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MEMORANDUM

SUBJECT: 370

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Imazalil - Report of the Cancer Assessment Review Committee

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The Cancer Assessment Review Committee met on October 27, 1999 to evaluate the carcinogenic potential of Imazalil. Attached please find the Final Cancer Assessment Document.

cc: L. Brunsman
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CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF

IMAZALIL (THIRD REVIEW)

FINAL REPORT

7-DECEMBER-1999

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

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EXECUTIVE SUMMARY

Previously, the Cancer Peer Review Committee (CPRC; August 24, 1994) classified Imazalil as a Group C- possible human carcinogen and recommended the low dose linear extrapolation (Q_1*) approach for quantification of human cancer risk. This decision was based on an increased incidence of hepatocellular adenomas and combined adenomas and carcinomas in male Swiss albino mice at the high dose. The increase was statistically significant by pair-wise comparison, with a statistically significant trend. Females had a significant dose-related trend for hepatocellular carcinomas and combined adenomas/carcinomas. No apparent increase in tumors was noted in Wistar rats. The Committee determined that the dosing was not adequate for assessing the carcinogenic potential in the rat and therefore, recommended that a new 2-year chronic feeding/carcinogenicity study in rats using higher dose levels be performed.

At the July 22, 1998 CARC meeting, the Committee (September, 4, 1998) reaffirmed the CPRC's previous decision in classifying Imazalil as a Group C carcinogen and the use of a linear low-dose extrapolation approach for quantification of human cancer risk based on liver tumors in male mice.

On October 27, 1999, the CARC met to evaluate the newly completed rat chronic/carcinogenicity study. The CARC concluded that Imazalil was carcinogenic to male rats because: 1) There was a significant increase by pair-wise comparison of the 2400 ppm (134.8 mg/kg/day) group with the controls for hepatocellular adenoma and combined adenomas/carcinomas. 2) The incidence of hepatocellular adenomas was outside the range of the historical control data and the combined incidence was driven by the adenomas. 3) There were also significant increasing trends for hepatocellular adenomas and combined adenomas/carcinomas ; and 4) There was a significant increase by pair-wise comparisons of the 1200 and 2400 ppm (65.8 and 134.8 mg/kg/day, respectively) groups with the controls for thyroid follicular cell combined adenomas/carcinomas. There was also a significant increasing trend for thyroid follicular cell combined adenomas/carcinomas. The incidence of combined thyroid tumors was outside the historical control. No increases in liver and thyroid tumors were noted in females. The dosing at the highest dose in both sexes was considered to be adequate and not excessive based on decreases in body weight gains and non-neoplastic changes in the liver (both sexes) and thyroid (males only). The CARC considered the liver and thyroid tumors in males to be treatment-related.

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

1. There were increases in hepatocellular adenomas and combined liver adenomas/carcinomas in **male** Swiss albino mice and Wistar rats. In male rats, there was also an increased incidence of combined thyroid follicular cell adenomas/carcinomas.

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

3. It is structurally related to triazole compounds, which are hepatocarcinogens in mice.

The Committee recommended a linear low-dose (Q_1*) extrapolation approach for the quantification of human cancer risk based on the most potent liver tumors in mice. This approach is supported by the lack of confirmation of the mode of action.

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I. INTRODUCTION

The carcinogenic potential of Imazalil was previously discussed by the Carcinogenicity Peer Review Committee (CPRC, 1994) as well as CARC (1998). They evaluated the weight-of-the-evidence on Imazalil with regard to its carcinogenic potential. The CPRC classified Imazalil as Group C and recommended a linear low-dose (Q1*) approach for the quantification of human cancer risk. The CARC (1998) reaffirmed the CPRC's decision.

On October 27, 1999, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs met to reconsider the carcinogenicity classification of Imazalil in light of the new chronic/carcinogenicity study in Wistar rats under the draft Agency Risk Assessment Guidelines (1996; 1999) for the human cancer risk assessment.

II. EVALUATION OF CARCINOGENICITY AND OTHER EVIDENCE

The mouse data are discussed in the previous CPRC (August 24, 1994) and CARC documents on Imazalil [HED Document No. 012870, September 4, 1998] and are not reiterated here.

Prior to the October 27, 1999 meeting of the Cancer Assessment Review Committee [CARC], the Registrant submitted a new chronic /carcinogenicity study in Wistar rats. The study was submitted in response to CPRC previous recommendation to fulfil the guideline requirement. The new data were evaluated by the Committee and are discussed below.

1. Combined Oral Chronic Toxicity/Carcinogenicity Study with Imazalil in the SPF Wistar Rat.

   Reference: Dept. Toxicology, Janssen Research Foundation, 2340 Beerse, Belgium. Laboratory report number, 3817, June 8, 1999. MRID 44858001.

