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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

August 17, 1994

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review Meeting on **IMAZALIL**

FROM: Esther Rinde, Ph.D. E.R.
Manager, Carcinogenicity Peer Review
Health Effects Division (7509c)

TO: Addressees

Attached for your review is a package on **Imazalil** prepared by Dr. Henry Spencer.

A meeting to consider the carcinogenicity classification of this chemical is scheduled for **Wednesday Aug. 24, 1994, at 10:00 am in Room 817, CM2.**

Addressees

S. Irene
R. Engler
W. Burnam
K. Baetcke
M. Van Gemert
K. Dearfield
H. Pettigrew
B. Fisher
L. Brunsman
E. Doyle
H. Spencer
K. Hamernik
R. Hill
Y. Woo
L. Brennecke

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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: The Weight of the Evidence Evaluation for the Oncogenic Potential of Imazalil and the Sulfate Salt of Imazalil
Imazalil
Tox Chem Number 497AB
PC Code 111901
Imazalil sulfate
Tox Chem Number 497B
PC Code 111902

FROM: Henry W. Spencer, Ph.D. H.W. Spencer 8/17/94
Review Section 3
Toxicology Branch 1
Health Effects Division (H7509C)

TO: Esther Rinde, Ph.D.
Manager, Peer Review for Oncogenicity
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

THRU: Karen Hamernik, Ph.D.
Section Head
Review Section 3
Toxicology Branch 1
Health Effects Division (H7509C) K.H. 8/17/94

THRU: Karl Baetcke, Ph.D.
Chief
Toxicology Branch 1
Health Effects Division (H7509C) Karl P. Baetcke 8/17/94

Attached is a report prepared for the Carcinogenicity Peer Review Committee on Imazalil and Imazalil sulfate. Administration of Imazalil base in the diet appears to be associated with an increased incidence of adenomas at 200 ppm and combined adenomas/adenocarcinomas of the livers of male Swiss mice at 600 ppm. Female mice have significant dose-related increasing trends in hepatocellular adenomas and combined adenomas and/or carcinomas. There were no significant differences in the pair-wise comparisons of the dosed groups of females with the controls.

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These incidences of liver tumors in the male mice exceeded the historical controls of the testing laboratory.

Toxicology Branch 1 is requesting the Peer Review Committee to examine the weight of the evidence and to classify the chemical/s according to the Agency's guidelines.

Attachment:

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Attachments:

1. Imazalil Qualitative Risk Assessment Based on Charles River SPF Swiss Albino Mouse Dietary Study dated July 29, 1994. Slide reading MRID 420972001.

2. DER for Mouse Chronic/ Oncogenicity Study. MRID Number 42972001, Experiment No. 2194, dated June 8, 1994.

3. DER for Rat Chronic Toxicity/ Carcinogenicity Study. Dated August 9, 1994. MRID Number 470261012 Study No. B 82-0555 and Study Number 419.

4. DER for 6- month Subchronic Rat Feeding Study. Dated June 24, 1987, study number V83.186/220555 HED document 005957.

5. Historical Control Data for rat Study Number B82-0555. listed as MRID 41558501, Report No. 581, Rec No. 227.

6. Equivalency of Imazalil Base and the Sulfate Salt Listed as Exhibit # 1, Dated March 1, 1994.

7. DER for old 24 month study in the Wistar rat evaluating toxicity. Acc.No. 070091, HED document # 001337, dated Jan. 6, 1982.

8. DER for the drinking water Carcinogenicity Study in Mice with Imazalil sulfate, pp 8 of HED document 000057, dated 1980.

9. DER for the oral Carcinogenicity Study in Mice with Imazalil, study No. 23979, Acc. No. 097233, HED document # 1337,4795, dated 1982.

10. DER of 3 month Mechanistic Study in mice, MRID 43222601, and 43202402 dated August 9, 1994.

11. Memo of Judith Hauswirth to S. Lewis on the Dose Selection for Mouse Oncogenicity Study. HED document #007865.

12. Toxicology Branch One-Liner for Imazalil sulfate # 497B

13. Letter from Dr. L. Van Leemput, Janssen Pharmaceutica dated 02/05/94 on the metabolism of Imazalil sulfate.

C. Background Information:

Imazalil, 1-(2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl)-1H-imidazole is an imidazole fungicide registered on bananas and on citrus for post-harvest uses and on cotton, wheat and barley as a seed treatment. There are tolerances for secondary residues in cattle, horses, pigs, goats and milk.

Imazalil sulfate, 1-(2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl)-1H-imidazole sulfate presently has no registered uses.

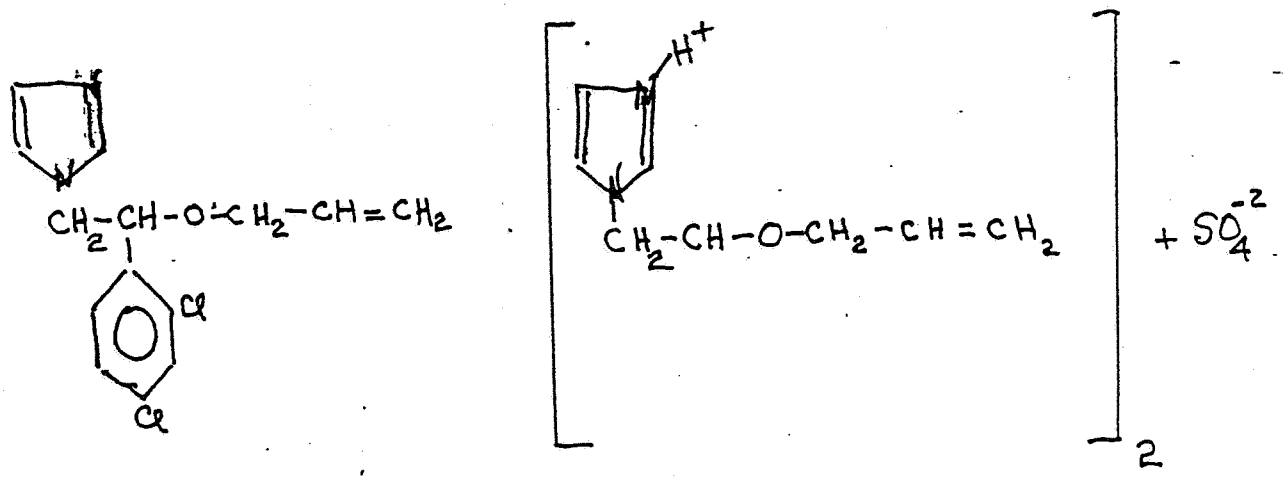
As a fungicide, Imazalil inhibits the cytochrome P450 14-alpha demethylation of sterols and prevents the formation of the essential cell wall component of ergosterol by conversion from lanosterol.

When Imazalil was reviewed for reregistration purposes in 1987, it was determined that Imazalil was not adequately tested for its carcinogenic potential in a 1975 mouse 18 month feeding oncogenicity study (Acc. No. 097233, HED document 001337, Attachment 7, 9). The study was to be rerun at doses higher than 400 ppm (see memo of J. Hauswirth attached, Attachment 11). In a new mouse 100-101 week feeding study, reviewed in 1994 (MRID 429720-01, Attachment 2), it was determined that Imazalil exhibited carcinogenic effects. A mouse drinking water carcinogenicity study completed in 1979 using Imazalil sulfate (Acc No. 099285, HED document 000057, Attachment 8) showed no convincing evidence of carcinogenicity at the highest dose tested of 40 mg/kg. An older (1975 or earlier) 2-year study in the rat performed with Imazalil (Acc. No. 099285 and HED document 001337, Attachment 7, 9) showed only equivocal effects for toxicity and little to no evidence of oncogenic potential. These older studies were considered to be minimum data at the times of their reviews but have been reevaluated and generally have been found to be insufficient in the testing regimen.

The Tox Chem Number of Imazalil free base is 497AB. The Chemical Abstracts Registry Number (CAS No.) is 35554-44-0.

The Tox Chem Number of Imazalil sulfate is 497B. The Chemical Abstracts Registry Number (CAS No.) is 58594-72-2.

The chemical structures follow:



Imazalil

Imazalil sulfate

D. Evaluation of Carcinogenicity Evidence:

1. Swiss Mouse Carcinogenicity Study

Reference: Verstraeten, A. (study director) "Evaluation of the carcinogenic potential of Imazalil base (R 23979) in SPF Albino Swiss mice", October 13, 1993. [MRID Number: 42972001, HED document# 011044, dated June 8, 1994.] Testing Facility: Department of Toxicology, Janssen Research Foundation, Beerse, Belgium. **(Attachment 2)**

a. Experimental Design

Imazalil base was administered in the diet to groups of 50 male and 50 female Swiss mice obtained from Charles River, France and treated for 100-101 weeks at nominal levels of 0, 50, 200, or 600 ppm. Dose selection was based on a 90 day feeding study in mice. Dosing material was composed of a mixture of 50% ai added to a mixture of equal parts of [redacted] and pelleted for feeding. Actual doses were 0, 6.76, 28, or

88 mg/kg/day for males and 0, 8.29, 34.8, or 110 mg/kg/day for females. Interim sacrifices were omitted in the study and the animals were either sacrificed in extremis, died on study or were sacrificed at 100-101 weeks on study. Food consumption, body weight changes, hematology, and organ weight data were recorded. Histopathological examinations were made on all animals available.

b. Discussion of Tumor Data

The histopathological evaluations of liver tumors were made by the registrant pathologist and, at the request of the registrant, an independent pathologist. However, based on the recommendation of HED's consulting pathologist, L. Brennecke, HED has chosen to use the evaluations produced by the independent pathologist who used a nomenclature and classification scheme which was more consistent with that currently used by the Agency.

Following the calculation of the qualitative risk assessment, the L. Brunsman, July 29, 1994 memo (see **Attachment #1)** notes that, "The statistical evaluation of mortality data indicated no significant incremental changes with increasing doses of Imazalil in male or female mice.

Male mice had significant dose-related increasing trends in hepatocellular adenomas, carcinomas, and combined adenomas and/or carcinomas. There was a significant difference in the pair-wise comparison of the 200 ppm dose group with the controls for hepatocellular adenomas. There were also significant differences in the pair-wise comparisons of the 600 ppm dose group with the controls for hepatocellular adenomas and combined adenomas and/or carcinomas.

Female mice had significant dose-related increasing trends in hepatocellular adenomas and combined adenomas and/or carcinomas. There were no significant differences in the pair-wise comparisons of the dosed groups with the controls" **(Attachment 1).**

Tables 3 and 4 below indicating the statistical findings are extracted from the memo of L.Brunsman dated July 29, 1994 on the Qualitative risk assessment of Imazalil in Swiss mice.

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)

	Dose (ppm)			
	0	50	200	600
Adenomas (%)	5/50 (10)	2/47 (4)	14[a]/50 (28)	13/48 (27)
p =	0.003**	0.244[n]	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[n]	0.088	0.006**

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

[n]Negative change from control.

[a]First adenoma observed at week 55, dose 200 ppm.

[b]First carcinoma observed at week 72, dose 600 ppm.

[c]Three animals in the 200 ppm dose group had both an adenoma and a carcinoma.

[d]Two 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 $p < 0.01$.

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)

	Dose (ppm)			
	0	50	200	600
Adenomas (%)	3/45 (7)	3[a]/48 (6)	0/45 (0)	7/47 (15)
p =	0.035*	0.630[n]	0.121[n]	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[n]	0.070

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

[n]Negative change from control.

[a]First adenoma observed at week 83, dose 50 ppm.

[b]First carcinoma observed at week 88, dose 600 ppm.

[c]One 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 $p < 0.01$.

c. Non-neoplastic Changes

Hematology: No treatment related effects were observed in the study.

Body weight: Reduced body weights were reported in males at the highest dose level (600 ppm) with a 5% to 7% reduction compared to controls (see table on pp 11). Body weights of females were reduced from 3% to 5% over the length of the study and generally only in the 600 ppm group.

Body weight gains were statistically significantly reduced at 600 ppm in males (see table 5 on pp 12). Weight gains were also reduced by 6% to 13% in females.

Clinical Chemistry: Reviewers did not consider there to be any treatment related changes in the study.

Organ Weights: Both absolute and relative liver weights were significantly increased (+18% absolute and +24% relative) in males at 600 ppm. Slight increases in absolute (+10) and relative (+14%) liver weights were observed in females at 600 ppm (see table 8 on pp 13). Macroscopic liver pathology is presented in Table 9 on page 13.

Histopathology: Analyses showed compound-related increases in the incidence of focal cellular changes of liver in males of all treatment groups (see table 12 on pp 14). Increased changes were greatest in the HDT (6/60 vas. 1/50 in controls).

Other changes included large vacuoles (1/50 in controls vs 9/50 in the HDT) and pigmentation (10/50 in controls vs 20/50 in the sinusoidal cells of the liver in males at 600 ppm). Hyperplasia of the liver was increased in males at 200 and 600 ppm by incidences of 12/50 and 8/50, respectively when compared to 4/50 in controls.

Females did not exhibit any cellular changes in the liver (see tables 10 and 12 on pp 15 and 14).

The study in mice produced data such that "At 200 ppm, males had a significant increase in the incidence of focal cellular changes, large vacuoles, and swollen sinusoidal cells in the liver. At the highest dose tested, males also had a significantly increased incidence of pigmentation in the sinusoidal cells of the liver and focal cellular changes in the pancreas, increased absolute (+18%) and relative (+24%) liver weight, and decreased body weight (-7%) and body weight gain (-18%). The **LOEL of 200 ppm, (28.0 mg/kg/day)** is based on the histopathology changes observed

in the livers of males. The **NOEL is 50 ppm (6.76 mg/kg/day)**. No LOEL was achieved in females (> 600 ppm or 110 mg/kg/day)" (excerpted from executive summary of HED document # 011044). The study was classified as Core Minimum.

There were no pertinent historical control data for this study because of the differences in nomenclature used by the testing laboratory and the Agency. There were only concurrent controls with which to compare the occurrences of tumors in this study.

TABLE 4. Mean Body Weight Data for Mice Ingesting Imazalil in the Diet for up to 23 Months[a,b]

Week	Mean Body Weight Data (g) by Dietary Level (ppm)			
	0	50	200	600
		Males		
12	37	37 (100)	36 (97)	35*** (95)
25	41	41 (100)	41 (100)	38** (93)
37	42	43 (102)	43 (102)	40* (95)
50	44	44 (100)	44 (100)	41*** (93)
76	44	45 (102)	45 (102)	41*** (93)
99	42	41 (98)	42 (100)	40* (95)
		Females		
12	30	31 (103)	30 (100)	30 (100)
25	34	34 (100)	33 (97)	33 (97)
37	36	36 (100)	35 (97)	35 (97)
50	38	38 (100)	37 (97)	36 (95)
76	39	40 (103)	39 (100)	38 (97)
99	38	40 (105)	38 (100)	37 (97)

[a] Data extracted from Study No. 2194, pp. 43-44.
[b] Numbers in parentheses indicate percent control.

* Significantly different from control, $p < 0.05$
** Significantly different from control, $p < 0.01$
*** Significantly different from control, $p < 0.001$

TABLE 5. Mean Cumulative Body Weight Gain Data for Mice Ingesting Imazalil in the Diet for up to 23 Months[a,b]

Week	Body Weight Gain (g) Data by Dietary Level (ppm)			
	0	50	200	600
		Males		
12	11	11 (100)	10* (91)	9*** (82)
25	15	15 (100)	15 (100)	12*** (80)
37	16	17 (106)	16 (100)	14** (88)
50	18	18 (100)	18 (100)	15*** (83)
76	18	19 (106)	19 (106)	15*** (83)
99	16	15 (94)	16 (100)	14** (88)
		Females		
12	8	8 (100)	7 (88)	7 (88)
25	11	11 (100)	10 (91)	10 (91)
37	13	14 (108)	13 (100)	12 (92)
50	15	15 (100)	14 (93)	13 (87)
76	17	18 (106)	16 (94)	15 (88)
99	16	18 (113)	15 (94)	15 (94)

[a] Data extracted from Study No. 2194, pp. 46-47.
[b] Numbers in parentheses indicate percent control.

* Significantly different from control, $p < 0.05$
** Significantly different from control, $p < 0.01$
*** Significantly different from control, $p < 0.001$

TABLE 8. Mean Liver Weight Data for Mice Ingesting Imazalil in the Diet for up to 23 Months[a,b]

	Mean Liver Weight Data by Dietary Level (ppm)			
	0	50	200	600
		Males		
absolute weight (mg)	2388 ± 878	2082 ± 611 (87)	2402 ± 712 (101)	2812 ± 1044* (118)
relative weight (mg/100 g)	5685 ± 1826	5112 ± 1521 (90)	5798 ± 1767 (102)	7060 ± 2521** (124)
		Females		
absolute weight (mg)	2143 ± 627	2264 ± 392 (106)	2088 ± 458 (97)	2359 ± 1541 (110)
relative weight (mg/100 g)	5509 ± 1246	5581 ± 664 (101)	5580 ± 1162 (101)	6269 ± 3745 (114)

[a] Data extracted from Study No. 2194, p. 72 and pp. 588-603.
[b] Numbers in parentheses indicate percent control.

* Significantly different from control, $p < 0.05$
** Significantly different from control, $p < 0.01$

TABLE 9. Incidence of Selected Macroscopic Liver Pathology Findings in Mice Ingesting Imazalil in the Diet for up to 23 Months[a]

	Incidence of Liver Pathology by Dietary Level (ppm)			
	0	50	200	600
		Males		
Liver focus	3/50	1/50	7/50	7/50
Liver mass	11/50	9/50	21/50	20/50
Liver nodule	1/50	4/50	7/50	3/50
		Females		
Liver focus	5/50	4/50	1/50	5/50
Liver mass	2/50	6/50	2/50	5/50
Liver nodule	0/50	4/50	0/50	3/50

[a] Data extracted from Study No. 2194, pp. 77-86.

TABLE 12. Incidence of Selected Histopathology by the Independent Pathologist in Mice Ingesting Imazalil in the Diet for up to 23 Months[a]

	Incidence Data by Dietary Level (ppm)			
	0	50	200	600
		Males		
Hepatocellular hyperplasia				
-total	4/50	2/49	12/50	8/50
-reported as neoplastic nodules by the study authors	3/50	1/49	7/50	3/50
Focus of alteration (basophilic or eosinophilic)				
-total	1/50	3/49	2/50	6/50
-reported as neoplastic nodules by the study authors	0/50	1/49	2/50	1/50
		Females		
Hepatocellular hyperplasia				
-total	1/50	1/50	0/50	3/50
-reported as neoplastic nodules by the study authors	1/50	0/50	0/50	2/50
Focus of alteration (basophilic or eosinophilic)	0/50	0/50	0/50	0/50

[a] Data extracted from Study No. 2194, pp. 277-290.

TABLE 10. Incidence of Selected Nonneoplastic Histopathology Findings in Mice Ingesting Imazalil in the Diet for up to 23 Months[a,b]

	Incidence of Histopathology by Dietary Level (ppm)			
	0	50	200	600
		Males		
Liver				
–Focal cellular changes	2/50 (1)	4/49 (1.3)	10/50* (1.2)	8/50 (1.5)
–Large vacuoles	1/50 (1)	0/49	8/50* (1)	9/50* (1)
–Pigmentation in sinusoidal cells	10/50 (1)	3/49 (1)	11/50 (1.2)	20/50* (1.2)
–Swollen sinusoidal cells	24/50 (1)	27/49 (1.1)	37/50* (1)	26/50 (1)
–Parenchymal cellular swelling	2/50 (1)	0/49	2/50 (1)	0/50
–Large vacuoles/vacuolization	5/50 (1)	3/49 (1)	8/50 (1)	12/50 (1)
Pancreas				
–Focal cellular changes	0/50	2/49 (1)	2/50 (1)	6/49* (1)
		Females		
Liver				
–Focal cellular changes	2/50 (1)	1/50 (1)	2/50 (1)	2/50 (1)
–Large vacuoles	0/50	1/50 (1)	1/50 (1)	5/50 (1.2)
–Pigmentation in sinusoidal cells	12/50 (1.2)	10/50 (1.1)	11/50 (1.3)	12/50 (1.3)
–Swollen sinusoidal cells	39/50 (1.1)	28/50* (1.1)	32/50 (1.1)	28/50* (1.1)
–Parenchymal cellular swelling	0/50	0/50	0/50	4/50 (1)
–Large vacuoles/vacuolization	1/50 (1)	1/50 (1)	3/50 (1)	9/50 (1)
Pancreas				
–Focal cellular changes	2/50 (1)	4/49 (1)	1/49 (1)	5/50 (1)
Vagina				
–Metaplasia	9/44 (1.4)	11/48 (1.5)	9/46 (1.7)	22/48* (1.6)

[a] Data extracted from Study No. 2194, pp. 95-117, and pp. 604-1004.
[b] Numbers in parentheses indicate average severity.

* Significantly different from control, $p < 0.05$ using Fisher's exact test performed by the reviewers

Adequacy of Dosing for Assessment of Carcinogenic Potential

The dosing was considered to be adequate for assessing the carcinogenic potential of Imazalil in the Swiss mouse.

Janssen Research Foundation completed a 90 day range finding study using doses of 0, 200, 400, or 800 ppm which showed 25% and 30 % weight gain reductions in male and female mice respectively as well as other degenerative changes in the livers at 400 ppm. These degenerative changes included decreased serum albumin, total bilirubin and phospholipid. Swollen centriolobular hepatocytes were noted, but were more pronounced at 800 ppm.

HED and Janssen Research Foundation concluded and agreed that the HDT was to be 600 ppm (memo of J. Hauswirth 5/1/89, **Attachment #11**).
Note: The only review of the 90-day study appears to be the Hauswirth memo (HED Doc 007865). A NOEL and LOEL were not commented on in that memo.

2. Wistar Rat Carcinogenicity Study
(This carcinogenicity study review is a composite of two studies which were both started at the same time, in the same laboratory, and performed using the same batch of test material).

References **(Attachment #3)**:

1. Til, H.P.; Lina, B.A.R. et al," Lifespan Oral Carcinogenicity study with Imazalil Base-R 23979 in Rats". Date: November 1985, MRID 470261012, also MRID 00162413, Study No. B82-0555,
Testing Facility: Netherlands Organization for Applied Scientific Research: Division for Nutrition and Food Research, 3700 AJ Zeist, Netherlands.

2. Til, H.P.; Lina, B.A.R. et al., "18-Month Oral Chronic Toxicity Study with Imazalil Base-R 23979 in Rats". Date: May 1984, MRID 00162412, Study Number: 419
Testing Facility : Netherlands Organization for Applied Scientific Research: Division for Nutrition and Food Research, 3700 AJ Zeist, Netherlands.

a. Experimental Design

In the 30 month chronic rat study (MRID 470261-012), Imazalil technical alone was provided to Cpb:WU Wistar rats in the diet at 0, 25, 100, or 400 ppm dosage levels until 43 weeks into the study. After 43 weeks, the test doses were

composed of the test material as a 50% mixture of Imazalil and a carrier, [redacted] In the 18 month chronic toxicity study (MRID 00162412), the composition of the test material was changed at approximately the same time as in the 30 month study.

The 30 month study used 50 animals/sex/dose while the 18 month study used 20 males and 20 females per dose level tested. Doses of test material administered in the two studies were quite similar.

When converted from ppm, the doses administered in the two studies were approximately 0, 1.0, 3.7, 15.5,mg/kg/day of the test material for males at the 0, 25, 100, and 400 ppm doses respectively. Values for compound ingestion by the female rats were 0, 1.2, 4.9, and 20 mg/kg/day for the 0, 25, 100, and 400 ppm dose levels respectively.

b. Discussion of Tumor Data:

There were minor increases in the epidermoid carcinomas of the uterus in females in the 30 month study but not in the 18 month study. This type of tumor is considered to be a rare tumor. Historical control data for 15 studies bracketing the time of the Imazalil study (see excerpted table on pp 18 and **Attachment #5**) indicates that this tumor type occurred in only one of the 15 studies (most of the control groups contained 50 animals). The Imazalil study showed an occurrence of 0/48, 1/50, 0/50, and 1/50 in the controls, low, mid and high dose levels. The range of tumors in historical controls is from 0 to 2%.

Other tumors which showed increases included the testicular Leydig cell tumors with occurrences of 1, 3, 4, and 4 in controls, 25, 100, and 400 ppm dose levels respectively (see table on pp 19). The incidences of these tumors were submitted and were found to be easily within the control ranges of 0-12.7% for Leydig cell tumors and 0-16% for uterine adenocarcinomas. Therefore, these two additional tumor types were not considered to be treatment related (see pp 18 and 20 and **Attachment #5**) for historical control data for Leydig cell tumors and uterine adenocarcinomas.

There were no significant increases in neoplasia in the 18 month study with only 20 animals/sex at each dose level.

c. Non-neoplastic Lesions:

The only non-neoplastic histopathology observed in the 30 month rat study was the slight increase in focal vacuolation

RIN 3394-95

IMAZALIL PEER REVIEW PACKAGE

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of hepatocytes in males at 400 ppm (see Table 16, pp 26).

Body weights were only slightly lower in females at the 400 ppm dose level (277.5 g in controls vs 253.5 g at 400 ppm) (tables 3 and 4 on pp 22 and 23) during the first 52 weeks on the 18 month study. The body weights were not different (265 g vs 269 g) at the same time point in the 30 month study at 400 ppm. The reviewers considered this effect in the 18 month study to be a palatability problem because the weights later in the study were not noticeably different from controls.

Organ weights: Absolute liver weights were slightly increased in high dose males in the 18 month (+5.4%) and the 30 month (+10.7%) studies. Absolute kidney weights of females were only slightly increased in both 100 and 400 ppm groups in the 18 month study (see table 7 on pp 24).

Males at 400 ppm in the 18 month study showed an increase in the multivacuolar hepatocytes (3/20 in controls vs 9/20 in 400 ppm animals).

d. Adequacy of Dosing for Assessment of Carcinogenic Potential:

Toxicology Branch questions whether the doses of 400 ppm were high enough in the rat study to adequately test the carcinogenicity of Imazalil since only minor signs of toxicity to the chemical were encountered and included slight weight gain reductions in females at 400 ppm after 78 weeks (see Table 5 on pp 25). Focal hepatocellular vacuolation in males was slightly increased (2/17 in controls and 6/17 in the 400 ppm animals) for those living to 30 months (see table 16 on pp 26). No other effects were considered to be related to dosing. Serum LDH values which were elevated over double the control group values in the 400 ppm females were discounted by the reviewers. Dosing levels for the 30 month study were based on the toxicity reported in a 6 month study (Tox doc #5957, **Attachment #4**). The 6 month study was reported to demonstrate a **NOEL of 100 ppm (approximately 10 mg/kg/day) based on an increase in relative kidney weights in high dose males (5.77 g/kg in controls vs 6.47 g/kg in 400 ppm animals) and female rats (6.51 g/kg in controls vs 7.34 g/kg at 400 ppm) and increased absolute liver weights (5.0 g in controls vs 6.0 g at 400 ppm) and relative liver weights in high dose females (22.9 g/kg in controls vs 27.1 g/kg).** The 6 month rat study is also reported with acute and subchronic studies in Section E.5.b. on pp 37 of this document. The Core Classification of the study is Minimum.

TABLE 3. Mean Body Weights at Representative Intervals in Rat Fed Imazalil for 30 Months

Dietary Level (ppm)	Mean Body Weight (g ± S.E.) at Week:						
	12	26	52	78	104	130
			Males				
0	65.7 ± 1.1	350.5 ± 5.2	423.7 ± 5.8	488.4 ± 7.3	513.8 ± 8.8	506.9 ± 10.6	426.3 ± 15.7
25	65.7 ± 0.8	343.4 ± 4.2	412.4 ± 5.6	472.6 ± 7.4	499.4 ± 9.2	480.6 ± 11.3	386.3 ± 11.6
100	65.9 ± 1.0	348.4 ± 4.7	424.7 ± 5.8	485.1 ± 7.7	524.9 ± 9.1	520.2 ± 9.4	412.1 ± 9.2
400	65.9 ± 0.9	330.1 ± 3.8**	409.4 ± 5.1	476.1 ± 6.7	514.1 ± 7.6	494.0 ± 11.9	435.1 ± 14.4
			Females				
0	66.9 ± 0.9	205.9 ± 2.5	238.8 ± 3.2	265.3 ± 4.3	302.0 ± 5.9	321.0 ± 7.9	270.6 ± 8.3
25	66.8 ± 1.0	209.0 ± 2.4	240.7 ± 3.1	273.1 ± 4.5	305.1 ± 5.8	314.8 ± 6.6	298.9 ± 11.6
100	66.5 ± 1.0	202.7 ± 2.2	234.2 ± 2.6	267.5 ± 4.5	303.4 ± 6.6	316.1 ± 8.1	278.0 ± 11.8
400	67.0 ± 0.8	207.8 ± 1.9	238.5 ± 2.1	269.5 ± 3.7	289.7 ± 4.9	304.0 ± 6.8	256.0 ± 13.6

**Significantly different from control value ($p < 0.01$).

TABLE 4. Mean Body Weights at Representative Intervals in Rats Fed Imazalil for 18 Months

Dietary Level (ppm)	Mean Weight (± S.E.) at Week:				
	0	12	26	52	78
	Males				
0	70.6 ± 1.5	342.8 ± 10.3	409.0 ± 12.0	471.8 ± 13.6	485.0 ± 14.1
25	70.3 ± 1.8	343.5 ± 6.8	414.0 ± 8.3	476.0 ± 102	477.4 ± 12.3
100	70.4 ± 1.3	337.7 ± 8.5	409.6 ± 10.1	479.4 ± 12.3	484.6 ± 12.2
400	70.4 ± 2.6	347.1 ± 8.4	424.1 ± 10.8	485.7 ± 12.8	498.8 ± 15.2
	Females				
0	64.7 ± 1.5	207.8 ± 4.1	241.9 ± 4.7	277.5 ± 4.4	323.5 ± 6.8
25	64.5 ± 1.4	211.9 ± 4.8	242.5 ± 6.0	277.8 ± 9.9	310.4 ± 14.3
100	64.5 ± 1.9	213.2 ± 5.0	246.4 ± 5.9	275.0 ± 7.8	302.1 ± 10.3
400	64.7 ± 1.7	206.3 ± 3.8	235.3 ± 3.9	254.5 ± 5.1*	279.7 ± 6.6**

*Significantly different from control value ($p < 0.05$).

**Significantly different from control value ($p < 0.01$).

$N^+ = 19$ or 20

TABLE 7. Liver and Kidney Weight Data (± S.E.) for Rats Fed Imazalil

Dietary Level (ppm)	18 months		30 months	
	g	g/kg	g	g/kg
Liver		**Males**		
0	11.89 ± 0.55	24.4 ± 0.7	11.86 ± 0.43	29.4 ± 1.1
25	11.57 ± 0.47	24.1 ± 0.5	10.99 ± 0.46	30.0 ± 1.6
100	11.49 ± 0.41	23.7 ± 0.6	12.05 ± 0.50	30.8 ± 1.0
400	12.54 ± 0.48	25.1 ± 0.5	13.13 ± 0.48	31.6 ± 1.0
	+5.4%		+10.7%	
		Females		
0	7.17 ± 0.14	23.5 ± 0.4	8.35 ± 0.41	32.4 ± 1.3
25	7.02 ± 0.3	23.7 ± 0.4	9.48 ± 0.63	33.4 ± 1.3
100	7.37 ± 0.35	25.6 ± 0.8*	8.86 ± 0.56	33.8 ± 1.3
400	6.76 ± 0.25	25.2 ± 0.4	8.35 ± 0.42	34.8 ± 1.5
Kidneys		**Males**		
0	2.91 ± 0.09	6.01 ± 0.13	3.36 ± 0.13	8.36 ± 0.40
25	2.88 ± 0.09	6.02 ± 0.07	3.12 ± 0.11	8.52 ± 0.49
100	2.87 ± 0.07	5.96 ± 0.11	3.41 ± 0.12	8.68 ± 0.30
400	2.95 ± 0.08	5.94 ± 0.13	3.45 ± 0.12	8.31 ± 0.26
		Females		
0	1.97 ± 0.05	6.48 ± 0.17	2.17 ± 0.08	8.55 ± 0.24
25	1.89 ± 0.05	6.47 ± 0.15	2.26 ± 0.06	8.10 ± 0.26
100	2.04 ± 0.07	7.17 ± 0.20*	2.14 ± 0.07	8.30 ± 0.25
400	2.07 ± 0.07	7.74 ±0.19**	2.22 ± 0.08	9.32 ± 0.48

*Significantly different from control value ($p < 0.05$).

**Significantly different from control value ($p < 0.01$).

TABLE 5. Mean Weight Gains (g) in Rats Fed Imazalil[a]

Dietary Level (ppm)	30-Month Study		18-Month Study	
	12 Weeks	78 Weeks	12 Weeks	78 Weeks
		Males		
0	284.8	448.1	272.2	414.4
25	277.7	433.7	273.2	407.1
100	282.7	459.0	267.3	414.2
400	264.2	448.2	276.7	428.4
		Females		
0	139.0	235.1	143.1	258.8
25	142.2	238.3	147.4	245.9
100	136.2	236.9	148.7	237.6
400	140.8	227.7	141.6	215.0

[a]Calculated by subtraction of means; values are not the means of individual animal weight gain.

TABLE 16. Representative Nonneoplastic Histologic Lesions of the Liver, Kidney, and Lungs of Surviving Rats Fed Imazalil for 30 Months[a]

	Dietary Level (ppm)							
	Males				Females			
Organ/Lesion	0	25	100	400	0	25	100	400
Kidney	(17)[b]	(10)	(16)	(17)	(16)	(13)	(17)	(9)
Nephrosis	17	9	16	17	14	13	16	9
- very slight/slight	8	4	5	7	8	13	15	6
- moderate/severe	9	5	11	10	6	0	1	3
Mononuclear cell infiltration	13	3	3	13	12	7	8	7
Mineralization	0	1	0	0	4	8	15	5
Liver[c]	(17)	(10)	(16)	(17)	(16)	(13)	(17)	(19)
Focal hepatocellular vacuolization	2	0	0	6	3	0	0	0
Bile duct sclerosis	4	0	0	4	3	0	0	1
Necrotic hepatocyte aggregates	9	0	0	5	7	0	0	2
Mononuclear cell infiltrate (portal)	4	0	0	6	4	0	0	2
Increased Kuppfer cells	0	0	0	0	1	0	0	2
Lung	(17)	(9)	(16)	(17)	(16)	(13)	(17)	(9)
Peribronchial lymphoid aggregates	2	1	3	1	12	11	10	8
Macrophage accumulation	2	0	2	1	6	3	2	2
Interstitial pneumonitis	3	3	3	3	7	2	4	3

[a]Preneoplastic lesions are not included, see Table 15.
[b]The numbers in parentheses represent the numbers of tissues histologically examined.
[c]Nonpreneoplastic lesions were not routinely examined in low- and mid-dose groups.

The study authors mentioned using a 12-month rat study (open literature reference: [Thienpont, D., et. al. (1981). The biological and toxicological properties of Imazalil, (Arzneim.-Forsch./Drug. Res. 31 (1) 2, 309-315)] to aid in establishing the dose levels used in the 18 and 30 month studies. The study was not submitted to the Agency in support of registration.

3. Mouse 18 month feeding/carcinogenicity study with Imazalil base. The testing laboratory was Janssen Pharmaceutica Research Laboratory, Belgium. Study # 27180. dated 8/30/75. (Acc No. 097233, 070090. 070089), **(Attachment 7,9).**

Doses of 0, 2.5, 10 or 40 mg/kg/day were administered for a period of 18 months in the Swiss mouse. In the brief reviews for this study the only finding mentioned appeared to be liver weight increases. There was no evidence of oncogenic potential reported. The study was classified as Minimum for oncogenicity. However, a copy of the microfiche was illegible so that any conclusions could not be readily verified.

4. Mouse oncogenicity study - 2 year drinking water study with Imazalil sulfate. The testing laboratory was Janssen Pharmaceutica Research Laboratory , Belgium, Study No. 666 dated 4/4/79. (MRID # 099285 and 245311) and **(Attachment #8).**

This drinking water study in the Swiss mouse used Imazalil sulfate technical at 0, 2.5, 10, or 40 mg/kg/day for a period of 2 years. Only increased mortality was reported in the study at the HDT.

The study was considered by HED reviewers in 1980 to exhibit a **NOEL of 10 mg/kg/day with a systemic LEL of 40 mg/kg/day based on increased mortality. No increase in tumor incidence was reported. The study was classified as Minimum for carcinogenicity testing in the One-Liners however the reference cited does not clearly support this (Attachment # 7,9).**

The copy of the study reproduced from the microfiche was illegible so that any conclusions could not be readily verified.

4. Chronic feeding Carcinogenicity 24 month study in Wistar rats, Acc. No. 099285 and **Attachment # 7,9.**

In the brief reviews available for this study, it was mentioned that the only findings were equivocal effects for toxicity in the liver. There was little to no evidence of oncogenic potential.

The study was considered to be core supplementary for systemic effects with **an LEL of 40 mg/kg/day based on increased liver and kidney weights in females. The NOEL is 10 mg/kg/day.** The study was considered to be core minimum for carcinogenicity.

The copy of the study reproduced from the microfiche was illegible so that any conclusions could not be readily verified.

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E. Additional Toxicology Data:

1. Metabolism

When Imazalil was tested in Wistar rats in a metabolism study (MRID 420120-03) it was found to be rapidly absorbed, distributed and almost completely metabolized following an oral dose. Up to 93% of the doses to both sexes were excreted in 24 hours. T 1/2 values were not calculated, but at 96 hours tissues contained 1% of the 14C dose. Imazalil is metabolized to as many as 25 metabolites with no conjugated forms found in the urine. The liver contained the highest levels of test material (about 110 ppb) after repeated dosing at 1.25 mg/kg/day when analyzed after 96 hours. This study was considered to be supplementary but upgradeable with the further characterization of urinary metabolites.

A 3-month oral dosing mechanistic study (MRID 42972001) was performed in Wistar rats of both sexes to study the effects of Imazalil base on the liver. (See **Attachment # 10)** Nominal doses of 0, 50, 200, or 600 ppm were provided in the diet to 25 animals/sex/dose and approximated 0, 9.5, 38.6, or 115 mg/kg/day in males and 0, 11.3, 45.6, or 138 mg/kg/day in females, respectively. Additional groups of 15 animals/sex/dose were administered the test doses and sacrificed after 1 month. Observations recorded in the study included: routine clinical signs, body weights, food consumption, and gross necropsy. Determinations of levels of liver enzymes, organ weights, histopathology of only the gall bladder and liver, electron microscopy, liver microsomal protein and P450 enzyme content, seven P450 isoenzymes activities, liver testosterone metabolism and serum levels of Imazalil were completed after sacrifice.

Treatment related histopathological effects were only noted at 200 ppm and above in males and females as early as 1 month including an increased incidence and severity of "centrilobular clearer aspect", and of large and/or small vacuoles in the hepatocytes (see table 4 on pp 29,30). Increased liver microsomal protein in males and females was reported by 1 month in both higher dose levels. Increased cytochrome P450 content in males and females was also noted (see tables 5 and 6 on pp 31-34). At 600 ppm increased alkaline phosphatase in males, increased absolute liver weights and relative liver/body weights and increased individual cell necrosis in livers of males, as

TABLE 4: Selected Liver Histopathology for Mice Given Imazalil in the Diet for 1 Month or 3 Months

1 Month, Males

Observation	Control	50 ppm	200 ppm	600 ppm
No. examined	10	10	10	10
Reticuloendothelial system aggregates	3[1] (0.30)[2]	2 (0.20)	2 (0.20)	3 (0.30)
Centrilobular clearer aspect	1 (0.10)	2 (0.20)	4 (0.40)	10 (1.00)***
Diffuse hepato swelling	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Focal necrosis (hepatocytes)	2 (0.20)	0 (0.00)	1 (0.10)	3 (0.40)
Individual cell necrosis	0 (0.00)	2 (0.20)	0 (0.00)	1 (0.10)
Large vacuoles (hepatocytes)	0 (0.00)	0 (0.00)	0 (0.00)	6 (0.60)**
Prominent Kupffer cells	2 (0.20)	2 (0.20)	1 (0.10)	1 (0.10)
Small vacuoles (hepatocytes)	0 (0.00)	0 (0.00)	8 (0.80)***	10 (1.00)***

[1] Incidence (no. with effect/no. exam.); not statistically analyzed

[2] Mean severity score (max. score = 5; based on all animals examined); statistically analyzed

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

TABLE 4 (Continued): Selected Liver Histopathology for Mice Given Imazalil in the Diet for 1 Month or 3 Months

1 Month, Females

Observation	Control	50 ppm	200 ppm	600 ppm
No. examined	10	10	10	10
Reticuloendothelial system aggregates	2[1] (0.20)[2]	2 (0.20)	4 (0.50)	0 (0.00)
Centrilobular clearer aspect	2 (0.20)	1 (0.10)	8 (0.80)**	7 (0.70)*
Diffuse hepato swelling	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Focal necrosis (hepatocytes)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Individual cell necrosis	1 (0.10)	1 (0.10)	0 (0.00)	0 (0.00)
Large vacuoles (hepatocytes)	0 (0.00)	0 (0.00)	0 (0.00)	3 (0.30)
Prominent Kupffer cells	3 (0.30)	1 (0.10)	3 (0.30)	5 (0.50)
Small vacuoles (hepatocytes)	5 (0.50)	3 (0.30)	8 (1.00)	10 (1.70)**

[1] Incidence (no. with effect/no. exam.); not statistically analyzed

[2] Mean severity score (max. score = 5; based on all animals examined); statistically analyzed

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

RIN 3394-95

IMAZALIL PEER REVIEW PACKAGE

Page_____ is not included in this copy.

