogenicity) when tested up to overtly toxic and cytotoxic doses in this *in vivo/in vitro* mouse hepatocyte UDS/RDS assay.

2. Unscheduled DNA synthesis (UDS) assay in primary rat hepatocytes

Reference: Fautz, R. (1990). Unscheduled DNA Synthesis in Primary Rat Hepatocytes of Male Rats *in vitro* with Imazalil. Cytotest Cell Research GmbH & Co., Federal Republic of Germany. CCR Project No. 192600/Janssen Report No. 920. August 20, 1990. MRID 43780201.

Results: There was no evidence that imazalil caused increased unscheduled DNA synthesis as determined by radioactive tracer procedures (nuclear silver grain counts) at any dose in either trial in this study.

3. Mammalian cells in culture: gene mutation in Chinese hamster lung V79 cells

Reference: Van Gompel, J. (1995). In vitro Mammalian Gene Mutation Assay with Imazalil. Janssen Research Foundation, Beerse, Belgium. Experiment No. 3470. April 27, 1995. MRID 43735003.

Results: There was no evidence that Imazalil was mutagenic at any dose under the conditions of this assay.

The CARC concluded that, overall, these mutagenicity data indicate that imazalil does not induce mutagenic activity. There was, however, clear evidence of cellular proliferation (mitogenic activity) in the *in vivo* assay conducted in mouse liver.

B. Structure-Activity Correlations

A tabulated summary of the carcinogenic and mutagenic findings and current classifications for ten triazole fungicides previously evaluated by the CPRC is presented in Table 5.

The CPRC, on August 24, 1994, concluded that imazalil is structurally related to the triazole fungicides. The triazole fungicides include triadimefon, triadimenol, uniconazole, tebuconazole, propiconazole, cyproconazole, hexaconazole, etaconazole, bitertanol and azaconazole. Imazalil, however, contains 2 nitrogen atoms in an imidazole ring whereas the triazole fungicides contain 3 nitrogen atoms in a triazole ring. Most of these structural analogs of imazalil (triadimefon, triadimenol, uniconazole, tebuconazole, propiconazole, cyproconazole, and etaconazole) have been shown to induce hepatocellular adenomas, carcinomas, or both in male and/or female mice. Hexaconazole and azaconazole were negative in mouse carcinogenicity studies, but the highest dose levels tested in these studies were inadequate. Bitertanol was negative in a mouse carcinogenicity study at dietary concentrations up to 500 ppm. In rat carcinogenicity studies, hexaconazole induced a treatment-related increase in benign Leydig cell tumors in the testes of male rats and triadimefon induced a borderline increase in thyroid follicular cell adenomas in male rats.

The remaining triazole fungicides were either negative (triadimenol, uniconazole, tebuconazole, propiconazole, bitertanol), not tested at adequate doses (cyproconazole), or

not tested at all (etaconazole, azaconazole) in rat carcinogenicity studies.

In mutagenicity studies, positive results were observed only with uniconazole (increased chromosomal aberrations in CHO cells and positive micronucleus study in male mice) and cyproconazole (increased chromosomal aberrations in CHO cells). Other triazole fungicides (triadimefon, triadimenol, tebuconazole, hexaconazole) were negative in a battery of mutagenicity studies, but some of these compounds (triadimefon, hexaconazole) also had data gaps. One compound (propiconazole) was negative in mutagenicity studies, but a major metabolite was positive. Mutagenicity data on etaconazole, bitertanol and azaconazole were not readily available.

V. WEIGHT OF THE EVIDENCE CONSIDERATIONS

The CARC considered the following observations regarding the toxicology of imazalil for a weight of the evidence determination on its carcinogenic potential.