   A. Experimental Design

   In this 24-month toxicity/carcinogenicity study (MRID 44858001), Imazalil (≥97.4% a.i.) was administered in the diet to groups of 50 male and 50 female Hannover substrain (SPF) Wistar-derived rats at concentrations of 0, 50, 200, 1200, or 2400 ppm (equivalent to 0.0, 2.7, 10.8, 65.8, and 134.8 mg/kg/day for males and 0.0, 3.6, 14.6, 85.2, and 168.8 mg/kg/day for females) for two years.

   B. Discussion of Tumor Data

   Based on HED's assessment (Brunsman, 1999; Table 1 and 2), male rats had significant differences in the pair-wise comparisons of the 2400 ppm dose group with the controls

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for hepatocellular adenomas, and combined adenomas/carcinomas, both at $p < 0.05$. The incidence of adenomas exceeded the historical control range (adenomas: 6%-10%; mean: 7.5%; carcinomas: 0%-4%, mean: 1%) and the increase in the combined incidence was driven by the adenomas. There were also significant increasing trends for hepatocellular adenomas, and combined adenomas/carcinomas, both at $p < 0.01$. In addition, there were significant differences in the pair-wise comparisons of the 1200 and 2400 ppm dose groups with the controls, for combined thyroid follicular cell adenomas/carcinomas, at $p < 0.05$. The incidences of these tumors at 1200 and 2400 ppm exceeded the range for the historical controls (adenomas; 6%-16%; mean 11.5%; carcinomas: 0%-4%; mean 2% and combined: 6%-16%; mean 12.5%). The increased incidence of thyroid adenomas at 50 and 200 ppm was not considered by the CARC to be biologically significant because of lack of dose response. There was also a significant ($p<0.05$) increasing trend for the combined thyroid follicular cell adenomas/carcinomas.

There were no compound-related increases in tumors in female rats.

C. Non-Neoplastic Lesions

The absolute liver weight of male rats in the 2400 ppm group was increased while it was decreased in female rats. The associated relative liver weights of male and female rats in the 1200 and 2400 ppm groups were significantly increased 9-26%. In addition, the absolute and relative thyroid weights of male but not female rats in the 1200 and 2400 ppm groups were increased.

The incidence of clear cell and basophilic foci was equivocal from controls for male rats in the 2400 ppm group, while the presence of eosinophilic foci was clearly and statistically increased ($p<0.001$). In contrast, the incidence of clear cell and basophilic foci in female rats in the 2400 ppm group was significantly decreased ($p<0.01$) from control rats while the incidence of eosinophilic foci was equivocal. Also noted were the incidences of hepatocyte fatty vacuolation and pigmentation. The incidence of hepatocyte fatty vacuolation of male rats in the 1200 ppm and 2400 ppm groups was significantly ($p<0.001$) increased but was not increased in female rats of these groups. In contrast, the incidence of hepatocyte pigmentation was significantly ($p<0.05$) increased in female rats of the 200, 1200, and 2400 ppm groups but not in male rats of these groups. Finally, the location of hepatocellular hypertrophy was distinctly different between the two sexes. Female rats in the 1200 and 2400 ppm groups had significant increases ($p<0.05$) in slight to moderate centriacinar and periacinar hypertrophy while male rats in these groups had slight to marked centriacinar hypertrophy ($p<0.01$) only. Nevertheless, the liver appears to be the target organ of toxicity.

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Table 1. Imazalil - SPF Wistar Rat Study
Male Hepatocellular Tumor Rates[+] and Exact
Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)				
	0	50	200	1200	2400
Adenomas (%)	4/48 (8)	2/47 (4)	3[a]/50 (6)	4/49 (8)	13/49 (27)
p =	0.000**	0.349[n]	0.477[n]	0.631	0.017*
Carcinomas (%)	1[b]/48 (2)	0/47 (0)	0/50 (0)	0/49 (0)	1/49 (2)
p=	0.403	0.505[n]	0.490[n]	0.495[n]	0.747
Combined (%)	4[c]/48 (8)	2/47 (4)	3/50 (6)	4/49 (8)	13[c]/49 (27)
p =	0.000**	0.349[n]	0.477[n]	0.631	0.017*

[+]Number of tumor-bearing animals/Number of animals examined, excluding those that died before week 53.
[a]First adenoma observed at week 103, dose 200 ppm.
[b]First carcinoma observed at week 105, dose 0 ppm.
[c]One animal in each of the 0 and 2400 ppm dose groups had both an adenoma and a carcinoma.
[n]Negative change from control.

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 2. Imazalil - SPF Wistar Rat Study

Male Thyroid Follicular Cell Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)				
	0	50	200	1200	2400
Adenomas (%)	4[a]/48 (8)	8/47 (17)	5/50 (10)	9/49 (18)	10/49 (20)
p =	0.059	0.167	0.526	0.124	0.079
Carcinomas (%)	0/48 (0)	0/47 (0)	2[b]/50 (4)	2/49 (4)	2/49 (4)
p=	0.093	1.000	0.258	0.253	0.253
Combined (%)	4/48 (8)	8/47 (17)	6[c]/50 (12)	11/49 (22)	12/49 (24)
p =	0.017*	0.167	0.397	0.049*	0.029*

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

[a]First adenoma observed at week 81, dose 0 ppm.