Pages 32 through 35 are not included.

The material not included contains the following type of information:

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well as diffuse swelling of hepatocytes in females were reported. EM showed increased lipid droplets in hepatocytes. Imazalil increased some P450 enzymes activities while inhibiting others. Testosterone hydroxylase levels were increased in both males and females by approximately 2X.

The **LOEL was 200 ppm based on increased incidence and severity of histological effects and increased P450 content in both males and females. The NOEL is 50 ppm (approximately 9.5 mg/kg/day in males and 11.3 mg/kg/day in females. The LEL is 200 ppm (approximately 38.6 mg/kg/day in males and 5.6 mg/kg/day in females).**

2. Equivalency of Imazalil and Imazalil sulfate

A document **(Attachments #6 and #13)** on the absorption of Imazalil base and its salts was submitted which argued that Imazalil salt will dissociate at the pH in the stomach to a protonated Imazalil which, when absorbed, is converted to Imazalil base at the pH of the blood. In a letter from Wm. Goodwine, Janssen Pharmaceutica representative, dated March 1, 1994, the equivalency of the Imazalil base and the sulfate salt was noted to have been recognized by the Ecological Effects and the Environmental Fate Branches, OPP, in a memo from Cutis Laird, EEB/HED. Based on the acid-base equilibria noted in **Attachment #6** and other available information, including some acute toxicity data for the technical **(Attachment #12)**, Toxicology Branch believes it is reasonable to expect that Imazalil and its sulfate salt would behave in a toxicologically similar fashion.

The registrant has verbally requested that the database for Imazalil be interchangeable with that of Imazalil sulfate. A complete set of acute data for Imazalil sulfate is not available for comparison.

3. Mutagenicity:

All the required mutagenicity testing for Imazalil has been completed with acceptable studies.

a. Salmonella assay, MRID 407293-01- Results were negative for increasing reversions at doses into the toxic range (25-500 ug/plate), both in the presence and absence of activation.

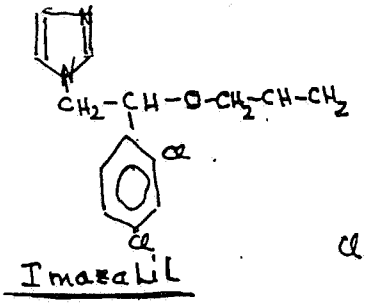
b. In vitro cytogenetics, MRID 40729302, 41026602- Results were negative for inducing chromosomal aberrations at concentrations of 125-5000 ug/culture and 22-909 ug/ml.

c. Micronucleus test, MRID 40729303 - Results were negative for induction of micronuclei in bone marrow cells of mice treated up to toxic doses (320 mg/kg).

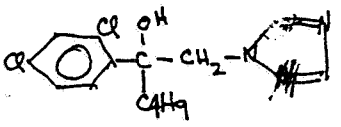
There was no evidence of genotoxicity in the Imazalil studies.

4. Structure-Activity Correlations

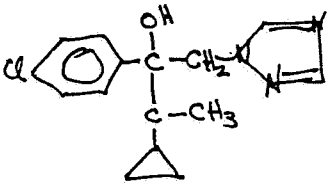
A search in the HED Cancer Peer Review files netted a total of four chemicals which might be considered to be structurally similar to Imazalil. These are: Hexaconazol, Uniconazole, Propiconazole, and Cyproconazole (structures appear below). All of these are currently classified as Class C carcinogens, although only Ciproconazole was found to be mutagenic (+ for chromosomal aberrations in the presence and absence of a microsomal activation system). However, the nitrogen-containing rings in Imazalil and the other chemicals differ. In Imazalil, the ring is an imidazole ring. In the other chemicals, the ring is a triazole ring. The Cancer Peer Review Committee is asked to comment on the whether it is appropriate or not to consider the triazole ring-containing chemicals to be structurally similar to Imazalil.



Imazalil



Hexaconazole



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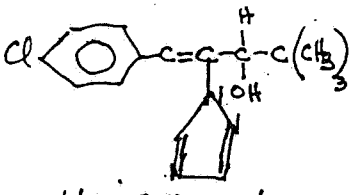
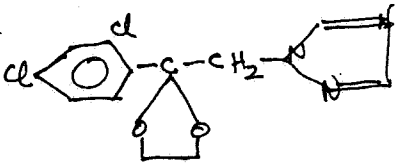


Uniconazole



Propiconazole

5. Acute, Subchronic and Chronic Toxicity Studies

a. Acute data

Imazalil free base technical Oral LD 50 in rats - 343 mg/kg in males and 227 mg/kg in females (Acc. No. 099285);

Imazalil sulfate technical Oral LD 50 in rats- 350 mg/kg in males and 309 mg/kg in females (Acc. No. 099285);

Imazalil free base technical Dermal LD 50 in rats - 4200 mg/kg in males and 4880 mg/kg in females (Acc. No. 097233).

b. Subchronic data- 6 month Wistar rat study (MRID 47026101)

In a 6 month rat study, 10 animals/sex/dose were administered diets containing 0, 25, 100, or 400 ppm of Imazalil. Results indicated there was no effect on food intake, body weights, hematological parameters or clinical chemistry attributable to the test material with the possible exception of an increase at 400 ppm of serum LDH values (271 in controls vs 585 U/L) in females. Kidney weights were increased at 400 ppm in males and females and lung, liver, and thymus weights were increased at 400 ppm in females. No histopathological changes occurred with the weight changes. An LEL of 400 ppm (approximately 40 mg/kg/day) was based on increased kidney and liver weights. The NOEL was 100 ppm (approximately 10 mg/kg/day). The core classification is Minimum.

F. Weight of Evidence Considerations:

The Committee is asked to consider the following regarding the toxicological data on Imazalil and its sulfate salt in a weight of the evidence determination of carcinogenic potential.

1. Administration of Imazalil for 23 months at 200 and 600 ppm in the diet of Swiss mice (MRID 429720-01) (approximately 28.0 and 88 mg/kg/day, respectively, in males and 34.8 and 110 mg/kg/day, respectively, in females) was associated with increases in combined hepatocellular adenomas/carcinomas at 600 ppm in males and increased numbers of adenomas at both 200 ppm ($p < 0.02$) and 600 ppm ($p < 0.027$). The incidence of carcinomas was increased but not statistically significantly so at 600 ppm, thus only suggesting a progression of adenomas to carcinomas in this sex.

Female mice also showed an increased incidence (not statistically significant) of adenomas, carcinomas, and combined tumors at 600 ppm compared to concurrent controls.

There were no historical control data excepting the concurrent controls with which to compare theses occurrences of tumors because of the differences in nomenclature between the testing laboratory and the Agency.

2. Imazalil was not found to be carcinogenic at the doses tested in the 30 month (MRID 470261-012) and 18 month (MRID 00162412) combined rat feeding study.

3. Imazalil was not found to be carcinogenic in the 1975 18 month mouse feeding/oncogenicity study (Acc No. 097233, HED document 001337) or the 1975 2-year rat feeding/oncogenicity study (Acc. No. 099285, HED document 001337), but these early studies were inadequate by today's standards.

4. Imazalil sulfate was not found to be carcinogenic in a 1979 drinking water study (Acc No. 099285, HED document 000057), the only carcinogenicity study available for this compound. This study study is also inadequate by today's standards.

5. The metabolism study of Imazalil in the Wistar rat indicated that the chemical when ingested is rapidly absorbed, metabolized to a large number of metabolites and excreted quite rapidly and completely in a period of 96 hours. Little (about 1% of the total ^{14}C dose) Imazalil remains in the animal carcass at 96 hours post-dosing.

6. There was no evidence of genotoxicity in the Salmonella assay both with and without activation, no evidence of the induction of chromosomal aberrations in an in vitro cytogenetics test, and no evidence of the induction of micronuclei in the bone marrow test in mice.

7. There is some evidence of some P450 enzyme activities being significantly induced in the male mice at both 200 and 600 ppm in a 3 month feeding study. Some isozymes were also inhibited in this same study.

Questions for the Committee to Address

Based on the above findings, the Cancer Peer Review Committee is asked to address the following:

1. the Carcinogenic potential of Imazalil based on the mouse study;

2. the adequacy of dosing in the rat carcinogenicity study and whether another rat study should be required which incorporates a higher dose level(s);

3. whether the Imazalil free base and Imazalil sulfate might be expected to behave in a toxicologically similar fashion and whether the carcinogenicity database for the Imazalil free base should be used interchangeably with that of Imazalil sulfate in assessing the carcinogenic potential of these two compounds;

4. whether the triazole ring compounds should be used in the structure-activity assessment of Imazalil and its sulfate salt;

5. whether it is appropriate, as the registrant has suggested, that the cancer risk assessment of Imazalil and its sulfate salt be performed using a threshold/NOEL approach.

Attachment 1

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

JUL 29 1994

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Imazalil Qualitative Risk Assessment Based On Charles River SPF Swiss Albino Mouse Dietary Study

Caswell No. 497AB

TO: Henry W. Spencer, Pharmacologist
Review Section III
Toxicology Branch I
Health Effects Division (7509C)

FROM: Lori L. Brunsman, Statistician
Statistics Section
Science Analysis Branch
Health Effects Division (7509C)

Lori L. Brunsman
7/29/94

THROUGH: Hugh M. Pettigrew, Section Head
Statistics Section
Science Analysis Branch
Health Effects Division (7509C)

Hugh M. Pettigrew
7/29/94

Summary

This qualitative risk assessment of Imazalil was based upon a chronic carcinogenicity feeding study conducted in Charles River SPF Swiss Albino mice. The animals were fed 0, 50, 200, or 600 ppm of Imazalil for 100 weeks. A re-read of the liver slides was conducted to indicate the most appropriate pathology nomenclature. The results of the re-read are presented in this qualitative risk assessment.

The statistical evaluation of mortality indicated no significant incremental changes with increasing doses of Imazalil in male or female mice.

Male mice had significant dose-related increasing trends in hepatocellular adenomas, carcinomas, and combined adenomas and/or carcinomas. There was a significant difference in the pair-wise comparison of the 200 ppm dose group with the controls for hepatocellular adenomas. There were also significant differences in the pair-wise comparisons of the 600 ppm dose group with the controls for hepatocellular adenomas and combined adenomas and/or carcinomas.

Female mice had significant dose-related increasing trends in hepatocellular adenomas and combined adenomas and/or carcinomas. There were no significant differences in the pair-wise comparisons of the dosed groups with the controls.

Background

A chronic carcinogenicity feeding study in Charles River-SPF Swiss Albino mice was conducted by the Department of Toxicology, Janssen Research Foundation, Beerse, Belgium, for Janssen Pharmaceutica N.V., Beerse, Belgium, and dated October 13, 1993 (Experiment No. 2194; MRID No. 429720-01). A re-read of the liver slides was conducted by S. Sparrow, Director of Pathology, Pharmaco LSR, Limited, Suffolk, England, and dated October 4, 1993 (Pharmaco LSR Report No. 93/JED003/0793; MRID No. 429720-01). The re-read was performed to employ a more common pathology nomenclature. The results of the re-read are presented in this qualitative risk assessment.

The study design allocated groups of 50 mice per sex to dose levels of 0, 50, 200, and 600 ppm of Imazalil for 100 weeks.

Survival Analyses

The statistical evaluation of mortality indicated no significant incremental changes with increasing doses of Imazalil in male or female mice. See Tables 1 and 2 for mortality test results.

The statistical evaluation of mortality was based upon the Thomas, Breslow and Gart computer program.

Tumor Analyses

Male mice had significant increasing trends in hepatocellular adenomas ($p < 0.01$), carcinomas ($p < 0.05$), and combined adenomas and/or carcinomas ($p < 0.01$). There was a significant difference in the pair-wise comparison of the 200 ppm dose group with the controls for hepatocellular adenomas ($p < 0.05$). There were also significant differences in the pair-wise comparisons of the 600 ppm dose group with the controls for hepatocellular adenomas ($p < 0.05$) and combined adenomas and/or carcinomas ($p < 0.01$).

Female mice had significant increasing trends in hepatocellular adenomas and combined adenomas and/or carcinomas, both at $p < 0.05$. There were no significant differences in the pair-wise comparisons of the dosed groups with the controls.

These statistical analyses were based upon the Exact trend test and the Fisher's Exact test for pair-wise comparisons. See Tables 3 and 4 for tumor analysis results.

Table 1. Imazalil - Charles River SPF Swiss Albino Mouse Study

Male Mortality Rates[+] and Cox or Generalized K/W Test Results

	Weeks				
Dose(ppm)	1-26	27-52	53-78	79-101[f]	Total
0	0/50	0/50	6/50	9/44	15/50 (30)
50	1/50	1/49	5/48	18/43	25/50 (50)*
200	0/50	0/50	4/50	13/46	17/50 (34)
600	1/50	0/49	5/49	16/44	22/50 (44)

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[f]Final sacrifice at week 100.

( )Percent.

Note: Time intervals were selected for display purposes only.

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 - Charles River SPF Swiss Albino Mouse Study

Female Mortality Rates[+] and Cox or Generalized K/W Test Results

	Weeks				
Dose(ppm)	1-26	27-52	53-78	79-100[f]	Total
0	0/50	3/50	5/47	19/42	27/50 (54)
50	1/50	1/49	7/48	19/41	28/50 (56)
200	1/50	1/49	8/48	16/40	26/50 (52)
600	0/50	1/50	12/49	19/37	32/50 (64)

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[f]Final sacrifice at week 100.

( )Percent.

Note: Time intervals were selected for display purposes only.

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 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)

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

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

[n]Negative change from control.

[a]First adenoma observed at week 55, dose 200 ppm.

[b]First carcinoma observed at week 72, dose 600 ppm.

[c]Three animals in the 200 ppm dose group had both an adenoma and a carcinoma.

[d]Two 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 $p < 0.01$.

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)

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

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

[n]Negative change from control.

[a]First adenoma observed at week 83, dose 50 ppm.

[b]First carcinoma observed at week 88, dose 600 ppm.

[c]One 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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Gart, J.J., D. Krewski, P.N. Lee, R.E. Tarone, and J. Wahrendorf (1986) The Design and Analysis of Long-Term Animal Experiments. In: Statistical Methods in Cancer Research, Volume III. IARC Scientific Publications No. 79. Lyon, France: International Agency for Research on Cancer, p. 18.

Peto, R., M. Pike, N. Day, R. Gray, P. Lee, S. Parish, J. Peto, S. Richard, and J. Wahrendorf (1980) Guidelines for Simple, Sensitive, Significant Tests for Carcinogenic Effects in Long-Term Animal Experiments. In: Monographs on the long-term and short-term screening assays for carcinogens: a critical appraisal. IARC Monographs, Supplement 2. Lyon, France: International Agency for Research on Cancer, pp. 311-426.

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New Mouse

Attachment 2

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

C11044

JUN 8 1994

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Imazalil Reregistration; Reregistration Case No. 2325; Review of Carcinogenicity Study in Mice, Guideline 83-2(b). 6(a)(2) Issue.

DP Barcode D196488
Case 816389
Submission S452505

Tox. Chem. No. 497AB
PC Code No. 111901
MRID No. 429720-01

FROM: Edwin R. Budd, Toxicologist
Review Section III
Toxicology Branch I
Health Effects Division (7509C)

Budd 6/2/94

TO: Kathryn Davis/Linda Deluise/Kathy Depukat
Chemical Review Manager Team 52
Reregistration Branch
Special Review and Reregistration Division (7508W)

THRU: Karen Hamernik, Ph.D., Section Head
Review Section III
Toxicology Branch I
Health Effects Division (7509C)

K Hamernik 6/2/94

K/B 6/2/94

Please find attached the Data Evaluation Report (DER) for a carcinogenicity study in mice using Imazalil base as the test material (MRID No. 429720-01). The study was sponsored by Janssen Pharmaceutica N.V. (Beerse, Belgium) and performed by the Janssen Research Foundation (Beerse, Belgium). The DER was prepared by Clement International Corporation (Fairfax, Virginia) and approved by Edwin Budd M.A. and Marion Copley D.V.M. of Toxicology Branch I.

Statistically significant increased incidences of hepatocytic neoplasms were observed in male and female mice in this study.

Because of the neoplastic response observed, this chemical is being scheduled for review by the HED Cancer Peer Review

Committee. A risk assessment will then be conducted. The results of this evaluation process will be addressed in a separate memorandum. It cannot be determined at this time whether the RfD for Imazalil will be affected.

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

At 200 ppm, males had a significant increase in the incidence of focal cellular changes, large vacuoles, and swollen sinusoidal cells in the liver. At the highest dose tested, males also had a significantly increased incidence of pigmentation in the sinusoidal cells of the liver and focal cellular changes in the pancreas, increased absolute (+18%) and relative (+24%) liver weight, and decreased body weight (-7%) and body weight gain (-18%). The LOEL of 200 ppm (28.0 mg/kg/day) is based on the histopathology changes observed in the livers of males. The NOEL is 50 ppm (6.76 mg/kg/day). No LOEL was achieved in females ($\geq$ 600 ppm or 110 mg/kg/day).

There was evidence of a carcinogenic potential. Hepatocytic neoplasms were increased in males in the 200 and 600 ppm groups (50% in both groups versus 26% in controls) and in females at 600 ppm (22% versus 8% in controls). Of the hepatocytic neoplasms, hepatic neoplastic nodules were increased in males in the 200 and 600 ppm groups (46% at 200 ppm, 34% at 600 ppm versus 16% in controls). Trends for increases in total hepatocytic neoplasms and neoplastic nodules were observed in both males and females. A possible increase in hepatocytic carcinomas was observed in males at 600 ppm (22% versus 10% in controls). The dose levels used in this study (0, 50, 200, and 600 ppm in the diet) were previously agreed to by Toxicology Branch I based upon depressed body weight gain seen in males (-25%) and females (-30%) at 800 ppm in a 90-day range finding study in mice.

This study is Core Minimum for carcinogenicity and satisfies the guideline requirement for a carcinogenicity study in mice, Guideline 83-2(b).

FINAL

DATA EVALUATION REPORT

011044

Imazalil

Study Type: 23 Month Carcinogenicity - Mouse

Prepared for:

Office of Pesticide Programs
Health Effects Division
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Jennifer Alexander for Carrie Rebe, Ph.D. Date 5/16/94

Independent Reviewer William L. McLellan William McLellan, Ph.D. Date 5/16/94

QA Reviewer Carol Maczka, Ph.D. Date 5/16/94

Contract Number: 68D10075
Work Assignment Number: 3-45
Clement Number: 173
Project Officer: Caroline Gordon

EPA Reviewer: Edwin Budd, M.A. Signature: Edwin Budd
Review Section III, Toxicology Branch I (7509C) Date: 5/31/94

EPA Section Head: Marion Copley, D.V.M. Signature: Marion Copley
Review Section IV, Toxicology Branch I (7509C) Date: 5/31/94

DATA EVALUATION REPORT

011044

STUDY TYPE: 23 Month carcinogenicity study - Mouse (83-2)

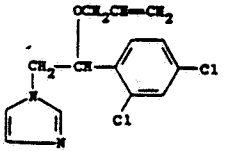
TOX. CHEM. NUMBER: 497AB

P.C. CODE: 111901

MRID NUMBER: 429720-01

TEST MATERIAL: Imazalil base

SYNONYMS: R 23979; enilconazole



STUDY NUMBER: Experiment No. 2194

SPONSOR: Janssen Pharmaceutica N.V.
Beerse, Belgium

TESTING FACILITY: Department of Toxicology,
Janssen Research Foundation
Beerse, Belgium

TITLE OF REPORT: Evaluation of the carcinogenic potential of Imazalil base (R 23979) in SPF Albino Swiss mice

AUTHOR: A. Verstraeten (study director; author not identified)

REPORT ISSUED: Not reported (latest date of signatures was October 13, 1993)

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

At 200 ppm, males had a significant increase in the incidence of focal cellular changes, large vacuoles, and swollen sinusoidal cells in the liver.

At the highest dose tested, males also had a significantly increased incidence of pigmentation in the sinusoidal cells of the liver and focal cellular changes in the pancreas, increased absolute (+18%) and relative (+24%) liver weight, and decreased body weight (-7%) and body weight gain (-18%). The LOEL of 200 ppm (28.0 mg/kg/day) is based on the histopathology changes observed in the livers of males. The NOEL is 50 ppm (6.76 mg/kg/day). No LOEL was achieved in females (≥ 600 ppm or 110 mg/kg/day).

There was evidence of a carcinogenic potential. Hepatocytic neoplasms were increased in males in the 200 and 600 ppm groups (50% in both groups versus 26% in controls) and in females at 600 ppm (22% versus 8% in controls). Of the hepatocytic neoplasms, hepatic neoplastic nodules were increased in males in the 200 and 600 ppm groups (46% at 200 ppm, 34% at 600 ppm versus 16% in controls). Trends for increases in total hepatocytic neoplasms and neoplastic nodules were observed in both males and females. A possible increase in hepatocytic carcinomas was observed in males at 600 ppm (22% versus 10% in controls). The dose levels used in this study (0, 50, 200, and 600 ppm in the diet) were previously agreed to by Toxicology Branch I based upon depressed body weight gain seen in males (-25%) and females (-30%) at 800 ppm in a 90-day range-finding study in mice.

This study is Core Minimum for carcinogenicity and satisfies the guideline requirement for a carcinogenicity study in mice (83-2).

Special Review Criteria (40 CFR 154.7) None at this time, pending evaluation by the HED Carcinogenicity Peer Review Committee.

A. MATERIALS

1. Test Material: Imazalil base

   Description: Not reported

   Lot/Batch #: G3A031 (from Janssen Chemical Manufacturing Plant No. 3)

   Purity: 96.9% (obtained from a separate submission)

   Stability of compound: stability was tested by the sponsor prior to initiation of the study (data not reported)

   CAS #: 35554-44-0

2. Vehicle and/or positive control: Test material was incorporated into the diets as a 50% premix (50% imazalil and 50% of a mixture of equal parts of [redacted] Vehicle controls received diets containing 600 ppm of the vehicle (equal parts of [redacted] The 50% premix was stored at room temperature.

INERT INGREDIENT INFORMATION IS NOT INCLUDED

3. Test animals: Mouse

Strain: SPF Swiss

Age and weight at study initiation: approximately 5-6 weeks; males weighed 24-28 g and females weighed 20-25 g.

Source: Charles River, France

Housing: Individually in macrolon cages

Environmental conditions:
Temperature: Monitored but not reported
Humidity: Monitored but not reported
Air changes: Not reported
Photoperiod: Not reported

Acclimation period: approximately 10 days

B. STUDY DESIGN

1. Animal assignment

Animals were stratified by weight and assigned randomly to the test groups in Table 1.

TABLE 1: STUDY DESIGN

Test Group	Dose in diet (ppm)	male	female
1 (Vehicle Control)	0	50	50
2 (Low)	50	50	50
3 (Mid)	200	50	50
4 (High)	600	50	50

Mice received treated diets from 2/7/90 to 1/10/92 (23 months).

Dose selection was based on the results of a 90-day range-finding study in mice (Janssen Research Foundation, Experiment No. 2020, 12/2/88) in which decreased body weight gain was observed in males (-25%) and females (-30%) at 800 ppm. Hepatocellular vacuolar degeneration, and decreases in albumin, phospholipids, and total bilirubin in males and females were observed at 800 ppm. In addition, females at 800 ppm had decreases in AST.

Note - The dose levels used in this study were previously agreed to by Toxicology Branch I based upon the depressed body weight

gain in males and females observed in this 90-day range-finding study. See memorandum from Judith W. Hauswirth, Ph.D. to Susan Lewis, dated 5/1/89 (Appendix 1).

2. Diet preparation and analysis

Fresh diet was prepared every month by mixing the appropriate amounts of the premix (50% test material and 50% of a mixture of equal parts of [redacted] with Huybrechts powdered rodent feed. The powdered diet was then formed into pellets using a Buhler Maig pellet machine and was stored at room temperature in closed containers. Measurement of the stability and actual concentration of the test material in both the powdered and pelleted diets was conducted using a method described only as #ST-GC90-34. Homogeneity was not tested. The concentration of test material in the diet was verified at approximately 3-month intervals. Data were combined for the dietary levels that were tested at each interval and the percent of nominal was reported as a range.

Results

Homogeneity analysis: Not reported

Stability analysis: At 7 weeks after preparation, the concentration of test material in powdered feed was approximately 91-93% of the concentration of the same batch measured 5 weeks earlier.

Concentration analysis:

The authors stated that the rather low values observed during the first 6 months of the study were because the analytical method was under development during that period. Subsequently, an established analytical methodology (#ST-GC90-34) provided somewhat higher values.

The authors also attributed the lower values in the pelleted feed to incomplete extraction of test material from the pelleted feed. This conclusion was based on previous experience with extraction of other test material from pelleted versus powdered feed. Insufficient data were provided to verify this conclusion or to determine the achieved dietary concentrations at each dietary level.

Due to the several uncertainties regarding actual dietary concentrations achieved in this study, the nominal dose levels of 50, 200, and 600 ppm in this study have been adjusted downward to 83.7% of nominal for each dose level. This value was calculated from the data presented for pelleted diet by averaging together all low and high range values for each time of analysis (at

approximately 3-month intervals) throughout the study. See Table 2.

TABLE 2. CONCENTRATION ANALYSIS DATA

Month	Concentration in percentage of the nominal concentration (range) Powdered Feed	Pelleted Feed
0	84.8–88.5	67.7–68.7
3	95.0–98.3	80.6–93.1
6	91.0–97.1	86.1–91.4
9	93.5–106.0	85.3–104.0
12	90.1–107.0	72.0–93.0
15	94.5–125.0	78.0–87.0
16	97.1–112.0	81.3–92.6
19	87.8–103.0	69.2–91.5
22	96.0–103.0	82.0–83.0
Mean range	92.2–104.4	78.0–89.4
Average	98.3	83.7

[a]Data extracted from study number 2194, page 24

3. Diet - Animals were given food (Huybrechts rodent feed) and received water *ad libitum*.

4. Statistics - Body weight, body weight gain, food consumption, clinical pathology values, and organ weight data were analyzed for differences from control using the Mann-Whitney U test. Mortality, clinical observations, and pathology were analyzed using the Chi-square test. In addition, mortality and neoplastic histopathology data were analyzed using a Peto analysis.

5. A signed and dated quality assurance statement was present. A signed and dated GLP statement was present.

C. METHODS AND RESULTS

1. Observations

Animals were inspected once daily for signs of *toxicity*, *morbidity*, or *mortality*.

Results - No treatment-related effects on mortality were observed. At termination of the study at 23 months, survival at 0, 50, 200, and 600 ppm was 70%, 50%, 66%, and 56%, respectively in males and 46%, 44%, 48%, and 36%, respectively in females. A trend toward early deaths in the groups fed imazalil was not observed.

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No treatment-related clinical signs indicative of toxicity of the test material were observed. However, as seen in Table 3, food wastage was increased in all treated groups (statistically significant only in the low-dose groups). The study authors noted that this is a common finding in mice and suggested that it may have been related to unpalatability of the food. While this finding does not indicate toxicity of the test material, it has repercussions for calculating food consumption and test material intake (see below).

TABLE 3: INCIDENCE OF FOOD WASTAGE[a,b]

Dietary Level	Males	Females
0	21/50 (9±11)	21/50 (10±9)
50	34/50* (18±21)	32/50* (22±23)
200	29/50 (23±28)	31/50 (16±24)
600	31/50 (18±19)	29/50 (25±26)

[a]Data extracted from study number 2194, pages 40-41 and 420-483
[b]Values in parentheses represent the mean (±S.D.) number of times food wastage was observed among the affected animals; values calculated by the reviewers.

2. Body Weight

Animals were weighed on day 0, weekly through week 52, and every 4 weeks, thereafter.

Results - Starting at study week 2, both the body weight and cumulative body weight gain of the high-dose males were consistently significantly lower than controls (Tables 4 and 5). The body weight of high-dose males averaged approximately 7% lower than controls and cumulative body weight gain averaged approximately 18% lower than controls. The body weight of high-dose females averaged approximately 3% lower than controls and cumulative body weight gain averaged 7% lower than controls (not statistically significant).

3. Food Consumption and Compound Intake

Food consumption for each animal was determined weekly until week 52, and every 4 weeks, thereafter. Mean weekly diet consumption was calculated as kg food/week. Compound intake (mg/kg/day) values were calculated as time-weighted averages using the nominal concentrations of test material in the diet and food consumption and body weight data.

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TABLE 4. Mean Body Weight Data for Mice Ingesting Imazalil in the Diet for up to 23 Months[a,b]

	Mean Body Weight Data (g) by Dietary Level (ppm)			
Week	0	50	200	600
		Males		
12	37	37 (100)	36 (97)	35*** (95)
25	41	41 (100)	41 (100)	38** (93)
37	42	43 (102)	43 (102)	40* (95)
50	44	44 (100)	44 (100)	41*** (93)
76	44	45 (102)	45 (102)	41*** (93)
99	42	41 (98)	42 (100)	40* (95)
		Females		
12	30	31 (103)	30 (100)	30 (100)
25	34	34 (100)	33 (97)	33 (97)
37	36	36 (100)	35 (97)	35 (97)
50	38	38 (100)	37 (97)	36 (95)
76	39	40 (103)	39 (100)	38 (97)
99	38	40 (105)	38 (100)	37 (97)

[a] Data extracted from Study No. 2194, pp. 43-44.
[b] Numbers in parentheses indicate percent control.

* Significantly different from control, $p < 0.05$
** Significantly different from control, $p < 0.01$
*** Significantly different from control, $p < 0.001$

TABLE 5. Mean Cumulative Body Weight Gain Data for Mice Ingesting Imazalil in the Diet for up to 23 Months[a,b]

Week	Body Weight Gain (g) Data by Dietary Level (ppm)			
	0	50	200	600
		Males		
12	11	11 (100)	10* (91)	9*** (82)
25	15	15 (100)	15 (100)	12*** (80)
37	16	17 (106)	16 (100)	14** (88)
50	18	18 (100)	18 (100)	15*** (83)
76	18	19 (106)	19 (106)	15*** (83)
99	16	15 (94)	16 (100)	14** (88)
		Females		
12	8	8 (100)	7 (88)	7 (88)
25	11	11 (100)	10 (91)	10 (91)
37	13	14 (108)	13 (100)	12 (92)
50	15	15 (100)	14 (93)	13 (87)
76	17	18 (106)	16 (94)	15 (88)
99	16	18 (113)	15 (94)	15 (94)

[a] Data extracted from Study No. 2194, pp. 46-47.
[b] Numbers in parentheses indicate percent control.

* Significantly different from control, $p < 0.05$
** Significantly different from control, $p < 0.01$
*** Significantly different from control, $p < 0.001$

Results

a. Food consumption - Mean food consumption values among treated males and females were frequently significantly greater than those of controls (Table 6). The magnitude of this effect was greater in males than in females. However, this effect was not dose-related; the wastage was greatest in both males and females at the lowest dose. Because body weight data were comparable between controls and most treated groups, and increased feed wastage was observed as a frequent clinical sign among all treated groups (see Clinical Signs above), the study authors concluded that actual food consumption was not greater than that of controls. Based on this conclusion, the study authors used the mean food consumption of the control groups of each sex as default values for food consumption among all groups of the same sex.

b. Compound consumption (time-weighted average) - Compound consumption values are presented in Table 7 as both the values determined using the measured mean food consumption in each group and as the values determined after assuming that food consumption in all groups was equivalent to that of controls. Presented data is based on nominal concentrations of test material in the diet and also as adjusted to 83.7% of nominal (for reasons previously discussed on page 4 of this DER under "Diet preparation and analysis".)

4. Ophthalmoscopic examination

Ophthalmoscopic examinations were not conducted.

5. Clinical pathology

Blood for hematology was collected via the tail vein from all animals at weeks 51-52. In addition, blood for hematology and clinical chemistry was collected via the carotid artery from all surviving animals at weeks 100-101. Whenever possible, blood was collected from all animals sacrificed in a moribund condition for hematological analysis. It was not stated whether blood samples were collected from fasted animals. The CHECKED (X) parameters were examined. A differential leukocyte count was performed only when the number of white cells exceeded 25,000/mm^3.

TABLE 6. Mean Weekly Food Consumption Data for Mice Ingesting Imazalil in the Diet for up to 23 Months[a,b]

Week(s)	Mean Food Consumption Data (g/mouse) by Dietary Level (ppm)			
	0	50	200	600
		Males		
12	44	51** (116)	50* (114)	49 (111)
25	45	55*** (122)	59** (131)	50 (111)
37	46	59*** (128)	50 (109)	51 (111)
50	46	55** (115)	51 (106)	52* (108)
73-76	216	268** (124)	246 (114)	275** (127)
97-99	166	183 (110)	158 (95)	183 (110)
Total	4726	5712** (121)	5254 (111)	5560* (118)
		Females		
12	51	54 (106)	53 (104)	52 (102)
25	45	50 (111)	50 (111)	50 (111)
37	53	51 (96)	58 (109)	55 (104)
50	47	46 (98)	48 (102)	52 (111)
73-76	254	282 (111)	278 (109)	266 (105)
97-99	167	189 (113)	176 (105)	162 (97)
Total	4955	5565 (112)	5515 (111)	5329 (108)

[a] Data extracted from Study No. 2194, pp. 51-52.
[b] Numbers in parentheses indicate percent control.

* Significantly different from control, $p < 0.05$
** Significantly different from control, $p < 0.01$
*** Significantly different from control, $p < 0.001$

TABLE 7. TIME-WEIGHTED AVERAGE COMPOUND CONSUMPTION

Dietary Level (ppm)	Compound Consumption[a,b] (mg/kg/day) Males	Females
	Without correcting for feed spillage	
50	9.78 ± 1.05	11.07 ± 1.24
200	37.12 ± 4.04	46.25 ± 5.03
600	123.85 ± 13.78	140.87 ± 16.86
	After correcting for feed spillage[b]	
50	8.08	9.91
200	33.4	41.6
600	105	131
	After correcting for feed spillage and adjusted concentration of test material[c]	
50	6.76	8.29
200	28.0	34.8
600	88	110

[a]Data from Study number 2194, pages 54-57
[b]Values represent mean intake values corrected for differences in total food consumption.
[c]Values represent mean intake values corrected for differences in total food consumption and for differences in compound concentration in feed versus nominal values.

a. Hematology

X Hematocrit (HCT)*
X Hemoglobin (HGB)*
X Leukocyte count (WBC)*
X Erythrocyte count (RBC)*
X Platelet count*
Blood clotting measurements
(Thromboplastin time)
(Clotting time)
(Prothrombin time)
X Leukocyte differential count*
X Mean corpuscular HGB (MCH)
X Mean corpusc. HGB conc. (MCHC)
X Mean corpuscular volume (MCV)
Reticulocyte count

* Required for subchronic and chronic studies

Results - No treatment-related effects were observed. Statistically significant changes were observed in a number of parameters in mid- and/or high-dose mice after 1 year, but the changes either were within historical control ranges, or were not observed at the end of the study and had no supporting histopathology.

b. Clinical Chemistry

Electrolytes
X Calcium*
X Chloride*
Magnesium*
X Phosphorus*
X Potassium*
X Sodium*

Enzymes
X Alkaline phosphatase*
X Cholinesterase
X Creatine phosphokinase*
Lactic acid dehydrogenase
X Serum alanine aminotransferase (also SGPT)*
X Serum aspartate aminotransferase (also SGOT)*
Gamma glutamyl transferase
Glutamate dehydrogenase

Other
X Albumin*
X Blood creatinine*
X Blood urea nitrogen*
X Cholesterol*
X Globulins (Haptoglobin)
X Glucose*
X Total bilirubin*
X Total serum protein*
X Triglycerides
Serum protein electrophores

* Required for subchronic and chronic studies

Results - No treatment-related effects were observed.

6. Urinalysis

Urinalysis was not conducted.

7. Sacrifice and Pathology

All animals that died on study, were sacrificed *in extremis*, or were sacrificed on schedule were subject to gross and histopathological examination. The CHECKED (X) tissues were collected for histological examination. All of the checked tissues were examined in the control and high-dose animals. In addition, the adrenals, epididymides, gall bladder, kidneys, liver, lung, mesenteric lymph nodes, mammary gland, pancreas, pituitary, salivary gland, spleen, testis, thymus, ovary, uterus, and vagina were examined histopathologically in all low- and mid-dose animals. The CHECKED (XX) organs were also weighed.

Digestive System	Cardiovasc./Hemat.	Neurologic
Tongue*	X Aorta*	XX Brain*
X Salivary glands*	XX Heart*	X Periph. nerve*
X Esophagus*	X Bone marrow*	X Spinal cord (3 levels)
X Stomach*	X Lymph nodes*	X Pituitary*
X Duodenum*	XX Spleen	X Eyes (optic n.)*
X Jejunum*	XX Thymus*	Glandular
X Ileum*	Urogenital	XX Adrenal gland*
X Cecum*	XX Kidneys*+	Lacrimal gland
X Colon*	X Urinary bladder*	X Mammary gland*
X Rectum*	XX Testes*+	X Parathyroids*++
XX Liver*+	X Epididymides	X Thyroids*++
X Gall bladder*	X Prostate	Other
XX Pancreas*	X Seminal vesicle	X Bone*
Respiratory	XX Ovaries*+	X Skeletal muscle*
X Trachea*	X Uterus*	X Skin*
XX Lung*	X Vagina	X All gross lesions and masses*
Nose		
Pharynx		
Larynx		

* Required for subchronic and chronic studies
\+ Organ weight required in subchronic and chronic studies
++ Organ weight required in nonrodent studies

Results -

a. Organ weight - Both absolute and relative liver weights were significantly increased in males at 600 ppm (Table 8). Slight increases in absolute and relative liver weights were observed in females at 600 ppm (not statistically significant).

b. Gross pathology - No statistically significant differences from controls were noted. However, the study authors noted that slight increases were observed in liver foci or nodules and masses in male mice at 200 and 600 ppm (Table 9). These

TABLE 8. Mean Liver Weight Data for Mice Ingesting Imazalil in the Diet for up to 23 Months[a,b]

	Mean Liver Weight Data by Dietary Level (ppm)			
	0	50	200	600
		Males		
absolute weight (mg)	2388 ± 878	2082 ± 611 (87)	2402 ± 712 (101)	2812 ± 1044* (118)
relative weight (mg/100 g)	5685 ± 1826	5112 ± 1521 (90)	5798 ± 1767 (102)	7060 ± 2521** (124)
		Females		
absolute weight (mg)	2143 ± 627	2264 ± 392 (106)	2088 ± 458 (97)	2359 ± 1541 (110)
relative weight (mg/100 g)	5509 ± 1246	5581 ± 664 (101)	5580 ± 1162 (101)	6269 ± 3745 (114)

[a] Data extracted from Study No. 2194, p. 72 and pp. 588-603.
[b] Numbers in parentheses indicate percent control.

* Significantly different from control, $p < 0.05$
** Significantly different from control, $p < 0.01$

TABLE 9. Incidence of Selected Macroscopic Liver Pathology Findings in Mice Ingesting Imazalil in the Diet for up to 23 Months[a]

	Incidence of Liver Pathology by Dietary Level (ppm)			
	0	50	200	600
		Males		
Liver focus	3/50	1/50	7/50	7/50
Liver mass	11/50	9/50	21/50	20/50
Liver nodule	1/50	4/50	7/50	3/50
		Females		
Liver focus	5/50	4/50	1/50	5/50
Liver mass	2/50	6/50	2/50	5/50
Liver nodule	0/50	4/50	0/50	3/50

[a] Data extracted from Study No. 2194, pp. 77-86.

findings corresponded to treatment-related histopathology findings.

c. Microscopic pathology -

1) Non-neoplastic - Histopathologic analyses showed dose-related increases in the incidence and/or severity of focal cellular changes, large vacuoles, and pigmentation or swelling in the sinusoidal cells of the liver in males at 200 and/or 600 ppm (Table 10). In addition, males at 600 ppm had a significantly increased incidence of focal cellular changes in the pancreas. Females did not show any increases in liver histopathology compared to controls. However, vaginal metaplasia was increased in the 600-ppm group.