A. Evaluation of Available Mode of Action Data on Mice

At the August 24, 1994 meeting, the CPRC considered the experimental data in the 3-month oral mechanistic toxicity study in mice (in 4 parts, MRID 43222601 and 43202402). In this study, designed to study effects on the liver, imazalil was administered in the diet for 1 or 3 months to male and female Swiss albino mice at dietary concentrations of 0, 50, 200 or 600 ppm. 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, and increased incidence and severity of "centrilobular clearer aspect" and of large and/or small vacuoles in the hepatocytes of males and females. At 600 ppm, the following treatment-related effects were observed: increased alkaline phosphatase in males, 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, and increased diffuse swelling of hepatocytes in females. Increased microsomal protein and increased microsomal cytochrome P450 content were observed in the livers of males and females at 200 and 600 ppm. Regarding liver enzymatic activities of seven P450 isoenzymes, the results indicated a mixed type of induction at 200 and 600 ppm. Dosing with imazalil significantly induced some enzymatic activities but also had an inhibitory effect on other enzyme activities. The total activity of testosterone hydroxylases was increased in both males and females at 600 ppm. 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 in the

hepatocytes of males and females at 600 ppm. Low levels of imazalil were detected in the serum of only some males and females and only at 600 ppm. In this study, imazalil was clearly demonstrated to be a mixed type of liver microsomal enzyme inducer at 200 and 600 ppm.

At the current meeting on imazalil (July 22, 1998), the CARC also considered the results in the recently submitted (1996) in vivo/in vitro unscheduled DNA synthesis (UDS) assay and replicative DNA synthesis (RDS) assay in primary mouse hepatocytes (MRID 43965702). In this study, there was no genotoxic response, but there was clear evidence of increased cellular proliferation (mitogenicity) in the mouse liver cells following treatment with imazalil. Overall, imazalil was non-genotoxic in a battery of six acceptable mutagenicity studies, but was also clearly demonstrated to be mitogenic (causing cellular proliferation) in mouse liver cells.

With respect to a potential mode of action leading to an increased incidence of hepatocellular tumors in imazalil-treated mice, the CARC is aware that tumorigenic findings in the livers of mice are oftentimes associated with non-genotoxic, mitogenic compounds that induce the liver microsomal enzyme system. The CARC, however, does not believe liver enzyme induction to be sufficiently well correlated to tumor induction to be considered as a mode of action for liver tumorigenicity. The CARC is also aware of the current scientific controversy regarding the potential relationship of such findings, particularly mitogenicity, to the induction of liver tumors in mice. At this time, the CARC does not consider this potential relationship to be sufficiently well established to conclude that such findings clearly indicate a mode of action leading to hepatocellular tumors in the livers of mice. Hence, at this time, the CARC does not consider the available mechanistic data on imazalil to be convincing evidence for a mode of action for the carcinogenicity of this compound.

B. Additional Observations Regarding the Toxicology of Imazalil for a Weight of the Evidence Determination of its Carcinogenic Potential

1. Evidence of Carcinogenicity in Mice and Rats

- a. Male and female Swiss albino mice were fed 0, 50, 200 or 600 ppm (0, 6.8, 28.0 or 88 mg/kg b.w./day in males and 0, 8.3, 34.8 or 110 mg/kg b.w./day in females) of imazalil base for 23 months. The highest dietary concentration tested in this study, 600 ppm, was determined to be sufficiently high for carcinogenicity testing. This determination was based on decreased body weights in males and females, decreased body weight gains in males and females, increased absolute and relative liver weights in males and females, and increased histopathologic lesions in the livers of males (focal cellular changes, hyperplasia, and large vacuoles and pigmentation of sinusoidal cells) at 600 ppm. There was no treatment-related effect on mortality.