[b]First carcinoma observed at week 105, dose 200 ppm.

[c]One animal in the 200 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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The other treatment-related effect was a significant increase ($p<0.05$) in the incidence of thyroid follicular cell hyperplasia in male rats at 1200 and 2400 ppm. No treatment-related effect on the thyroid was observed in female rats.

D. Adequacy of Dosing for Assessment of Carcinogenic Potential

The test material induced slight clinical chemistry and hematological changes, as well as hepatocellular hypertrophy and fatty liver changes in male and female SPF Wistar rats treated with Imazalil at ~200 to 800 ppm for three months (MRID # 43965704). In a 13-week study (MRID # 43965705), the test material at 3200 ppm induced decreases in body weight gains (13-26% in males and 7-19% in females after 13 weeks), decreases in food consumption at doses ≥800 ppm, as well as hepatocellular hypertrophy and fatty liver changes.

In the carcinogenicity study, there were no mortalities related to treatment. At 1200 ppm, body weight gain in males and females was 5-10% and 10-28%, respectively, less than their respective controls. At 2400 ppm, body weight gain in males and females was 15-20% and 19-38% less than the controls, respectively. Food consumption in female rats at 1200 ppm, and in male and female rats at 2400 ppm was significantly less ($p<0.05$; 7-14%) than the controls throughout the study. Gross and microscopic changes were noted in the liver in both sexes and in the thyroid in male rats at >1200 ppm.

E. ADDITIONAL TOXICOLOGY DATA

A. Mutagenicity

The mutagenicity studies on Imazalil were previously evaluated at the August 24, 1994 CPRC and July 22, 1998 CARC meetings. Overall, the available data indicate that Imazalil does not induce mutagenic activity.

Mode of Action

In the published literature, Imazalil was found to induce glutathione S-transferase positive (GST-P) foci in rat liver (Hasegawa and Ito, 1992). Rats were given a 200 mg/kg injection of diethylnitrosamine (DEN), followed two weeks later by a diet containing 1000 ppm Imazalil (for six weeks) and partial hepatectomy at week three. Numbers and areas of GST-P foci per unit area of liver were increased significantly ($p<0.001$) in the group treated with DEN and then fed Imazalil compared with the group treated with DEN only. The CARC **concluded** that the available data are not adequate to establish the role of Imazalil as a tumor promoter.

III. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

The new acceptable rat study supports the previous assessment of the carcinogenic potential of Imazalil, in that Imazalil displays evidence for carcinogenicity in rats as well as in mice. The review of both *in vitro* and *in vivo* mutagenicity studies indicate a non genotoxic effect.

Previously, triazole fungicides have been listed as structurally similar to Imazalil. These include triadimefon, triadimenol, uniconazole, tebuconazole, propiconazole, cyproconazole, hexaconazole, etaconazole, bitertanol and azaconazole. Seven of these ten structural analogs have been shown to induce hepatocellular tumors in one or both sexes of mice.

IV. CLASSIFICATION OF CARCINOGENIC POTENTIAL

Under the Draft Guidelines for Carcinogen Risk Assessment (July, 1999), the Committee classified Imazalil into the category **"Likely to be carcinogenic to humans"** based on the following weight-of-the-evidence:

1. There was an increase (both trend and pair-wise) in combined liver adenomas/carcinomas in **male** Swiss albino mice and **male** Wistar rats and an increase in combined thyroid follicular adenomas/carcinomas in **male** Wistar rats.

2. Imazalil was **negative** in *in vivo* and *in vitro* mutagenicity assays.

3. It is structurally related to triazole compounds, which are hepatocarcinogens in mice.

V. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The Committee recommended a linear low-dose(Q_1*) extrapolation approach for the quantification of human caner risk based on the most potent liver tumors in mice. This approach is supported by the lack of confirmation of the mode of action.

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VI. BIBLIOGRAPHY

MRID No.	CITATION
44858001	Van Deun, K. 1999. Combined oral chronic toxicity/carcinogenicity study with Imazalil in the SPF Wistar rat. Dept. Toxicology, Janssen Research Foundation, 2340 Beerse, Belgium. Laboratory report number, 3817, June 8, 1999.
--------	Brunsman, LL. (1999). Imazalil Qualitative Risk Assessment based on SPF Wistar Rat Dietary Study. A memorandum from Lori L. Brunsman, Science Analyses Branch, Health Effects Division to Abdhallah Khasawinah, Reregistration Branch 4, Health Effects Division, dated September 9, 1999. Document no. 0137227
--------	CPRC (1994). Carcinogenicity Peer Review of Imazalil. A memorandum from Henry Spencer, Toxicology Branch 1 and Esther Rinde, Science Analyses Branch, Health Effects Division to Cynthia Giles-Parker, Reregistration Division and Jay Ellenberger, Special review and Reregistration Division, dated Dec. 27, 1994.
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