Reanalysis of the liver sections by an independent pathologist failed to find any statistically significant nonneoplastic changes in livers of treated mice.

2) Neoplastic - Dose-related increases in the incidence of hepatic neoplasms were observed in both male and female mice (Table 11a). The incidence of hepatic neoplasms was significantly increased in mid- and high-dose males and in high-dose females. The incidence of hepatic neoplastic nodules was also significantly increased in mid- and high-dose males. The incidence of hepatic neoplastic nodules was not significantly increased in any group of females, but a statistically significant positive trend was observed. High-dose males also had a slight increase in the incidence of hepatic carcinomas (not statistically significant).

Reanalysis of liver sections by an independent pathologist found similar results, but because of differences in nomenclature and diagnostic criteria, somewhat lower numbers of benign tumors were reported overall (Table 11b). The differences in the number of benign tumors were attributed to the inclusion by the performing laboratory of some lesions designated by the independent pathologist as "hepatocellular hyperplasia" or "foci of alteration (basophilic or eosinophilic)." Table 12 shows the incidence of hepatocellular hyperplasia and foci of alteration (basophilic or eosinophilic) that were observed by the independent pathologist. The number of these findings that were classified as neoplastic nodules by the performing laboratory are also noted. No increased incidence of tumors other than liver tumors were observed in the imazalil-treated animals in this study.

TABLE 10. Incidence of Selected Nonneoplastic Histopathology Findings in Mice Ingesting Imazalil in the Diet for up to 23 Months[a,b]

	Incidence of Histopathology by Dietary Level (ppm)			
	0	50	200	600
		Males		
Liver				
-Focal cellular changes	2/50 (1)	4/49 (1.3)	10/50* (1.2)	8/50 (1.5)
-Large vacuoles	1/50 (1)	0/49	8/50* (1)	9/50* (1)
-Pigmentation in sinusoidal cells	10/50 (1)	3/49 (1)	11/50 (1.2)	20/50* (1.2)
-Swollen sinusoidal cells	24/50 (1)	27/49 (1.1)	37/50* (1)	26/50 (1)
-Parenchymal cellular swelling	2/50 (1)	0/49	2/50 (1)	0/50
-Large vacuoles/vacuolization	5/50 (1)	3/49 (1)	8/50 (1)	12/50 (1)
Pancreas				
-Focal cellular changes	0/50	2/49 (1)	2/50 (1)	6/49* (1)
		Females		
Liver				
-Focal cellular changes	2/50 (1)	1/50 (1)	2/50 (1)	2/50 (1)
-Large vacuoles	0/50	1/50 (1)	1/50 (1)	5/50 (1.2)
-Pigmentation in sinusoidal cells	12/50 (1.2)	10/50 (1.1)	11/50 (1.3)	12/50 (1.3)
-Swollen sinusoidal cells	39/50 (1.1)	28/50* (1.1)	32/50 (1.1)	28/50* (1.1)
-Parenchymal cellular swelling	0/50	0/50	0/50	4/50 (1)
-Large vacuoles/vacuolization	1/50 (1)	1/50 (1)	3/50 (1)	9/50 (1)
Pancreas				
-Focal cellular changes	2/50 (1)	4/49 (1)	1/49 (1)	5/50 (1)
Vagina				
-Metaplasia	9/44 (1.4)	11/48 (1.5)	9/46 (1.7)	22/48* (1.6)

[a] Data extracted from Study No. 2194, pp. 95-117, and pp. 604-1004.
[b] Numbers in parentheses indicate average severity.

* Significantly different from control, $p < 0.05$ using Fisher's exact test performed by the reviewers

TABLE 11a. Tumor Incidence Data for Mice Ingesting Imazalil in the Diet for up to 23 Months[a]

	Tumor Incidence Data by Dietary Level (ppm)				
	0	50	200	600	p-value[b] (for trend)
		Males			
Hepatocytic neoplasms	13/50	10/49	25/50*	25/50*	0.0002
–Hepatic neoplastic nodule	8/50	5/49[c]	23/50**[d]	17/50*[e]	0.0006
–Hepatic neoplastic nodule only[f]	8/50	3/49	19/50	14/50	—
–Hepatic carcinoma	5/50	7/49	6/50	11/50	0.0685
		Females			
Hepatocytic neoplasms	4/50	6/50	2/50	11/50*	0.0230
–Hepatic neoplastic nodule	4/50	6/50[g]	0/50	10/50[g]	0.0475
–Hepatic neoplastic nodule only[f]	4/50	5/50	0/50	9/50	—
–Hepatic carcinoma	0/50	1/50	2/50	2/50	0.1000

[a] Data extracted from Study No. 2194, pp. 122-125.
[b] The p-value obtained by Peto-analysis for a dose-related trend in tumor incidence.
[c] Two animals also had an hepatic carcinoma.
[d] Four animals also had an hepatic carcinoma.
[e] Three animals also had an hepatic carcinoma.
[f] Data for hepatic neoplastic nodule only was not statistically analyzed.
[g] One animal also had an hepatic carcinoma.

* Significantly different from control, $p < 0.05$ (pairwise analysis using one-tailed Chi-square test)
** Significantly different from control, $p < 0.01$ (pairwise analysis using one-tailed Chi-square test)

TABLE 11b. Tumor Incidence Data from the Independent Pathologist for Mice Ingesting Imazalil in the Diet for up to 23 Months[a]

	Tumor Incidence Data by Dietary Level (ppm)			
	0	50	200	600
		Males		
Hepatocytic neoplasms	10/50	8/49	17/50	22/50*
–Hepatic adenoma[b]	5/50	2/49	14/50*[c]	13/50[d]
–Hepatic adenoma only[e]	5/50	2/49	11/50	11/50
–Hepatic carcinoma	5/50	6/49	6/50	11/50
		Females		
Hepatocytic neoplasms	3/50	5/50	2/50	9/50
–Hepatic adenoma[b]	3/50	3/50	0/50	7/50[f]
–Hepatic adenoma only[e]	3/50	3/50	0/50	6/50
–Hepatic carcinoma	0/50	2/50	2/50	3/50

[a] Data extracted from Study No. 2194, pp. 277-290.
[b] See text for an explanation.
[c] Three animals also had an hepatic carcinoma.
[d] Two animals also had an hepatic carcinoma.
[e] Data for hepatic adenomas only was not statistically analyzed.
[f] One animal also had an hepatic carcinoma.

* Significantly different from control, $p < 0.05$ (pairwise analysis using one-tailed Chi-square test)

TABLE 12. Incidence of Selected Histopathology by the Independent Pathologist in Mice Ingesting Imazalil in the Diet for up to 23 Months[a]

	Incidence Data by Dietary Level (ppm)			
	0	50	200	600
		Males		
Hepatocellular hyperplasia				
-total	4/50	2/49	12/50	8/50
-reported as neoplastic nodules by the study authors	3/50	1/49	7/50	3/50
Focus of alteration (basophilic or eosinophilic)				
-total	1/50	3/49	2/50	6/50
-reported as neoplastic nodules by the study authors	0/50	1/49	2/50	1/50
		Females		
Hepatocellular hyperplasia				
-total	1/50	1/50	0/50	3/50
-reported as neoplastic nodules by the study authors	1/50	0/50	0/50	2/50
Focus of alteration (basophilic or eosinophilic)	0/50	0/50	0/50	0/50

[a] Data extracted from Study No. 2194, pp. 277-290.

E. DISCUSSION

Review of the final report and supporting data indicates that the conduct of the study was adequate and the reporting of the results was accurate. However, no evidence of systemic toxicity was observed in females at the highest dose tested. Other study deficiencies are listed below. The liver was the primary target organ for imazalil toxicity in mice. This finding is consistent with the results of the range-finding study (Experiment No. 2020). In the current study, modest histopathological changes (an increased incidence of hepatic focal cellular changes, large vacuoles, and swollen sinusoidal cells) were observed in livers of mid-dose males. At the highest dose tested, males also had increased absolute and relative liver weights, an increased incidence of pigmentation of sinusoidal cells, and decreased body weight and body weight gain. In addition, high-dose males had an increased incidence of focal cellular changes in the pancreas.

Based on the histopathological changes observed in livers of mid-dose males, the LOEL for systemic toxicity in males is 200 ppm (28.0 mg/kg/day). The NOEL for systemic toxicity in males is 50 ppm (6.76 mg/kg/day). The NOEL for systemic toxicity in females is 600 ppm (110 mg/kg/day); no LOEL was established in females.

Statistically significant increases in incidences of hepatic neoplasms were observed in both males (mid- and high-dose) and females (high-dose). Hepatic neoplastic nodules exhibited a significant positive dose-related trends in both males and females, but only males (mid- and high-dose) had statistically significant increases in incidence. High-dose males also had a slight (but not statistically significant) increase in the incidence of hepatic carcinoma. Reanalysis of the hepatic lesions by an independent pathologist found similar results. Historical data for liver tumors in the testing laboratory are presented in Table 13.

F. STUDY DEFICIENCIES are as follows:

- No information was provided regarding the homogeneity of the diet.

- No information was provided regarding the method of analysis for the concentration of test material in the diet and insufficient concentration data were presented to determine the achieved dietary concentrations at each dietary level.

- Feed spillage precluded determining actual food consumption and test article intake. The study author used the food consumption of the control groups as default values based on the conclusion that since body weights of the treated mice (excepting high-dose males) were comparable to controls, the food consumption of treated mice was not greater than that of controls.

TABLE 13. Historical Control Data for Liver Neoplasm Incidence in Mice at the Testing Facility[a,b]

	Incidence for Each Experiment Number				
Exp. No.	1649	1881	1987	2030	1927
Start Date	10/24/86	3/1/88	3/16/88	6/2/88	8/28/88
Duration (months)	25	19	19	18	18
		Males			
Hepatocellular neoplasia	14/50 (28)	11/50 (22)	11/49 (22)	7/50 (14)	3/50 (6)
–Hepatic neoplastic nodule	10/50 (20)	7/50 (14)	9/49 (18)	3/50 (6)	3/50 (6)
–Hepatocytic carcinoma	6/50 (12)	6/50 (12)	3/49 (6)	6/50 (12)	0/50 (0)
		Females			
Hepatocellular neoplasia	0/50 (0)	1/50 (2)	1/49 (2)	0/50 (0)	1/50 (2)
–Hepatic neoplastic nodule	0/50 (0)	1/50 (2)	1/49 (2)	0/50 (0)	0/50 (0)
–Hepatocytic carcinoma	0/50 (0)	0/50 (0)	0/49 (0)	0/50 (0)	1/50 (2)

[a] Data extracted from Study No. 2194, pp. 294 and 296.
[b] Numbers in parentheses indicate percent incidence.

- Complete histopathological examinations were not conducted on animals from the low- and mid-doses that died on study. This may be a minor deviation because a large number of organs (including those that exhibited treatment-related pathological changes in high-dose animals) were examined in all animals at all dose levels.

- Females exhibited no significant systemic toxicity at any of the doses tested.

This study is classified as **Core Minimum**. The study deficiencies described above were not considered serious enough to warrant a lower classification or to require that the study be repeated.

CASWELL FILE

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

Appendix 1

5/1/89

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Imazalil - Dose Selection for Mouse Oncogenicity Study. Submitted by Janssen Pharmaceutica by Fax on April 7, 1989.

Tox. Chem. No.: 497AB

TO: Susan Lewis
Product Manager #21
Registration Division (H7505C)

FROM: Judith W. Hauswirth, Ph.D., Chief
Toxicology Branch I - IRS
Health Effects Division (H7509C)

Judith W. Hauswirth 4/28/89

THRU: William L. Burnam, Acting Director
Health Effects Division (H7509C)

WLB 5/1/89

The registrant has sent a desk copy of a recently completed 90 day mouse study on imazalil to aid in the dosage selection for an oncogenicity study. Subsequent to this submission, Dr. H. J. van Cauteren of Janssen Pharmaceutica in Belgium called to discuss the study and to set appropriate dosage levels for the oncogenicity study. This telephone conversation took place during the week of March 20, 1989. He then faxed his version of this conversation to this reviewer along with his proposed high dosage level for the mouse study (A copy of the faxed material is attached for information).

Dr. van Cauteren proposed a high dose of 600 ppm for the oncogenicity study. Toxicology Branch I agrees to this dosage level based upon depressed body weight gain seen in males and females at 800 ppm in the 90 day range finding study. The percentage body weight decrement at this dosage level was by Toxicology Branch's calculations 30% in females and 25% in males. The differences in body weight gains were statistically significant at several time points during the 90 day study for females but not for males. Toxicology Branch notes that although the food consumption table in the report indicates that the dosed groups ate more than the control group, the report states that food wastage was a problem in this study. We also note that the mice were housed 2-3 per cage which could have contributed to wastage. The report further states that there could have been a palatability problem with the treated diet. Based upon the values given in the table for food consumption, it is difficult to determine whether this was a problem. We urge that csre be taken in the long term study to determine whether there is a palatability problem at 600 ppm.

Other effects seen at 800 ppm in the range finding study were hepatocellular

vacuolar degeneration, a decrease in albumin, phospholipids, and total bilirubin in males and females and a decrease in AST in females only.

JANSSEN

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DATE : April 7, 1989

FAX 00/1/703 557 ~~2233~~ 3106

ATTENTION : Dr. J.W. HAUSWIRTH - Arlington VA 22202 -U.S.A.

FROM : Dr. H. VAN CAUTEREN - Janssen Pharmaceutica - BELGIUM

IN CASE YOU DO NOT RECEIVE ANY OF THESE PAGES PROPERLY, PLEASE CALL : (14) 60 24 80.

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TELEFAX - No.703-5570233
TELEPHONE NUMBER: 703-5577397

TO: Dr. Judith W. HAUSWIRTH - Arlington VA 22202 - U.S.A.

FROM: Dr. H. Van Cauteren - Janssen Pharmaceutica - Belgium

DATE: April 7, 1989

SUBJ.: Imazalil
Dose levels for mouse carcinogenicity study

Dear Dr. Hauswirth,

Referring to correspondence dated March 8, 1989, from Bill Goodwine to you, a desk copy of a subchronic feeding study in mice (Exp.2020, December 2, 1988) was submitted for review and comment.

Subsequently, we spoke by phone and agreed that the finding, with regard to MTD, supported a high dose between 400 and 800 ppm.

Please find below, my dose level suggestions and justification for the 24-month mouse carcinogenicity study.

Protocol
In general, the protocol of this mouse carcinogenicity study will be fully compliant with the EPA guidelines (1984). More specifically, we will meet the criteria of the test procedures with regard to age (5 weeks at start), sex and number of mice (50 males and females/group), clinical observations (daily, weekly), measurements of body weight and of food consumption (weekly, monthly), clinical pathology (at 12 and 18 months and terminally), gross necropsy (including organ weights in terminal animals), and histopathology.

Route of administration and dose level selection
Imazalil will be admixed into the diet at levels of 50, 200 and 600 ppm. These levels have been selected based upon the following:
- Fifty ppm is an appropriate low dose since it is estimated to be a no-toxic effect level (NOEL). This level is in the same order of magnitude as the medium dose of the previously conducted mice carcinogenicity study (25 ppm in the drinking water is approximately equivalent to 50 ppm into the feed assuming mice drink about the double of the dry feed they consume).
- As an intermediate dose, 200 ppm will be used. It is estimated to be at the borderline of toxicity based upon a 3-month dose range finding study (Exp.No.2020) whereby dosing at 200 ppm resulted in slightly decreased aspartate aminotransferase, cholesterol and phospholipid values in the serum of females and in a vacuolar degeneration in the liver of males. This dose level also falls in the same order of magnitude of the high dose of the previously conducted mice carcinogenicity study (100 ppm in the drinking water is approximately

16
/ 5

- The high dose will be 600 ppm. In a 3-month dose range finding study (Exp.2020), dosing at 400 ppm resulted in toxicity which was characterized by some altered serum parameters (decrease of albumin, total bilirubin and phospholipids in males and decrease of cholesterol, phospholipids, total bilirubin and AST in females) and by histological modifications on the liver (vacuolar degeneration and centrilobular swelling). These effects were also, but more pronounced, present at 800 ppm. In addition, the liver weight was increased and macroscopically showed a swollen and dark aspect at this dose. In females, dosing at 800 ppm also resulted in a lower body weight gain (about 12%). This study indicated that the dose level of 400 and 800 ppm into the diet are toxic with cholesterol and lipid metabolism and the liver as potential targets.
Since it could not be fully excluded that survival might not be adversely affected at 800 ppm, it was decided to select 600 ppm as the intermediate between 400 and 800 ppm.

Prior to initiating the study, we would like to receive verification, from you, that the subchronic feeding study (Exp.2020) has been reviewed and supports the proposed dose levels. Bill suggested that you might handle this by way of an Internal Memorandum to the Registration Division, with a copy faxed to Janssen, U.S.A. at (201)524-9895, so that we may proceed with our plans to initiate the study later this month. Alternatively, we would be most grateful to receive a letter directly from you on this matter by way of facsimile. We will leave it up to you to follow the best route.

I will ask Bill Goodwine to follow-up this letter within the next week.

Sincerely,

Herman Van Cauteren

New Rat

Attachment #3

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

011161

AUG 12 1994

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: **Submission of rat Carcinogenicity study (combination review of both MRID 470261-01 the 30 month study and MRID 00162412 the 18 month toxicity study) in the Wistar rat, and the One year, Chronic toxicity Study in dogs MRID 413288-02**

Tox Chem No.: 497AB
HED Project No.: 0-1704
Record No.: 267943
PC. Code: 111901
DP 206361
S 452505

TO: James Stone
PM. # 21
Fungicide Branch
Registration Division (H7505C)

FROM: Henry Spencer, Ph.D. 8/9/94
Pharmacologist
Review Section 3
Toxicology Branch 1
Health Effects Division (H7509C)

THRU: Karen Hamernik, Ph.D. 8/9/94 KB 8/10/94
Section Head
Review Section 3
Toxicology Branch 1
Health Effects Division (H7509C)

CONCLUSION:

The rat carcinogenicity study did not have sufficient toxicity in the high dose for evaluating carcinogenicity of the chemical, Imazalil.

However, minor effects of relatively non-toxicologic significance were reported in the review at the HDT (20 mg/kg/day), establishing a minimal effect (LEL) at 20 mg/kg/day for the chronic carcinogenicity study.

The supplemental (MRID 41558501) histopathological information on the historical incidence of leydig cell testicular tumors, and adenocarcinoma or epidermoid carcinomas of the uterus adequately answered the concerns of the significance of the tumor incidences seen in the 30 month rat

study. The **testicular tumors** are **not** considered to be associated with administration of the test chemical based on the lack of both statistically significant increased incidence and the lack of a dose related increase in tumors. However, uterine **epidermoid carcinomas** occurred twice in this same study - though only 1/50 for each dose group occurrence. The question remains whether the occurrence of these two tumors far exceeds the historical incidence of 1/50 for only one test dose in 15 studies. Results of the historical data submitted are attached at the end of the rat study DER.

Data will be submitted to the HED Carcinogenicity Peer Review committee with regard to whether an MTD or adequate dosing regimen was actually attained and also whether in fact a concern exists over the incidences of the above tumor types reported in the study. Conclusions reported above may be altered by the committee report.

The 12 month Chronic feeding study in the dog (MRID 413288-02) showed increased vomiting and food wastage, mean body weight gains and mean body weights were depressed at the HDT of 20 mg/kg/day.

The LEL is 20 mg/kg/day and the NOEL is 5 mg/kg/day. (DER attached).

Toxicology Branch concludes that the chronic rat study data does allow for the establishment of only a **minimal LOEL at 400 ppm with a NOEL established at 4.7 mg/kg/day in the females based on liver effects.**

The dog study is adequate to fulfill the G1 83-1b.

Executive summaries are attached to the end of the DERs.

[redacted]

EPA No.: 68D80056
DYNAMAC No.: 276-A,C
TASK No.: 2-76A,C
May 3, 1990

DATA EVALUATION RECORD

IMAZALIL

Oncogenicity Feeding Study in Rats

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: _____

Date: _____

This DER is considered FINAL even though there are NO contractor signatures.

HW Spencer 6/21/94
MC 5/3/94

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REVIEWED BY:

William L. McLellan, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: _____
Date: _____

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: _____
Date: _____

APPROVED BY:

Roman J. Pienta, Ph.D.
Department Manager
Dynamac Corporation

Signature: _____
Date: _____

Henry Spencer, Ph.D.
EPA Reviewer, Section II
Toxicology Branch I
(H-7509C)

Signature: Henry M Spencer
Date: 6/21/94

Marion P. Copley, D.V.M., D.A.B.T.
EPA Section Head,
Section II
Toxicology Branch I
(H-7509C)

Signature: Marion Copley
Date: 8/3/94

DATA EVALUATION RECORD

GUIDELINE §83-2, 83-1

STUDY TYPE: Chronic oncogenicity feeding study in rats.

MRID NUMBERS: 470261-012 (30-month study); Nist. Cont. 41558501
00162412 (18-month study).

Tox. Chem# 497 AB P.C. Code 111901

TEST MATERIAL: Imazalil base-R 23979, technical.

SYNONYM: 1-[2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl]-1H-imidazole.

STUDY NUMBER: B82-0555.
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SPONSOR: Janssen Pharmaceutica, Beerse, Belgium.

TESTING FACILITY: Netherlands Organization for Applied Scientific Research; Division for Nutrition and Food Research, 3700 AJ Zeist, Netherlands.

TITLES OF REPORTS:
1. Lifespan Oral Carcinogenicity Study with Imazalil Base-R 23979 in Rats.
2. 18-Month Oral Chronic Toxicity Study with Imazalil Base-R 23979 in Rats.

AUTHORS: Til, H. P.; Lina, B.A.R.; van Nesselrooij; J.H.J.; Beems, R. B.; Falke, H. E.

REPORTS ISSUED: May 1984 (18-month report);
November 1985 (30-month report).

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CONCLUSIONS: Executive summary is at end of DER

Under the conditions of the present studies Imazalil was not carcinogenic when fed to Cpb:WU Wistar rats for 18 or 30 months at dietary levels of 25, 100, or 400 ppm (approximately 1.0, 3.7, or 15.5 mg/kg/day in males and 1.2, 4.9, or 20.0 mg/kg/day in females). Leydig cell tumors of the testes were increased at 30 months in all dosed groups of males (6 to 8%) when compared to controls (2%); although this is not considered to be of toxicologic importance, it cannot be fully evaluated until the sponsor provides laboratory historical data for incidence in rats of comparable age.

Minimal decrements in body weight gains in females receiving 400 ppm were observed after 52 weeks, and slight nonsignificant increases in liver weights were seen in males receiving 400 ppm (18 and 30 months). Focal hepatocellular vacuolation was increased in high-dose males that survived to 30 months (6/17 versus 2/17 controls), and at 18 months there was an increased incidence of intracytoplasmic inclusion bodies in hepatocytes (5/20 versus 0/20 for controls) and an increased severity of hepatocyte vacuolization in high-dose males. No other effects of toxicologic importance related to dosing were observed in the 400-ppm groups, and no toxic effects were observed at 25 or 100 ppm. The rats could probably have tolerated a higher dose.

Core Classification: The 30-month study is CORE supplementary for an oncogenicity study (Guideline 83-2). The classification may be upgraded to minimum after review of laboratory historical incidence of Leydig cell tumors of the testis and clarification of the rationale for dose selection. The 18-month study is CORE Supplementary for a chronic toxicity study (Guideline 83-1). This classification may be upgraded based on results of 6- and 12-month studies referred to by the authors, which were not reviewed in this DER. Submitted - see attached I

Subsequent Review of these studies does not support the high dose of the 18 or 30 month studies. MC 9/8/94

A. MATERIALS:

1. Test Compound: Imazalil base-R 23979; description: slightly yellow to brown crystalline mass (solidified oil); batch No.: D41/03; purity: 98.1%, [redacted] From week 43, the technical material was attached to a carrier (equal volumes of [redacted] so that a 50% powder was obtained (purity 48%). Feeding levels were based on technical Imazalil.

2. Test Animals: Species: rat; strain: Cpb:WU (Wistar random); age: 3.5 weeks at receipt and 4.5 weeks at initiation; weight: 35 to 50 g at receipt; mean group weights at initiation--lifetime study: 65.7 to 65.9 g (males) and 66.5 to 67.0 g (females), 18-month study: 70.3

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to 70.6 g (males) and 64.5 to 64.7 g (females); source: Central Institute for the Breeding of Laboratory Animals, TNO, Zeist, Netherlands.

B. STUDY DESIGN:

1. Animal Assignment: Animals were acclimated for 7 days and received a health examination. Animals were assigned to the following test groups by computer randomization:

Test Group	Dose in Diet (ppm)	Lifetime Study (30 months) Males	Lifetime Study (30 months) Females	18-Month Study Males	18-Month Study Females
1 Control	0	50	50	20	20
2 Low (LDT)	25	50	50	20	20
3 Mid (MDT)	100	50	50	20	20
4 High (HDT)	400	50	50	20	20

Doses were based on a previous carcinogenicity study (667-79-04.20). A previous 6-month study (CIVIO V83.186, 1983) used the same dose levels as the present study.

2. Diet Preparation: High-dose diets were prepared by mixing an appropriate amount of test compound with powdered diet for 2 minutes in a Stephan mechanical blender. Mid- and low-dose diets were prepared by diluting the high-dose diets with additional feed, and mixing. After week 43, a 50/50 mixture of test compound and a carrier [redacted] was used to prepare diets in an attempt to improve homogeneity; the amount of test compound was corrected for the carrier. Carrier was added to control diets. Fresh diets were prepared every 2 to 3 weeks. Samples were collected monthly and stored for analysis. Homogeneity of samples prepared with Imazalil was assayed at 0, 2, and 6 months, and homogeneity of diets prepared with carrier was assayed at 10 months. Stability of test compound in diets was determined at 2 and 6 months by analysis before and after storage of the diets at room temperature for 4 weeks. Test compound in the diets was analyzed at 10 intervals over 18 months and 14 times over 30 months.

Results: Test compound was completely stable in the diets stored for 1 month at room temperature. The homogeneity was not acceptable for low- and mid-dose diets analyzed at

EXACT INGREDIENT INFORMATION IS NOT INCLUDED

0 and 2 months; the coefficients of variation (five samples) were 27.5% and 16.5% at 25 ppm and 22.7 and 5.4% at 100 ppm, but were 3.5 and 3.1% for the high-dose diets. For diets prepared with Imazalil/carrier, the coefficients of variation for all diets ranged between 1.2 and 2.2%. Table 1 summarizes data for analyses of Imazalil in diets. Analyzed values were generally about 90% of target levels.

TABLE 1. Data on Dietary Analysis of Imazalil

	Analyzed Levels as Percent of Nominal ± C.V.		
	11 months (N = 6)	18 months (N = 10)	30 months (N = 14)
25	95.2 ± 10.4	94.0 ± 10.0	94.4 ± 8.8
100	87.6 ± 6.0	90.0 ± 8.2	90.9 ± 7.9
400	85.1 ± 10.7	89.8 ± 11.7	92.2 ± 11.5

3. Food and Water Consumption: Animals received the laboratory's cereal-based stock diet (mixed by Van Eck, Cothen, Netherlands) ad libitum. All rats had free access to nonfluoridated tapwater by means of an automatic watering system. However, this system malfunctioned during weeks 1 and 15; therefore, water was supplied in glass bottles from week 15.

4. Statistics: The following procedures were utilized in analyzing the numerical data. Body weight, food intake, food efficiency, plasma albumin, and organ weight data were evaluated by analysis of variance, analysis of covariance (organ weights), followed by multiple comparison tests (Dunnett), or by the L.S.D. test (for food intake and food efficiency). Total and differential white blood cell counts were analyzed by the Mann/Whitney U-test. Data on mortality and histopathological changes were evaluated using Fisher's exact probability test (one-tailed).

5. Quality Assurance: Quality assurance statements were signed and dated May 14, 1984, for the 18-month study and November 12, 1985, for the 30-month study.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected daily for general behavior and condition and for mortality and morbidity.

Detailed examinations and palpation of masses were conducted every 4 weeks for the first 12 months and biweekly thereafter.

Results: Table 2 presents data on mortality and percent survival. No effect of dosing was seen. Survival in the 18-month study was between 90 and 100% in all groups. In the 30-month study, survival at 104 weeks was 66 to 76% in male groups and 68 to 78% in female groups; at 130 weeks, group survival ranged between 20 and 34%.

No overt signs of toxicity were observed that were considered related to dosing. Symptoms of aging developed late in the study but the incidence of unthrifty animals did not differ between groups. Symptoms indicating respiratory tract infections such as sniffing, grunting, and encrustation about the nose were present in all groups including controls, and their incidence increased with time into the study. In the 78-week study, sniffing was observed in 16 to 19 rats/group, grunting in approximately 6/group, and nose encrustation in about 4/group. In the 30-month study, sniffing was observed in 16 to 24 males/group and 12 to 18 females/group between weeks 53 and 65, but was seen in more than 90% of males and females in all groups in the period between 92 and 130 weeks. The number of animals with palpable masses and the number of masses observed were not affected by dosing.

2. Body Weight: Body weights were recorded weekly for the first 12 weeks of treatment and biweekly thereafter.

Results: Tables 3, 4, and 5 summarize mean body weight data at representative intervals in the 30-month and 18-month studies. Mean weights of high-dose males were significantly lower in the first 16 weeks for the 30-month study than in controls, suggesting a palatability problem. The weight gain in the first week was 13.6 g for the high-dose males as compared to 24.0 g for controls. Interpretation of this initial decrease is complicated by the fact that the automatic watering system failed in the first week; this caused a lower than normal food consumption in all groups. For weeks 1 to 12 of the 30-month study, the weight gain in high-dose males (264 g) was 93% of the control gain, and for the first 78 weeks, weight gains were nearly identical in control and high-dose males (Table 5). In females (30-month study), mean body weights were similar in all groups until week 66 when a decrement was seen at the high dose. No significant differences were seen, but at 130 weeks the mean weights in high-dose females were 5.4% lower than in controls.

TABLE 2. Mortality and (Percent Survival) for Rats Fed Imazalil for 30 Months or 18 Months

Dietary Level (ppm)	30-Month Study					18-Month Study
	Week 52	78	104	118	130	78
			Males			
0	2 (96)	3 (94)	14 (72)	27 (46)	33 (34)	1 (95)
25	0 (100)	7 (86)	17 (66)	28 (44)	39 (22)	1 (95)
100	2 (90)	4 (92)	14 (72)	20 (60)	33 (34)	1 (95)
400	0 (100)	4 (92)	12 (76)	26 (46)	33 (34)	0 (100)
			Females			
0	2 (96)	5 (90)	11 (78)	27 (46)	34 (32)	1 (95)
25	0 (100)	8 (84)	13 (74)	30 (40)	37 (26)	1 (95)
100	1 (98)	1 (98)	15 (70)	26 (48)	33 (34)	0 (100)
400	0 (100)	3 (94)	16 (68)	31 (38)	40 (20)	2 (90)

TABLE 3. Mean Body Weights at Representative Intervals in Rat Fed Imazalil for 30 Months

Dietary Level (ppm)	Mean Body Weight (g ± S.E.) at Week:						
	12	26	52	78	104	130
			Males				
0	65.7 ± 1.1	350.5 ± 5.2	423.7 ± 5.8	488.4 ± 7.3	513.8 ± 8.8	506.9 ± 10.6	426.3 ± 15.7
25	65.7 ± 0.8	343.4 ± 4.2	412.4 ± 5.6	472.6 ± 7.4	499.4 ± 9.2	480.6 ± 11.3	386.3 ± 11.6
100	65.9 ± 1.0	348.4 ± 4.7	424.7 ± 5.8	485.1 ± 7.7	524.9 ± 9.1	520.2 ± 9.4	412.1 ± 9.2
400	65.9 ± 0.9	330.1 ± 3.8**	409.4 ± 5.1	476.1 ± 6.7	514.1 ± 7.6	494.0 ± 11.9	435.1 ± 14.4
			Females				
0	66.9 ± 0.9	205.9 ± 2.5	238.8 ± 3.2	265.3 ± 4.3	302.0 ± 5.9	321.0 ± 7.9	270.6 ± 8.3
25	66.8 ± 1.0	209.0 ± 2.4	240.7 ± 3.1	273.1 ± 4.5	305.1 ± 5.8	314.8 ± 6.6	298.9 ± 11.6
100	66.5 ± 1.0	202.7 ± 2.2	234.2 ± 2.6	267.5 ± 4.5	303.4 ± 6.6	316.1 ± 8.1	278.0 ± 11.8
400	67.0 ± 0.8	207.8 ± 1.9	238.5 ± 2.1	269.5 ± 3.7	289.7 ± 4.9	304.0 ± 6.8	256.0 ± 13.6

**Significantly different from control value ($p < 0.01$).

TABLE 4. Mean Body Weights at Representative Intervals in Rats Fed Imazalil for 18 Months

Dietary Level (ppm)	Mean Weight (± S.E.) at Week: 0	12	26	52	78
			Males		
0	70.6 ± 1.5	342.8 ± 10.3	409.0 ± 12.0	471.8 ± 13.6	485.0 ± 14.1
25	70.3 ± 1.8	343.5 ± 6.8	414.0 ± 8.3	476.0 ± 102	477.4 ± 12.3
100	70.4 ± 1.3	337.7 ± 8.5	409.6 ± 10.1	479.4 ± 12.3	484.6 ± 12.2
400	70.4 ± 2.6	347.1 ± 8.4	424.1 ± 10.8	485.7 ± 12.8	498.8 ± 15.2
			Females		
0	64.7 ± 1.5	207.8 ± 4.1	241.9 ± 4.7	277.5 ± 4.4	323.5 ± 6.8
25	64.5 ± 1.4	211.9 ± 4.8	242.5 ± 6.0	277.8 ± 9.9	310.4 ± 14.3
100	64.5 ± 1.9	213.2 ± 5.0	246.4 ± 5.9	275.0 ± 7.8	302.1 ± 10.3
400	64.7 ± 1.7	206.3 ± 3.8	235.3 ± 3.9	254.5 ± 5.1*	279.7 ± 6.6**

*Significantly different from control value ($p < 0.05$).

**Significantly different from control value ($p < 0.01$).

$N^+ = 19$ or 20

TABLE 5. Mean Weight Gains (g) in Rats Fed Imazalil[a]

Dietary Level (ppm)	30-Month Study		18-Month Study	
	12 Weeks	78 Weeks	12 Weeks	78 Weeks
		Males		
0	284.8	448.1	272.2	414.4
25	277.7	433.7	273.2	407.1
100	282.7	459.0	267.3	414.2
400	264.2	448.2	276.7	428.4
		Females		
0	139.0	235.1	143.1	258.8
25	142.2	238.3	147.4	245.9
100	136.2	236.9	148.7	237.6
400	140.8	227.7	141.6	215.0

[a]Calculated by subtraction of means; values are not the means of individual animal weight gain.

In the 18-month study, no effect of dosing on body weights was observed in males; mean body weights in high-dose females were significantly lower ($p < 0.01$) than controls from weeks 60 to 78. At week 78, the mean body weights were 14% lower in high-dose females than in controls and the weight gain was 17% lower than in controls.

3. Food Consumption and Compound Intake: Food consumption was determined weekly on a cage basis, and weekly dietary consumption per rat was calculated. Efficiency was calculated from the consumption and body weight gain data for the first 12 weeks.

   Results: No effects of dosing on overall food consumption were observed. Food consumption was markedly depressed in all groups in weeks 1 and 15 when the watering system failed. Values for all groups were approximately 55 to 70% of the values in the preceding or following weeks. Food consumption was significantly decreased during week 1 (30-month study) when high-dose males (49.4 g/week) were compared to controls (62 g/week), and food efficiency was depressed. Food consumption in high-dose males tended to be consistently marginally lower than in controls (2 to 3 g/rat/week) in the 30-month study but not in the 18-month study. Food efficiency (weeks 1 to 12) was similar in dosed and control groups in both studies. Mean compound intake, calculated on the basis of overall mean body weights and food consumption, and the analyzed levels of Imazalil in the diets were reported, and the data are summarized in Table 6.

4. Ophthalmological Examinations: Ophthalmological examinations were performed on animals in the control group and high-dose group at study initiation and during weeks 52 and 104. Examinations were not conducted for the 18-month study.

   Results: No effects related to dosing were observed.

5. Hematology and Clinical Chemistry: Blood was collected from the tip of the tail of all rats in the 18-month study at week 77 for hematology. Blood was also collected from 10 rats/sex/group at the 78-week necropsy for clinical chemistry. The CHECKED (X) parameters were examined:

TABLE 6. Compound Intake in Rats Fed Imazalil

Dietary Level (ppm)		Intake (mg/kg/day)			
		18-Month Study		30-Month Study	
Nominal	Actual	Males	Females	Males	Females
25	23.6	1.0	1.2	1.0	1.2
100	90.9	3.9	5.1	3.6	4.7
400	368.8	15.9	20.3	15.0	19.7

a. Hematology:

X	Hematocrit (HCT)†	X	Leukocyte differential count[a]
X	Hemoglobin (HGB)†	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)†	X	Mean corpuscular HGB concentration (MCHC)
X	Erythrocyte count (RBC)†	X	Mean corpuscular volume (MCV)
X	Platelet count†		Coagulation: thromboplastin time (PT)
	Reticulocyte count (RETIC)		
	Red cell morphology		

Results: Platelet counts were significantly increased ($p < 0.01$) in high-dose females (1039 ± 39 10E9/L) when compared to controls (885 ± 37 10E9/L). But the values were reported within the historical range and no changes were seen in males. No changes were seen in other parameters. Hematology parameters were not examined in the 30-month study.

b. Clinical Chemistry:

	Electrolytes		Other
X	Calcium†	X	Albumin†
X	Chloride†		Albumin/globulin ratio
	Magnesium†	X	Blood creatinine†
X	Phosphorus†	X	Blood urea nitrogen†
X	Potassium†	X	Cholesterol†
X	Sodium†		Globulins
		X	Glucose†
	Enzymes	X	Total bilirubin†
X	Alkaline phosphatase (ALP)		Direct bilirubin
	Cholinesterase		Total protein†
	Creatine phosphokinase†		Triglycerides
X	Lactic acid dehydrogenase	X	Haptoglobulin
X	Serum alanine aminotransferase (SGPT)†		
X	Serum aspartate aminotransferase (SGOT)†		
	Gamma glutamyltransferase (GGT)		

In the 30-month study, blood was collected from 10 rats/sex/group at week 131 for analysis of plasma albumin.

Results: The level of albumin was decreased in males receiving 400 ppm at 18 months, but the value was within one standard deviation of the control value and no corresponding change was seen at week 131. Serum GPT was slightly decreased (not significant) in males receiving 400 ppm (18 months) when compared to controls. All other parameters were similar in control and dosed groups.

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†Recommended by Subdivision F (November 1984) Guidelines.

[a]Control and high-dose groups only.

6. Urinalysis: Urine was collected from fasted animals at 77 weeks in the 18-month study (10/sex/group). The CHECKED (X) parameters were examined:

X	Appearance†	X	Glucose†
X	Volume†	X	Ketones†
X	Specific gravity†	X	Bilirubin†
X	pH	X	Blood†
X	Sediment (microscopic)†		Nitrate
X	Protein†	X	Urobilinogen

Results: Values were comparable for volume and specific gravity among groups, and there were no changes in composition. Urinalysis was not performed in the 30-month study.

7. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subject to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed:

	Digestive System		Cardiovasc./Hemat.		Neurologic
X	Tongue	X	Aorta†	XX	Brain†
X	Salivary glands†	XX	Heart†	X	Peripheral nerve (sciatic nerve)†
X	Esophagus†	X	Bone marrow†	X	Spinal cord (2 levels)
X	Stomach†	X	Lymph nodes†	XX	Pituitary†
X	Duodenum†	XX	Spleen†	X	Eyes (optic nerve)†
X	Jejunum†	XX	Thymus†		
X	Ileum†				
X	Cecum†				
X	Colon†				
X	Rectum†		Urogenital		Glandular
XX	Liver†	XX	Kidneys†	XX	Adrenals†
	Gallbladder†	X	Urinary bladder†	X	Lacrimal gland
XX	Pancreas†	XX	Testes†	X	Mammary gland†
		X	Epididymides	XX	Thyroids†
		X	Prostate	XX	Parathyroids† (weighed with thyroids)
		X	Seminal vesicle		
	Respiratory	XX	Ovaries	X	Harderian glands (coagulating glands)
X	Trachea†	X	Uterus†		
XX	Lung†				
					Other
				X	Bone (sternum and femur)†
				X	Skeletal muscle†
				X	Skin†
				X	All gross lesions and masses†

†Recommended by Subdivision F (November 1984) Guidelines.