- (i) In male mice, based on a PWG re-evaluation of liver slides from all male mice in the study and statistical analyses of this re-evaluation, treatment-related increased incidences of hepatocellular adenomas and combined adenomas/carcinomas were observed in the 200 and 600 ppm dietary concentration groups. A statistically significant pairwise increase in adenomas was observed at 200 ppm (19/50, $p < 0.05$) compared to controls (8/50). The increase in adenomas at 600 ppm (14/48, $p = 0.093$), however, was not statistically significant by pairwise comparison to controls. A statistically significant pairwise increase in combined adenomas/carcinomas was observed at 200 ppm (22/50, $p < 0.05$) and at 600 ppm (22/48, $p < 0.05$) compared to controls (13/50). Statistically significant positive trends were also observed for adenomas ($p < 0.05$) and for combined adenomas/carcinomas ($p < 0.01$). For carcinomas, neither a statistically significant pairwise increase nor a statistically significant positive trend was noted. For adenomas, the percentage incidence in male mice was 16%, 9%, 38% and 29% for the 0, 50, 200 and 600 ppm dietary concentration groups respectively. The incidence in the single comparable historical control group submitted by the registrant was 10%. For carcinomas, the percentage incidence in male mice was 10%, 11%, 6% and 17% for the 0, 50, 200 and 600 ppm dietary concentration groups respectively. The incidence in the single comparable historical control group submitted by the registrant was 16%.
- (ii) In female mice, based on a PWG re-evaluation of liver slides from all female mice in the study and statistical analyses of this re-evaluation, no statistically significant pairwise increased incidences of liver neoplasms were observed in the study. An increase in adenomas at 600 ppm (9/47, $p = 0.133$) was not statistically significant by pairwise comparison to controls (4/45). Similarly, an increase in combined adenomas/carcinomas at 600 ppm (10/47, $p = 0.086$) was not statistically significant by pairwise comparison to controls (4/45). Statistically significant positive trends were observed, however, for adenomas ($p < 0.05$) and for combined adenomas/carcinomas ($p < 0.05$). For carcinomas, neither a statistically significant pairwise increase nor a statistically significant positive trend was noted. For adenomas, the percentage incidence in female mice was 9%, 10%, 0% and 19% for the 0, 50, 200 and 600 ppm dietary concentration groups respectively. The incidence in the single comparable historical control group submitted by the registrant was 6%. For carcinomas, the percentage incidence in female mice was 0%, 2%, 4% and 2% for the 0, 50, 200 and 600 ppm dietary concentration groups respectively. The incidence in the single comparable historical control group was 2%.

- b. In a 30-month carcinogenicity study in male and female Wistar rats, imazalil base was administered in the diet at dietary concentrations of 0, 25, 100 or 400 ppm (0, 1.0, 3.6 or 15.0 mg/kg b.w./day in males and 0, 1.2, 4.7 or 19.7 mg/kg b.w./day in females). In an associated 18-month chronic feeding study in male and female Wistar rats, imazalil base was administered in the diet at dietary concentrations of 0, 25, 100 or 400 ppm (0, 1.0, 3.7 or 15.5 mg/kg b.w./day in males and 0, 1.2, 4.9 or 20.0 mg/kg b.w./day in females). Both studies were initiated at the same time in the same testing laboratory using the same batch of test material and the same strain of rats from the same supplier. In the initial review of these studies by the CPRC on August 24, 1994, it was concluded that the dosing in the 30-month carcinogenic study was not sufficiently high to adequately evaluate the carcinogenic potential of imazalil in rats since only minor signs of toxicity were observed at the highest dietary concentration tested in this study, 400 ppm, or in other feeding studies in rats at similar dietary concentrations. Accordingly, the CPRC recommended that a new 2-year chronic feeding/carcinogenicity study in rats using higher dose levels be performed unless the registrant could properly justify the dose levels which were used in this study. At the present meeting of the CARC on July 22, 1998, after considering additional data from two 90-day feeding (dose-range finding and mechanistic toxicity) studies in rats, a 6-month interim report on an ongoing 24-month chronic feeding/carcinogenicity study in rats, and additional information provided by the registrant, the prior conclusions and recommendations of the CPRC were reaffirmed. Further, based on a consideration of all the available data and information, the present CARC determined that the ongoing 24-month chronic feeding/carcinogenicity study in rats should be continued to completion, including full pathological evaluation of all surviving animals at termination of the study. The CARC recommended that the registrant's request for a waiver of this study be denied.

A treatment-related increase in neoplasms was not observed in either the 30-month carcinogenicity study or in the 18-month chronic feeding study. Although increased incidences of testicular Leydig cell tumors, uterine adenocarcinomas, and uterine epidermoid carcinomas (rare tumor type) were observed in the 30-month carcinogenicity study, the incidences of these tumors were considered to be within the historical control range and not related to treatment with the imazalil.

2. Evaluation of Mutagenic Potential

Imazalil was not mutagenic in a battery of six genotoxicity studies submitted by the registrant. These studies were a Salmonella gene mutation assay, an *in vitro* gene mutation assay in Chinese hamster V79 cells, an *in vitro* cytogenetics assay in human lymphocytes, a micronucleus test in bone marrow cells of mice, an unscheduled DNA synthesis (UDS) assay in primary rat hepatocytes, and an *in vivo/in vitro* unscheduled

DNA synthesis (UDS) assay in primary mouse hepatocytes. In an associated replicative DNA synthesis (RDS) assay in primary mouse hepatocytes, however, a clear dose-related statistically significant increase in the percentage of cells in S-phase was obtained from hepatocytes harvested 48 hours posttreatment. The results in this latter study presented clear evidence of cellular proliferation (mitogenic activity) in this assay conducted in mouse liver.

3. Structure Activity Relationships

Imazalil is structurally related to the triazole fungicides, which include triadimefon, triadimenol, uniconazole, tebuconazole, propiconazole, cyproconazole, hexaconazole, etaconazole, bitertanol and azaconazole. Seven of these ten structural analogs of imazalil have been shown to induce hepatocellular adenomas, carcinomas, or both in male and/or female mice. Two were negative in mouse carcinogenicity studies, but the highest dose levels tested in these studies were inadequate. One was negative in a mouse carcinogenicity study at dietary concentrations up to 500 ppm. In rat carcinogenicity studies, one (hexaconazole) induced a treatment-related increase in benign Leydig cell tumors in the testes of male rats and one (triadimefon) induced a borderline increase in thyroid follicular cell adenomas in male rats. The remaining triazole fungicides were either negative, not tested at adequate doses, or not tested at all in rat carcinogenicity studies.

In mutagenicity studies, positive results were observed only with uniconazole and cyproconazole. Four of the ten triazole fungicides were negative in a battery of mutagenicity studies, but two of these same compounds also had data gaps. One compound (propiconazole) was negative in mutagenicity studies, but a major metabolite was positive. Mutagenicity data on three compounds (etaconazole, bitertanol and azaconazole) were not readily available. A tabulated summary of the carcinogenic and mutagenic findings and current classifications for these triazole fungicides is presented in Table 5.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

The CARC concluded there was no compelling reason to deviate from the conclusion reached by the CPRC on August 24, 1994, when the CPRC classified imazalil as a Group C Carcinogen (possible human carcinogen). The CARC also determined that the available mechanistic data do not provide a definitive mode of action with respect to the hepatocellular tumors observed in imazalil-treated mice. This determination was based on an evaluation of the mechanistic data on mice provided by the Registrant. It was concluded that more data/information will be necessary before a definitive mode of action can be delineated. These conclusions were based on the following factors:

1. Statistically significant pairwise increases in hepatocellular adenomas and combined hepatocellular adenomas/carcinomas in the livers of male Swiss Albino mice. In addition, statistically significant positive trends for hepatocellular adenomas and combined hepatocellular adenomas/carcinomas in the livers of male and female Swiss Albino mice.
2. Although not statistically significant by pairwise or trend statistical analyses, the increase in hepatocellular carcinomas in the livers of male Swiss Albino mice added concern to the increased incidence of combined tumors.
3. Lack of an acceptable carcinogenicity study in rats at this time with dose levels high enough to adequately evaluate the carcinogenic potential of imazalil.
4. Lack of mutagenic activity in a battery of six mutagenicity studies, but clear evidence of cellular proliferation (mitogenic activity) in the liver in an in vivo assay conducted in mice.
5. Structural relationship of imazalil to at least seven triazole fungicides which also induce hepatocellular adenomas, carcinomas or both in male and/or female mice.
6. Lack of a sufficiently well established causal relationship between non-genotoxic, mitogenic compounds that induce the liver microsomal enzyme system and hepatocellular tumors in mice.
7. Relevance of the observed tumors to human exposure cannot be dismissed since a definitive mode of action for these tumors in animals has not been established.