In the 18-month study, the liver and kidneys of all animals in all groups (20/sex/group) were examined microscopically. The complete list of tissues (above) was examined for 10 males and 10 females in the control- and high-dose groups, and when possible for animals that died or were sacrificed moribund. Neoplasms, preneoplastic and hyperplastic lesions, as well as other types of nonneoplastic lesions, were recorded.

In the 30-month study, all of the listed organs were examined in the high-dose and control groups for the presence of hyperplastic, preneoplastic, and neoplastic changes. A limited set of organs (liver, kidneys, lungs, spleen, brain, testes, adrenals, thyroid and pituitary) of animals in the low- and mid-dose groups were examined for hyperplastic, preneoplastic, and neoplastic lesions. Only gross lesions suspected of being tumors were examined microscopically, and if the abnormality was not a neoplasm it was not necessarily recorded. For the livers of all survivors of the control- and high-dose groups and the kidneys and lungs of all survivors in all groups, all types of nonneoplastic lesions were reportedly recorded.

Results:

a. Organ Weights: At 18 months and 30 months, the liver weights showed a tendency to be increased in males receiving 400 ppm, but the increases were not significant when compared to controls; the absolute weights of livers were not affected in females, but the relative weights were slightly increased in 400-ppm females at both 18 and 30 months (Table 7). The absolute kidney weights were not affected in males, but the relative kidney weights were significantly increased at 18 months in females receiving 400 ppm and non-significantly increased at 30 months in the female group receiving 400 ppm (Table 7). These organ weight changes were not considered of toxicologic importance by the study authors, since there were no correlating histologic changes. The absolute and relative adrenal weights were increased in all dosed groups of females at 18 months (Table 8), but this was attributed to the control value being abnormally low in comparison to the laboratory historical control weight in other studies performed in the same time period. No weight changes in adrenals were noted at 30 months (Table 8). At 18 months, the brain-to-body weight ratio was increased in high-dose females when compared to controls, but no effects of dosing were noted on absolute brain weights in females or on absolute or relative brain weights in males. At 30 months, there were slight but significant decreases in brain weight in males receiving 100 and 400 ppm, but changes in relative brain weights were not significant (Table 8).

TABLE 7. Liver and Kidney Weight Data (± S.E.) for Rats Fed Imazalil

Dietary Level (ppm)	18 months		30 months	
	g	g/kg	g	g/kg
Liver		**Males**		
0	11.89 ± 0.55	24.4 ± 0.7	11.86 ± 0.43	29.4 ± 1.1
25	11.57 ± 0.47	24.1 ± 0.5	10.99 ± 0.46	30.0 ± 1.6
100	11.49 ± 0.41	23.7 ± 0.6	12.05 ± 0.50	30.8 ± 1.0
400	12.54 ± 0.48	25.1 ± 0.5	13.13 ± 0.48	31.6 ± 1.0
		Females		
0	7.17 ± 0.14	23.5 ± 0.4	8.35 ± 0.41	32.4 ± 1.3
25	7.02 ± 0.3[illegible]	23.7 ± 0.4	9.48 ± 0.63	33.4 ± 1.3
100	7.37 ± 0.35	25.6 ± 0.8*	8.86 ± 0.56	33.8 ± 1.3
400	6.76 ± 0.25	25.2 ± 0.4	8.35 ± 0.42	34.8 ± 1.5
Kidneys		**Males**		
0	2.91 ± 0.09	6.01 ± 0.13	3.36 ± 0.13	8.36 ± 0.40
25	2.88 ± 0.09	6.02 ± 0.07	3.12 ± 0.11	8.52 ± 0.49
100	2.87 ± 0.07	5.96 ± 0.11	3.41 ± 0.12	8.68 ± 0.30
400	2.95 ± 0.08	5.94 ± 0.13	3.45 ± 0.12	8.31 ± 0.26
		Females		
0	1.97 ± 0.05	6.48 ± 0.17	2.17 ± 0.08	8.55 ± 0.24
25	1.89 ± 0.05	6.47 ± 0.15	2.26 ± 0.06	8.10 ± 0.26
100	2.04 ± 0.07	7.17 ± 0.20*	2.14 ± 0.07	8.30 ± 0.25
400	2.07 ± 0.07	7.74 ±0.19**	2.22 ± 0.08	9.32 ± 0.48

*Significantly different from control value ($p < 0.05$).
**Significantly different from control value ($p < 0.01$).

TABLE 8. Brain and Adrenal Gland Weight Data for Rats Fed Imazalil

Dietary Level (ppm)	18 Months		30 Months	
Brain	g	g/kg	g	g/kg
		Males		
0	2.10 ± 0.03	4.37 ± 0.10	2.19 ± 0.03	5.47 ± 0.18
25	2.09 ± 0.03	4.42 ± 0.10	2.15 ± 0.02	5.85 ± 0.13
100	2.10 ± 0.03	4.37 ± 0.09	2.11 ± 0.02*	5.39 ± 0.12
400	2.13 ± 0.02	4.32 ± 0.10	2.09 ± 0.02**	5.07 ± 0.16
		Females		
0	1.90 ± 0.02	6.25 ± 0.12	1.87 ± 0.02	7.43 ± 0.27
25	1.86 ± 0.03	6.41 ± 0.20	1.90 ± 0.03	6.86 ± 0.31
100	1.88 ± 0.02	6.65 ± 0.19	1.88 ± 0.03	7.42 ± 0.33
400	1.91 ± 0.02	7.20 ± 0.15**	1.93 ± 0.03	8.13 ± 0.37
Adrenals	mg	mg/kg	mg	mg/kg
		Males		
0	53 ± 2	109 ± 5	66 ± 5	167 ± 14
25	53 ± 3	112 ± 5	66 ± 3	179 ± 11
100	53 ± 2	110 ± 5	69 ± 2	177 ± 7
400	51 ± 2	105 ± 4	74 ± 3	181 ± 11
		Females		
0	51 ± 2	168 ± 8	86 ± 5	347 ± 26
25	61 ± 3	209 ± 9*	83 ± 5	297 ± 14
100	64 ± 4*	227 ± 14**	76 ± 4	298 ± 21
400	62 ± 3	234 ± 13**	82 ± 7	349 ± 37

*Significantly different from control value ($p < 0.05$).
**Significantly different from control value ($p < 0.01$).

b. Gross Pathology (18-Month Study): Table 9 summarizes gross findings in the 18-month study. The incidence of masses suspected of being tumors was low. An increase in livers with a prominent lobular pattern was observed in males and females receiving 100 and 400 ppm. No clear dose-related increase in other findings was apparent.

c. Microscopic Pathology (18-Month Study):

1) Nonneoplastic: Table 10 summarizes microscopic findings in the liver and kidneys. An increase in the incidence of intracytoplasmic inclusion bodies and an increase in the severity of vacuolization of hepatocytes was observed in the liver of males receiving 400 ppm. The vacuolization of hepatocytes was mainly in the periportal area and correlated with the pronounced lobular pattern of the liver. The inclusion bodies in the vacuoles were eosinophilic, did not stain for glycogen or fat, and were considered related to dosing since they were not found in controls. An increase in the severity of bile duct proliferation was also observed in high-dose males.

Nephrosis was present and more frequent in male groups than in females; mineralization of the kidneys was more frequent in females than males. There was no increase in the incidence or severity of kidney findings in dosed rats. Other organs were examined for only 10 rats/sex in control- and high-dose groups and for rats that died in the mid- and low-dose groups. Frequent findings or those that were increased or decreased in high-dose rats when compared to controls are presented in Table 11. These findings were about equally distributed in groups, were mostly of minimum or slight grade, are common findings in rats of this age, and were not considered related to dosing.

2) Neoplastic: Table 12 summarizes neoplastic findings in the 18-month study. There was no indication of neoplasia related to dosing. The most frequent finding was fibromatous polyps of the uterus. There was a good correlation between gross masses and histologic findings. One uterine mass seen grossly in controls was not found to be a tumor, and for one control female (No. 21) there was no histologic entry for a uterine mass. Only 2 of 19 masses in females had no histologic entry and only 1 of 3 in males (100-ppm male No. 6, a mass in the axillary lymph node).

TABLE 9. Gross Findings in Rats Fed Imazalil for 18 Months

Site/Finding	Dietary level (ppm)							
	Males				Females			
	0	25	100	400	0	25	100	400
Suspected tumors								
Axillary lymph node	0	0	1	0	0	0	0	0
Adrenals	0	0	0	0	0	0	2	1
Mammary gland	0	0	0	0	0	1	2	1
Epididymis	1	0	0	0	--	--	--	--
Clitoris	--	--	--	--	0	0	1	0
Uterus	--	--	--	--	3	2	2	4
Zymbal gland	1	0	0	0	0	0	0	0
Other Gross Findings								
Liver								
Prominent lobular pattern	2	2	4	7	1	2	3	3
Pale	1	1	3	2	0	1	0	0
Enlarged	0	1	0	0	0	1	0	0
Lungs								
Atelectasis	1	3	2	0	0	0	0	0
Chronic resp. disease	1	2	0	0	1	0	0	0

(continued)

TABLE 9. (continued)

Site/Finding	Dietary level (ppm)							
	Males				Females			
	0	25	100	400	0	25	100	400
Mammary gland								
Secretory activity	--	--	--	--	5	4	4	2
Adrenal								
Enlarged	0	0	0	0	0	1	1	2
Ovaries								
Cyst(s)	--	--	--	--	2	5	5	1
Eyes								
Inflammation	0	0	0	0	0	3	0	2

TABLE 10. Nonneoplastic Histologic Findings in the Liver and Kidneys of Rats Fed Imazalil for 18 Months

Organ/Finding	Males				Females			
	0	25	100	400	0	25	100	400
Liver	(20)[a]	(20)	(20)	(20)	(20)	(20)	(20)	(20)
Pronounced lobular pattern	13	12	9	19	0	0	0	0
Multivacuolar hepatocytes	16	19	17	18	4	5	5	4
- very slight/slight	13	16	13	9	4	5	5	4
- moderate	3	3	4	9	0	0	0	0
Univacuolar hepatocytes	13	11	11	18	2	0	1	3
Intracytoplasmic inclusion bodies	0	0	0	5	0	0	0	0
Bile duct proliferation	12	16	13	18	8	9	5	11
- very slight/slight	11	14	11	15	8	9	5	11
- moderate/severe	1	1	2	3	0	0	0	0
Single cell necrosis	13	14	12	11	0	0	0	0
Kidney	(20)	(20)	(20)	(20)	(20)	(20)	(20)	(20)
Nephrosis	20	19	20	20	6	12	10	9
- very slight/slight	12	12	16	12	6	10	8	8
- moderate/severe	8	7	4	8	0	2	2	1
Urothelial hyperplasia	0	0	0	0	4	4	6	7
Mononuclear cell infiltration	13	18	12	18	10	13	13	15
Mineralization	0	0	0	0	5	9	8	8

[a]The numbers in parentheses represent the number of animals with tissues examined.

TABLE **. Representative Nonneoplastic Histologic Findings in Control and High-Dose Rats Fed Imazalil for 18 Months[a]

Organ/Finding	Dietary Level (ppm)			
	Males		Females	
	0	400	0	400
Lungs	(11)[b]	(10)	(10)	(11)
Peribronchial lymphoid aggregation	9	9	5	4
Mineralization, arterial wall	3	4	0	0
Pneumonitis	2	1	0	1
Bronchiolitis	0	1	4	1
Trachea	(11)	(10)	(10)	(11)
Tracheitis	6	9	5	6
Nasal Cavity	(10)	(10)	(10)	(10)
Rhinitis	3	6	7	2
Mononuclear cell infiltrate	10	10	3	5
Lacrymal glands, exorbital	(11)	(10)	(10)	(11)
Proplasia	6	9	1	0
Mononuclear cell infiltrate	2	6	2	1
Adrenal	(11)	(10)	(10)	(11)
Cortical degeneration	0	0	1	3
Pituitary	(11)	(10)	(10)	(10)
Focus of cellular alteration	3	5	1	1
Thyroid	(11)	(10)	(10)	(9)
Parafollicular cell proliferation	2	3	1	0
Pancreas	(11)	(10)	(10)	(10)
Fatty atrophy	1	1	4	3

(continued)

TABLE 11. (continued)

Organ/Finding	Dietary Level (ppm)			
	Males		Females	
	0	400	0	400
Spleen	(11)	(10)	(10)	(10)
Brown pigment accumulation	7	6	10	10
Extramedullary hematopoiesis	1	2	3	4
Stomach	(11)	(10)	(10)	(11)
Glandular dilatation, fundus	7	10	3	5
Testis	(11)	(10)	--	--
Interstitial edema	11	10	--	--
Interstitial cell proliferation	1	3	--	--
Tubular atrophy	0	2	--	--
Mammary glands	--	--	(10)	(12)
Duct ectasia	--	--	5	1
Ovaries	--	--	(10)	(11)
Cyst(s)	--	--	0	4
Urinary bladder	(11)	(10)	(10)	(11)
Proteinaceous material	4	5	0	0

[a]Does not include liver and kidneys.

[b]The numbers in parentheses represent the number of animals with specific tissue examined.

TABLE 12. Neoplastic Lesions in Rats Fed Imazalil for 18 Months

	Dietary level (ppm)							
	Males				Females			
Organ/Neoplasm	0	25	100	400	0	25	100	400
Liver	(20)[a]	(20)	(20)	(20)[a]	(20)	(20)	(20)	(20)
Cholangiosarcoma	0	0	0	0	0	1	0	0
Adrenal glands	(11)	--	(1)	(10)	(10)	--	(1)	(11)
Pheochromocytoma	0	0	0	1	0	0	1	0
Pituitary	(11)	--	(2)	(10)	(10)	(1)	(1)	(10)
Hemorrhagic tumor	0	--	1	0	0	1	0	0
Mammary gland					(10)	(1)	(2)	(12)
Fibroadenoma					0	1	2	0
Papillary carcinoma					0	0	0	1
Uterus					(10)	(3)	(3)	(13)
Fibromatous polyp					1	2	2	4
Adenocarcinoma/carcinoma					0	1	0	1
Zymbal gland	(11)	--	--	(9)	(8)	--	--	(9)
Squamous cell carcinoma	1	--	--	0	0	--	--	0

[a]The numbers in parentheses represent the number of animals with organ examined.

d. Gross Pathology (30-Month Study): Table 13 summarizes the incidence of animals with masses suspected of being tumors and rats with organ enlargement. No dose-related trends were apparent. Other randomly distributed, frequently occurring gross findings included spotted or discolored surface of the pancreas, stomach, adrenal, kidneys, liver, and lungs; atrophy of the testes; increased secretory activity of the mammary glands; hydrothorax; pronounced lobular pattern in the liver, granular surface of the kidneys and spleen, and thrombi of the heart (Table 14). For all these changes there was no indication of a treatment-related effect.

e. Histopathology (30-Month Study):

1) Nonneoplastic: Table 15 summarizes the incidence of hyperplastic and preneoplastic lesions. The mid- and low-dose groups were routinely examined for only a limited set of organs (liver, kidneys, lungs, spleen, brain, testes, adrenal, thyroid, and pituitary); for controls and high-dose groups, a complete complement of tissues was examined for hyperplastic and preneoplastic lesions. No significant ($p < 0.05$) increases were seen, and there was a fairly large fluctuation in incidence between groups. In males receiving 400 ppm, the incidence of skin warts, hypertrophic and hyperplastic cell foci in the pituitary, and parafollicular cell proliferation in the thyroids were observed. These changes were not considered (by the authors) to be related to dosing. Other nonprecancerous-nonneoplastic lesions were recorded for the liver, kidney, and lungs of surviving rats (all groups); Table 16 summarizes the findings. No significant changes or dose-related changes were observed. Nephrosis was seen in practically all survivors, and the severity was greater in males than females. Mononuclear cell infiltration was fairly frequent in the kidneys and liver of all groups of survivors; peribronchial and perivascular lymphoid aggregates, focal accumulation of alveolar macrophages, and interstitial pneumonitis in the lung occurred in all groups. None of the changes were considered to be related to dosing.

TABLE 13. Gross Findings in Rats Fed Imazalil for 30 Months (Mass or Enlarged)

	Dietary level (ppm)							
	Males				Females			
Organ/Neoplasm	0	25	100	400	0	25	100	400
				Mass or Suspected Tumor				
Liver	1	0	2	0	0	2	0	0
Kidneys	1	2	0	2	0	0	1	0
Spleen	0	0	0	1	0	0	0	0
Testes	0	0	1	2	--	--	--	--
Adrenals	2	3	0	0	4	2	2	0
Thyroid	1	3	1	1	2	2	2	1
Pituitary	1	3	3	0	7	6	7	7
Abdominal cavity	0	0	2	0	1	1	4	1
Nasal cavity	0	3	0	0	0	0	0	0
Mammary gland	--	--	--	--	20	22	17	17
Skin/subcutis	2	7	7	4	0	0	1	0
Thymus	0	0	0	0	0	3	1	1
				Enlarged				
Liver	3	4	3	3	1	0	0	0
Kidneys	4	3	0	1	1	0	0	0
Spleen	2	3	1	1	3	1	3	3
Testes	1	2	1	0	--	--	--	--

(continued)

TABLE 13. (continued)

Organ/Neoplasm	Dietary level (ppm)							
	Males				Females			
	0	25	100	400	0	25	100	400
				Enlarged (continued)				
Adrenals	7	3	1	3	12	5	8	6
Thyroids	3	5	2	1	0	2	1	2
Pituitary	0	1	3	1	1	2	0	1
Lymph nodes, axillary	1	0	3	0	1	0	1	4
Lymph nodes, mediastinal	3	2	4	3	3	0	1	2
Lymph nodes, mesenteric	2	2	4	3	3	0	1	2
Heart	6	5	3	5	1	0	1	2
Prostate	0	3	1	3	--	--	--	--
Thymus	2	0	0	0	1	0	1	0

TABLE 14. Gross Findings in Rats Fed Imazalil for 30 Months (Other Findings)

	Dietary level (ppm)							
	Males				Females			
Organ/Neoplasm	0	25	100	400	0	25	100	400
Liver								
Pronounced lobular pattern	9	8	10	10	9	7	8	7
Spotted	1	5	3	1	1	4	2	1
Discolored	5	8	9	3	4	3	8	1
Lungs								
Spotted	9	12	9	8	0	0	0	0
Inflammation	6	5	1	3	1	1	2	0
Atelectasis	6	9	6	7	5	6	5	5
Discolored	7	3	3	12	10	5	5	9
Kidneys								
Granular	25	15	20	26	7	7	1	7
Discolored	17	10	8	15	7	6	2	3
Spleen								
Granular	7	0	2	2	1	0	2	2

(continued)

TABLE 14. (continued)

Organ/Neoplasm	Dietary level (ppm)							
	Males				Females			
	0	25	100	400	0	25	100	400
Spotted tissue	6	3	3	3	16	10	13	10
Discolored	5	3	3	3	5	5	5	9
Heart								
Thrombus	4	6	7	4	1	1	2	4
Testis								
Atrophy	9	12	10	3				
Mammary gland								
Secretory activity					28	29	25	27
Thorax								
Hydrothorax	2	5	3	1	5	1	2	5

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TABLE 15. Hyperplastic and Preneoplastic Histologic Findings in Rats Fed Imazalil for 30 Months[a]

Organ/Lesion	Dietary level (ppm)							
	Males				Females			
	0	25	100	400	0	25	100	400
Liver	(50)[b]	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Focus of cellular alteration	11	8	4	8	3	6	7	1
Bile duct proliferation	18	9	11	11	10	5	5	11
Lungs	(50)	(48)	(50)	(49)	(50)	(50)	(50)	(50)
Increased septal cellularity	5	0	1	0	2	0	0	0
Kidneys	(50)	(50)	(50)	(49)	(50)	(50)	(50)	(50)
Urothelial hyperplasia	5	3	2	7	14	10	13	11
Adrenals	(50)	(49)	(48)	(50)	(50)	(48)	(50)	(49)
Focus of cellular alteration (cortex)	6	7	9	10	12	13	13	6
Basophilic focus (medulla)	2	4	2	4	2	0	0	0
Mammary glands	(46)	--	--	(45)	(49)	(27)	(23)	(49)
Lobular hyperplasia	3	--	--	3	25	10	8	19
Pituitary	(47)	(49)	(48)	(47)	(48)	(49)	(50)	(46)
Hypertrophy, focal	11	12	12	18	2	4	2	1
Hyperplasia, focus	2	5	2	5	5	3	2	3

(continued)

TABLE 15. (Continued)

Organ/Lesion	Dietary level (ppm)							
	Males				Females			
	0	25	100	400	0	25	100	400
Thyroid	(47)	(45)	(46)	(49)	(49)	(47)	(49)	(48)
Parafollicular cell proliferation	5	9	4	13	8	12	12	9
Uterus					(50)	(23)	(24)	(50)
Endometrial hyperplasia					12	6	8	5
Squamous metoplasia					0	0	0	2
Cecum	(49)	--	--	(46)	(49)	--	--	(47)
Hyperplasia, Peyer patches	4	--	--	3	6	--	--	3
Heart	(50)	--	--	(50)	(50)	--	--	(50)
Cartilaginous metaplasia	2	--	--	0	8	--	--	1
Pancreas	(48)	--	--	(48)	(50)	--	--	(50)
Acinar cell nodule	0	--	--	3	2	--	--	1
Skin	(49)	--	--	(46)	(47)	--	--	(49)
Wart	1	--	--	5	0	--	--	0
Zymbal gland	(39)	--	--	(47)	--	--	(41)	--
Squamous metaplasia	1	--	--	0	--	--	0	--

[a] Findings occurring at a very low frequency (<4%) are not necessarily presented.
[b] The numbers in parentheses represent the number of tissues examined microscopically.

TABLE 16. Representative Nonneoplastic Histologic Lesions of the Liver, Kidney, and Lungs of Surviving Rats Fed Imazalil for 30 Months[a]

	Dietary Level (ppm)							
	Males				Females			
Organ/Lesion	0	25	100	400	0	25	100	400
Kidney	(17)[b]	(10)	(16)	(17)	(16)	(13)	(17)	(9)
Nephrosis	17	9	16	17	14	13	16	9
- very slight/slight	8	4	5	7	8	13	15	6
- moderate/severe	9	5	11	10	6	0	1	3
Mononuclear cell infiltration	13	3	3	13	12	7	8	7
Mineralization	0	1	0	0	4	8	15	5
Liver[c]	(17)	(10)	(16)	(17)	(16)	(13)	(17)	(19)
Focal hepatocellular vacuolization	2	0	0	6	3	0	0	0
Bile duct sclerosis	4	0	0	4	3	0	0	1
Necrotic hepatocyte aggregates	9	0	0	5	7	0	0	2
Mononuclear cell infiltrate (portal)	4	0	0	6	4	0	0	2
Increased Kuppfer cells	0	0	0	0	1	0	0	2
Lung	(17)	(9)	(16)	(17)	(16)	(13)	(17)	(9)
Peribronchial lymphoid aggregates	2	1	3	1	12	11	10	8
Macrophage accumulation	2	0	2	1	6	3	2	2
Interstitial pneumonitis	3	3	3	3	7	2	4	3

[a]Preneoplastic lesions are not included, see Table 15.
[b]The numbers in parentheses represent the numbers of tissues histologically examined.
[c]Nonpreneoplastic lesions were not routinely examined in low- and mid-dose groups.

2) Neoplastic: Table 17 summarizes the neoplastic lesions in the 30-month study. There were no dose-related increases in neoplasms at any site with the possible exception of Leydig cell tumors of the testis. The tumor types observed were reported to be common findings in this strain of rat, and were considered related to aging.

D. STUDY AUTHORS' CONCLUSIONS:

The continuous feeding of Imazalil to Wistar-derived rats at levels of 25, 100, or 400 ppm for 18 or 30 months did not cause any distinct deleterious effects. Mortality was low in all groups and was 28 and 22% for control males and females respectively after 24 months. A minimal toxic dose of 400 ppm was indicated by slightly lower body weights in males in the 30-month study without an effect on food consumption. In the 18-month study, there was a slight decrease in mean body weight in high-dose females. Increases in relative weights of adrenals, kidneys, liver, and heart of high-dose females in the 18-month study were not considered of toxicologic importance. A slight tendency (not significant) for increased relative liver weights of high-dose rats of both sexes (30 months) and of the relative kidney weight in high-dose females at 30 months were not considered of toxicologic importance because there were no correlating histologic changes in liver and kidneys. Histopathologic liver changes in males receiving 400 ppm in the 18-month study and vacuolation of hepatocytes of surviving high-dose males at 30 months were considered an adaptive response to the test compound. No treatment-related effect on total tumor incidence or the incidence of the various tumor types was seen. It was concluded that Imazalil was not carcinogenic and the no-toxic-effect level was 100 ppm fed in the diet for 18 or 30 months.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

It is our assessment that the two reports present sufficient data to adequately evaluate the carcinogenic potential and chronic toxicity of Imazalil at the doses tested. The study design, however, does not completely conform to Guideline 83-1 (Chronic Toxicity), 83-2 (Oncogenicity Study), or 83-5 (Combined Chronic Toxicity/Oncogenicity Study).

The 18-month study is nearly adequate for assessing chronic toxicity, except that the duration is insufficient for rats according to guideline requirements and clinical laboratory examinations were only at termination. Liver and kidneys were examined in all rats (20/sex) in all groups, but lungs were examined histologically only for 10/sex in the control and

TABLE 17. Representative Neoplasms in Rats Fed Imazalil for 30 Months

Organ/Lesion	Dietary level (ppm)							
	Males				Females			
	0	25	100	400	0	25	100	400
Kidney	(50)	(50)	(50)	(49)	(50)	(50)	(50)	(50)
Adenoma	0	0	0	0	0	0	0	1
Nephroblastoma	0	0	0	1	0	0	0	0
Lipomatous tumor	1	1	0	0	0	0	1	0
Mesenchymal tumor	0	2	0	1	0	0	0	0
Liver	(50)	(50)	(50)	(50)	(50)	(50)	(50)	(50)
Cholangioma (cystic)	0	0	0	0	0	0	2	0
Cholangiocarcinoma	0	0	3	0	0	0	0	0
Neoplastic nodule	0	0	0	0	0	1	0	0
Hepatocellular carcinoma	1	0	0	0	0	0	0	0
Lungs	(50)	(48)	(50)	(49)	(52)	(50)	(50)	(50)
Adenoma	0	1	0	0	0	0	0	0
Pituitary	(47)	(49)	(48)	(47)	(48)	(49)	(50)	(46)
Solid tumor	2	0	0	1	0	0	2	1
Spongiocytic tumor	1	0	0	0	0	0	1	1
Hemorrhagic tumor	6	2	10	4	7	10	7	4
Pleomorphic hemorrhagic tumor	4	7	5	2	4	4	6	4
Pars intermedia tumor	0	0	0	0	0	0	1	0

(continued)

TABLE 17. (continued)

Organ/Lesion	Dietary level (ppm)							
	Males				Females			
	0	25	100	400	0	25	100	400
Testes	(50)	(50)	(50)	(47)				
Leydig cell tumor	1	3	4	4				
Uterus					(50)	(23)	(24)	(50)
Multiple polyps					13	12	12	18
Adenocarcinoma					1	4	5	3
Epidermoid carcinoma					0	1	0	1
Adrenal	(30)	(49)	(48)	(50)	(50)	(48)	(50)	(49)
Pheochromocytoma	15	7	8	9	0	2	0	3
Mammary gland					(49)	(27)	(23)	(49)
Fibroma/adenoma					2	1	1	1
Fibroadenoma					22	16	16	15
Adenocarcinoma (multiple)					1	3	3	3
Papillary carcinoma					2	2	0	1
Carcinoma					0	0	0	1
Skin	(49)	(9)	(8)	(46)				
Benign[b]	2	4	6	0	0	0	0	0
Malignant[c]	2	4	1	1	0	0	0	0

(continued)

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TABLE 17. (continued)

Organ/Lesion	Dietary level (ppm)							
	Males				Females			
	0	25	100	400	0	25	100	400
Thyroid	(47)	(45)	(46)	(49)	(49)	(47)	(49)	(48)
C-cell adenoma	3	4	4	1	3	6	2	1
C-cell carcinoma	3	6	2	1	1	3	3	2
Follicular adenoma	1	1	1	0	0	0	0	0
Papillary cystadenoma	0	0	2	1	0	0	2	1
Brain	(48)	(49)	(50)	(50)	(50)	(50)	(50)	(50)
Glioma	1	2	0	0	1	0	1	0

[a]The numbers in parentheses represent the number of animals with the specific tissue examined microscopically.

[b]Lipoma, fibroma, keratoacanthoma.

[c]Squamous cell carcinoma, leiomyosarcoma, sarcoma, fibrous histiocytoma.

high-dose groups. Neoplastic lesions and all types of nonneoplastic lesions were examined. Pathology was complete for the 7 rats that died (all groups) and for 10 survivors/sex in the control and high-dose groups. A complete complement of tissues for 20/sex in high-dose and controls is suggested by guidelines.

The 30-month study is deficient for an oncogenicity study since all tissues were not examined histologically for low- and mid-dose rats that died or were sacrificed moribund. We do not consider this a serious deficiency, since all possible target organs in these groups were examined for hyperplasia and preneoplastic and neoplastic lesions, and all gross lesions suggestive of neoplasia were also examined.

A tissue inventory was not available. The reviewers used the individual pathology sheets for rats in the control- and high-dose groups in the 30-month study to evaluate tissue accountability, and concluded that it was acceptable. Table 18 presents our list of organs in which insufficient tissue was available for evaluation or tissues were missing or autolyzed and not examined histologically. With a few minor exceptions, it supports the number of tissues recorded in Table 17 of the report (Summary of Hyper- and Pre-neoplastic Histologic Findings).

Liver, lungs, and heart were examined in all animals. All kidneys except one (high-dose male No. 70) were examined. Tissue loss was low for all sites except thymus, parathyroid, eyes and lacrimal gland, and urinary bladder. Autolysis of several tissues (13 to 17) was found in 3 control males (Nos. 16, 88 and 90) and 1 high-dose male (No. 70, 12 tissues including kidney). Two high-dose females (Nos. 63 and 67) had several tissues autolyzed (14 in each). Some tissues that were noted as autolyzed were examined histologically (high-dose males Nos. 16 and 18 and high-dose females Nos. 31, 77, and 85; 4 to 8 tissues/animal). We assess that the numbers of tissues missing and autolyzed did not have an impact on interpretation of the histopathology data.

We conclude that the rats could have tolerated a somewhat higher dose, since there were no effects on survival and no indication of any important toxic effects. The body weight data do not convincingly support a maximum tolerated dose (MTD). Although there was a significant decrease ($p < 0.01$) in mean body weight in males receiving 400 ppm at week 12 of the 30-month study, the decrease was minimal (a 5.8% lower mean weight than control and a 7.2% lower weight gain from weeks 1 to 2). The decrease may have been an artifact caused by failure of the automatic watering system. A corresponding effect was not seen at 12 weeks in the 78-week study. In the 30-month study, mean body weights were comparable in control and high-dose males from weeks 52 to 130, and cumulative weight gains were identical at 78 weeks in both groups (see Table 5). No effects were seen for body weights in dosed females, except that the mean weights in high-dose females were slightly lower

TABLE 18. List of Tissues Not Examined Microscopically in 30-Month Rat Study With Imazalil[a]

	Dietary Level (ppm)			
	Males		Females	
Tissue	0	400	0	400
Liver	0	0	0	0
Kidneys	0	1	0	0
Lungs	0	0	0	0
Heart	0	0	0	0
Aorta	0	1	0	1
Spleen	0	1	0	0
Brain	1	1	2	1
Adrenal	0	0	0	2
Thyroid	3	1	1	2
Pituitary	3	3	2	4
Testes/ovaries	0	3	6	4
Esophagus	3	2	0	2
Stomach	0	2	0	0
Small intestine	1	4	0	3
Cecum	1	4	1	3
Colon	1	4	0	3
Salivary gland	4	0	1	3
Lacrimal gland	6	0	0	2
Eyes	5	3	0	3
Pancreas	2	2	0	0
Spinal cord	1	0	1	2
Bladder	0	1	4	5
Parathyroid	9	6	6	8
Thymus	11	13	14	18

[a]Prepared by our reviewers from individual animal pathology sheets.

than controls (5 to 7%) from weeks 104 to 130. In the 18-month study, body weights and gains were comparable in controls and high-dose males throughout the study, but mean weights of high-dose females were decreased 18% and 13.6% when compared to controls at weeks 52 and 78.

A previous 12-month rat study at levels of up to 800 ppm was referenced by the study authors (Thienpont, 1981), but was not available for review. No data on body weights were provided, but it was reported that microscopic liver changes of a probable adaptive nature occurred in males at 800 ppm. There was no indication that 800 ppm was excessive. It would be helpful if the study authors gave additional information on the rationale for dose selection or clarified the effects of higher doses on body weight gain, liver weights, and liver histopathology in the 12-month study.

The only neoplasm that appeared to be increased in the 30-month study in dosed animals was Leydig cell tumors of the testes, 6, 8, and 8.5% at 25, 100, and 400 ppm, respectively, compared to 2% for controls. Laboratory historical control data would be useful for comparison purposes. Data from RCC for Wistar KFM-HAN rats indicates a control incidence of 6/234 (2.6%) and 7/149 (4.8%) in two studies of rats <110 weeks old and 12/199 (6.0%) for rats <138 weeks old.

Based on minimal changes in liver weights and minimal body weights changes in the present studies, the LOEL can be tentatively set at 400 ppm and the NOEL at 100 ppm.

(ACTIVE INGREDIENT INFORMATION IS NOT INCLUDED)

EXECUTIVE SUMMARY:

In a 30 month carcinogenicity study, Imazalil technical was administer as a 50% mixture with 50% of equal parts of [redacted] in the diet to Cpb: Wu Wistar rats, 50/sex/dose at levels of 0, 25, 100, or 400 ppm . Approximate doses were 1.0, 3.6, and 15 mg/kg/day for males and 1.2, 4.7, and 19.7 mg/kg/day for females.

Minor losses in body weight gains in females (-3.4%) were seen at 400 ppm after 52 weeks in the 30 month study while a -16% body weight gain loss was noted in the 18 month study.. Slight increases in liver weights ie. + 2.9% after 18 months and +10.7% in the 30 month study were also noted in males at that dose. Focal hepatocellular vacuolation was increased in males at 400 ppm that lived to 30 months. No other effects consider to be related to dosing were reported. The rats were thought to have been able to tolerate a higher dose. The **review DER notes that a minimal LEL could be established at 400 ppm with a NOEL established at 100 ppm (4.7 mg/kg/day in females) based on the liver effects and slight body weight gain reductions (3.6 mg/kg/day in males)**

There is a question of whether the rat study was tested at a high enough dose to evaluate Imazalil carcinogenicity potential.
The Toxicology Branch considers this study to not fulfill the requirements of GL -83-1a by not being tested at high enough dosages.

Carcinogenic potential is questionable in the study with the unusual epidermoid carcinoma of the uterus in the female rats. (Referred to Peer Review Committee).
This study is currently classified as core Supplementary pending the cancer Peer Review Committee evaluation and currently does not satisfy the Guideline requirement for a carcinogenicity study (83-2a).

1. EXECUTIVE SUMMARY

In a chronic feeding study, Imazalil technical (97% ai) was administer by capsule to 4/sex/dose of beagle dogs aged 7 to 8 months for a period of 12 months. Dosages were 0, 1.25, 2.5, or 20 mg/kg/day.

At 20 mg/kg/day mean body weights were slightly depressed (approx. 12.4%in males and 9.4%)in females at 52 weeks. Increased vomiting and food wastage were recorded. Males were more affected than females.
Serum alkaline phosphatase was markedly increased at 20 mg/kg/day after 29 weeks in females with 74 U/L vs 144 U/L and 92 U/L in controls vs 211 U/L at 20 mg/kg/day in males at 52 weeks., relative liver weights at 2.5 mg/kg/day in males were increased but without histopathological correlates. **The LEL is 20 mg/kg/day based on vomity and lakaline phosphastase increases with increased liver weights. The NOEL is established at 2.5 mg/kg/day.**

This study is minimum for chronic toxicity and satisfies the guideline requirements for a 83-1b chronic study in the dog.

EPA No.: 68D80056
DYNAMAC No.: 276-D
TASK No.: 2-76D
September 12, 1990

DATA EVALUATION RECORD

IMAZALIL

Chronic Toxicity Study in Dogs

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: September 12, 1990

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EPA No.: 68D80056
DYNAMAC No.: 276-D
TASK No.: 2-76D
September 12, 1990

DATA EVALUATION RECORD

IMAZALIL

Chronic Toxicity Study in Dogs

REVIEWED BY:

William L. McLellan, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: William L. McLellan
Date: Sept. 10, 1990

Margaret E. Brower, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Margaret E Brower
Date: Sept. 10, 1990

APPROVED BY:

Nicolas P. Hajjar, Ph.D.
Department Manager
Dynamac Corporation

Signature: Nicolas P. Hajjar
Date: September 10, 1990

Henry Spencer, Ph.D.
EPA Reviewer, Section II
Toxicology Branch I
(H-7509C)

Signature: Henry Spencer
Date: June 15, 1994

Marion Copley, D.V.M.,
D.A.B.T.
EPA Section Head, Section ~~IV~~ II
Toxicology Branch I
(H-7509C)

Signature: Marion Copley
Date: June 15, 1994

DATA EVALUATION RECORD

GUIDELINE § 83-1

STUDY TYPE: Chronic toxicity feeding study in dogs.

MRID NUMBER: 413288-02.

PC Code: 111901 Tox. Chem#: 497 AB

TEST MATERIAL: Imazalil base (R23979).

SYNONYM: 1-[2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl]-1H-imidazole.

STUDY NUMBER: 1899.

SPONSOR: Janssen Pharmaceutica N.V., 2340 Beerse, Belgium.

TESTING FACILITY: Department of Toxicology, Janssen Research Foundation, 2340 Beerse, Belgium.

TITLE OF REPORT: Imazalil base: R23979, Experiment No. 1899 (November 6, 1989) Chronic Toxicity Study in Beagle Dogs: Repeated Dosage for 12 Months.

AUTHORS: Verstraeten, A.; Teuns, G.; Van Cauteren, H.; Vandenberghe, J.; Marsboom, R.

REPORT ISSUED: November 6, 1989.

Executive Summary at end of DER

CONCLUSIONS: Beagle dogs were administered Imazalil by capsule for 1 year at daily doses of 0, 1.25, 2.5 or 20 mg/kg/day. Mean body weights were slightly depressed at 20 mg/kg/day and weight gains were depressed compared to controls primarily in the first 12 weeks of the study. Increased vomiting and food wastage were observed in dogs receiving 20 mg/kg/day; the incidence was more frequent in the males than in the females. Salivation also occurred in the high-dose males. These signs were most frequent in the last three months of the study. Serum alkaline phosphatase was markedly increased at 20 mg/kg/day and relative liver weights were increased but there were no correlating histologic changes. No compound related adverse effects were observed at 1.25 or 2.5 mg/kg/day. The LOEL in this study is 20 mg/kg/day and the NOEL is 2.5 mg/kg/day.

CORE Classification: The study is CORE minimum for chronic toxicity (Guideline 83-1). The classification may be upgraded on provision of data validating dosing.

A. MATERIALS:

1. Test Compound: Imazalil base (R23979); description: slightly yellow to brown crystalline mass (solidified oil); batch No.: ZR023979BEB211; purity: 98.8%; 97.2% by gas chromatography.

2. Test Animals: Species: dog; strain: beagle (non inbred); age: between 7 and 8 months at initiation; weight: males--7.6 to 14.6 kg, females--6.1 to 11.4 kg; mean group weights 10.3 to 10.6 g (males and females combined); source: Janssen Research Foundation laboratory colony.

B. STUDY DESIGNS:

1. Animal Assignment: Dogs had been vaccinated against parvovirus (5, 7, and 9 weeks of age) and distemper and canine hepatitis virus (12 weeks of age). They were acclimated to laboratory conditions for at least 2 weeks, given complete pretest examinations, and assigned on the basis of initial weight to give approximately equal mean weights in the following groups:

Test Group	Dosage (mg/kg/day)[a]	Males	Females
1 Control	0	4	4
2 Low (LDT)	1.25	4	4
3 Mid (MDT)	2.5	4	4
4 High (HDT)	20.0	4	4

[a]By capsule.

Dogs were individually caged in barriered testing rooms with positive pressure and approximately 13 air changes/hour. Temperature and humidity were constantly monitored.

Rationale for Dosage Selection: Available data on pharmacology, kinetics, and toxicology were used to set the dose levels. In a previous 12-month dog study, doses of 1.25, 5, and 20 mg/kg/day were given by capsule. No effects were observed at 1.25 mg/kg/day; at 5 mg/kg/day, a slight decrease in appetite and lowered body weight gain were observed. At 20 mg/kg/day, a decreased appetite, increased incidence of salivation and vomiting, and decreased body weight gain were observed. Decreased serum calcium and increased alkaline phosphatase and slight histologic liver changes were observed at the high dose.

2. Capsule Preparation: The test material was synthesized at the test facility and was given orally as a powder in gelatin capsules. Dosages were prepared 1 week in advance, based on the weekly recorded body weight except during two vacation periods: 1. Dosage capsules for weeks 23, 24, 25, and 26 were prepared based upon the body weight of week 22. 2. Dosage capsules for week 48 were prepared based on the body weight of week 46. Capsules were stored at room temperature.

   Results: Data on stability, homogeneity, and concentrations were not provided.

3. Food and Water Consumption: Animals received food ("Hendrix" pelleted dog food) and tapwater ad libitum. Food consumption was not recorded because food wastage problems prevented an accurate measure of this parameter.

4. Statistics: The following procedures were utilized in analyzing the numerical data: Data for males and females were combined for statistical analysis. The Mann-Whitney

U test was utilized to assess the significance of any intergroup differences for the following parameters: ECG and heart rate, body weight, hematology, clinical chemistry, urinalysis, organ weight, and histopathology. The Chi-square test was used to analyze data on mortality and gross pathology.

The reviewers also tabulated the following data separately by sex: mean body weight, body weight gain, organ weight, and selected parameters in hematology, clinical chemistry, and histopathology. Body weight gain data were analyzed by ANOVA and the significance of a trend tested using linear regression. The significance of liver weight data was evaluated utilizing Kruskal-Wallis nonparametric ANOVA.

5. Quality Assurance: A quality assurance statement was signed and dated November 6, 1989.
6. No confidentiality statement was supplied.
7. A GLP statement was dated Dec. 8, 1989.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected at least twice a day for health, abnormal behavior, unusual appearance, clinical effects, morbidity, and mortality.

Results: All dogs survived the 12-month study. The only clinical observations that were considered possibly related to dosing by the study authors were seen in the group receiving 20 mg/kg/day; these were increased vomiting, salivation, wasting of food, and soft feces. These observations occurred primarily in high-dose males between weeks 41 and 47. In this study interval, moderate vomiting was observed 6 to 7 times in all 4 males receiving 20 mg/kg/day, and moderate food wastage was seen 10 times in 2 of these males. Slight to moderate salivation was seen 3 and 5 times in 2 males and on 1 occasion in the other 2 males of the high-dose group. Salivation was not seen in high-dose females. Vomiting occurred once during week 45 or 46 in all high-dose females; however moderate vomiting also was noted at week 47 in two control females. Since dosing records were not available, it could not be determined if vomiting was due to misdosage. It was also noted that vomiting occurred in two high-dose males at day 23 and in the other two at day 29. Soft feces were not frequent but were seen only at 20 mg/kg/day (two males at both weeks 5 and 52; two females at both weeks 43 and 53). One female (No. 121 in the 2.5-mg/kg group) experienced sporadic epileptic seizures (severe to moderate) on five occasions (weeks 6, 14, 27, 29, and 38), but this was not considered related to dosing since a slight seizure was also observed prior to dosing (day 1).

2. Body Weight: Body weights were recorded weekly.

Results: Data for group mean body weights and weight gain were presented for males and females combined in the study report. Mean weights were slightly depressed in the group receiving 20.0 mg/kg/day, but none of the decreases were significant. Cumulative mean body weight gain was significantly ($p < 0.05$) lower than in controls at 42 of 52 weekly study intervals in the high-dose group. Tables 1 and 2 present data for mean body weights and weight gains for males and females separately at representative study intervals. Weight gains were decreased in both high-dose males and females in the first half of the study and in the last 3 months of the study. In the period between days 283 and 325, when vomiting was frequently seen there was a mean weight loss of 75 g in high-dose males compared to a gain of 875 g for controls and 325 g for low- and mid-dose males.

3. Food Consumption and Compound Intake: Consumption was not recorded. An accurate reading was not possible because of food wastage.

Results: No data were provided; it was estimated that food consumption was approximately 250 g/day. Compound intake could not be verified.

4. Ophthalmological Examinations: Ophthalmic examinations were performed on all dogs prior to study initiation, at least once during the study, and at the end of the study.

Results: There were no abnormal findings for any of the dogs in the study.

5. Electrocardiograms and Heart Rate: Analyses were conducted on all dogs at weeks -2, 6, 14, 29, 38, and 52.

Results: It was reported that no relevant adverse effects were observed. All values were within the normal range (provided), and sporadically significant changes in some values were seen but they were inconsistent over intervals of analysis and were not dose related.

6. Hematology and Clinical Chemistry: Blood was collected from the jugular vein of all animals, twice prior to study initiation, after 2 weeks of dosing, and monthly thereafter for hematology and clinical chemistry analysis. However, no hematology and clinical chemistry was performed in weeks 21 through 28. The CHECKED (X) parameters were examined:

TABLE 1. Mean Body Weights at Selected Intervals for Dogs Fed Imazalil for 1 Year[a]

Dietary Level (ppm)	Mean Body Weight (kg ± S.D.) at Study Week: Pretest	12	26	36	52
			Males		
0	11.2 ± 2.40	12.6 ± 2.73	13.2 ± 2.49	12.8 ± 2.89	13.7 ± 3.00
1.25	11.1 ± 1.91	12.9 ± 1.73	13.2 ± 1.87	13.0 ± 1.71	14.0 ± 2.11
2.5	11.1 ± 2.88	12.9 ± 3.88	13.4 ± 4.38	13.4 ± 4.26	14.3 ± 5.37
20.0	10.7 ± 1.71	11.8 ± 2.28	11.9 ± 2.30	11.8 ± 2.21	12.0 ± 2.57
			Females		
0	10.0 ± 1.13	11.5 ± 1.70	11.9 ± 1.85	11.8 ± 1.89	12.7 ± 1.99
1.25	9.8 ± 1.14	11.1 ± 1.26	11.4 ± 1.02	11.6 ± 0.95	13.1 ± 1.42
2.5	9.5 ± 2.37	10.3 ± 2.82	10.6 ± 2.80	10.5 ± 2.84	11.5 ± 3.40
20.0	10.1 ± 0.68	10.7 ± 0.79	10.7 ± 1.16	10.8 ± 1.55	11.5 ± 1.73

[a]Calculated and statistically analyzed by reviewers.

TABLE 2. Mean Body Weight Gains (kg ± S.D.) at Selected Intervals for Dogs Fed Imazalil for 1 Year[a]

Dietary Level (mg/kg)	Mean Body Weight Gain (kg/day ± S.D.)			
	0-12	12-26	26-36	36-52
		Males		
0	1.4 ± 0.70	0.6 ± 0.43	-0.4 ± 0.57	0.9 ± 0.57[T]
1.25	1.8 ± 0.41	0.3 ± 0.32	-0.2 ± 0.37	1.0 ± 0.48
2.5	1.7 ± 1.03	0.6 ± 0.61	-0.03 ± 0.45	0.8 ± 1.12
20.0	1.0 ± 0.65	0.2 ± 0.26	-0.1 ± 0.35	0.3 ± 0.41
		Females		
0	1.5 ± 0.60[T]	0.3 ± 0.29	-0.1 ± 0.36	1.0 ± 0.51
1.25	1.3 ± 0.55	0.3 ± 1.00	0.2 ± 0.26	1.6 ± 0.52
2.5	0.9 ± 0.53	0.3 ± 0.37	-0.1 ± 0.61	1.1 ± 1.06
20.0	0.6 ± 0.12*	0.1 ± 0.57	0.1 ± 0.45	0.7 ± 0.40

[a]Calculated and statistically analyzed by the reviewers.

*Significantly different from control value ($p < 0.05$) by pairwise comparison.

[T]Significant trend by linear regression ($p < 0.05$).

a. Hematology:

X	Hematocrit (HCT)†	X	Leukocyte differential count
X	Hemoglobin (HGB)†	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)†	X	Mean corpuscular HGB concentration (MCHC)
X	Erythrocyte count (RBC)†	X	Mean corpuscular volume (MCV)
X	Platelet count†	X	Coagulation: thromboplastin time (PT)
	Reticulocyte count (RETIC)		
	Red cell morphology		

Results: Total white cell counts tended to be increased in dosed groups as compared to controls in a dose-related manner. However, for males and females combined, practically all mean values were within one standard deviation of the historical mean value of 13.4 ± 2.9 thousand/mm^3. The authors reported significant increases at 1.25 mg/kg/day in weeks 32 and 48, at 2.5 mg/kg/day in weeks 4, 12, 16, 32, 36, 48, and 52, and at 20 mg/kg/day in weeks 8, 12, 16, 20, and 48 (sexes combined). Table 3 shows mean values ± S.D. for the sexes separated. The effects were considered of minor importance. Differential white counts showed a trend toward a slightly increased percentage of segmented neutrophils and a corresponding slight decrease in lymphocytes of females receiving 20 mg/kg/day. Mean values, however, were within the normal laboratory range, 57.2 ± 7.9% and 31.2 ± 7.9% for segmented neutrophils and lymphocytes, respectively. Other hematologic changes in dosed groups that differed from controls were infrequent and considered to be incidental.

b. Clinical Chemistry:

	Electrolytes		Other
X	Calcium†	X	Albumin†
X	Chloride†		Albumin/globulin ratio
	Magnesium†	X	Blood creatinine†
X	Phosphorus†	X	Blood urea nitrogen†
X	Potassium†	X	Cholesterol†
X	Sodium†		Globulins
		X	Glucose†
	Enzymes	X	Total bilirubin†
X	Alkaline phosphatase (ALP)		Direct bilirubin
X	Cholinesterase	X	Total protein†
	Creatine phosphokinase†	X	Triglycerides
X	Lactic acid dehydrogenase (LDH)	X	Haptoglobin
X	Serum alanine aminotransferase (SGPT)†	X	Phospholipids
X	Serum aspartate aminotransferase (SGOT)†		
	Gamma glutamyltransferase (GGT)		

†Recommended by Subdivision F (November 1984) Guidelines.

TABLE 3. Mean Leukocyte Counts ($100/mm^3$) in Dogs Fed Imazalil for 1 Year[a]

Interval (week)	Dietary Level (mg/kg)							
	Males				Females			
	0	1.25	2.5	20.0	0	1.25	2.5	20.0
0	13.9 ± 2.1	14.8 ± 4.2	13.8 ± 2.6	17.5 ± 7.3	15.0 ± 3.4	13.9 ± 2.0	14.4 ± 1.2	13.0 ± 3.7
2	11.9 ± 0.9	14.8 ± 0.7	14.0 ± 1.5	13.7 ± 1.3	15.2 ± 4.1	11.8 ± 1.0	16.0 ± 2.2	12.7 ± 3.7
4	11.8 ± 1.1	14.9 ± 0.8	14.5 ± 1.7	16.4 ± 2.5	13.4 ± 1.8	11.6 ± 1.1	14.6 ± 1.5	12.9 ± 2.7
8	11.4 ± 0.8	13.3 ± 1.9	12.8 ± 2.4	14.8 ± 1.4	11.8 ± 0.6	11.0 ± 0.3	11.9 ± 1.1	12.3 ± 2.7
12	14.4 ± 0.7	18.1 ± 3.8	15.6 ± 2.3	17.1 ± 4.0	13.1 ± 1.8	13.6 ± 1.7	15.8 ± 0.7	15.6 ± 1.7
16	12.4 ± 1.1	14.0 ± 1.5	14.4 ± 1.8	16.9 ± 2.9	12.5 ± 1.2	13.3 ± 1.3	14.6 ± 2.2	16.1 ± 5.4
20	11.8 ± 1.1	15.1 ± 2.8	14.7 ± 4.4	16.5 ± 2.3	13.5 ± 1.2	12.8 ± 0.3	15.6 ± 3.0	16.0 ± 4.6
29	11.4 ± 0.8	13.5 ± 1.4	13.1 ± 2.1	15.3 ± 2.3	13.4 ± 1.6	13.8 ± 2.2	14.2 ± 2.7	13.6 ± 3.1
32	10.2 ± 0.4	14.3 ± 1.9	13.3 ± 4.2	16.1 ± 3.5	11.9 ± 1.8	11.7 ± 0.4	13.5 ± 1.3	12.6 ± 4.2
36	10.0 ± 0.8	12.8 ± 1.5	12.6 ± 1.2	15.0 ± 3.0	11.9 ± 0.9	10.9 ± 1.0	13.4 ± 1.8	12.1 ± 3.0
40	11.2 ± 1.2	13.4 ± 1.7	13.0 ± 12.4	14.7 ± 2.8	12.5 ± 0.7	13.7 ± 4.2	15.8 ± 3.5	13.0 ± 2.6
44	10.5 ± 1.2	13.1 ± 1.2	15.5 ± 2.8	13.0 ± 1.1	11.5 ± 1.7	10.2 ± 2.5	12.2 ± 1.7	14.4 ± 5.8
48	10.7 ± 0.5	14.0 ± 0.5	13.4 ± 1.7	15.6 ± 4.4	11.0 ± 0.9	11.3 ± 1.5	14.1 ± 2.4	15.7 ± 2.2
52	12.5 ± 0.9	13.0 ± 0.7	15.8 ± 5.3	14.6 ± 1.1	11.3 ± 0.7	13.8 ± 4.6	13.5 ± 0.5	13.8 ± 4.6

[a]Means and standard deviations were calculated by the reviewers.

Results: The authors reported that slight decreases in calcium and marked increases in alkaline phosphatase may have been related to dosing but that other changes in clinical chemistry parameters were not dose related, were often transient or not consistent over time, or were due to sporadically high or low values in one animal. Most values were also within the normal range, and the changes were not considered of toxicologic importance. Data were not separated by sex for analysis by the study authors. The values for serum calcium were significantly decreased in the 20-mg/kg/day group at weeks 2, 4, and 12 ($p < 0.05$) and at week 16 ($p < 0.001$). All values were within the normal range. Alkaline phosphatase (AP) activity was significantly ($p < 0.05$) increased in the 20-mg/kg group (sexes combined) at weeks 4, 12, 20, 29, 32, 36, and 44. Alkaline phosphatase data separated by sex are summarized in Table 4. Pretest values for individual dogs had a fairly large range, 102 to 164 U/L and 107 to 220 U/L in control males and females, respectively, and 113 to 184 U/L and 137 to 168 U/L in high-dose males and females, respectively. However, the pretest mean values (± S.D.) for the two groups, 131 ± 38 U/L, compares to 125 ± 50 U/L for laboratory historical controls (n=1615). The control values tended to decrease compared to pretest values as the dogs aged (normal finding). The mean values in both sexes receiving 20 mg/kg/day were increased compared to controls throughout the study, but no effects were seen at the lower doses. Examination of the individual animal data indicated a response in two of four high-dose males and three of four high-dose females. Examination of data for all other clinical chemistry parameters did not indicate any effects of dosing or any apparent trends. Infrequent significant decreases occurred for sodium, potassium, total protein, cholesterol, urea nitrogen, blood creatinine, and total bilirubin. Infrequent significant increases occurred for chloride, haptoglobin, glucose, triglycerides, SGOT, GGT, and LDH.

7. Urinalysis: Urine was collected from fasted animals before study initiation, after 1 month of dosing, and thereafter at 3-month intervals. The CHECKED (X) parameters were examined:

X	Appearance†	X	Glucose†
	Volume†		Ketones
X	Specific gravity†	X	Bilirubin†
X	pH	X	Blood†
X	Sediment (microscopic)†		Nitrate
X	Protein†	X	Urobilinogen
		X	Acetone bodies
		X	Creatinine

†Recommended by Subdivision F (November 1984) Guidelines.

TABLE 4. Alkaline Phosphatase Values (U/L ± S.D.) in Dogs Fed Imazalil for 1 Year[a]

Interval (week)	Dietary Level (mg/kg)							
	Males				Females			
	0	1.25	2.5	20.0	0	1.25	2.5	20.0
0	134 ± 28.4	144 ± 29.5	126 ± 20.2	144 ± 30.1	159 ± 48.8	154 ± 10.7	163 ± 59.4	152 ± 12.9
2	153 ± 44.8	166 ± 34.9	139 ± 29.7	177 ± 44.4	188 ± 66.2	168 ± 20.1	210 ± 94.6	237 ± 30.5
4	149 ± 47.3	158 ± 29.4	142 ± 29.8	195 ± 51.9	185 ± 66.0	161 ± 26.4	217 ± 121.0	300 ± 84.7
8	126 ± 42.3	154 ± 28.6	128 ± 24.7	194 ± 80.4	210 ± 74.0	197 ± 89.4	160 ± 71.5	198 ± 80.2
12	115 ± 31.7	133 ± 36.4	121 ± 28.3	174 ± 78.4	150 ± 52.7	144 ± 48.9	205 ± 163.0	317 ± 99.0
16	114 ± 46.7	125 ± 46.8	121 ± 34.7	170 ± 64.0	159 ± 57.3	139 ± 40.0	174 ± 139.8	285 ± 114.4
20	104 ± 43.0	107 ± 29.5	107 ± 33.7	178 ± 81.8	150 ± 56.8	123 ± 62.0	146 ± 80.7	324 ± 170.6
29	74 ± 23.7	80 ± 20.7	80 ± 24.0	143 ± 69.1	133 ± 24.5	108 ± 63.4	147 ± 116.8	223 ± 72.6
32	79 ± 32.2	121 ± 77.8	94 ± 26.0	212 ± 144.3	105 ± 34.8	91 ± 38.2	154 ± 105.8	267 ± 106.8
36	86 ± 38.4	80 ± 23.5	81 ± 23.1	164 ± 98.1	116 ± 30.7	89 ± 31.6	156 ± 107.7	265 ± 90.8
40	88 ± 42.7	89 ± 32.9	80 ± 26.9	180 ± 113.1	127 ± 40.0	108 ± 39.0	135 ± 96.5	257 ± 90.8
44	87 ± 51.3	89 ± 29.8	94 ± 27.2	226 ± 182.3	126 ± 60.1	143 ± 105.1	109 ± 58.1	291 ± 128.0
48	82 ± 42.5	82 ± 20.6	83 ± 28.7	149 ± 67.8	135 ± 50.4	101 ± 30.1	115 ± 56.5	226 ± 116.0
52	74 ± 37.5	72 ± 19.7	81 ± 27.0	144 ± 70.6	117 ± 61.4	92 ± 36.0	99 ± 56.7	211 ± 103.6

[a]Data separated by sex, calculated by reviewers.

Results: No effects of toxicologic importance were observed for any urinary parameters. Decreased values for creatinine and specific gravity were observed in high-dose animals at week 37; however, the values were within the normal range.

8. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subject to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination. In addition, the (XX) organs were weighed:

	Digestive System		Cardiovasc./Hemat.		Neurologic
X	Tongue	X	Aorta†	XX	Brain
X	Salivary glands†	X	Heart†	X	Peripheral nerve (sciatic nerve)†
X	Esophagus†	X	Bone marrow†	X	Spinal cord (3 levels)
X	Stomach†	X	Lymph nodes†	XX	Pituitary†
X	Duodenum†	XX	Spleen	X	Eyes (optic nerve)†
X	Jejunum†	XX	Thymus		
X	Ileum†				
X	Cecum†				
X	Colon†				
X	Rectum		Urogenital		Glandular
XX	Liver†	XX	Kidneys†	XX	Adrenals†
X	Gallbladder†	X	Urinary bladder†		Lacrimal gland
XX	Pancreas†	XX	Testes†	X	Mammary gland†
		X	Epididymides	XX	Thyroids†
		XX	Prostate	X	Parathyroids†
			Seminal vesicle		Harderian glands
		XX	Ovaries		
	Respiratory	X	Uterus		
X	Trachea†	X	Vagina		
XX	Lung†				
					Other
					Bone (sternum and femur)†
				X	Skeletal muscle†
				X	Skin
				X	All tissues showing abnormality

†Recommended by Subdivision F (November 1984) Guidelines.

Results:

a. Organ Weights: Imazalil had no adverse effect on the weight (absolute or relative) of lungs, spleen, heart, pancreas, kidneys, brain, thymus, adrenals, thyroids, testes/ovaries, pituitary, or prostate. The absolute and relative liver weights were increased in a dose-related manner for both sexes combined, and the relative liver weight was significantly ($p < 0.01$) greater at 20 mg/kg/day (269 g/10 kg) than in controls (211 g/10 kg). Table 5 presents data for liver weights separated by sex. The relative liver weight was significantly increased ($p < 0.05$) in males receiving 2.5 and 20 mg/kg/day when compared to controls but increased in dosed females.

b. Gross Pathology: There were no changes related to dosing. Increased incidences in high-dose females of swollen ovaries, uterus, and vagina were considered by the study authors to be related to the estrus cycle. Urinary bladder nodules were observed in three of four high-dose male dogs. Chronic inflammation was present for each dog upon histological evaluation. The study authors reported that this finding was due to catheterization during urine sampling.

c. Microscopic Pathology: Table 6 presents selected nonneoplastic lesions. Chronic inflammation of the prostate, prostatic urethra, and urinary bladder were observed with increased incidence in high-dose groups. The study author concluded that the finding was due to frequent catheterization of the animals. Congestion of the splenic red pulp in male dogs and dilated lumen of the uterus in female dogs were observed in all dose groups but not in the control groups. Slight mineralization (grade 1) of the kidney was seen in all animals. Hyaline casts in the kidney and chronic inflammation in the epididymis were present for one male dog in each dose group. In addition, the following incidental findings were observed: reticuloendothelial cell aggregates in the liver were seen in 4/4 low-dose male dogs; cystic pituitary was recorded for 3/4 high-dose male and mid-dose female dogs, and 4/4 low-dose female dogs. Most of the histologic findings were graded slight (grade 1), and none were graded severe (grade 4). No neoplastic findings were observed.

TABLE 5. Mean Liver Weights (g ± S.D.) and Liver-to-Body Weight Ratios (g/10 kg) for Dogs Fed Imazalil for 1 Year[a]

Dietary Level (mg/kg)	Liver Weight	Liver-to-Body Weight Ratio
	Males	
0	269.8 ± 37.99	200.5 ± 23.53[T]
1.25	316.0 ± 65.13	224.3 ± 22.14
2.5	347.5 ± 105.17	248.3 ± 18.37*
20.0	313.5 ± 64.07	261.5 ± 19.49*
	Females	
0	276.3 ± 32.07	221.0 ± 41.93[TT]
1.25	267.0 ± 75.06	201.8 ± 38.06
2.5	251.5 ± 75.84	219.3 ± 23.98
20.0	312.0 ± 7.07	227.0 ± 40.99

[a]Data calculated and analyzed by the reviewers.

*Significantly different from control value ($p < 0.05$); Kruskal-Wallis nonparametric ANOVA, analyses by reviewers.

[T]Significant trend, $p = 0.05$.

[TT]$p = 0.01$.

TABLE 6. Incidence of Selected Nonneoplastic Lesions in Dogs Fed Imazalil for 1 Year[a]

Organ/Finding	Males				Females			
	0	1.25	2.5	20.0	0	1.25	2.5	20.0
Epididymis	(4)	(4)	(4)	(4)				
Chronic inflammation	0	1	1	1				
Periarteritis	0	1	0	1				
Kidney	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Hyaline casts	0	1	1	1	0	0	0	0
Mineralization	4	4	4	4	4	4	4	4
Liver	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
RES aggregates[b]	2	1	1	0	2	4	3	1
Pigmented sinusoidal cells	0	1	0	2	1	3	1	1
Round cell in interstitium	0	0	0	1	0	0	0	0
Pituitary Gland	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Cystic (trace)	0	1	1	3	2	4	3	2
Prostate	(4)	(4)	(4)	(4)				
Chronic inflammation	2	3	1	3				
Spleen	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Congestion (trace)	0	3	2	2	2	0	2	1
Urinary Bladder	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Chronic inflammation	2	2	0	3	0	2	2	2
Uterus					(4)	(4)	(4)	(4)
Dilated lumen (trace)					0	2	3	2

[a]Based on four dogs/sex/group.

[b]Reticuloendothelial cell.

D. STUDY AUTHORS' CONCLUSIONS:

Imazalil base (R23979), when given orally to dogs, was well tolerated without mortality up to 20 mg/kg/day. At 1.25 and 2.5 mg/kg/day, no compound-related adverse effects were noted. Toxicity in dogs receiving 20 mg/kg/day was characterized by a slightly lowered body weight gain accompanied by decreased appetite, occasional vomiting and softened feces, and some salivation. Serum calcium was slightly increased, and serum alkaline phosphatase was markedly increased at 20 mg/kg/day. Liver weight was increased at the highest dose, but no correlating histologic findings were observed in the liver. The NOEL was 2.5 mg/kg/day and based on a slight decreased appetite and weight gain in a previous study, the lowest effect level in dogs was considered to be 5.0 mg/kg/day.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The conduct and reporting of the study were acceptable with the exception that mean data were provided for males and females combined. Separation of the data by sex and statistical reanalysis of selected parameters, however, did not substantially affect data interpretation.

Since there were no analytical data on the capsules used for dosing and no records were provided validating the dosing, it cannot be determined if the vomiting in the high-dose groups and salivation in high-dose males were related to pharmacokinetic parameters or were the result of an overdosing. Vomiting that occurred in two control females at week 47 may have been related to a dosing error, but this cannot be determined. In addition, dosage capsules were prepared for weeks 23 to 27 based on week 22 body weights and stored for up to 4 weeks, and no analytical or stability data were provided.

Weight gain data show a definite decrease in both males and females receiving the highest dose when compared to controls; the effect is most marked in the first 12 weeks of the study, when vomiting was infrequent but was also apparent in the last 16 weeks of the study. Food efficiency could not be calculated, since there was food wastage and consumption data were not recorded. Hematology parameters were normal throughout the study despite some changes in mean values. The effects on alkaline phosphatase may be related to dosing; however, there were no degenerative histological changes that correlated with the increases or any meaningful changes in activity of other serum enzymes. Similarly, the increases in liver weights and liver-to-body weight ratio were not accompanied by any histologic findings. There was a dose-related trend for relative liver weight in both males and females. When data were combined for males and females, the increase at 20.0 mg/kg/day was significant ($p < 0.01$); when separated by sex, the increase

was significant for males at 2.5 and 20.0 mg/kg/day, but not in females at either dose. The apparent increase in the absolute liver weight in males receiving 2.5 mg/kg/day was caused by an abnormally high value (477 g) for one male that weighed 21.4 kg. We assess that the LOEL for the study is 20 mg/kg/day Imazalil, and the NOEL is 2.5 mg/kg/day.

Attachment 1

COMMENTS:

Janssen Pharmaceutical submitted supplemental historical control tumor incidence for the Wistar rat in the Civo Institutes TNO, 3700 AJ Zeist, Netherlands Laboratories. Approximately 15 studies were listed which bracketed the Imazalil study dates by 2 to 4 years. (MRID 41553501)

RESULTS:

Testicular Tumors:

The range of Leydig cell tumors was from 0/50 to 6/47 in any of the control groups of the 15 studies reported. The Imazalil study contained 1/50 in controls and 3/50, 4/50, and 4/47 in the low to high dosage groups respectively.
The incidences reported in the Imazalil study easily fall within the historical incidence and as such are not considered to be chemically induced.

Uterine Tumors:

The incidence of adenocarcinomas of the uterus in the 15 studies reported as historical controls ranged from 0/50 to 8/50 with 0only 3/15 studies showing no tumors in the controls. In the present study the incidence of adenocarcinomas of the uterus was 1/50, 4/50, 5/50 and 3/50 for controls, low, mid and high dose levels tested and are well within the range of the historical controls. Therefore, it is concluded that this type of tumor is not considered to be the result of exposure to the chemical.

Epidermoid carcinomas:

This type of uterine tumor is considered to be a rare tumor. Historical data presented by the registrant indicates that in 15 studies bracketing the Imazalil study only 1 tumor in 50 control animals in only one study was reported. In the Imazalil study the occurence of this type tumor was reported as 0/50, 1/50, 0/50, and 1/50 in the controls, low, mid, and high dosage groups respectively. The fact that more than one tumor occurred in the study alone is sufficient to question the significance but that a tumor occurred only in treatment groups and included the highest dose lends further concern when coupled with the opinion that the dose levels were probably not tested at sufficiently high levels.

Attachment 4
CASWELL FILE

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

6-Month Rat

JUN 24 1987

005957

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Imazalil - EPA Registration No. 43813-2
Related Actions - PP#5F3250, 6G3308, 4F3096

TOX Chem No.: 497AB

FROM: Carlos A. Rodriguez Carlos A. Rodriguez 6/23/87
Review Section VI, Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Lois A. Rossi, PM 21
Fungicide-Herbicide Branch
Registration Division (TS-767C)

THRU: Judith W. Hauswirth, Ph.D., Section Head
Review Section VI, Toxicology Branch
Hazard Evaluation Division (TS-769C) Judith W. Hauswirth 6/23/87

6/24/87

APPLICANT: Janssen Pharmaceutica
Bear Tavern Road
P.O. Box 344
Washington Crossing, NJ 08560

Requested Action

Review and evaluate a 6-month oral feeding toxicity study with Imazalil Base-R23979 in rats.

Janssen Pharmaceutica also requests that the 18-month chronic feeding study in rats, 30-month feeding/oncogenic study in rats, and mutagenicity and teratology studies in rabbits with imazalil reviewed by B. Jaeger, Toxicology Branch (TB) for JMPR to establish a permanent ADI for CODEX be cited as an acceptable review of studies for TB.

6 mo.

Conclusions

The 6-month oral feeding study in rats is acceptable and classified "Core-Minimum." Systemic NOEL = 100 ppm. Systemic LEL = 400 ppm (increase in relative kidney weights in both male and female rats, increased absolute and relative liver weights in female rats).

The study will be part of the files of EPA Registration No. 43813-2.

The 18-month rat feeding/oncogenic study was classified Acceptable by WHO[1] for systemic toxicity and Supplementary for oncogenicity. The 30-month rat oncogenic study is classified as Acceptable by WHO.[1]

The rabbit teratology study demonstrated no embryotoxic or teratogenic effects in rabbit fetuses at dose levels of 1.25, 2.5, and 5 mg/kg/day. The slightly reduced body weight gain (5.13%) at the high-dose level (5 mg/kg/day) when compared to the control group appears to be of no significant toxic effect to the pregnant rabbits. The dose levels tested should have been higher since a maximum tolerated dose (MTD) may not have been reached. The study was accepted by WHO.[1]

The mutagenicity studies - in vitro mutagenicity screening by microsomal activation bacterial assays, micronucleus test in rats, and sex-linked recessive lethal test in Drosophila melanogaster with imazalil were found to be unacceptable by WHO[1] because the data submitted were insufficient to warrant the final conclusion that imazalil is not a mutagenic agent.

The following additional battery of tests for mutagenicity assessment of imazalil are required: Gene mutation and unscheduled DNA synthesis or DNA damage/repair.

TB defers to RCB regarding Janssen request to consider citrus tolerances on separate food factors, i.e., citrus pulp and citrus rind.

---

[1]Reference March 5, 1984 letter from John W. Melone, Director, Hazard Evaluation Division, EPA, to Bill Burnam, Chief, Toxicology Branch, EPA, in which he sets forth the policy for use of WHO monographs as final EPA reviews. This memorandum is attached.

(111)

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DATA REVIEW

Study Type: Subchronic Oral Feeding

Accession No.: Not available

Study Report No.: V83.186/220555

Sponsor: Janssen Pharmaceutica
Beerse, Belgium

Testing Facility: Department of Toxicology
Janssen Pharmaceutica

Title of Report: 6-Month Oral Toxicity Study with Imazalil Base-R23979 in Rats

Authors: Lina, B.A.R.; Til, H.P.; van Nesselrooij, J.H.J.; Kuper, C.F.; Falke, H.E.

Report Date: September 1983

Test Material: Imazalil Base-R23979
Purity 98.1%

Introduction:

At the request of Janssen Pharmaceutica, Beerse, Belgium, Imazalil Base-R23979 was examined in a 6-month oral toxicity study in rats. The study was part of a chronic oral toxicity/carcinogenicity study with this test substance.

Materials and Methods:

The test material was Imazalil Base-R23979, an agricultural fungicide. Chemical name: 1-[2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl]-1H-imidazole.

Groups of 10 male and 10 female healthy SPF Wistar rats approximately 4 to 5 weeks old were fed diets containing imazalil (base technical grade) of 0, 25, 100, and 400 ppm for 6 months. The rats were housed under conventional conditions, five per cage, and separated by sex and dose. The animals were checked daily for clinical signs. Body weights were recorded initially, then weekly for the first 12 weeks and then every 2 weeks. Food consumption was recorded weekly. The following hematological parameters were evaluated on all male rats on day 174 and on all female rats on day 175 of the study: hemoglobin, packed cell volume, red blood cells, thrombocytes, white blood cells, differential white blood cell count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentrate (MCHC).

(112)

The following urinalysis parameters were measured on all animals after fasting on day 177 of the study: volume, density, pH, protein, glucose, occult blood, ketones, uribilinogen, bilirubin, and sediment for the presence of erythrocytes, leukocytes, epithelial cells, amorph material, crystals, casts, sperm cells, and worm cells.

The following chemistry parameters were measured from blood collected at autopsy on day 183 on all male rats and on day 184 on all female rats:

- Glucose
- Total protein
- Albumin
- Haptoglobin
- Alkaline phosphatase (ALP)
- Glutamic oxalacetic-transaminase (GOT)
- Glutamic-pyruvic transaminase (GPT)
- Lactate dehydrogenase (LDH)
- Urea
- Creatinine
- Total bilirubin
- Cholesterol
- Inorganic phosphatase
- Calcium (Ca)
- Chloride (Cl)
- Potassium (K)
- Sodium (Na)

At sacrifice, gross pathological observations were made and the following organs from all male and female rats were weighed: adrenals, brain, heart, kidneys, liver, lungs (with mainstem bronchi), ovaries, pancreas, pituitary, spleen, testes, thymus, and thyroid. Histopathological examinations on the following tissues were conducted on all male and female rats of the top-dose group and all control rats: adrenals, aorta, axillary lymph node, brain, cecum, cervical lymph node, coagulating glands, colon, duodenum, epididymides, external auditory canal, extra-orbital lacrimal glands, eyes, heart, ileum, jejunum, kidneys, larynx, liver, lungs, oesophagus, ovaries, pancreas, poratid salivary glands, pituitary, preputial glands, prostate, rectum, sciatic nerve, seminal vesicles, skeletal muscle, skin (flank), spinal cord (two levels), spleen, sternum (with bone marrow), stomach (cardia, fundus, and pylorus), sublingual salivary glands, submaxillary salivary glands, tongue, testes, and thymus. Microscopic examination of the kidneys was conducted in all animals. Statistical analysis was performed on body weight, food intake, red blood cell, volume and density of urine, clinical chemistry, organ weights by analysis of (co-)variance followed by multiple comparison test (Dunnett) or the LSD test (for food intake and food efficiency). Total and differential white blood cell counts by the Mann/Whitney U-test. The histopathological changes by Fisher's exact probability test.

Reported Results:

1. Food Consumption - Food consumption in all dosed groups was comparable with that of the control group during the study.

2. Mortality - None of the animals died during the study.

3. No dose-related increase in toxic signs was observed among individual groups.

4. Body Weights - The group mean body weights of the males of the top-dose group (400 ppm) were slightly but not statistically significantly lower than those of the control group throughout the study. (Table 3 of the report.)

Body Weights of Rats Fed Imazalil for 6 Months

Dietary Level (ppm)	Group Mean Body Weight (g) at Week 0	13	26
Males			
0	72.6	373.5	405.2
25	72.7	356.6	392.0
100	72.8	365.5	347.9
400	72.7	347.9	382.3
Females			
0	64.3	214.4	218.9
25	64.1	213.8	220.6
100	64.1	214.8	224.5
400	64.2	218.1	221.9

5. Hematology - Hematological determinations of hemoglobin and WBC at study termination (week 26) are presented in the following table:

	0 ppm	25 ppm	100 ppm	400 ppm
Hemoglobin				
Males	9.4	9.2	9.4	9.4
Females	9.2	9.5*	9.4	9.2
WBC (10E9/L)				
Males	12.2	14.0	14.2**	14.2**
Females	9.2	10.7	11.2	10.7

*Significantly different from control at $p < 0.05$.
**Significantly different from control at $p < 0.01$.

7. Organ Weights - (Tables 12 and 13 of report) No significant differences were noted among the test groups with respect to testes, pancreas, pituitary, thyroid, adrenals, brain, heart, spleen, and ovaries. However, an increase in the relative weight of the kidney in male rats and in the absolute and relative weights of the kidneys and liver in female rats were observed in the top-dose group. An increase in absolute weight of the thymus and the relative weight of the lungs were observed in female rats of the top-dose group and are summarized as follows:

Group Mean Absolute and Relative Organ Weights (g/kg)

Dietary Levels (ppm)	Kidneys		Lungs		Liver		Thymus	
	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.
Males								
0	2.34	5.77	1.50	3.71	9.85	24.3	0.220	0.55
25	2.28	5.82	1.58	4.04	9.61	24.4	0.240	0.61
100	2.26	5.77	1.51	3.90	9.85	25.0	0.189	0.49
400	2.48	6.47**	1.45	3.79	9.65	25.2	0.206	0.54
Females								
0	1.42	6.51	1.05	4.79	5.00	22.9	0.146	0.67
25	1.49	6.77	1.06	4.81	5.38	24.4	0.183	0.83
100	1.47	6.56	1.09	4.87	5.35	23.8	0.196	0.88
400	1.62**	7.34**	1.16	5.27*	6.00**	27.1**	0.216*	0.97

[Statistics: ANOVA + Dunnett Tests, *p < 0.05 ** p < 0.01.]

The toxicological significance of the increase of liver weights in females is questionable since no changes in clinical chemistries ALP and SGPT and no histopathological changes in the liver were observed. No significant increases in male liver weights were observed.

The increase in the relative weight of the kidneys in male rats and in the absolute and relative weight of the kidneys in female rats are also of questionable toxicological significance since no changes in clinical chemistries, urinalysis, or histopathology suggest kidney pathology.

The observed increase in absolute weight of the thymus and the relative weight of the lungs in female rats of the top-dose group appear not to be associated

with the administration of the test material since the histological examination of these tissues did not reveal any abnormalities or variation that suggest thymus or lung pathology.

8. Macroscopic Pathology - Gross examination revealed no compound-related effects in the tissues examined.

Incidental findings included:

Male Rats

Small intestine	- pronounced Peyer plaques in one control rat and in one rat at 100 ppm dose level.
Kidney	- cyst in one rat at 25 ppm dose level.
Skin	- alopecia in three rats at 25 ppm dose level.
Cervical glands	- enlarged in one rat at 100 ppm dose level.
Liver	- prominent lobular pattern in one rat at 400 ppm dose level.

Female Rats

Skin	- alopecia in two rats at 100 ppm dose level and in one rat at 400 ppm dose level.
Abdominal cavity	- nodule in fat tissue in one control rat and in one rat at 25 ppm dose level.
Thymus	- suspected tumor in one rat at 25 ppm dose level.
Preputial gland	- cystic nodule in one rat at 400 ppm dose level.

9. Histologic Examination - The organs investigated histologically from the rats that received the test material exhibited no essential differences from controls. The presence of one thymoma tumor in the thymus of one low-dose female rat was observed.

Conclusions:

A sytsemic NOEL at 100 ppm is demonstrated.

LEL - increase in relative kidney weights in high-dose male and female rats (400 ppm). Increased absolute and relative liver weights in high-dose females (400 ppm).

Classification: Core-Minimum Study.

RIN 3394-95

IMAZALIL PEER REVIEW PACKAGE

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____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

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Pages 155 through 163 are not included.

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____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

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The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Attachment 8

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

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000057

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE: October 27. 1980

SUBJECT: Imazalil (active ingredient). Fungaflor (Imazalil - containing formulation) 1-(2-(2,4-dichlorophenil)-2-(2-propenylonxy)-ethyl) - 1H-imidazole. CASWELL NO. 497AB

FROM: Carlos A. Rodriguez
Toxicology Branch/HED (TS-769)

TO: Mr. Henry M. Jacoby, PM 21
Registration Division (TS-767)

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EPA File Symbol 43812-R

PP# OF2331 and Food Additive Petition OH5254

Petitioner: Janssen R & D, Inc.
501 George Street
New Brunswick, N.J. 08903

Action Requested

1. Applicant requests a tolerance of 10 ppm of Imazalil in whole citrus fruit and 0.2 ppm in the edible pulp.

2. Applicant requests the following food additive tolerances of Imazalil resulting from post-harvest treatment of citrus.