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

Since a definitive mode of action for the carcinogenicity of imazalil in mice has not been established, the CARC concluded there was no compelling reason to deviate from the conclusion of the CPRC on August 24, 1994 that for the purpose of risk characterization, a linear low dose extrapolation model be applied to the animal data for quantification of human risk (Q1*). The CARC also re-affirmed the prior conclusion by the CPRC that the quantification of human risk be based on the total liver tumors (combined adenomas/carcinomas) in the male mouse and recommended that the previously calculated Q1* continue to be used to characterize the carcinogenic risk to humans.

VIII. CITATIONS

1. Carcinogenicity Peer Review of Imazalil, Memo, December 27, 1994.
2. Rationale for Proceeding with the Second Carcinogenicity Peer Review for Imazalil, February 3, 1997, MRID 44269601.
3. Overall Assessment of Rodent Chronic Toxicity and Oncogenicity Data for Imazalil for Purposes of Carcinogenicity Peer Review, April 28, 1997, MRID 44269601.
4. Relevance of Liver Tumor Formation in Mice, February 20, 1996, MRID 43965703.
5. Waiver Request from Conducting an Additional Oncogenicity Study in Rats with Imazalil, December 16, 1996, MRID 44269601.

Table 5: Structure Activity Relationship -Classifications of Triazole Fungicide by HED's Carcinogenicity Peer Review Committee

Chemical Meeting Dates	Species/Duration/Doses/Adequacy of Doses Tested	Evidence of Carcinogenicity	Evidence of Mutagenicity	Current Classification and Basis for Classification
Triadimefon (Bayleton)	<u>Mice</u> #1 (CF1-W74) 2 years HDT = 1800 ppm (adequate)	<u>Negative</u> (re-eval of liver slides requested, but adequate number of slides were not available)	<u>Negative</u> in 4 studies	1) HC adenomas in M and F mice at HDT
1) 6/6/90	#2 (NMRI) 21-months HDT = 1800 ppm (adequate)	<u>Positive</u> Liver M: HC adenomas at HDT F: HC adenomas at HDT	<u>Data gap</u> CPRC recom. <u>in vivo/in vitro</u> UDS assay (not yet received?)	2) Borderline † in thyroid follicular cell adenomas/ adenomas multiple in M rats. Negative in F rats 3) Negative in 4 muta studies (data gap) 4) SAR data are supportive; [Iridimenol (major metabolite)
2) 4/24/96	<u>Rats</u> #1 (Wistar) 21-months HDT = 5000 ppm (excessive) MDT inadequate			Classification/Quantification: Group C, no Q1* (RfD approach risk assessment)
	#2 (Wistar) 24-months HDT = 1800 ppm (Adequate)	<u>Positive (?)</u> <u>Thyroid</u> M: borderline † follicular cell adenomas /adenomas multiple F: negative		