Dried Citrus Pulp	20 ppm
Citrus Oil	20 ppm

2. Application: In dips, wash tanks, and drenches, dilute with water to give a treating concentration of 500-700 ppm imazalil. (1:1364 dilution)

In non-recovery spray dilute with water to give a treating concentration of 1000 ppm imazalil. (1:681 dilution)

For water spray brush application, dilute with water to give a treating concentration of 1000 ppm imazalil. (1:681 dilution)

Foamer, dilute with ready to use detergent material to give a treating concentration of 2,000 ppm of imazalil. (1:341 dilution)

In citrus wax, dilute with citrus wax to give a treating concentration of 2000 ppm imazalil. (1:341 dilution)

E. Toxicity Studies for Imazalil submitted with Pesticide Petition - 8E2100, July 17, 1978 are the following:

Technical Product (Imazalil Free-Base

Study	Species	P.I., NEL, LD50 mg/kg, etc.	95% C.L. mg/kg.
Acute Oral	Rat (M)	320	253-405
Acute Dermal	Rat (M&F)	4,200 (M) 4,880 (F)	2,966-5,498 3,144-7,575
Acute Oral	Dog (M&F)	640	- -
Acute Inhalation (20% E.C.)	Rat (M&F)	>16 mg/l	- -
Eye Irritation (1,000 and 2,000 ppm)	Rabbit	slight irritation (use dilution)	
Eye Irritation (98%)	Rabbit	severe irritation	
6 months, 1&2 years feeding	Rat (M&F)	N.E.L. needs to be re-evaluated based on submission of additional data.	

Study	Species	P.I., NEL, LD50 mg/kg, etc.	95% C.L. mg/kg
3-Generation (5,20, & 80 mg/kg)	Rat	Reproduction performance not affected	
Feeding (4 weeks) (53,116,&120 g/animal/day)	Hen		Egg production & reproduction not affected.
Cumulative 4 week Oral (0,100,200 mg/kg)	Rat	NOEL = 200 mg/kg	
Mutagenic Studies (Dominant lethal) (0,10,40 & 160 mg/kg) - Male Mice (0,20 & 160 mg/kg) - Female Mice		NO LETHAL MUTATIONS INDUCED	
Acute Oral	Rat (M&F)	LD50 = 374(284-488) mg/kg	
Acute (I.P.)	Rat (M&F)	LD50 = 287 mg/kg (M) LD50 = 154 mg/kg (F)	
Oral Fertility (5,20 and 80 mg/kg)	Rat (M&F)	Fertility not affected	
2 Year Feeding Study	Dog (M&F)	NOEL = not determined, pending submission of additional information.	
Oral-3 Generation Study (0,5,20 and 80 mg/100g food)	Rat (F)	NOEL = 80 mg/kg	
Teratogenic and Embryotoxicity	Rat (F)	No teratogenic or embryotoxic effects	
Metabolism	Rat	No retention in fatty tissues, little tissue retention.	
Subacute Dermal (2 weeks) (40.160, and 640 mg/kg)	Rat	At 40 mg/kg - no effects; at 160mg/kg temporary irritation. At 640 mg/kg scale and crusts at the end of 2 wks; reversible after 2 weeks.	
Oral Dietary (14 weeks) (0,5,20 and 80 mg/100g food)	Rat	NOEL = 20 mg/100 g food	

Study	Species	LD50 mg/kg	95% C.L. mg/kg

Technical-Imazalil Sulphate - R 27180

Acute Oral — Rat (M&F) — LD50 = 550 (421-719) mg/kg.

Technical-Imazalil Nitrate

Acute Oral — Rat (M) — LD50 = 343 (262-448) mg/kg
(F) — LD50 = 288 (221-377) mg/kg.

F. Comparative Acute Oral Toxicity Studies in Rats Using Different Imazalil Salts, (Dept. of Pharmacology, Janssen Pharmaceutica, Belgium, May 1979).

Imazalil nitrate (R 18531)
Imazalil base (R 23979)
Imazalil sulphate (R 27180)
Imazalil acetate (R 47657)

~~120 male~~ and 120 female inbred Wistar rats weighing 254 ± 19 g (males) and 245 ± 21 g (females) were used in this study. The dose levels for each compound (160, 320, and 640 mg/kg) were administered orally by gavage as an aqueous suspension at a ~~volume~~ of 1 ml/100g body weight. For each dose 10 male and 10 female rats were used. The rats were observed at regular intervals during 14 consecutive days for signs of toxicity and mortality.

Results:

Imazalil nitrate (R 18531):

At 160 mg/kg - ataxia, piloerection, hypotonia and hypothermia in male and females; tremors, salivation, lacrimation and excitation in females only.

At 320 mg/kg - same symptoms as 160 mg/kg, plus exophthalmia, palpebral ptosis and loss of the righting reflex, convulsions, diuresis and hyperemia in females only.

At 640 mg/kg - same symptoms as 320 mg/kg, plus diarrhea in female only.

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Comment: These symptoms differ from original study in 1973. No symptoms or abnormal behavior were observed or reported in the 160 and 320 mg/kg dosages. Please explain these differences.

Imazalil base (R 23979)

At 160 mg/kg: ataxia, piloerection, hypotonia, hypothermia, tremors and excitation in both sexes.

At 320 mg/kg: same symptoms as 160 mg/kg dose level, plus salivation, lacrimation, diuresis, diarrhea, palpebral ptosis and loss of righting in both sexes; excitation in males only, and hyperemia in females only.

At 640 mg/kg: same symptoms as 320 mg/kg, plus exophthalmia and lacrimation in females only.

Comment: slight tremors and clonic seizures were reported in the 1974 study, however, these symptoms were not reported in the present study. Please, explain these differences.

Imazalil Sulfate (R 27180)

At 160 mg/kg: Ataxia, piloerection, hypotonia, exophthalmia and tremors in male and female.

At 320 mg/kg: same symptoms as 160 mg/kg, plus hypothermia, lacrimation, salivation, diarrhea, diuresis, palpebral ptosis, excitation, loss of righting reflex in both sexes, sedation in males only. At 640 mg/kg: same symptoms as the 320 mg/kg dose level.

Imazalil acetate: (R 47657)

At 160 mg/kg: ataxia, hypotonia, tremors in both sexes; exophthalmia, piloerection, diarrhea, diuresis, lacrimation, salivation, hypothermia, hyperemia, excitation and loss of the righting reflex in females only.

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At 320 mg/kg: same symptoms as 160 mg/kg dose level for both sexes; convulsions in females only.

At 640 mg/kg: same symptoms as 320 mg/kg dose level for both sexes; lacrimation and diuresis in females only.

The LD50 of the compounds tested were as follows: (Rat - male and female)

Imazalil nitrate: LD50 = 343 (262-448) mg/kg (M)
= 288 (221-297) mg/kg (F)

Imazalil base: LD50 = 343 (262-448) mg/kg (M)
= 227 (174-297) mg/kg (F)

Imazalil Sulfate: LD50 = 355 (272-464) mg/kg (M)
= 309 (237-404) mg/kg (F)

Imazalil acetate: LD50 = 371 (284-485) mg/kg (M)
= 309 (237-404) mg/kg (F)

TOX Category: II

Classification: Core-Minimum Study

G. Toxicity studies submitted with this petition:

1. Oral Carcinogenicity in Wistar Rats with Imazalil base (R 23979), (R. Marsboom, V. Hann, Jansseen Research Labs; Belgium).

Four hundred young healthy Wistar rats with an initial body weight of ± 400 g (males) and ± 300 g (females) and whose initial age varied between 3 and 4 months were used in this study. Fifty male and fifty female rats were fed 0,2.5,10 and 40 mg/kg/day of Imazalil for 24 months. The compound was admixed with the basic laboratory diet. The dosed animals received the test material as a wettable powder (consisting of : 25% Imazalil base, [redacted] for seven days a week for 24 months. The test material plus food was prepared fresh once every two weeks and kept at room temperature. All rats were examined daily for signs of waning health, abnormal behavior, unusual appearance, clinical effects, toxic and pharmacological responses and survival.

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"THIS INDENT INFORMATION IS NOT INCLUDE

Terminal studies:

At the termination of the study, a full necropsy was performed on all surviving animals. A full necropsy was also performed on all animals which died during the course of the study. Autopsy was performed as soon as possible after death or sacrifice and any macroscopic pathological changes noted.

2. Oral Carcinogenicity Study in Mice with Imazalil-sulfate R 27180, (R. Marsboom, V. Herin, Janssen Research Labs, Belgium).

Four hundred young healthy Albino Swiss mice (200 male + 200 female) with an initial body weight ranging between 20 and 25 g and whose initial age varied between 7 and 8 weeks were used in this study. The four hundred mice were divided into 4 dosage groups each consisting of 50 males and 50 females and given the test material orally in aqueous solution via the drinking water seven days each week during 18 consecutive months. Approximate doses of 0,2.5, 10 and 40 mg/kg body weight were given. The solutions used were prepared fresh each week. All animals were observed daily for signs of waning health, abnormal behavior, unusual appearance, clinical effects, toxic and pharmacological responses and survival.

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Terminal Studies:

At the termination of the study, a full necropsy was performed on all surviving animals. A full necropsy was also performed on all animals which died during the course of the study. Autopsy was performed as soon as possible after death or sacrifice and any macroscopic pathological changes were noted.

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Results:

Please refer to the attached paper, Subject: Additional Pathologic data to experiment "Imazalil Residue Tolerance Petition" in rats and mice by Louis Kasza, Pathologist, Toxicology Branch.

3. Micronucleus Test in Rats with Imazalil (R 23979), (R. Marshboon, Ph. Vanparys, Janssen Research Labs; Experiment No. 916, 12-18-79)

The purpose of this study is to assay for the induction of structural chromosome aberrations in bone marrow cells by the test compound Imazalil (R 23979) when administered to rats intraperitoneally. Male rats were dosed with 10,40 and 160 mg/kg of test material per kg body weight at 30 hours and 6 hours prior to preparation of the bone marrow. A total of 2,500 erythrocytes per animal were screened for the presence of micronuclei. Structural chromosome mutations were evaluated by the enumeration of the micronuclei in polychromatic and normo-chromatic erythrocytes.

A positive control group of male rats were dosed I.P. with 40 mg/kg cyclophosphamide.

Results:

The rate of micronucleated polychromatic cells in the negative control and three Imazalil R 23979 dosage groups are considered normal spontaneous rates.

The dosing of male rats in a positive control group with 40 mg/kg cyclophosphamide intraperitoneally led to an increase in the number of micronucleated erythrocytes as a result of the chromosome - breaking effect of cyclophosphamide.

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Conclusion:

The test material Imazalil R 23979 when given at 10,40 and 160 mg/kg intraperitoneally was considered non-mutagenic in rats under these tests conditions.

4. Acute Oral Toxicity in Rats with Imazalil 68% w/w formulation (C.J.E. Niemegeers, Janssen Research Labs., Belgium, August 1979).

90 Wistar rats, 45 males weighing 247 ± 17 g and 45 females weighing 235 ± 21 g were used in this study. Five males and five females were used per dosage level of 158, 199, 251, 316, 398, 502, 632, 795 and 1001 mg/kg. The compound was administered by gavage, as a single oral dose. Gross behavioral effects and the number of deaths were recorded at regular time intervals after the drug administration.

Results

LD50 = 252 mg/kg (M&F)
= 299 mg/kg (M)
= 214 mg/kg (F)

Gross behavioral effects: at all levels

Exophtalmos and piloerection. At 199 and above-Ataxia and loss of righting reflex, tremors, salivation, hypotonia hypothermia, palpebral ptosis, lacrimation and diarrhea.

5. Determination of the Acute Oral Toxicity of Imazalil EC 68% W/W in Rats, (Spanjers, H.P. Til, Janssen Pharmaceutica, Belgium, 6-14-79).

30 young adult albino rats (Wistar derived), 15 males and 15 females weighing from 126 to 213 g for males and females from 101 to 150 g were used in this study. The test material was administered by gavage to groups of 3 males and 3 females as a 7.35% (W/W) aqueous dilution in single doses of 5.0, 7.0, 9.8, 13.7 or 19.2 ml/kg body weight. After treatment the rats were observed for signs of intoxication

during a 14-day period. Autopsies were carried out on the survivors. The LD50 values after 24 hours and after 14 days were calculated by the method of Weil (Biometrics 8 (1952) 249-263).

Results:

LD50 = 286 (264-309) mg/kg

The rats showed sedation, tremors and convulsions within few hours after treatment.

In the two highest dose groups diarrhea was observed. Deaths occurred between 3 hours and 3 days after dosing. Macroscopic examination of the survivors did not reveal any treatment related gross alterations.

Classification: Core-Minimum Study

TOX Category: II

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6. Acute Dermal Toxicity (LD50) in Rabbits with Imazalil R 23979 (68% W/W), (R. Marsboom, Ch. van Ravestyn, Experiment No. 932, 12-14-79, Janssen Research Laboratories)

28 New Zealand White rabbits, 14 male and 14 female weighing 2.65 to 4.8 kg were used in this study. Four rabbits, 2 male and 2 female, were used per dose level of 2 ml/kg (placebo), 1.0,1.4,2.0,2.8,4.0, and 5.6 ml/kg of Imazalil formulation. Two rabbits assigned to each dose level were abraded. On the day of treatment the fur was closely clipped from the upper back area. The trunk or back of each rabbit was covered. At the end of the 24 hour exposure, the bandages were removed and the test site flushed with tap water and gently dried. Rabbits were observed daily for signs of toxicity, mortality, body weights for 14 days. Necropsies were performed.

Results:

LD50 = 2.0 ml/kg

Body weight normal at 1.0 and 1.4 ml/kg. At 2 ml and above, mortality prevented further recording.

The formulation caused erythema and edema ranging from moderate to severe. Observation during the second week revealed dry and/or hardening of skin in the treated area, crustforming eschar and exfoliation. In all cases the lesions were reversible.

Postmortem observations were generally the same at lower dosages, more pronounced at highest dose level showing a pale discoloration of the liver.

Classification: Core-Minimum Study
TOX Category: II

7. Acute Inhalation Toxicity in Rats with 20% Solution of Imazalil EC 68% W/W in Water, (L.M. Appelman, Report No. R 6126, Janssen Research Labs, June 20, 1979).

The acute inhalation toxicity of a 20% solution of Imazalil EC 68% W/W in water was studied in 5 male and 5 female rats by exposing them for a period of 4 hours to an atmosphere in which the material was finely dispersed at the maximum attainable concentration of 65 mg/m^3 of air. After the exposure period the animals were observed for 2-weeks for toxic signs, body weights, mortality and gross examination at autopsy .

Results:

After 4 hours exposure the animals were slow in their movements. No mortality occurred during the 4-hour exposure period and subsequent 14 day observation period.

Classification: Core-Minimum

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8. Ocular Irritation Study in Rabbits with Imazalil R 23979, (Experiment No. 895, 7-11-79, Marsoom, Ch. Van Ravestyn, Janssen Research Labs., Belgium).

0.1 ml of the test material was instilled into the lower conjunctival sac of one eye of each of six rabbits weighing about 4 kg. The opposite eye of each rabbit remained untreated and served as the control. Observations of ocular lesions were made at 1,2,3,4,5,6,7 and 10 days after instillation. Study was terminated at 10 days observation period.

Results:

Corneal opacity (Grade 2) in 6/6 animals was observed at 24 hours. The corneal opacity persisted throughout the study (Grade 4) was observed in 4/6 and (Grade 3) in 2/6 animals at the 10th day observation period. Due to corneal opacity no scoring could be done on the iris. Severe Conjunctival irritation persisted throughout the study.

Classification: ~~Core-Minimum~~ Study

TOX Category: I

9. Primary Skin Irritation in Rabbits with Imazalil R 23979, (Experiment No. 894, 7-10-79, Marboom, Ch. Van Ravestyn, Janssen Research Labs., Belgium).

0.5 ml of the test material was applied to the intact and abraded skin of six male albino rabbits weighing about 4 kg. The sample was introduced under a double gauze layer to an area of skin 1" x 1" square. The patches were then covered ~~and maintained~~ in contact with the skin for 24 hours. After 24 hours the patches were removed and the skin reactions evaluated.

Results:

Primary Irritation Index = 1.75. Very slight to well defined erythema and very slight edema were noted at 24 and 72 hours.

Classification: Core-Minimum Study.

TOX Category: III

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Conclusion:

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For reasons stated by Dr. Kasza, in his attached evaluation, the Oral Carcinogenicity Studies in Mice and Rats contain significant deficiencies. Information and data requested by Dr. Kasza must be addressed by the Registrant. An individual tabulation of lesions noted should be reported and separated according to whether they are neoplastic or non-neoplastic.

It is further noted that the Wistar rats used were + 400 and + 300 grams (M or F) (reportedly 3-4 months old) when started on test. The recommended age for rodents is "as soon as possible af[ter] weaning and acclimatization and preferably, before the ani[mals] are 6 weeks old". These animals were not within the recommended bracket and may account for the mortality rate observed and which could further obscure the oncogenic potential of the material tested. Nevertheless, the tumor incidences reported for rats and mice (and noted by Dr. Kasza) have been statistically evaluated by B. Litt.

Rat Study

Of the tumors noted by Dr. Kasza, only the incidence of hypophyseal tumors in treated rats appeared significantly different from control ($p < .05$). However, considering the age of the animals at the start (3-5 months) this incidence at termination (18-24 months) could be more related to the aging process in rats - as such, spontaneous tumor formation in the hypophysis is common in aging Wistar rats. Pituitary tumors may be found in association with ovarian abnormalities and spontaneous tumors of the breast - which was also observed in these animals. In this study, such rats would have been 23 to 29 months old. The time-related observance did not appear to differ from control animals; indicating that the compound did not stimulate or shorten the latent period for hypophyseal tumors.

Considering the nature and degree of the deficiencies/inadequacies of this study as well as the high mortality rate and poor health of all animals, a negative finding for oncogenicity in this strain of rat cannot be supported by the data presented. Therefore, another two-year rat oncogenicity study, properly designed and performed, must be submitted.

Mouse Study

Statistical evaluation of the incidence of lymphoma, histocytic lymphoma and thymoma did not indicate significant differences from control.

The same conclusions with regard to the negative finding for carcinogenic potential in this strain of mouse are equally true for this mouse study as for the rat study previously stated. Therefore, a separate mouse study, properly designed and performed, must be submitted.

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In addition, a rereview of the chronic toxicity studies (Rats and Dogs) reveals the following:

Rat 2-Year Chronic Feeding Study

1. No individual histopathology reports submitted. These are needed and should be supplied in a readable format.

2. There were only 10M and 10F rats per dose and with subsequent 40-70% mortality; 10 test animals were not autopsied at 24 months; and average of 60% (M) and 70% (F) of the test animals (not control) were autopsied at 20 months or longer.

Mortality

Dose	Male	Female
0	4/10	3/10
5	7/10	4/10
20	4/10	3/10
80	5/10	4/10

Why the high mortality and why no autopsy reports?

3. There was insufficient histopathology of suspected target organs. There was also no histopath of the spinal cord. Considering the severity of the acute toxicity symptoms (i.e. ataxia, hypotonia, clonic seizures, hypothermia, loss of righting reflex, tremors convulsions), more complete histopath of the CNS is needed. The clonic seizures, tremors and hypothermia are considered associated with a CNS disturbance.

I believe this is just the feeding part of the oncogenic study reported earlier in this paper on page 7. Thus the levels are really 0, 2.5, 5.0 and 40 mg/kg body wt/day.

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These acute effects, which were also noted in the 2-year dog study, were inadequately evaluated histopathologically for CNS effects. Specific areas of the CNS should have been sectioned, as well as the spinal cord. These were not performed. Why not? If these tissues are available they should be sectioned and evaluated by a pathologist.

Dog 2-Year Chronic Feeding

1. Individual gross and histopath reports were not submitted. These are needed and must be submitted in a readable format.

2. The values (clinical) for male and female dogs were pooled. This is an unacceptable practice and tends to average out the extremes in both cases. Therefore, the values obtained for males and females, per dose must be segregated and reported separately. Also, historical baseline data for the colony of dogs used must be submitted.

3. The dogs showed a declining body weight gain associated with dose for both male and female dogs with marginal effects noted at 1.25 mg/kg in females, but significant for males.

Males

Dose	Initial Weight	Final Weight	Av. Wt.	Gain
0	35.45	51.10	16 kg	5.3
1.25	35.00	48.00	13	4.3
5	34.30	43.65	9	3
20	36.40	40.90	4	1

Females

Dose	Initial Weight	Final Weight	Av. Wt.	Gain
0	30.00	43.45	13	4.3
1.25	30.40	42.55	12	4
5	30.50	36.80	6	2
20	32.10	35.40	3	1

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Examination of the study revealed that the dogs experienced emesis shortly after dose administration (gelatin capsule) and, therefore, the chemical interfered with proper dietary intake of nutrients since there was free access to food and water. Also, since the chemical caused emesis(not noted in controls) there is no adequate calculation as to the average daily dose received. It must be assumed that a dose smaller than that administered was received. Therefore, the 1.25 mg/kg dose is not an accurate reflection of the NEL. Also, an epileptic seizure was noted in 1 of 3 dogs at 5 mg/kg. This observation coincides with the CNS effects noted in rats from acute ingestion.

Again, as in the rat, there is inadequate evaluation of the CNS with emphasis on the specific areas of the brain associated with motor control and dopaminergic pathways.

Because of the decrease in body weight gain and emesis noted at all dose levels (other than control), 1.25 mg/kg cannot be considered a NEL for the dog 2-year feeding study.

Recommendations

Based on the above, the toxicity data are insufficient to support the requested tolerances; pending requested data, and resolution of the many deficiencies noted.

TS-769:RODRIGUEZ:slv:CM#2:RM.816:X73710:10/4/80

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Attachment 7,9

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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE: JAN 6 1982

SUBJECT: Imazalil, EPA Reg. No. 43813-R, PP #0F2331, 8E2100 and FAP #0H5254. Response to Company Submittal of Additional Data and Information.

FROM: R. Bruce Jaeger, Section Head
Review Section #1
Toxicology Branch/HED (TS-769)

TO: Henry M. Jacoby, PM-21
Registration Division (TS-769)

The data submitted by Janssen Pharmaceutica, Inc. to support registration of Imazalil have been reviewed by several persons which resulted in a meeting with Company Representatives 8/28/81. At that meeting, Janssen Pharmaceutica clarified some points and agreed to provide additional data to clarify the remaining discrepancies. The most recent submittal of data is information requested by Tox. branch at that 8/28/81 meeting. Some of this data was previously submitted and reviewed.

The following conclusions pertain to the total data package submitted to date with respect to registration of Imazalil.

1. 2-Year Dog Feeding Study

Janssen has presented convincing evidence that the decrease in body weight gain for the beagle dog is not significantly different from control ($p > .05$) at 1.25 mg/kg. Therefore, the body weight gain is satisfactorily resolved with the statistical presentation. The NOEL for this effect is considered to be 1.25 mg/kg.

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It must be recognized, and it has been stated in previous correspondence (both written and oral), that the 2 yr. chronic feeding study in dogs was presented in a "piece meal" manner which created some of the "discrepancies" that we have subsequently satisfactorily resolved. If all of the necessary data had been presented originally, much of the ensuing confusion could have been avoided. Tox. Branch remains of the opinion that the high dose group (20 mg/kg) cannot be considered useful for pathology, histopathology, etcetera because of poor or inadequate compound incorporation and uptake by those dogs on the high dose. Nonetheless, sufficient data have been presented to Tox. Branch to conclude that the NOEL for this study is 1.25 mg/kg with an LEL of 5 mg/kg for decreased body weight gain.

Classification: Core Minimum

2. Rat/Mouse Carginogenicity Studies

The individual animal data requested by Dr. L. Kasza as pertains to the rat and mouse carcinogenicity experiments have been submitted and reviewed. Tox. Branch concludes that there were no significant differences in the number, type or severity of lesions in any of the treatment groups versus the controls, in either experiment. There was also no significant effect on the lateny period for development of the lesions observed in the treatment groups versus the control (e.g. rat, < 18 month and > 18 months; mouse <18 months and at 18 months).

Janssen has presented information entitled "Life Expectancy, Wistar Rat" (Oct. 1981) which appears to support their contention that in short living rat strains, such as the Janssen Wistar substrain, tumors will appear earlier than in longer living strains. Toxicology Branch, nonetheless, contends that the most suitable strain of laboratory rat be used in carcinogenicity studies and that such a strain be exposed to test material for at least 2 years. Obviously, the Janssen Wistar substain does not live that long and geriatric effects only compound the interpretation of such data. Toxicology Branch therefore, recommends that future rat carcinogenicity studies utilize a strain of rat with sufficient longevity to allow for such a treatment schedule without complication by geriatrics effects.

Toxicology Branch does not believe Janssen has adequately addressed the point 2.H. in their 5/20/81 letter, e.g. use of 3-4 month old rats. Janssen has presented data (Life Expectancy - Wistar Rat, October 1981) which would indicate that carcinogenicity studies performed in their lab during the period 1976-1980 utilized rats which were 8 weeks old at the start of the study (see Table 2, September 1981 in reference above). This contradicts their previous

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statement that "at the start of the study (8/12/76) no recommendations for carcinogenicity studies existed. Since the official proposals were published, they only use animals of 6 weeks of age when starting a carcinogencity study. "Toxicology Branch wants these disparities clarified and again asks specifically why were 3-4 month rats used rather then their recommended starting age of 8 weeks?

Furthermore, with respect to their reply (5/20/81 letter) to item 2.H., life expectancy tables presented could argue against using older rats (i.e., older than 8 weeks) in that at 24 months their is 94.3% and 89.5% mortality in males and females respectively. These rats obviously can't live to 27 or 28 months as purported by Janssen. (only a few died)

Also, true lifetime tumorigenesis appears to occur in the Janssen Wistar substrain at 18-20 months for females and males, respectively. That being the case, animals should have been on test at an earlier age, but as such were exposed for only 14 months.

In the above discussion Toxicology Branch has tried to point out some of the deficiencies in the design and execution of the rat carcinogenicity study and would therefore, for those reasons, not encourage the same type of data to be submitted in the future.

Nonetheless, after carefully evaluating the data presented Toxicology Branch does not believe that imazalil is carcinogenic in the Janssen Wistar rat substrain or in the Albino Swiss Mouse at the highest dose tested, respectively. TB bases this conclussion on the reported number and frequency of lesions which occurred in the control and treatment groups.

TB requests that Janssen provide calculations for reputed compound intake of 2.5, 10 and 40 mg/kg body wt./day for both rats and mice. The Food Consumption tables (A1.1 - A1.24 Acc. #070089), do not lend themselves to such determinations and would indicate that males and females were not receiving the same amount of imazalil (on a mg/kg body wt. basis).

Classification (Both Studies): Core Minimum

3. 2-Year Chronic Rat Feeding Study

Toxicology branch has thoroughly evaluated all available data pertaining to the 2 year rat chronic feeding study and reiterates its previous conclusion that the study design and conduct are deficient in several areas.

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Tox Branch remains of the opinion that insufficient number of animals survival 24 months to provide meaningful interpretation (gross, histo, rel/abs. organ wt.) by themselves. There were too few male rats evaluated at the high dose (e.g. 2, 3, etc.). However, the 6 month, 12 month and 24 month data does provide same useful information in that there are significant relative organ weight increases for liver and kidney in the females at the high dose. These occurred at each time interval (e.g.6, 12, 24 months). There was also a significant increase in relative liver weight for males at the Mid- and High doses at 12 months; but insufficient number of animals examined at 24 months (also, relative organ weights were not determined or included, only absolute).

There were compound-related effects on the hypophysis in the female rats, both at 12 months and 24 months in the high dose group:

12 months: HD - 2/8 chromophobe tissue
Control 0/7 " "

24 month: HD - 5/5 chromophobe tissue
Control 0/6 " "
HD - 3/5 hyperplasia (adenoma)

Control 1/6 hyperplasia (adenoma)

[Note: there was no histopathology of the low- or mid-dose group animals].

There was also an apparent effect on the lung in the HD animals for reticuloendothelial sareoma (hyperplasia):

HD 4/12
Control 1/16

The company has still not supplied complete autopsy information. Animal number 191 (HD, male) was not subjected to histopathological examination and no indication of his fate. Please clarify.

In most instances the relative organ weights for thyroid and thymus were incorrectly presented. See Table at A6.67, A6.66, A 6.107, A6.27, A6.146 and A6.227. For example, the mean of 0.8638 and 0.1249 cannot be equal to 0.1056. The Company must please correct these tables.

Furthemore, TB requests the company please submit calculations and determinations of compound ingestion (daily) on a mg/kg body weight/ day basis.

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The study, as submitted is considered to be CORE: Supplementary data due to the deficiencies noted above. However, when considered in conjunction with the data provided in the 2 year rat oncogenicity study, sufficient pathology is provided to determine a NOEL for the study; by itself, the study will not support such a determination. The NOEL for the 2 yr. rat chronic feeding study is conservatively 5 mg/100 grams food with an LEL = 20 mg/100 grams food for liver effects (relative liver weight increase) in males and marginal effects in females. Similarly, at the HD there are effects on the hypophysis, kidneys and lungs.

Classification: Core Supplementary

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Attachment 10

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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Imazalil Reregistration; Reregistration Case No. 2325; Review of Reports Submitted by Registrant Regarding 1) Relevance of Liver Tumor Formation in Mice and 2) 3-Month Oral Mechanistic Toxicity Study in Mice

DP Barcode D203268
Case 816389
Submission S465342

Tox. Chem. No. 497AB
PC Code No. 111901
MRID No. 432024-01
432024-02
432226-01

FROM: Edwin R. Budd, Toxicologist
Review Section III
Toxicology Branch I
Health Effects Division (7509C)

Budd 7/29/94

TO: Kathryn Davis/Kathleen Depukat
Chemical Review Manager Team 52
Reregistration Branch
Special Review and Reregistration Division (7508W)

THRU: Karen Hamernik, Ph.D., Section Head
Review Section III
Toxicology Branch I
Health Effects Division (7509C)

K. Hamernik 8/9/94

Background:

The registrant of Imazalil, Janssen Pharmaceutica, recently submitted to EPA a carcinogenicity study in mice using Imazalil Base as the test material (MRID No. 429720-01). In this study, statistically significant increased incidences of hepatocytic neoplasms were observed in male and female mice (see memorandum from Edwin R. Budd, HED, to Kathryn Davis, SRRD, dated June 8, 1994). Because of the neoplastic response observed in this study, this chemical is being scheduled for review by the HED Cancer Peer Review Committee.

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Requested Action:

The registrant, on its own initiative, has how submitted for review the following additional information regarding the carcinogenicity of Imazalil in mice:

1) Safety Assessment of Imazalil (Enilconazole), Relevance of Liver Tumor Formation in Mice, Janssen Research Foundation, Report No. PPD-4, March 30, 1994 (MRID No. 432024-01), and

2) a 3-Month Oral Mechanistic Toxicity Study in Mice, Janssen Research Foundation, No. 3140/FK1600/FK1682, February, 1994 (MRID No. 432226-01 and 432024-02)

Conclusions:

1) Receipt of the first document (MRID No. 432024-01) by Toxicology Branch I is acknowledged. In this document, the registrant presented a position statement, based on the available toxicological information, on the human relevance of the liver tumor formation in mice. A brief summary of the statements in this document is presented in Attachment #1 to this memorandum. Information in this document will be considered, as appropriate, by the HED Cancer Peer Review Committee.

2) The second submission, a 3-month mechanistic study in mice using Imazalil Base as the test material (MRID No. 432226-01 and 432024-02), has been reviewed and a DER for this study is included in this memorandum. Information in this study also will be considered, as appropriate, by the HED Cancer Peer Review Committee. The Executive Summary for this study is presented below.

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

No treatment-related effects on mortality, clinical signs, body weights, body weight gains or food consumption were observed. At 200 ppm, the following treatment-related effects were observed: increased incidence of dark liver at gross necropsy in males, increased incidence and severity of "centrilobular clearer aspect" and of large and/or small vacuoles in the hepatocytes of males and females, increased liver microsomal protein in males and females, and increased microsomal cytochrome P450 content in males and females. At 600 ppm, the following treatment-related effects were observed: increased incidence of dark livers at gross necropsy in males and females, increased absolute liver weights and relative liver/body weight ratios in males and females, increased incidence and severity of "centrilobular clearer aspect" and of large and/or small vacuoles in the hepatocytes of males and females, increased individual cell necrosis in hepatocytes of males, increased diffuse swelling of hepatocytes in females, increased liver microsomal protein in males and females, and increased microsomal cytochrome P450 content in males and females. Electron microscopy revealed increased numbers of lipid droplets in hepatocytes, which corresponded with the increased vacuolization observed by light microscopy, and a morphologically changed rough endoplasmic reticulum (RER) in the hepatocytes of 600 ppm males and females. Regarding liver enzymatic activities of 7 P450 isoenzymes, dosing with Imazalil at 200 ppm and 600 ppm significantly induced certain enzymatic activities but also had an inhibitory effect on other metabolic activities. At 600 ppm, the total activity of testosterone hydroxylases was increased in both males and females. Low levels of Imazalil were detected in the serum of some males and females at 600 ppm only.

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

This study was not classified in accordance with the Core Grading System since it is a non-guideline study.

ATTACHMENT #1

Toxicological information referenced and/or discussed in the first document (MRID No. 432024-01) included the following (as interpreted by the registrant):

Carcinogenicity studies in mice

a. a first study (# 666, 1976/77) in which mice were given imazalil sulfate in the drinking water for up to 18 months at doses up to 100 ppm (40 mg/kg/day). Results in this study were said by the registrant to be negative for carcinogenicity.

b. a second study (# 2194, 1993) in which mice were given imazalil base in pelleted food for up to 23 months at doses up to 600 ppm (105 and 131 mg/kg/day in males and females, respectively). [This is the same study referred to on page 1 of this memorandum, i.e. MRID No. 429720-01.] In this study, significant increases in hepatocytic neoplasms were observed in males and females. The registrant again presented the liver tumor incidence data in this study and claimed the large majority of these tumors occurred late in the study and that there was no dose-related shortening of time to tumors. Further, liver tumor formation was observed only at doses at which non-neoplastic pathological changes were also observed in the liver (see mechanistic study below). Historical control data for liver tumors, including data for 2 new studies not previously presented, were also included.

Mechanistic studies in mice

a. an oral study (# 3140, 1994) in which mice were given imazalil base in pelleted food for up to 3 months at doses up to 600 ppm. [This study, MRID No. 432226-01), is reviewed in this memorandum.] In this study, which focused on effects of imazalil on mouse liver, hepatotoxicity was indicated by increased liver weight, increased serum alkaline phosphatase and/or increased alanine aminotransferase, histopathological changes in the liver and electron microscopic changes in hepatocytes. The registrant stated that the liver effects observed in this mechanistic study "indicate that 200 ppm represented the maximum tolerated dose level of imazalil with regard to hepatotoxicity in mice, and revealed that 600 ppm definitely exceeded the MTD (maximum tolerated dose), resulting in significant hepatocellular injury." (quoted from page 5 of the submission).

Liver enzyme induction studies in mice

a. Induction of xenobiotic metabolizing enzymes: a further study (# RO23979/FK1600, 1994) using microsomes derived from livers of mice in the above mechanistic study . [This study, MRID No. 432024-02,is reviewed in this memorandum.] The induction/inhibition of several xenobiotic metabolizing enzymes was assayed in this study. Dose-related increases in relative liver weight, microsomal protein and cytochrome P450 contents were observed. Induction of certain iso-enzyme activities representative of particular P450 gene subfamilies was observed, but inhibition of certain other iso-enzyme activities was also noted.

b. Effects on testosterone metabolism: a further study (# RO23979/FK1600, 1994) again using microsomes derived from livers of mice in the above mechanistic study to determine the possible effects of imazalil on regio- and stereo-selective hydroxylations of testosterone. [This study, MRID No. 432024-02, is also reviewed in this memorandum.) In this study, testosterone catabolism was enhanced and hydroxylated metabolites of testosterone increased 1.8 and 2.0 fold in male and female mice, respectively.

Genotoxic potential studies

"Imazalil and/or its metabolites were devoid of genotoxic potential, when investigated for induction of primary DNA-damage, gene mutations and chromosome aberrations in a variety of in vitro and in vivo test systems." (quoted from page 6 of the submission).

The following "Conclusion and Assessment" and "References" was copied from pages 6 and 7 of the submission.

Conclusion and Assessment

There is abundant experimental evidence to suggest that non-genotoxic liver enzyme inducers may increase the incidence of hepatocytic tumors in rodents after long-term administration of maximum tolerated and higher doses (Newberne et al., 1987). Formation of liver tumors appears to be closely related to liver enlargement; it does not occur at dose levels or exposures where the hepatomegaly is minimal or absent . For the mouse, in particular, there is a strong association between liver hypertrophy and the development of liver neoplasia (Butterworth, 1990; Grasso et al., 1991). These events have been linked to proliferative stimuli inducing enhanced replicative DNA-synthesis of "resting" hepatocytes (Goodman et al., 1991). The mitogenic potential is stimulated further by cytotoxicity and liver cell necrosis which, by increasing cell turnover, may give rise to compensatory hyperplasia terminating in neoplasia (Grasso and Hinton, 1991).

Imazalil preferentially and significantly increased the incidence of benign liver tumors (hepatic neoplastic nodules) in male mice at 200 and 600 ppm, and in female mice at 600 ppm. Survival was not affected by tumor development and the tumors occurred only towards the end of the study period. The dose level of 50 ppm, corresponding to a daily intake of 8 mg/kg body weight in males and 10 mg/kg body weight in females, was a clear NOAEL.

Mechanistic studies conducted at the same dietary dose levels for one and three months, identified 200 ppm as the maximum tolerated dose. At 200 ppm liver enlargement and microsomal enzyme induction occurred, accompanied by histological alteration of hepatocytes. At 600 ppm, hepatotoxicity was pronounced and all parameters measured suggested that this dose level exceeded the capacity of the hepatocytes to effectively eliminate or detoxify the chemical. There was evidence of accumulation of imazalil in liver microsomes and inhibition of initially induced P450-dependent enzyme activities resulted.

In mice, the toxicological data indicate a NOAEL at 50 ppm for hepatic effects and an MTD for liver neoplasia at 200 ppm. At 600 ppm, excessive or pronounced hepatotoxicity is exerted. Negative results were obtained in a spectrum of short-term mutagenicity tests. A non-genotoxic threshold mechanism is postulated to operate for the mouse liver tumors and there is no evidence that these effects are of human relevance. Therefore, for the quantification of human risk, it is appropriate to use a safety factor approach, and to base the factor to be determined on all the safety data available.

References

Butterworth, B.E. (1990). Consideration of both genotoxic and nongenotoxic mechanisms in predicting carcinogenic potential. Mutation Res. 293, 117-132.

Goodman, J.I., Ward, J.M., Popp, J.A., Klaunig, J.E., and Fox, T.R. (1991). Symposium overview: Mouse liver carcinogenesis: Mechanisms and relevance. Fundam. Appl. Toxicol. 17, 651-665.

Grasso, P., and Hinton, R.H. (1991). Evidence for and possible mechanisms of non-genotoxic carcinogens in rodent liver. Mutation Res. 248, 271-290.

Grasso, P., Sharratt, M., and Cohen, A.J. (1991). Role of persistent, non-genotoxic tissue damage in rodent cancer and relevance to humans. Annu. Rev. Pharmacol. Toxicol. 31, 253-287.

Lubet, R.A., Nims, R.W., Ward, J.M., Rice, J.M., and Diwan, B.A. (1989). Induction of cytochrome P450b and its relationship to liver tumor promotion. J. Amer. Coll. Toxicol. 8, 259-268.

Newberne, P.M., Suphakarn, V., Punyarit, P., and de Camargo, J. (1987). Nongenotoxic mouse liver carcinogens. In: Banbury Report 25: Nongenotoxic Mechanisms in Carcinogenesis. Cold Spring Harbor Laboratory, pp.165-178.