Chemical Meeting Dates	Species/Duration/ Doses/Adequacy of Doses Tested	Evidence of Carcinogenicity	Evidence of Mutagenicity	Current Classification and Basis for Classification
Triadimenol (Baytan) (plant & animal metabolite of triadimefon) 1) 10/1/87	<u>Mice</u> 2 years HDT = 2000 ppm (adequate)	<u>Positive Liver</u> M: Negative F: HC adenomas at HDT	<u>Negative</u> in 5 studies	1) HC adenomas in F mice at HDT. Negative in M mice 2) Negative in rats 3) Negative in 5 muta studies 4) SAR data are supportive Classification/Quantification: Group C, no Q1* (RfD approach)
	<u>Rats</u> 2 years HDT = 2000 ppm (adequate)	<u>Negative</u>		
<u>Uniconazole</u> 1) 7/25/90	<u>Mice</u> 78-weeks HDT = 1500 ppm (adequate) (F may have tolerated higher doses)	<u>Positive Liver</u> M: HC adenomas, carcinomas & comb adenomas/carcinomas F: Negative	<u>Positive</u> (Clastogenic) ↑ chromo aberr in CHO cells (only with activ)	1) HC adenomas, carcinomas and comb adenomas/ carcinomas in M mice. Negative in F mice 2) Negative in rats 3) Positive in 2 studies Negative in 3 studies 4) SAR data are supportive Classification/Quantification: Group C, no Q1* (RfD approach)
	<u>Rats</u> 2 years HDT = 1000 ppm (adequate)	<u>Negative</u>	<u>Positive</u> in mouse micronucleus assay in M <u>Negative</u> in 3 studies	

Chemical Meeting Dates	Species/Duration/ Doses/Adequacy of Doses Tested	Evidence of Carcinogenicity	Evidence of Mutagenicity	Current Classification and Basis for Classification
<p>Tebuconazole</p> <p>1) 5/26/93</p>	<p><u>Mice</u></p> <p>#1 (NMR1) 21 months HDT = 180 ppm (inadequate)</p> <p>#2 (NMR1) 91 weeks 0, 500 & 1500 ppm (HDT excessively toxic in M & F)</p> <p><u>Rats</u> 2 years HDT = 1000 ppm (adequate)</p>	<p><u>Negative</u></p> <p><u>Positive</u> <u>Liver</u> M: HC adenomas, carcinomas and comb adenomas/ carcinomas at HDT F: HC adenomas, carcinomas and comb adenomas/ carcinomas at HDT (CPRC considered these tumors to be cmpd-related in spite of excessive toxicity at HDT)</p> <p><u>Negative</u></p>	<p><u>Negative</u> in 4 studies</p>	<p>1) HC adenomas, carcinomas and comb adenomas/ carcinomas in M and F mice at HDT</p> <p>2) Negative in rats</p> <p>3) Negative in 4 muta studies</p> <p>4) SAR data are supportive</p> <p>Classification/Quantification: Group C, no Q1* (RfD approach)</p>

Chemical Meeting Dates	Species/Duration/ Doses/Adequacy of Doses Tested	Evidence of Carcinogenicity	Evidence of Mutagenicity	Current Classification and Basis for Classification
<p>Propiconazole</p> <p>1) 1/15/87 SAP Mtg (3/2/88)</p> <p>2) 3/30/88 3) 4/26/89 4) 7/12/89 5) 4/15/92</p>	<p><u>Mice</u> 2 years 0, 100, 500 & 2500 ppm (excessive toxicity at 2500 & 500 inadequate) (CPRC determined MTD is 850 ppm at 5th meeting)</p>	<p><u>Positive</u> <u>Liver</u> M: HC adenomas, carcinomas & comb adenomas/carcinomas at 2500 ppm and <u>decreased latency</u> F: Negative</p>	<p><u>Negative</u> in 7 studies</p> <p>Note: Triazolyl alanine (common plant metab-olite for almost all triazole fungicides) was positive for muta activity</p>	<p>1) HC adenomas, carcinomas and comb adenomas/ carcinomas and decreased latency in M mice at HDT (excessive) Negative in F mice Negative in rats</p> <p>2) Negative in 7 muta studies (but metabolite is positive)</p> <p>3) SAR data are supportive</p> <p>4) Suggestion of promotor activity in rats</p> <p>5)</p>
<p><u>Rats</u> #1 2 years 0, 100, 500 & 2500 ppm (adequate)</p> <p>#2 Special promotor activity study</p>	<p><u>Negative</u></p>	<p>Suggestion of promotor activity in M and F rats pretreated with DENA</p>		<p>Classification/Quantification: Group C, no Q1* (RfD approach) (No dose on which to base a Q1*)</p> <p>Note: A new mouse study (with intermediate doses) was later required by the Reregistration Peer Review Committee)</p>