Reviewed by: Edwin R. Budd, M.A. Budd 7/29/94
Review Section III, Toxicology Branch I (7509C)
Secondary Reviewer: Karen Hamernik, Ph.D., Section Head
Review Section III, Toxicology Branch I (7509C)

DATA EVALUATION REPORT

Study Type: 3-Month Oral Mechanistic Toxicity Study, Mice (Non-Guideline Study)

Test Material: Imazalil Base (R 23979), Technical Grade

Tox. Chem. No. 497AB
PC Code No. 111901

MRID No.: 432226-01 (PART 1)
432024-02 (PART 2)
432024-02 (PART 3)
432024-02 (PART 4)

Testing Facility: Janssen Research Foundation
Beerse, Belgium

Sponsor: Janssen Pharmaceutica N.V.
Beerse, Belgium

PART 1 Three-month oral mechanistic toxicity study with one month interim sacrifice in SPF Albino Swiss mice with imazalil base (R 23979); Experiment No. 3140: February 18, 1994; Reported by Department of Toxicology; K. Van Deun, D.V.M. (Study Director)

PART 2 Study on the possible induction and/or inhibition of hepatic drug metabolizing enzymes by imazalil in male and female SPF Albino Swiss mice, after oral administration through the food for one and three consecutive months; Report No. R 23979/FK1600; February 11, 1994; Reported by Department of Drug Metabolism and Pharmacokinetics; M. Vermeir (Study Director)

PART 3 Effects of three months of imazalil administration on mouse liver microsomal protein and cytochrome P450 content, and testosterone metabolism; Report No. R 23979/FK1600; February, 1994; Reported by Department of Comparative Biochemistry; Gustaaf Willemsens (Principal Investigator)

PART 4 Toxicokinetics of imazalil (R 23979) in SPF Albino Swiss mice at the end of a 3-month oral mechanistic toxicity study (Exp. No. 3140) with an imazalil-

medicated diet at intended dose levels of 10, 40 or 120 mg/kg/day; Study No. R 23979/FK 1682; December 9, 1993; Reported by Department of Drug Metabolism and Pharmacokinetics; P. Sterkens (Study Director)

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

No treatment-related effects on mortality, clinical signs, body weights, body weight gains or food consumption were observed. At 200 ppm, the following treatment-related effects were observed: increased incidence of dark liver at gross necropsy in males, increased incidence and severity of "centrilobular clearer aspect" and of large and/or small vacuoles in the hepatocytes of males and females, increased liver microsomal protein in males and females, and increased microsomal cytochrome P450 content in males and females. At 600 ppm, the following treatment-related effects were observed: increased incidence of dark livers at gross necropsy in males and females, increased absolute liver weights and relative liver/body weight ratios in males and females, increased incidence and severity of "centrilobular clearer aspect" and of large and/or small vacuoles in the hepatocytes of males and females, increased individual cell necrosis in hepatocytes of males, increased diffuse swelling of hepatocytes in females, increased liver microsomal protein in males and females, and increased microsomal cytochrome P450 content in males and females. Electron microscopy revealed increased numbers of lipid droplets in hepatocytes, which corresponded with the increased vacuolization observed by light microscopy, and a morphologically changed rough endoplasmic reticulum (RER) in the hepatocytes of 600 ppm males and females. Regarding liver enzymatic activities of 7 P450 isoenzymes, dosing with Imazalil at 200 ppm and 600 ppm significantly induced certain enzymatic activities but also had an inhibitory effect on other metabolic activities. At 600 ppm, the total activity of testosterone hydroxylases was increased in both males and females. Low levels of Imazalil were detected in the serum of some males and females at 600 ppm only.

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

This study was not classified in accordance with the Core Grading System since it is a non-guideline study.

PART 1

A. Purpose of Study: "The purpose of this experiment was to study the potential effects of imazalil base on liver- morphology, -enzymes and -metabolism when administered daily orally to SPF Albino Swiss mice for a period of one and three months." (quoted from page 13 of the study report)

B. Test Material: Imazalil Base (R 23979), technical grade.

Batch no. ZR023979G3A561, from Janssen chemical manufacturing plant No. 3, purity not stated, Premix Batch no. GGI-141.

C. Test Animals: Mice, SPF Albino Swiss, males and females.

Description: from Charles River, France; approximately 10 day acclimation period; approximately 5-6 weeks old at initiation of dosing; males weighed 29 ± 1 g and females weighed 22 ± 1 g.

Environment: individual housing in macrolon cages; temperature, humidity, air changes and photoperiod were monitored (but not reported)

D. Study Design: Animals were assigned to treatment groups as shown below. Animals were fed control or treated pelleted diet for 3 months; interim kill animals (15/sex/group) were sacrificed at 1 month. Feeding with test material was initiated on September 13, 1993. The 1 month interim sacrifice was on October 11-12, 1993 and the 3 month terminal sacrifice was on December 13-16, 1993.

Dose Level of Imazalil (ppm)	3-Month Sacrifice Males	3-Month Sacrifice Females	1-Month Sacrifice Males	1-Month Sacrifice Females
0 (veh cont)[1]	25	25	15	15
50	25	25	15	15
200	25	25	15	15
600	25	25	15	15

[1] 600 ppm of unmedicated premix (containing equal volumes [redacted]

Dose levels employed in this mechanistic study, the method of diet preparation and all other experimental parameters, as much as possible, were identical to those employed in a

previously conducted 23-Month carcinogenicity study in mice at the same laboratory (MRID No. 429720-01).

At the 1 month interim sacrifice, 10/sex/group were subjected to gross necropsy, organ weight determinations and histopathological examination (PART 1) and also to the study designed to assay liver enzymatic activities of several P450 isoenzymes (PART 2). The additional 5/sex/group were used to examine the influence of Imazalil on testosterone metabolism in the liver (PART 3). At the 3 month sacrifice, 20/sex/group were subjected to the PART 1 procedures, including 10/sex/group which were subjected to the PART 2 procedures, and the additional 5/sex/group were subjected to the PART 3 procedures. In addition, at 3 months, serum samples were analyzed for Imazalil concentration (PART 4).

E. Diet Preparation and Achieved Dosages: Test material was incorporated into the feed as a 50% premix (50% imazalil and 50% of a mixture of equal parts [redacted]. Basic diet was Huybrechts powdered rodent feed. Diets were then pelleted. Fresh diet was prepared once every month. Homogeneity and stability analyses were satisfactory.

Concentration Analyses: Concentrations of test material in the powdered feed were satisfactory. After pelleting, however, concentrations of test material in the pellets averaged about 83% of the nominal concentration. Accordingly, in calculations for intake of test material (see below), an adjustment of -17% was made by this reviewer to account for this finding.

Intake of Test Material: For animals sacrificed at 1 month, the intake of test material was calculated to be 11.1, 44.0 and 134 mg/kg/day for males and 13.6, 53.5 and 165 mg/kg/day for females for the 50, 200 and 600 ppm groups respectively. For animals sacrificed at 3 months, the intake of test material was calculated to be 9.5, 38.6 and 115 mg/kg/day for males and 11.3, 45.6 and 138 mg/kg/day for females for the 50, 200 and 600 ppm groups respectively.

F. Quality Assurance and GLP Compliance: Signed statements were included in the study report.

G. Observation and Results:

1. Mortality and Clinical Signs: No mortalities or relevant signs of toxicity were observed in the male or female animals given Imazalil for up to 3 months in the diet.

2. Body Weights and Body Weight Gains: No relevant differences in body weights or body weight gains were observed between male or female control animals and animals of the same sex given Imazalil during the 3 month duration of this study.

3. Food Consumption: Considerable food wastage in all groups, particularly in the Imazalil treated groups, obscured food consumption data. Considering the highly variable wastage, no obvious differences in food consumption between control groups and groups given Imazalil were observed for either male or female animals during the 3 month study.

4. Clinical Chemistries: The following 3 clinical chemistry determinations were made on all 15 animals sacrificed at 1 month and on all 25 animals sacrificed at 3 months: alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase. Results are presented in Table 1. Although alkaline phosphatase was significantly increased in 600 ppm males ($p < 0.001$) at 1 month, it was not significantly increased in females at 1 month or in either sex at 3 months. Although alanine aminotransferase was significantly increased in 600 ppm males ($p < 0.05$) and in 600 ppm females ($p < 0.01$) at 1 month and in 600 ppm males ($p < 0.001$) at 3 months, the increases were very slight and not impressive. The increased liver enzyme values at 600 ppm in both males and females are not clearly treatment related.

5. Gross Necropsy: Gross necropsies were conducted on 10 mice/sex/group at 1 month and on 20 mice/sex/group at 3 months. Results are presented in Table 2. A statistically significant increased incidence of dark livers was observed in 600 ppm males ($p < 0.001$) and in 600 ppm females ($P < 0.01$) at 1 month and in 600 ppm males ($p < 0.001$) and in 600 ppm females ($p < 0.001$) at 3 months. In addition, it is likely that a dark liver observed in one 200 ppm male (not significant) at 3 months was treatment related. The increased incidences of dark livers at 200 ppm in males and at 600 ppm in both males and females are considered to be treatment related.

6. Organ Weights and Organ/Body Weight Ratios: Mean organ weights and organ/body weight ratios were determined for the following organs on 10 mice/sex/group at 1 month and on 20 mice/sex group at 3 months: lungs, spleen, liver, heart, pancreas, kidneys, brain, thymus, adrenals and gonads. Results are presented Table 3. Significantly increased absolute liver weights and

liver/body weight ratios were consistently observed in 600 ppm males and females at 1 month and at 3 months. The increased liver weights and liver/body weight ratios at 600 ppm in both males and females are considered to be treatment related.

7. Histopathology: The only organs examined by light microscopy were the gall bladder and liver (10/sex/group at 1 month and 20/sex/group at 3 months). In addition to incidence data, observed lesions were graded on a scale from 0 (no change) to 5 (severe change). Results are presented in TABLE 4. No treatment related effects were observed for gall bladder. For liver, increased incidences of "centrilobular clearer aspect" were frequently observed in 200 ppm and 600 ppm males and females at 1 month and at 3 months. Mean severity scores were similarly increased (oftentimes statistically significant). Large and/or small vacuoles were also frequently observed in the hepatocytes of 200 ppm and 600 ppm males and females at 1 month and at 3 months. Mean severity scores were again similarly increased (usually statistically significant). Other probably treatment related liver lesions were increased individual cell necrosis in 600 ppm males at 3 months and diffuse hepatocytic swelling in 600 ppm females at 3 months. The above liver lesions at 200 and 600 ppm in both males and females were considered to be treatment related.

8. Electron Microscopy of Liver Cells: From the animals sacrificed at 1 month, the livers of one control and one 600 ppm male and one control and one 600 ppm female were prepared and examined by electron microscopy. The results indicated for the 600 ppm male and female an increased number of lipid droplets in the hepatocytes, particularly in the periportal areas. This increase corresponded with the increase in hepatocytic vacuolation previously described by light microscopy. In addition, for hepatocytes showing a large number of lipid droplets, the morphology of the rough endoplasmic reticulum (RER) in these cells was different from that in control animals. Rather than the RER being arranged in parallel stacks of cisternae (normal architecture), the RER in these cells appeared as small vesicles diffusely spread over the cytosol.

TABLE 1: Mean Clinical Chemistry Values for Mice Given Imazalil in the Diet for 1 Month or 3 Months [1]

1 Month

Parameter	Dosage group (ppm) Males Vehicle	50	200	600	Females Vehicle	50	200	600
ALP: Alkal. phosphatase U/l	91	95	94	123 ***	122	117	119	139
AST: Aspartate aminotr. U/l	83	84	96	88	98	110	113	83
ALT: Alanine aminotran. U/l	33	33	35	38 *	30	35	33	38 **

Significance computed by Mann-Whitney U test (two tailed) : * P < .05 ** P < .01 *** P < .001

3 Months

Parameter	Dosage group (ppm) Males Vehicle	50	200	600	Females Vehicle	50	200	600
ALP: Alkal. phosphatase U/l	58	53	57	65	86	102	83	79
AST: Aspartate aminotr. U/l	90	121	95	95	115	124	114	135
ALT: Alanine aminotran. U/l	50	52	39	52 ***	36	39	36	45

Significance computed by Mann-Whitney U test (two tailed) : * P < .05 ** P < .01 *** P < .001

[1] Data copied from pages 43 and 44 of Study Report 3140

TABLE 2: Gross Necropsy Observations on Mice Given Imazalil in the Diet for 1 Month or 3 Months [1]

1 Month, Males

Organ or tissue : observation	Males			
	Dosage group (ppm)			
	Vehicle	50	200	600
	X/ N	X/ N	X/ N	X/ N
Kidney : dilated	2/10	0/10	4/10	3/10
Liver : dark	0/10	0/10	0/10	9/10***
Liver : more pronounced lobulation	0/10	0/10	0/10	1/10
Lung : aspiration bleedings	0/10	0/10	1/10	0/10
Testis : small	0/10	0/10	0/10	1/10
Urinary bladder (content) : mucus	0/10	1/10	1/10	0/10

Significance computed by Chi square test (two tailed) : * P < .05 ** P < .01 *** P < .001

X : Number of positive animals
N : Total number of animals

1 Month, Females

Organ or tissue : observation	Females			
	Dosage group (ppm)			
	Vehicle	50	200	600
	X/ N	X/ N	X/ N	X/ N
Kidney : dilated	1/10	0/10	0/10	0/10
Kidney : small	0/10	0/10	0/10	1/10
Liver : dark	0/10	0/10	0/10	8/10**
Liver : more pronounced lobulation	0/10	0/10	1/10	0/10
Ovary : cyst	1/10	2/10	1/10	3/10
Ovary : cyst, hemorrhagic, +	1/10	1/10	0/10	0/10
Pancreas : small	0/10	1/10	0/10	0/10
Spleen : swollen	1/10	0/10	0/10	0/10
Uterus : swollen	1/10	2/10	2/10	2/10
Uterus : swollen, content, watery, +	0/10	1/10	1/10	0/10

Significance computed by Chi square test (two tailed) : * P < .05 ** P < .01 *** P < .001

+ This observation is already included in the major observation of this tissue

X : Number of positive animals
N : Total number of animals

[1] Data copied from pages 53 and 54 of Study Report 3140

TABLE 2 (Continued): Gross Necropsy Observations on Mice Given Imazalil in the Diet for 1 Month or 3 Months [1]

3 Months, Males

Organ or tissue : observation	Dosage group (ppm)			
	Vehicle	50	200	600
	X/ N	X/ N	X/ N	X/ N
General : obesitas	1/20	0/20	0/20	0/20
Heart : dilated	1/20	0/20	0/20	0/20
Kidney : changed surface, rough	0/20	0/20	1/20	0/20
Kidney : dilated	5/20	4/20	2/20	5/20
Kidney : swollen	0/20	0/20	1/20	1/20
Liver : dark	0/20	0/20	1/20	11/20***
Liver : more pronounced lobulation	0/20	0/20	0/20	1/20
Liver : swollen	0/20	0/20	0/20	1/20
Lung : aspiration bleedings	2/20	0/20	1/20	0/20
Lung : nodule, white	0/20	0/20	1/20	0/20
Spleen : small	0/20	1/20	0/20	1/20
Testis : focus, hemorrhagic	0/20	1/20	0/20	0/20
Ureter : dilated	0/20	0/20	1/20	0/20
Urinary bladder (content) : mucus	2/20	0/20	0/20	0/20
Urinary bladder : content, mucus	1/20	1/20	0/20	1/20

3 Months, Females

Organ or tissue : observation	Dosage group (ppm)			
	Vehicle	50	200	600
	X/ N	X/ N	X/ N	X/ N
Abdominal mesothelia : nodule, hemorrhagic	0/20	1/20	0/20	0/20
Adrenal gland : cyst, hemorrhagic	0/20	1/20	0/20	0/20
Adrenal gland : swollen	0/20	1/20	0/20	0/20
Kidney : dilated	0/20	0/20	0/20	1/20
Liver : dark	0/20	0/20	0/20	12/20***
Liver : more pronounced lobulation	0/20	0/20	0/20	1/20
Lung : aspiration bleedings	0/20	0/20	1/20	1/20
Lung : nodule, white	0/20	1/20	0/20	0/20
Lymph node(s) : swollen	1/20	1/20	0/20	0/20
Ovary : cyst	2/20	4/20	5/20	5/20
Ovary : cyst, hemorrhagic, +	1/20	2/20	2/20	0/20
Ovary : swollen	0/20	1/20	0/20	0/20
Spleen : swollen	0/20	1/20	0/20	0/20
Uterus : swollen	1/20	1/20	4/20	4/20
Uterus : swollen, content, watery, +	1/20	0/20	0/20	2/20

Significance computed by Chi square test (two tailed) : * P < .05 ** P < .01 *** P < .001

+ This observation is already included in the major observation of this tissue

X : Number of positive animals
N : Total number of animals

[1] Data copied from pages 55 and 56 of Study Report 3140

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TABLE 3 (Continued): Mean Organ Weights and Organ/Body Weight Ratios for Mice Given Imazalil in the Diet for 1 Month or 3 Months [1]

3 Months

Parameter		Dosage group (ppm) Males Vehicle	50	200	600	Females Vehicle	50	200	600
WGT: Body weight	g	44	42	42	42	29	30	30	30
LNG: Lungs	mg	329	326	337	315	264	271	271	275
	mg / 100 g	755	769	798	751	914	919	916	921
SPL: Spleen	mg	109	109	109	104	112	124	111	114
	mg / 100 g	250	257	258	248	387	420	375	382
LIV: Liver	mg	2362	2201 *	2307	2697 ***	1551	1561	1585	1784 **
	mg / 100 g	5399	5192	5487	6413 ***	5347	5266	5332	5968 **
HRT: Heart	mg	193	193	199	196	150	151	149	156
	mg / 100 g	442	458	473 **	466	520	513	505	523
PNC: Pancreas	mg	444	448	436	422	389	388	391	373
	mg / 100 g	1020	1060	1036	1004	1347	1309	1314	1248 *
KDN: Kidneys	mg	675	672	704	672	390	406	413	417
	mg / 100 g	1546	1590	1672 *	1600	1354	1375	1397	1396
BRN: Brain	mg	504	507	508	509	499	496	511	512
	mg / 100 g	1158	1201	1210	1215	1739	1686	1731	1717
THY: Thymus	mg	42	40	36 *	37 *	38	38	39	40
	mg / 100 g	97	92	86 *	88	132	129	133	135
ADR: Adrenals	mg	8	7	7	7	12	13	13	12
	mg / 100 g	18	17	17	18	42	45	43	40
GON: Gonads	mg	259	274	279	271	47	49	48	44
	mg / 100 g	596	649	664	645	165	166	161	148

Significance computed by Mann-Whitney U test (two tailed) : * P < .05 ** P < .01 *** P < .001

[1] Data copied from page 49 of Study Report 3140

TABLE 4: Selected Liver Histopathology for Mice Given Imazalil in the Diet for 1 Month or 3 Months

1 Month, Males

Observation	Control	50 ppm	200 ppm	600 ppm
No. examined	10	10	10	10
Reticuloendothelial system aggregates	3[1] (0.30)[2]	2 (0.20)	2 (0.20)	3 (0.30)
Centrilobular clearer aspect	1 (0.10)	2 (0.20)	4 (0.40)	10 (1.00)***
Diffuse hepato swelling	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Focal necrosis (hepatocytes)	2 (0.20)	0 (0.00)	1 (0.10)	3 (0.40)
Individual cell necrosis	0 (0.00)	2 (0.20)	0 (0.00)	1 (0.10)
Large vacuoles (hepatocytes)	0 (0.00)	0 (0.00)	0 (0.00)	6 (0.60)**
Prominent Kupffer cells	2 (0.20)	2 (0.20)	1 (0.10)	1 (0.10)
Small vacuoles (hepatocytes)	0 (0.00)	0 (0.00)	8 (0.80)***	10 (1.00)***

[1] Incidence (no. with effect/no. exam.); not statistically analyzed

[2] Mean severity score (max. score = 5; based on all animals examined); statistically analyzed

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

TABLE 4 (Continued): Selected Liver Histopathology for Mice Given Imazalil in the Diet for 1 Month or 3 Months

1 Month, Females

Observation	Control	50 ppm	200 ppm	600 ppm
No. examined	10	10	10	10
Reticuloendothelial system aggregates	2[1] (0.20)[2]	2 (0.20)	4 (0.50)	0 (0.00)
Centrilobular clearer aspect	2 (0.20)	1 (0.10)	8 (0.80)**	7 (0.70)*
Diffuse hepato swelling	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Focal necrosis (hepatocytes)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Individual cell necrosis	1 (0.10)	1 (0.10)	0 (0.00)	0 (0.00)
Large vacuoles (hepatocytes)	0 (0.00)	0 (0.00)	0 (0.00)	3 (0.30)
Prominent Kupffer cells	3 (0.30)	1 (0.10)	3 (0.30)	5 (0.50)
Small vacuoles (hepatocytes)	5 (0.50)	3 (0.30)	8 (1.00)	10 (1.70)**

[1] Incidence (no. with effect/no. exam.); not statistically analyzed

[2] Mean severity score (max. score = 5; based on all animals examined); statistically analyzed

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

TABLE 4 (Continued): Selected Liver Histopathology for Mice Given Imazalil in the Diet for 1 Month or 3 Months

3 Months, Males

Observation	Control	50 ppm	200 ppm	600 ppm
No. examined	20	20	20	20
Reticuloendothelial system aggregates	8[1] (0.40)[2]	13 (0.65)	14 (0.75)*	13 (0.65)
Centrilobular clearer aspect	8 (0.40)	10 (0.50)	8 (0.40)	14 (0.70)
Diffuse hepato swelling	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.05)
Focal necrosis (hepatocytes)	0 (0.00)	1 (0.05)	0 (0.00)	2 (0.10)
Individual cell necrosis	0 (0.00)	3 (0.15)	3 (0.15)	7 (0.35)**
Large vacuoles (hepatocytes)	1 (0.05)	0 (0.00)	7 (0.50)*	14 (1.05)***
Prominent Kupffer cells	0 (0.00)	4 (0.20)*	3 (0.15)	4 (0.20)*
Small vacuoles (hepatocytes)	4 (0.20)	6 (0.30)	15 (0.80)***	20 (1.70)***

[1] Incidence (no. with effect/no. exam.); not statistically analyzed

[2] Mean severity score (max. score = 5; based on all animals examined); statistically analyzed

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

TABLE 4 (Continued): Selected Liver Histopathology for Mice Given Imazalil in the Diet for 1 Month or 3 Months

3 Months, Females

Observation	Control	50 ppm	200 ppm	600 ppm
No. examined	20	20	20	20
Reticuloendothelial system aggregates	13[1] (0.65)[2]	9 (0.45)	8 (0.40)	7 (0.35)
Centrilobular clearer aspect	0 (0.00)	1 (0.05)	4 (0.20)*	11 (0.55)***
Diffuse hepato swelling	0 (0.00)	0 (0.00)	0 (0.00)	3 (0.15)
Focal necrosis (hepatocytes)	1 (0.05)	2 (0.10)	1 (0.10)	0 (0.00)
Individual cell necrosis	0 (0.00)	0 (0.00)	2 (0.10)	1 (0.05)
Large vacuoles (hepatocytes)	0 (0.00)	0 (0.00)	6 (0.30)**	15 (1.05)***
Prominent Kupffer cells	3 (0.15)	6 (0.30)	2 (0.10)	1 (0.05)
Small vacuoles (hepatocytes)	14 (0.70)	17 (0.90)	16 (0.80)	20 (1.65)***

[1] Incidence (no. with effect/no. exam.); not statistically analyzed

[2] Mean severity score (max. score = 5; based on all animals examined); statistically analyzed

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

PART 2 (Liver Enzymatic Assays)

A. Materials and Methods:

1. Samples of livers from male and female mice from the PART 1 1-month interim sacrifice (10/sex/group) and the 3-month terminal sacrifice (10/sex/group) were collected and stored frozen until used for the preparation of microsomal suspensions by standard procedures (described on pages 9-10 of the study report). Liver samples from 2 mice were pooled in order to prepare 5 microsomal suspensions per group.

2. The following analytical procedures were subsequently performed on each microsomal suspension (details described on pages 10-15 of the study report). Appropriate assay validation procedures using microsomal preparations from non-induced rats and from rats pretreated with inducers belonging to specific cytochrome P-450 inducer classes were performed concurrently for each analytical procedure.

   a. microsomal protein content
   b. cytochrome P-450 content
   c. microsomal enzymatic activities
      - aniline hydroxylation
      - N-demethylation of N-ethylmorphine
      - 7-ethoxyresorufin O-deethylation
      - 7-pentoxyresorufin O-dealkylation
      - lauric acid hydroxylation
      - 7-ethoxycoumarin O-deethylation
      - UDP-glucuronosyltransferase towards 4-nitrophenol

B. Results: The results for mice sacrificed at 1 month are presented in TABLE 5 and for mice sacrificed at 3 months in TABLE 6 (both tables copied from the study report). The following was also copied from the Abstract for PART 2 (page 1 of Report no. R 23979/FK1600).

> **The relative liver weight and the hepatic protein and cytochrome P-450 contents were significantly increased in a dose-dependent way in both male and female mice after one and three months treatment with imazalil. Treatment with imazalil for one month also induced the *N*-ethylmorphine *N*-demethylase and 7-pentoxyresorufin *O*-dealkylase activities, whereas the aniline hydroxylation and 7-ethoxyresorufin *O*-deethylation were decreased in both male and female mice. After dosing for three months, the *N*-ethylmorphine *N*-demethylation was virtually not affected, and the aniline hydroxylation, 7-pentoxyresorufin *O*-dealkylation, lauric acid hydroxylation and especially the 7-ethoxyresorufin *O*-deethylation were decreased for both sexes. The 7-ethoxycoumarin *O*-deethylation was induced in males after one month dosing, and both in males and females after three months dosing. The UDP-glucuronosyltransferase activity was not affected in male mice, and slightly induced after one month treatment and inhibited after three months treatment in female mice.**

2000

RIN 3394-95

IMAZALIL PEER REVIEW PACKAGE

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PART 3 (Liver Testosterone Metabolism)

A. Materials and Methods:

1. Samples of livers from male and female mice from the PART 1 1-month interim sacrifice (5/sex/group) and the 3-month terminal sacrifice (5/sex/group) were collected and stored frozen until used for the preparation of microsomal suspensions by standard procedures (described on pages 33-34 of the study report). Five microsomal suspensions per group (1/mouse) were assayed.

2. The following analytical procedures were subsequently performed on each microsomal suspension (details described on page 34 of the study report).

   a. microsomal protein content
   b. cytochrome P-450 content
   c. microsomal enzymatic activities
      - 9 separate assays for P450-dependent regio- and stereoselective hydroxylations of testosterone

B. Results: For mice sacrificed at 1 month, 600 ppm imazalil in the diet increased the liver microsomal protein content per gram of liver 2.2- and 2.0-times in males and females respectively. Also, the liver microsomal P450 content, expressed as nmol/mg protein, increased 2.3- and 1.6-times in males and females respectively. The results for testosterone metabolism are presented in TABLE 7 (table copied from the study report). At 600 ppm imazalil in the diet, the total activity of testosterone hydroxylases was increased 1.9- and 1.5-times in males and females respectively. No effects were seen in mice dosed at 50 or 200 ppm.

For mice sacrificed at 3 months, 200 ppm imazalil in the diet increased the liver microsomal P450 content, expressed as nmaol/mg protein, 2.1-fold in females. At 600 ppm, the liver microsomal protein content per gram of liver increased 1.7- and 1.5-times in males and females respectively. Also, at 600 ppm the liver microsomal P450 content increased 1.8- and 2.4-times in males and females respectively. The results for testosterone metabolism are presented in TABLE 8 (table copied from the study report). At 600 ppm, the total activity of testosterone hydroxylases was increased 1.8- and 2.0-times in males and females respectively. No effects, other than increased P450 content in females at 200 ppm, were seen in mice dosed at 50 or 200 ppm.

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PART 4 (Imazalil Levels in Serum)

A. Materials and Methods: Samples of serum from male and female mice from the PART 1 3-month terminal sacrifice (20/sex/group) were pooled and analyzed for imazalil concentration by gas chromatography (quantification limit of 1.0 ng/sample).

B. Results: The results are presented in TABLE 9 (copied from the study report). At 50 ppm and 200 ppm, concentrations of imazalil in the serum of both males and females were at or below the level of detection (i.e. $\leq$ 1.0 ng/sample). At 600 ppm, imazalil was detected in the serum of some males (up to 2.8 ng/sample) and in that of some females (up to 3.3 ng/sample).

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CASWELL FILE
Attachment 11

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

007865

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Imazalil - Dose Selection for Mouse Oncogenicity Study. Submitted by Janssen Pharmaceutica by Fax on April 7, 1989.

Tox. Chem. No.: 497AB

TO: Susan Lewis
Product Manager #21
Registration Division (H7505C)

FROM: Judith W. Hauswirth, Ph.D., Chief
Toxicology Branch I - IRS
Health Effects Division (H7509C)

Judith W. Hauswirth 4/28/89

THRU: William L. Burnam, Acting Director
Health Effects Division (H7509C)

WLB 5/1/89

The registrant has sent a desk copy of a recently completed 90 day mouse study on imazalil to aid in the dosage selection for an oncogenicity study. Subsequent to this submission, Dr. H. J. van Cauteren of Janssen Pharmaceutica in Belgium called to discuss the study and to set appropriate dosage levels for the oncogenicity study. This telephone conversation took place during the week of March 20, 1989. He then faxed his version of this conversation to this reviewer along with his proposed high dosage level for the mouse study (A copy of the faxed material is attached for information).

Dr. van Cauteren proposed a high dose of 600 ppm for the oncogenicity study. Toxicology Branch I agrees to this dosage level based upon depressed body weight gain seen in males and females at 800 ppm in the 90 day range finding study. The percentage body weight decrement at this dosage level was by Toxicology Branch's calculations 30% in females and 25% in males. The differences in body weight gains were statistically significant at several time points during the 90 day study for females but not for males. Toxicology Branch notes that although the food consumption table in the report indicates that the dosed groups ate more than the control group, the report states that food wastage was a problem in this study. We also note that the mice were housed 2-3 per cage which could have contributed to wastage. The report further states that there could have been a palatability problem with the treated diet. Based upon the values given in the table for food consumption, it is difficult to determine whether this was a problem. We urge that csre be taken in the long term study to determine whether there is a palatability problem at 600 ppm.

Other effects seen at 800 ppm in the range finding study were hepatocellular

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vacuolar degeneration, a decrease in albumin, phospholipids, and total bilirubin in males and females and a decrease in AST in females only.

JANSSEN
PHARMACEUTICA

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DATE : April 7, 1989

FAX 00/1/703 557 ~~2233~~ 3106

ATTENTION : Dr. J.W. HAUSWIRTH - Arlington VA 22202 -U.S.A.

FROM : Dr. H. VAN CAUTEREN - Janssen Pharmaceutica - BELGIUM

IN CASE YOU DO NOT RECEIVE ANY OF THESE PAGES PROPERLY, PLEASE CALL : (14) 60 24 80.

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TELEFAX - No.703-5570233
TELEPHONE NUMBER: 703-5577397

TO: Dr. Judith W. HAUSWIRTH - Arlington VA 22202 - U.S.A.

FROM: Dr. H. Van Cauteren - Janssen Pharmaceutica - Belgium

DATE: April 7, 1989

SUBJ.: Imazalil
Dose levels for mouse carcinogenicity study

Dear Dr. Hauswirth,

Referring to correspondence dated March 8, 1989, from Bill Goodwine to you, a desk copy of a subchronic feeding study in mice (Exp.2020, December 2, 1988) was submitted for review and comment.

Subsequently, we spoke by phone and agreed that the finding, with regard to MTD, supported a high dose between 400 and 800 ppm.

Please find below, my dose level suggestions and justification for the 24-month mouse carcinogenicity study.

Protocol
In general, the protocol of this mouse carcinogenicity study will be fully compliant with the EPA guidelines (1984). More specifically, we will meet the criteria of the test procedures with regard to age (5 weeks at start), sex and number of mice (50 males and females/group), clinical observations (daily, weekly), measurements of body weight and of food consumption (weekly, monthly), clinical pathology (at 12 and 18 months and terminally), gross necropsy (including organ weights in terminal animals), and histopathology.

Route of administration and dose level selection
Imazalil will be admixed into the diet at levels of 50, 200 and 600 ppm. These levels have been selected based upon the following:
- Fifty ppm is an appropriate low dose since it is estimated to be a no-toxic effect level (NOEL). This level is in the same order of magnitude as the medium dose of the previously conducted mice carcinogenicity study (25 ppm in the drinking water is approximately equivalent to 50 ppm into the feed assuming mice drink about the double of the dry feed they consume).
- As an intermediate dose, 200 ppm will be used. It is estimated to be at the borderline of toxicity based upon a 3-month dose range finding study (Exp.No.2020) whereby dosing at 200 ppm resulted in slightly decreased aspartate aminotransferase, cholesterol and phospholipid values in the serum of females and in a vacuolar degeneration in the liver of males. This dose level also falls in the same order of magnitude of the high dose of the previously conducted mice carcinogenicity study (100 ppm in the drinking water is approximately equivalent to 200 ppm into the diet).

- The high dose will be 600 ppm. In a 3-month dose range finding study (Exp.2020), dosing at 400 ppm resulted in toxicity which was characterized by some altered serum parameters (decrease of albumin, total bilirubin and phospholipids in males and decrease of cholesterol, phospholipids, total bilirubin and AST in females) and by histological modifications on the liver (vacuolar degeneration and centrilobular swelling). These effects were also, but more pronounced, present at 800 ppm. In addition, the liver weight was increased and macroscopically showed a swollen and dark aspect at this dose. In females, dosing at 800 ppm also resulted in a lower body weight gain (about 12%). This study indicated that the dose level of 400 and 800 ppm into the diet are toxic with cholesterol and lipid metabolism and the liver as potential targets.
Since it could not be fully excluded that survival might not be adversely affected at 800 ppm, it was decided to select 600 ppm as the intermediate between 400 and 800 ppm.

Prior to initiating the study, we would like to receive verification, from you, that the subchronic feeding study (Exp.2020) has been reviewed and supports the proposed dose levels. Bill suggested that you might handle this by way of an Internal Memorandum to the Registration Division, with a copy faxed to Janssen, U.S.A. at (201)524-9895, so that we may proceed with our plans to initiate the study later this month. Alternatively, we would be most grateful to receive a letter directly from you on this matter by way of facsimile. We will leave it up to you to follow the best route.

I will ask Bill Goodwine to follow-up this letter within the next week.

Sincerely,

Herman Van Cauteren

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
Feeding/oncogenic-2 year Species: mice Janssen Pharmaceutica Res. Lab 636; 3/4/79	Imazalil tech (sulfate).	099285 245311	Oncogenic NOEL > 40 mg/kg (HDT). Dosage levels: 2.5, 10 & 40 mg/kg in Swiss albino strain. Sys. NOEL = 10 mg/kg. Sys LEL = 40 mg/kg (increased mortality).		Supplementary 000057 Minimum 001337
Dermal-14 day Species: rat Janssen Pharmaceutica Res. Lab 636; 3/26/76	Imazalil tech (sulfate)	097234	NOEL = 160 mg/kg. LEL = 640 mg/kg (scale and crusts).		Minimum 000065
Dissimilation chemicals	Imazalil sulfate		Caswell 497AB Imazalil; 497C Imazalil acetate; 497D Imazalil nitrate		
Acute oral LD50 Species: rat Janssen Pharmaceutica Res. Lab 2778	Imazalil tech. (Sulfate)	097234	LD50 = 550 (421-719) mg/kg	3	Minimum 000065
Acute oral LD50 Species: rat Janssen Pharmaceutica Res. Lab 37; 5/79	Imazalil tech (Sulfate)	099285	LD50 = 350 (272-464) mg/kg (M). LD50 = 309 (237-404) mg/kg (F).	2	Minimum 000057
83-3(a) Developmental Toxicity Study Species: rat Janssen Pharmaceutica Res. Lab 2003/88-05; 7/5/88	Imazalil sulfate tech. 99.9%	410266-03	Sprague-Dawley rats (OFA-SD) dosed by gavage at: 0, 40, 80 & 120 mg/kg/d. Maternal NOEL & LEL not determined; < 40 mg/kg (LDT), based on decreased food consumption from days 6-16 gestation. Developmental NOEL = 40 mg/kg. Developmental LEL = 80 mg/kg, based on decreased fetal weights; in addition at 120 mg/kg decr. litter size and reduced number of live fetuses. Increased rudimentary ribs and resorbed fetuses.		Minimum 007865
83-3(b) Developmental Toxicity Study Species: rabbit Janssen Pharmaceutica Res. Lab 2615; 05/27/92	Imazalil sulphate 98.2-100% pure; batch# ZR027/80 PUA 631	425936-01	Imazalil sulphate (98.1-100% pure) was admin. by gavage groups of 15 pregnant albino rabbits at levels of: 0, 5, 10 or 20 mg/kg/day during gestation days 6-18. Maternal NOEL = 5 mg/kg/day. Maternal LOEL = 10 mg/kg/day, based on decreases in body weight gain. Death occured at 20 mg/kg/day. Developmental NOEL = 5 mg/kg/day. Dev. LOEL = 10 mg/kg/d, based on increased resorptions. Core Supplementary - Upgradeable.		Supplementary 010581

Attachment B

Fundamentals on the absorption of imazalil base and imazalil salts are described in the statement "On the pharmacokinetics of imazalil base and its various salts in mammals", prepared by Jos Heykants in May 1979. He argued that the salt form of imazalil has no or little influence on the pharmacokinetics of the substance. Orally administered imazalil is solubilized by protonation in the acid stomach (pH 2 to 3). The salts will dissolve and dissociate in the gastric juice giving protonated imazalil and the anion, this is sulphate in the case of imazalil sulphate. Upon passing from the stomach to the small intestine, the pH increases and protonated imazalil will be gradually neutralised to undissociated base, which can be absorbed. The small intestine thus is the principal site of absorption.

The dissociation in the stomach separates imazalil from the sulphate anions. From then on both entities behave independently. Sulphate is a normal constituent of the diet. It takes part in important biochemical pathways, for example in the metabolism of the sulphur-containing amino acids cysteine and methionine, by activation with ATP to APS (adenosine 5'-sulfatophosphate) and PAPS (adenosine 3'-phosphate 5'-sulfatophosphate; "active sulphate"), which in turn is of importance for the sulfation reactions facilitating the elimination of endogenous or xenobiotic substances from the body. Orally administered sulphate is absorbed in the gut by carrier transport. Sulphate is found in serum at concentrations in the mM-range (millimol per litre) depending on the animal species. The serum concentration is regulated by the kidneys, urine being the main route of elimination. A general overview of the behaviour of sulphate is given in the included reference (G. J. Mulder: Sulfate availability in vivo. Chapter 3 in Sulfation of Drugs and Related Compounds (G. J. Mulder, ed.), CRC Press Inc., Boca Raton, Florida, 1981, page 31 to 52).

Dr. Leo Van Leemput
Department of Pharmacokinetics
Janssen Research Foundation

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Sulfation of Drugs and Related Compounds

Editor

Gerard J. Mulder, Ph.D.

Associate Professor
Department of Pharmacology
University of Groningen
The Netherlands

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Chapter 3

SULFATE AVAILABILITY IN VIVO

G. J. Mulder

TABLE OF CONTENTS

I. INTRODUCTION

Sulfate availability to the organism may limit the role sulfation may play. In this chapter the various mechanisms by which sulfate is provided to the cell will be briefly discussed. In the next chapter the activation step, required for the synthesis of the donor of the sulfate group, adenosine 3'-phosphate 5'-sulfatophosphate (PAPS), will be discussed. Where possible, recent reviews will be referred to so that further information may be obtained from these sources.

Basically, there are two primary sources of inorganic sulfate: absorption of this anion from the gut and oxidation of the amino acid cysteine. Under some conditions, however, catabolism of sulfated macromolecules, especially glycosaminoglycans, may also provide inorganic sulfate.[1] Another possible route of administration is the absorption of inorganic sulfate from the airways, when it is applied as a spray.[2,3] In the isolated perfused rat lung it is rapidly absorbed after intratracheal administration, and causes bronchoconstriction due to local histamine release.

In some species inorganic sulfur may be converted by the microflora in the intestine to inorganic sulfate;[4] this occurs, for instance, in ruminants, but not in man or the rat and most other laboratory species.

II. ABSORPTION OF INORGANIC SULFATE FROM THE GUT

It would be expected that a primary source of inorganic sulfate in the body would be intake with the food and subsequent absorption from the gut. However, in many pharmacological handbooks the belief is expressed that inorganic sulfate salts tend to be absorbed minimally and hence, add water to the bowel, thus leading to diarrhea.[5] *Martindale's Pharmacopoeia*[6] states that sodium sulfate is "poorly absorbed from the gastronintestinal tract." This poor absorption is believed to explain the cathartic effect of oral administration of inorganic sulfate salts. Yet, the available data show that inorganic sulfate is excellently absorbed after oral administration, in spite of the supposedly poor membrane-permeating properties of this anion.[7] Therefore, the above explanation is apparently incorrect.

In the beginning of this century a strong controversy existed between those who contended that sulfate for sulfation only arose from sources other than absorbed inorganic sulfate (the sulfur-containing amino acids, for instance), and those that tried to prove that also inorganic sulfate in the food was available for sulfation.[8] At that time, of course, it was hard to prove that orally administered inorganic sulfate was used for sulfation, since no radioactive sulfate was available. Later it was shown that [^{35}S]-labeled inorganic sulfate was absorbed from the intestinal tract, and was incorporated into many endogenous and exogenous substances.[9]

One of the first papers that conclusively showed absorption of inorganic sulfate and its utilization for sulfation of drugs was published in 1931 by Hele.[10] When he fed his dogs Patsy and Pansy nonradioactive inorganic sulfate, he measured a great increase in their urinary output of sulfate; upon addition of phenol, by stomach tube, the amount of free inorganic sulfate in urine decreased, and it was replaced by a big increase in the amount of ethereal sulfate, presumably phenyl sulfate. Similar results were obtained with indole showing that it was converted in vivo *(inter alia)* to indoxyl sulfate.