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Chemical Meeting Dates	Species/Duration/ Doses/Adequacy of Doses Tested	Evidence of Carcinogenicity	Evidence of Mutagenicity	Current Classification and Basis for Classification
<p>Cyproconazole</p> <p>1) 6/20/90 2) 1/15/92 3) 10/21/92</p>	<p><u>Mice</u> 81(M)-88(F) weeks HDT = 200 ppm (adequate)</p> <p><u>Rats</u> 2 years HDT = 350 ppm (inadequate)</p>	<p><u>Positive</u> <u>Liver</u> M: HC adenomas & carcinomas & comb adenomas/ carcinomas F: HC adenomas & carcinomas & comb adenomas/ carcinomas</p> <p>(Negative at doses up to 350 ppm) (CPRC recom study be repeated) Study will <u>not</u> be repeated</p>	<p><u>Positive</u> (clastogenic) ↑ chromo aberr in CHO cells (with & <u>without</u> activ)</p> <p><u>Negative</u> in 4 studies</p>	<p>1) HC adenomas, carcinomas and comb adenomas/ carcinomas in M and F mice</p> <p>2) Inadequate doses in rat study (Negative up to 350 ppm) (Study will <u>not</u> be repeated)</p> <p>3) Clastogenic in 1 study Negative in 4 muta studies</p> <p>4) SAR data are supportive</p> <p>Classification/Quantification: Group B2 (with Q1*)</p>
<p>Hexaconazole</p> <p>1) 6/27/90</p>	<p><u>Mice</u> 2 years HDT = 200 ppm (inadequate)</p> <p><u>Rats</u> 2 years HDT = 1000 ppm (may be inadequate/could have tested higher)</p>	<p>(Negative at doses up to 200 ppm) (Marginal ↑ in HC tumors) (CPRC did <u>not</u> recom study be repeated)</p> <p><u>Positive</u> <u>Testes</u> M: ↑ Leydig cell tumors (benign) (only triazole chem to do this) F: Negative (CPRC did <u>not</u> recom study be repeated)</p>	<p><u>Negative</u> in 3 studies</p> <p><u>Data gap:</u> CPRC recom mouse lymphoma gene mutation assay</p>	<p>1) Inadequate doses in mouse study (Negative up to 200 ppm) (Study need not be repeated)</p> <p>2) Leydig cell tumors (benign) in testes of rats</p> <p>3) Negative in 3 muta studies (data gap) SAR data are supportive</p> <p>Classification/Quantification: Group C, with Q1*</p>

Chemical Meeting Dates	Species/Duration/ Doses/Adequacy of Doses Tested	Evidence of Carcinogenicity	Evidence of Mutagenicity	Current Classification and Basis for Classification
Etaconazole Not evaluated to date	<u>Mice</u> HDT = 3600 ppm	<u>Positive</u> <u>Liver</u> M: HC adenomas, carcinomas & comb adenomas/carcinomas F: HC adenomas, carcinomas & comb adenomas/carcinomas	-----	1) HC adenomas, carcinomas & comb adenomas/carcinomas in M and F mice Note: Registration voluntarily withdrawn. Not evaluated by CARC.
	<u>Rats</u>	-----	-----	1) Negative in mice at doses up to 500 ppm 2) Negative in rats at doses up to 500 ppm Has not been evaluated by CARC
Birtantanol (Baycor) Not evaluated to date	<u>Mice</u> HDT = 500 ppm	<u>Negative</u>	-----	1) Inadequate doses in mouse study (Negative up to 400 ppm) Has not been evaluated by CARC
	<u>Rats</u> HDT = 500 ppm	<u>Negative</u>	-----	
Azaconazole No Peer Review Mitg	<u>Mice</u> HDT = 400 ppm (inadequate) <u>Rats</u>	<u>Negative</u>	-----	

HDT = Highest Dietary concentration Tested

RAB1398:IMAZAL27.098

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