One of the problems at that time was the quantitative determination of inorganic sulfate and sulfate esters. Free inorganic sulfate was usually precipitated by barium chloride, and sulfur was determined in the precipitate. Total sulfur (without barium chloride precipitation) was also determined in the sample; subtraction yielded organic, presumably esterified, sulfur. This method, of course, gives inaccurate data because

several other sources of sulfur (e.g., mercapturates) are measured as ethereal sulfate. Yet Hele's findings[10] however, were fairly conclusive, and indicated that inorganic sulfate was absorbed from the gastrointestinal tract in the dog. He found that about 22 to 66% of the sodium sulfate administered (0.8 mmol/kg daily) was utilized for synthesis of phenyl sulfate or indoxyl sulfate.

The first use of radiolabeled sulfate was reported in 1937.[11] Radioactive sodium [^{35}S]-sulfate was administered orally to a human volunteer, and urine was collected for several days. Of the dose of sodium sulfate (6.3 mmol) 15% was recovered within 9 hr. in the urine and a further 32% in the following 15 hr. This clearly showed that sodium sulfate was absorbed to a high degree. Much later, Bauer[12] showed the same for a tracer dose of [^{35}S]-sulfate that was administered orally to fasted volunteers (Figure 1). Eighty percent or greater was recovered in the 24-hr urine. Sixty to one hundred min after oral administration, [^{35}S]-sulfate attained equilibrium and achieved a plasma concentration equivalent to the intravenously administered tracer. This again proves rapid and almost complete absorption of a low dose of inorganic sulfate from the gut in man.

After the second world war more work with radiolabeled [^{35}S]-sulfate was reported, and it was firmly established (although not always accepted)[13] that inorganic sulfate fed to animals, was available in the body for incorporation into endogenous and exogenous substances. Within 24 hr, for instance, Dziewiatkowski[14] recovered more the 70% of the oral dose of sodium [^{35}S]-sulfate (1 mmol/kg) in the urine of the rat. Morrow et al.[15] recovered 41 to 64% of a tracer dose of sodium sulfate in urine within 8 hr after oral administration to 24 hr fasted cats; similar findings were obtained in an intestinal loop *in situ*. In the rat, [^{35}S]-labeled sodium and calcium sulfate were absorbed from the gut to a high degree:[16,17] in feeding experiments the sulfates were administered with the food at two levels, 0.10 and 0.42% (w/w), and 60 to 80% of the sulfate salts was absorbed. Subsequently, [^{35}S]-radioactivity was excreted in urine, or incorporated into endogenous compounds such as glycosaminoglycans in cartilage. These results confirmed the absorption of sulfate from the gut. A difference observed between the rats fed calcium sulfate and sodium sulfate[17] may be fictitious, since both diets contained calcium carbonate, and ionization in the stomach would yield both salts. Using unlabeled sulfate, Wellers[18] found a similar increase in the urinary output of inorganic sulfate after adding sodium sulfate to the food of rats.

In sheep, a rapid and fairly complete absorption of a tracer dose of sodium [^{35}S] sulfate was observed.[19,20] The radioactivity reached a peak in the plasma at 6 hr, and approximately 75% was excreted in urine. In baby pigs, more than 60% of the dose was recovered in urine.[21,22]

More detailed measurements were performed in adult pigs by Berry et al.[23] They studied absorption, excretion, placental transfer, and maternal-fetal tissue distribution of (presumably) a tracer dose of [^{35}S]-labeled sodium sulfate. The swine (gilts and barrows) were placed in metabolism crates, and blood, urine, and feces were collected. At the end of the collection period the animals were slaughtered and the tissue distribution of the radioactivity was determined. A peak in [^{35}S]-radioactivity in serum was found about 2 to 3 hr after an oral dose (Figure 1). Within 48 hr about 50% of the radioactivity had been excreted in urine; after intravenous (i.v.) administration, this was about 60%. Less than 2% had been excreted in feces after 48 hr, indicating almost complete absorption.

Similar experiments were done in dogs[24] that were dosed orally with ammonium sulfate (about 0.5 to 1.0 mmol/kg). The plasma sulfate concentration, determined 3½ hr after administration, had increased from 1.4 m*M* in controls to 2.2 m*M* after ammonium sulfate administration. At the same time there was a strong increase in the urinary output of inorganic sulfate. Within 4 hr about 50% of the dose had already been absorbed from the gut; the remainder was still in the intestine.

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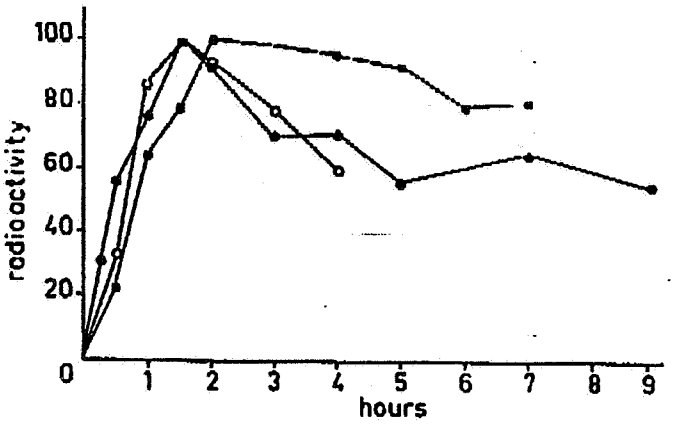

FIGURE 1. Absorption of a tracer dose of [^{35}S]-labeled sodium sulfate after oral administration in man, rat, and swine: plasma concentrations of radioactivity. The data for man[12] (○ – ○); for swine[20] (■ – ■); and for the rat[26] (● – ●). The highest value in each set of data has been taken to be 100, and the other values were calculated as percentage of this. The tracer dose was administered orally at t = 0.

In man, Meier and Schmidt-Kessen[25] also observed a rapid absorption of an orally administered mixture of (unlabeled) sodium-, magnesium-, and calcium-sulfate given in a total dose of 15 mmol (5 mmol of each salt). Their data suggest that the greater part of the dose is absorbed and ultimately excreted in urine in agreement with the results of Bauer.[12]

Finally in the rat, fairly complete data were obtained by Krijgsheld et al.[26] In the conscious, freely moving rat they administered various oral doses of sodium [^{35}S]-sulfate. At a relatively low dose (3 mmol/kg), over 80% of the radioactivity was recovered in urine, indicating almost complete absorption of the dose. At a higher dose, however, the recovery in urine decreased because of diarrhea, and sodium sulfate was lost in the watery feces. Approximately 2 hr following oral administration, a peak in the [^{35}S]-concentration in plasma was found (Figure 1). When 8 to 15 mmol/kg sodium sulfate were given, the serum concentration of inorganic sulfate increased from the normal level of 0.8 m*M* to 2.0 m*M* at the peak (Table 1). Interestingly, in control groups that received isoosmotic doses of sodium chloride a decrease in the serum level of sulfate was observed, for which as yet no explanation has been given.

The results described above permit several conclusions to be drawn. First, sodium sulfate and several other sulfate salts are rapidly absorbed from the gut in all species studied so far; since biological membranes are believed to be almost impermeable to sulfate, at least by diffusion, this passage would presumably require a carrier (facilitated diffusion?). The absorption of sulfate from the gut is almost quantitative, unless diarrhea occurs, preventing absorption. The serum sulfate concentration may increase two- to three-fold following oral administration of inorganic sulfate; further increases will presumably be prevented by renal excretion (see below).

The cathartic effect of sulfate solutions is probably not the result of a lack of absorption of sulfate from the gut. More likely however, the rapid transport of water into the gut, which is more rapid that that of sulfate from the gut into blood, causes accumulation of water in the gut, and thereby diarrhea.

Ammonia, calcium, potassium, and sodium salts of sulfate are used as food additives, The U.S. Food and Drug Administration (FDA) has proposed to give these food additives a generally recognized as safe (GRAS) status.[27] The inorganic sulfate content

Table 1
EFFECT OF ORAL ADMINISTRATION OF SODIUM SULFATE AND SODIUM CHLORIDE ON SERUM SULFATE IN THE RAT

Orally administered	Serum sulfate concentration (mM)
Nothing	0.77 ± 0.04
Sodium sulfate	
0.5 mmol	1.34 ± 0.06[a]
2.5 mmol	1.95 ± 0.15[a]
5.0 mmol	2.13 ± 0.09[a]
Sodium chloride	
0.15 mmol	0.66 ± 0.03[b]
2.5 mmol	0.57 ± 0.02[a]
7.5 mmol	0.58 ± 0.02[a]

Note: The dose was administered in a volume of 2 ml water to rats of 300-g body weight. Serum sulfate was determined 2 hr after the administration of the dose, by a turbidimetric method. Blood was withdrawn under ether anaesthesia. The mean ± S.E.M. for 6 rats per group is given.

[a] Significantly different from control at $p < 0.005$ (Wilcoxon's test).
[b] The same at $p < 0.05$.

From Krijgsheld, K. R., Frankena, H., Scholtens, E., Zweens, J., and Mulder, G. J., *Biochim. Biophys. Acta*, 586, 492, 1979. With permission.

in most food products is unknown, presumably because it seemed of marginal nutritional importance (but see Section V).

III. INTESTINAL CARRIER TRANSPORT OF INORGANIC SULFATE

Since the sulfate anion is not very lipid soluble, a carrier most likely is involved in the transport of this ion across biological membranes as has been reported for erythrocytes (see section VI). The first indication of such a carrier transport in the intestine was reported by Deyrup in 1963,[28] who found in vitro a sodium-dependent accumulation of [^{35}S]-sulfate in the lower ileum of the rat. These findings were confirmed by Anast et al.[29] using the everted sac technique with intestine from rat, rabbit, and hamster. Their results suggest that sulfate transport is energy-dependent. Dziewiatkowski[7] confirmed this in a study on the properties of the sulfate transport system in everted sacs from rat intestine; metabolic inhibitors such as sodium azide inhibited the transport. Whereas transport from mucosal side to serosal side was highest in the ileum, the reverse transport was highest in the jejunum. Batt,[30] using mouse intestinal ring segments, similarly observed highest activity of the carrier in the terminal ileum of the adult mouse. In the very young mouse sulfate transport was very active along the whole small intestine and in the colon; 3 weeks after birth, however, the adult pattern had established itself, with the highest transport activity in the ileum. No sex differences were observed for sulfate transport. Prior hypophysectomy decreased the activity of the sulfate carrier in the rat intestinal everted sac;[29] this could be counteracted by injection of bovine growth hormone. The rate of transport in everted sacs from guinea pig intestine was higher than that in the rat preparation.[31]

More evidence on the carrier-mediated sulfate transport in the intestine came from work of Cardin and Mason.[32,33] Using the everted sac technique with rat lower ileum, they observed that a concentration gradient of [^{35}S]-sulfate was established across the sac wall: the [^{35}S]-concentration inside (serosa side) increased by as much as nine times

over the concentration outside (mucosa side) at low concentration of sulfate. At higher sulfate concentrations (applied at the mucosa side) the gradient was less steep; this proved that sulfate accumulated against a concentration gradient. The apparent K_m of sulfate for the carrier was 0.4 to 0.6 m*M*, and the transport was saturable. Between the gut tissue and the solution inside in the sac there was no sulfate concentration gradient, indicating that sulfate could freely diffuse, once it had passed the mucosa cells. The sulfate transport was inhibited competitively by chemically related anions such as molybdate, tungstate, selenate, thiosulfate, and sulfite. Conversely, the transport of molybdate and tungstate was inhibited competitively by sulfate, and similarly was highest in the lower ileum of the rat. K_m values for the transport of these ions were of the same order of that for sulfate, but the V_{max} values were slightly lower. Similar transport phenomena were observed by Mason and Cardin in pieces from sheep intestine.[34] Recently, the sulfate transport in a marine gastropod, *Aplysia californica* intestine has been studied.[35]

The existence of sulfate transport across frog gastric mucosa has been suggested but has not been further substantiated.[36] Other sulfate carrier systems, such as occur in the erythrocyte, will be discussed in Section VI.

IV. GENERATION OF INORGANIC SULFATE FROM CYSTEINE AND METHIONINE

Most, if not all, of the sulfate requirements of mammals can be met with methionine and cysteine, ingested with the food in the form of proteins.[37] To this end, the -SH group of cysteine is oxidized, resulting in the release of SO_3^{2-}. Methionine can be converted into cysteine by transsulfuration. Reviews on the various aspects of conversion of sulfur in the amino acids into inorganic sulfate are available.[38-40] Here, the relevant data will be discussed only briefly.

The transsulfuration pathway, converting methionine into cysteine, proceeds according to the scheme shown in Figure 2.[39] Influences of the diet, hormones, age, and many other details on this pathway can be found in a review by Finkelstein.[38] Patients with a genetically deficient cystathionine synthesis have been described (see Finkelstein [41] for a review).

Through oxidation, cysteine is converted into alanine 3-sulfinate (Figure 3), presumably by cytosolic enzymes;[40] a dioxygenase incorporates both atoms of molecular oxygen into the substrate. Subsequently the sulfinate is transferred to the mitochondria and reacts with 2-oxoglutarate or oxaloacetate by transamination.[42] The 3-sulfinopyruvate spontaneously decomposes to sulfite. In the rat nicotinamide, hydrocortisone, and cysteine enhanced the cytosolic dioxygenase activity by an as yet unknown mechanism (Reference 43 and references therein).

In the rat, deprivation of sulfur-containing amino acids causes a dramatic decrease in the urinary excretion of inorganic sulfate, decreasing to near zero, shortly after beginning the sulfur-free diet.[44,45] The excretion of "ethereal" sulfate, though also decreases, continued long after free sulfate had virtually disappeared from the urine. At least 50 mg of methionine-sulfur per kilogram per day was required to sustain a positive growth rate and a positive sulfur balance. At that level however, inorganic sulfate in urine was still much below control. Yet, the urinary excretion of sulfate esters was about normal. Not only methionine, but also homocysteine could be used to provide sufficient sulfur to the rats. Further studies on methionine utilization have been reported by Almquist.[46] Uren et al.[47] have tried to decrease cysteine availability in vivo by injection of cyst(e)ine degrading enzymes in the rat; an i.v. injection of γ-cystathionase was briefly effective, and the γ-cystathionase inhibitor propargylglycine prevented this.

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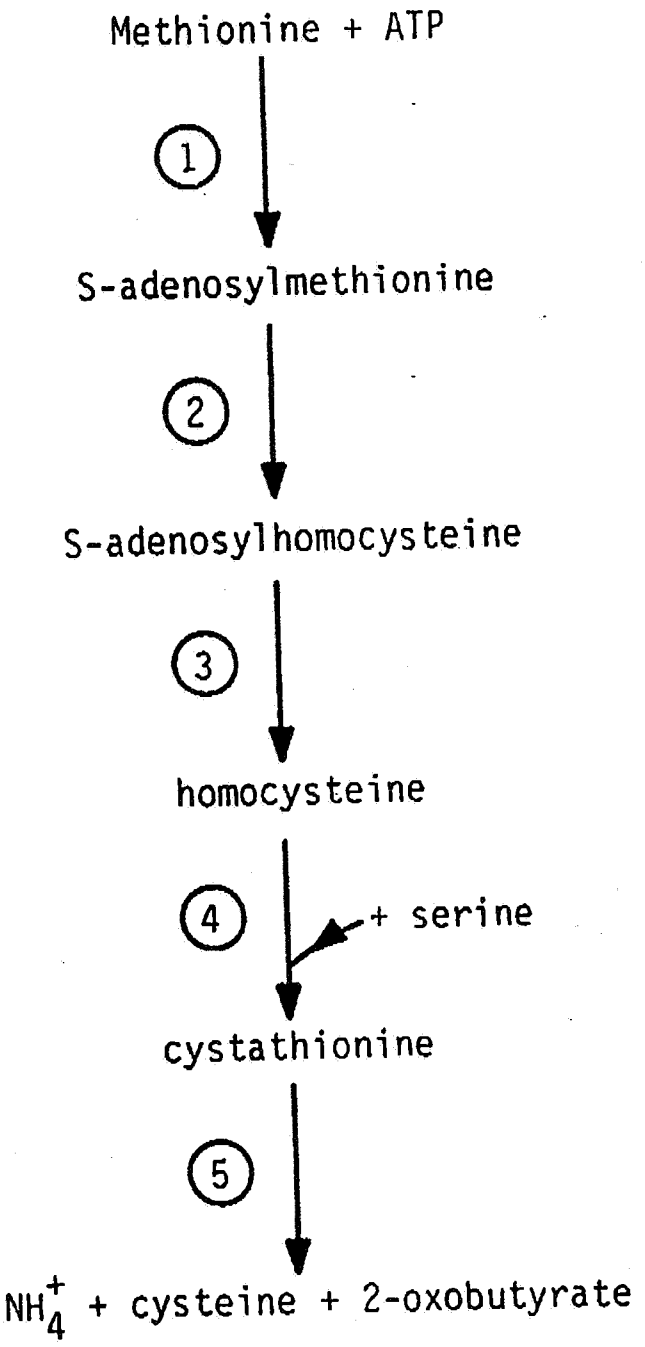

FIGURE 2. The cystathionine pathway. Enzymes in the scheme: 1. ATP : L-methionine *S*-adenosyltransferase (EC 2.5.1.6). 2. *S*-adenosyl-L-methionine : L-homocysteine *S*-methyltransferase (EC 2.1.1.10.). 3. *S*-adenosyl-L-homocysteine hydrolase (EC 3.3.1.1.). 4. Cystathionine β-synthetase (EC 4.2.1.22) (L-serine hydrolyase, adding homocysteine). 5. L-Cystathionine cysteine-lyase, deaminating (EC 4.4.1.1.).

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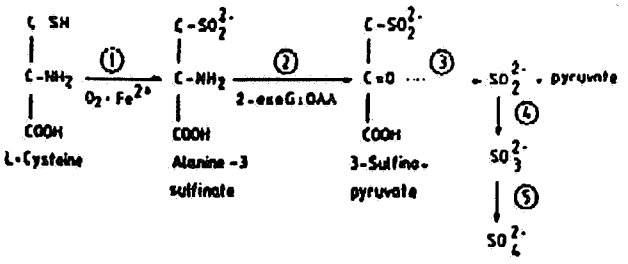

FIGURE 3. Sulfoxidation of cysteine to inorganic sulfate. The enzymes involved are 1. A cysteine dioxygenase (EC 1.13.11.20.), 2. Cysteine sulfinate transaminase, 3. and 4. are nonenzymatic, rapid steps, 5. Sulfite oxidase (EC 1.8.3.1). Abbreviations: 2-oxoG, 2-oxoglutarate; OAA, oxaloacetate.

So far there are only few studies on the effect of deleting the sulfur-containing amino acids from the food in man. In such a trial, Lakshmanan et al.[48] found the expected decrease of the urinary excretion of inorganic sulfate and methionine; surprisingly however, the plasma concentration of methionine was not yet decreased 8 days after the start of the diet. During this time period, the sulfate excretion in urine was decreased by approximately 65%. Yet, some inorganic sulfate was still found. Thus it seems that protein catabolism can provide for cysteine and methionine during some time; inorganic sulfate may be released from tissue pools by catabolism of various compounds such as glycosaminoglycans. Unfortunately however, the plasma levels of inorganic sulfate were not determined.[48] The fact that still inorganic sulfate was found in urine suggests that the plasma level may not have been appreciably decreased (see Section VI).

The relationship between sulfur-containing amino acids in the food and urinary elimination of inorganic sulfate has been investigated in man by Sabry et al.[49] Under conditions where these amino acids were limiting, they observed a linear relationship between intake of cysteine and methionine on one hand, and excretion of inorganic sulfate on the other. When they increased only methionine at a constant supply of the other amino acids, a higher percentage of this methionine was converted to inorganic sulfate, indicating that the body eliminated the surplus in methionine by sulfoxidation. Parallel to these findings, Lakshmanan et al.[50] reported that limiting threonine in the diet, resulted in a significant increase in the urinary excretion of inorganic sulfate. This finding thus suggests that an increased excretion of inorganic sulfate might be an indicator of an unbalanced diet.[51] The available literature, however, suggests that under normal conditions, the sulfur-containing amino acids in the food provide sufficient inorganic sulfate for the various sulfation reactions in the body, by oxidation of cysteine.[52,53]

Sulfite is the penultimate product of the oxidation pathway of cysteine to sulfate. The pharmacokinetics of (i.v. administered) sulfite have been investigated in several mammalian species.[54,55] In most species it is rapidly converted to sulfate; the sulfite oxidase activity however, may be rate limiting at high doses of sulfite. An inverse correlation between sulfite oxidation and bisulfite toxicity in rabbit, hamster, guinea pig, mouse, and rat has been found:[56] the highest rate of the oxidase is observed in the rat, which is least sensitive to sulfite toxicity. Superoxide anions may be involved in sulfite oxidation.[57]

Recently, two patients with an inherited disease of sulfite oxidation have been described, in whom the sulfite oxidase activity was extremely low or not present. Mudd et al.[58,61] clearly demonstrated a defective sulfite oxidase in a patient that died at 2½ years of age. The child had many neurological abnormalities at birth, such as severe

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mental retardation, seizures, and opisthotonos. Increased amounts of sulfite, thiosulfate, and an abnormal amino acid, S-sulfocysteine, were found in urine. In post-mortem liver, kidney, and brain no sulfite oxidase activity was observed.[41] Two more patients were reported in 1977.[42] When a diet low in sulfur-containing amino acids was introduced, there was some physical and mental improvement. In retrospect, it might have been worthwhile to add some inorganic sulfate, to ensure a sufficient supply for sulfation reactions; then even less sulfur-containing amino acids might have been given. It seems that the latter patients had a less complete block of sulfite oxidase than the patient reported by Mudd et al.,[38] because about 50% of total sulfur in urine was in the form of inorganic sulfate, whereas Mudd's patient excreted almost no sulfate at all. This genetic defect in sulfur metabolism has been extensively reviewed by Finkelstein,[43] who also discussed several more genetic deficiencies in sulfur metabolism in man. Despite the occurrence of this genetic deficiency in the handling of sulfite, inorganic sulfite salts have been considered relatively safe as food additives.[43]

Finally it should be mentioned that methionine in the food is an important source of "activated" methyl groups, and that cysteine is required for glutathione synthesis, and in many species is the only precursor of taurine. An early review on many aspects discussed in this and the following section appeared in 1966.[44]

V. NUTRITIONAL ASPECTS OF INORGANIC SULFATE FEEDING

The potential growth-promoting effect of sulfate feeding in chickens, sheep, and several other species has extensively been investigated (see for reviews References 4 and 65). The assumption is that a sufficient supply of inorganic sulfate in the food might decrease the need for sulfoxidation of cysteine; thereby, more cysteine and methionine would be available for protein synthesis, resulting in increased growth. Therefore, the effect of various dietary sulfate salts on growth rates has been determined in a variety of species. In the chicken, both a growth-promoting and a growth-inhibiting effect was observed, depending on the sulfate salt used and other conditions.[66-68] In sheep a positive sulfur balance is of economic importance, since wool contains a high percentage of sulfur-containing amino acids. Sulfate feeding promotes growth in sheep.[69-71] A similar effect has been observed in pigs and cattle;[72,73] 2500 mg sodium sulfate per liter is well tolerated by cattle in the drinking water for a period of at least 90 days.[74] Also in young men, the addition of inorganic sulfate to a diet of soybean protein caused an increase in the nitrogen retention, most likely by a sparing effect on growth-limiting sulfur-containing amino acids.[75]

In the rat, some more detailed experiments have been performed in order to determine the mechanism of the growth-promoting effect. Smith[76] has determined the production of $^{14}CO_2$ from [1-^{14}C]-labeled methionine in the food of rats, in the presence and absence of 0.02% (w/w) inorganic sulfate. A significant decrease in $^{14}CO_2$ production was observed when sulfate was fed, as compared to controls, indicating a decreased demethylation of methionine, presumably because less sulfoxidation was required. Thus, more methionine might be available for protein synthesis. Indeed a small increase in feeding efficiency has been observed in the rat by the addition of sodium sulfate to the food.[77] Of course such a growth-promoting effect can only be seen if the basic diet has a very low natural nonprotein sulfur or inorganic sulfate content. James and Hove[77] found only growth promotion by sodium sulfate in the food in rats when a commercial casein diet was extensively washed with water at pH 8.5; by this procedure they decreased the inorganic sulfate content from 0.074 to 0.003%, and only then did they observe the growth-promoting effect. Since in many of the studies on the efficiency of inorganic sulfate feeding the inorganic sulfate content of the various diets

was not reported (and since data on sulfate utilization and absorption usually are lacking),[16] it is often hard to evaluate the effects in terms of sulfate availability in blood in the animals. In conclusion however, the available data show that under conditions in which sulfur-containing amino acids are growth-limiting, inorganic sulfate feeding may have a growth-promoting effect, most likely because it increases the availability of the sulfur-containing amino acids for protein synthesis by decreasing the need for sulfoxidation. Although inorganic sulfate salts are relatively nontoxic and may be fed for prolonged periods of time, other changes may occur. For example sheep on a 1.27% (w/w) sodium sulfate monohydrate diet acquired bacteria in the ruminal fluid that produced more propionic-, butyric-, and higher fatty acids and less lactic acid than the gut flora in controls.[78]

Gut bacteria especially in ruminants may use inorganic sulfate for the synthesis of cysteine.[64,79-82] The gut bacteria reduce inorganic sulfate to sulfide, which subsequently is converted with *O*-acetylserine to cysteine.[64] Methionine can be synthesized from cysteine by further metabolism to cystathionine.[64,83] This reduction of inorganic sulfate requires the intermediary synthesis of adenosine 3'-phosphate 5'-sulfatophosphate (PAPS), which is further reduced.

Mammalian tissues lack a sulfate reducing system.[66] In germ-free rats therefore, no incorporation of sulfite or sulfate into amino acids or protein takes place. When in normal rats inorganic sulfate is fed, some sulfur may be incorporated into protein as a result of bacterial action. If sufficient sulfur-containing amino acids are present, however, the bacteria utilize these instead of synthesizing them from inorganic sulfate.

VI. SERUM CONCENTRATION, DISTRIBUTION, AND ELIMINATION OF INORGANIC SULFATE

There are wide variations in serum concentration of inorganic sulfate in various species[87] (Table 2), as measured by the turbidimetric method, according to Berglund and Sorbö.[88] In man, serum concentrations have been reported between 0.15 and 0.5 m*M*, with a mean of about 0.3 m*M*, clearly lower than in most other mammalian species.[25,88-92] Genetic factors may be involved in the species differences, but also large variations in serum sulfate concentration are to be expected, due to different feeding habits in animals and man. When rats are fed a standard diet, the variations are rather small.[87]

Meier and Schmidt-Kessen[25] have reported a circadian rhythm in the serum sulfate concentration in man with slightly higher levels at night than during daytime. A rather steep rise occurred between 3 and 5 p.m. Krijgsheld et al.[87] observed a circadian rhythm also in rats; a peak in the serum sulfate concentration occurred in the afternoon (Figure 4), and a rapid fall between 6 and 10 p.m. was coincident with the turning off of the light and resuming of the activity of the animals.

The serum sulfate concentration is a balance between absorption of inorganic sulfate and its production from cysteine, and sulfate elimination by (mainly) urinary excretion and incorporation into low molecular-weight substrates of sulfation. The serum sulfate concentration is primarily regulated by the kidneys, because below a certain threshold inorganic sulfate is completely reabsorbed from the primary urine in the proximal tubule.[93] If the sulfate concentration in serum increases above that threshold, urinary excretion will be increased. For that reason, high i.v. infusion rates of sodium sulfate result in an osmotic diuresis. Tubular secretion of sulfate has been considered[93] but seems of little importance in those species where it has been investigated. The urinary excretion mechanisms for sulfate have been reviewed by Mudge.[93]

In view of the above findings it is not very surprising that a great variation in urinary

Table 2
SERUM SULFATE CONCENTRATIONS IN VARIOUS SPECIES

Species	Sex	Inorganic sulfate[a] (mM)	Ref.
Man	m/f	0.3	See text, 87, 152
Monkey	m/f	0.46 ± 0.10	87
Rat			
Wistar	m	0.77 ± 0.04	87
Brown Norway	m	0.82 ± 0.02	87
WAG	m	0.72 ± 0.02	87
Mouse			
Swiss	m	1.26 ± 0.12	87, 152
C57 B1/6J	?	1.00 ± 0.09	152
Guinea pig	m/f	0.84 ± 0.04	87
Rabbit	m	2.01 ± 0.13	87
Cat	f	0.68 ± 0.03	87
Dog	f	1.39 ± 0.12	24
Sheep	f	1.24 ± 0.10	87
Goat	f	2.43	87
Shetland pony	f	1.06	87
Pig	f	0.75 ± 0.03	87
Cow	f	1.80	87
Hen	f	1.82 ± 0.32	87
Rooster	m	2.41 ± 0.13	87

[a]Mean ± S.E.M. or S.D.

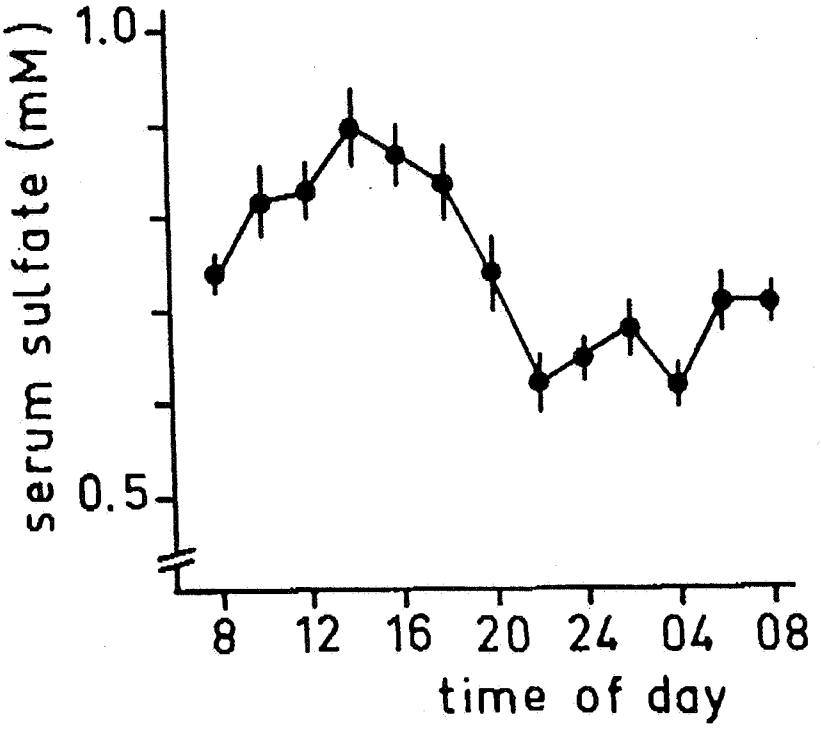

FIGURE 4. Circadian rhythm of serum sulfate in fed rats. Serum sulfate was determined turbidimetrically in blood collected from the aorta or the heart of rats anaesthesized with ether.[87] The rats had free access to food and water. Means ± S.E.M. are given of 10 rats at each point. The lights-on period was from 7 a.m. till 7 p.m.

output of inorganic sulfate is observed. First of all, it will be dependent on sulfate consumption and production. Secondly, the "sulfo-stat" in the kidneys will determine the rate of sulfate excretion in urine. Finally the blood flow to the kidneys is important: the lower the blood flow, the lower the urinary elimination. In man, the urinary inorganic sulfate excretion rate is about 20 to 25 mmol sulfate per 24 hr;[25,91,93,95-99] slightly lower values were reported for women than for men.[98] In children lower values were found:[100] about 18 mmol/24 hr for boys of 6 to 14 years of age, and 7 to 10 mmol/24 hr for girls of the same age. Considering the body weight of the children as compared to adults, it seems that their urinary excretion rate of inorganic sulfate is rather high. No data are available on age-dependent fluctations in serum sulfate levels and urinary excretion of inorganic sulfate. Upon fasting, the urinary excretion of inorganic sulfate tended to increase.[96]

Sabry et al.[99] observed a linear relationship between intake of sulfur-containing amino acids and urinary excretion of inorganic sulfate in man. Their results suggest that a surplus of cysteine or methionine is oxidized to inorganic sulfate. In agreement with these findings, Simmons[101] observed very low urinary inorganic sulfate levels in African children in a rural area as compared with an urban area. These findings correlated with a methionine-deficient diet in the rural area.

Very little data have been reported on the urinary excretion of inorganic sulfate in animals, and with the data that are available one has to keep in mind that there may be large variations because of differences in diet. For example, O'Connor and Summerill[24] found a basal excretion level of 300 μmol inorganic sulfate per hour in dogs (11 to 16 kg body weight). If these dogs were fed 10 g of meat per kilogram, however, this increased to 2400 μmol/hr, illustrating the effect of the diet. In fasted rabbits Bray et al.[102] measured an excretion rate of about 60 μmol/hr per rabbit. In the rat (body weight 250 to 300 g, males, nonfasted) about 18 μmol/hr was reported.[13] This value was confirmed by Büch et al.,[95] who compared the urinary excretion of inorganic sulfate in the rat and in male human volunteers. Their human volunteers excreted about 1000 μmol inorganic sulfate per hour (body weight 70 kg?), whereas the rats excreted 15 μmol/hr (body weight 250 to 300 g). This means that the excretion rate of inorganic sulfate on a weight-base in the rat is about four times that in man.

A high sulfate requirement for sulfate conjugation of administered paracetamol in the rat caused an almost complete disappearance of inorganic sulfate from urine.[95] The amount (expressed in μmol) of esterified sulfate (paracetamol sulfate) that appeared in urine was slightly higher than that of free inorganic sulfate excreted in controls. The latter suggests that a decrease in plasma sulfate may have occurred due to its depletion by sulfation of paracetamol. In human volunteers, a similar decrease in the urinary excretion of sulfate was found after paracetamol administration, although it was not as complete as in the rat presumably because the dose of paracetamol was much lower. These findings demonstrate that substrates for sulfation are another factor that affect urinary excretion of free sulfate.

The main route of elimination of sulfate in blood is urinary excretion;[9,12,14,101] biliary excretion is of little importance.[104,105] Bird[106] found only 6 to 8% of the radioactivity of [^{35}S]-labeled sodium sulfate in combined bile and pancreatic secretions in sheep, whereas 63 to 76% of the dose was recovered from urine. In a study in the isolated perfused rat liver, a slow rate of biliary excretion was also found:[107] at low concentration of sulfate in the perfusion medium (about 0.03 m*M*), the concentration of radioactivity in bile was higher than in the perfusion medium, but at 0.6 and 1.3 m*M* in the medium, the concentrations of radioactivity of inorganic sulfate in bile and in medium were equal. In an autoradiographical study[108] however, a significant amount of radioactivity was found in the intestinal lumen of the mouse after intravenous injection of

[^{35}S]-sodium sulfate. Kennedy et al.[109] established that some of intravenously administered [^{35}S]-sodium sulfate in sheep was excreted in the stomach and metabolized by the ruminal flora. Would secretion by the stomach mucosa into the gut play a role?

The distribution volume of inorganic sulfate in the body ("sulfate space" or "radiosulfate space") is an important parameter in studies on the pharmacokinetics of administered sulfate, and may be used to determine the physiological, endogenous sulfate pools. In addition, physiologists have used the radiosulfate space as a measure of the distribution volume of extracellular water. Different types of pharmacokinetics will be obtained with unlabeled and a tracer dose of [^{35}S]-labeled sulfate because of at least two reasons. In the first place an i.v. injection of a large dose of unlabeled sulfate, required to increase the serum concentration sufficiently in order to make this type of study possible, will increase the plasma concentration considerably above the absorption threshold in the kidney tubuli. Therefore, a rapid initial elimination will take place and this will lead to a correspondingly short halflife component in the eliminatory pattern of sulfate. A tracer dose of [^{35}S]-sulfate, labeled at high specific radioactivity, on the other hand, does not increase the total serum concentration of inorganic sulfate, and therefore will lack this rapid component. In the second place, if radio-labeled sulfate is used, the [^{35}S]-labeled sulfate will exchange with various (e.g., cellular) unlabeled sulfate pools. If unlabeled ions exchange with unlabeled ions, there is no net shift of sulfate from serum to cells or vice versa, but if a labeled ion in blood exchanges with unlabeled sulfate in cells, the radioactivity in blood decreases. Therefore, this effect will add a component to the elimination of [^{35}S]-sulfate as compared with unlabeled sulfate, where this self-exchange is not measured. Furthermore, in studies with [^{35}S]-labeled sulfate, usually no distinction has been made between inorganic sulfate and conjugated sulfate in the plasma samples: radioactivity in plasma has been counted indiscriminately. Of course most of the radioactivity will usually be present in the form of free sulfate, but under certain unfavorable conditions this may be different, for instance in patients with a high consumption of drugs that are metabolized by sulfate conjugation. If the pharmacokinetics of these conjugates are much different from that of free sulfate, the results in these patients must be misleading. In practice, so far only the pharmacokinetics of [^{35}S]-sulfate have been studied in several species, including man, where it has some clinical value. Usually, the tracer dose is injected intravenously, but it may also be administered orally.[12] Since a tracer dose of [^{35}S]-sulfate is not very rapidly excreted as long as plasma sulfate remains below the threshold for resorption, it may reach equilibrium and give a good estimate of the radiosulfate space. An indication of the time required for equilibrium may be the finding that after approximately 5 hr, the concentration in ascites fluid in man had equilibrated with the plasma concentration of [^{35}S]-sulfate.[110] Clearance of [^{35}S]-sulfate in man was 35 ml/min (per 1.73 m^2 surface area) and the distribution volume was 14.6% of body weight, with a range of 11.5 to 20.9%.[111] In dogs a higher distribution volume was found: 20.1% of body weight, with much less variation, probably because only a small proportion of their body weight is fat tissue. This value for the dog was confirmed, using nephrectomized dogs.[112] The distribution volume of radiosulfate slowly increases as a function of time after injection.[111] This may be due to slow penetration into cells, exchange of radiosulfate with unlabeled sulfate in cells, and incorporation into endogenous macromolecular compounds. These findings were confirmed in man by Ryan et al.[110] who used a slightly different method and found distribution volumes of 19.1 and 16.7% of body weight, respectively, for young soldiers and sedentary young males, and about 15% for elderly men and women. As is to be expected, various diseases affect this volume of distribution: during severe dehydration it may be decreased to 7% of body weight, and in patients with ascites, values of 23 to 26% have been reported.[110] In healthy human volunteers, Bauer[12] found a radiosulfate

space of 15.3% after oral administration of radiosulfate, and 16.8% after its i.v. administration.

In the rat, the radiosulfate space was calculated to be 34% of body weight by Sheatz and Wilde.[113] In the liver, the same percentage of the tissue was available to radiosulfate, but in muscle only 12%. The blood cells equilibrated very rapidly with plasma [^{35}S]-sulfate, and the ratio between the concentration inside and outside blood corpuscles was 0.44. Barratt and Walser reported a ratio of 0.35 for erythrocytes.[105] Later it was shown that in calves[114] and in the rat, the volume of the sulfate space was time-dependent: in the rat it was 23.6 + (1.2 × t) percent of body weight, where t indicates hours after i.v. injection of the radiosulfate.[105] This may explain why much higher volumes have been reported, such as 34.6% of body weight in the rat; this was calculated from a single blood sample taken 3 hr after injection of [^{35}S]-sulfate in nephrectomized rats.[115] The radiosulfate space in various tissue in the rat is given in Reference 105; an unknown part of the radioactivity in each tissue may have been incorporated in small conjugates or in macromolecules. The fractional distribution of total body sulfate has been calculated by Barratt and Walser[105] from the radiosulfate distribution at equilibrium.

Finally, Douglas et al.[116] have pointed out the importance of obtaining the complete plasma disappearance curve for radiosulfate, and not just the final phase, because analysis of the entire curve allows separation and measurement of each component of the elimination process. In the dog, both with intact and ligated kidneys, they observed the same sulfate space, namely about 17% of body weight. Further detailed analysis have been made in man with various diseases.[117]

VII. AVAILABILITY OF INORGANIC SULFATE IN BLOOD FOR INTRACELLULAR SULFATE POOLS; CARRIER TRANSPORT OF INORGANIC SULFATE

Since sulfation processes probably occur in all tissues in the body, sulfate must be available in the cells in each of them. Apart from the possibility that in some of the tissues this sulfate is provided exclusively by oxidation of cysteine, the possibility that inorganic sulfate in blood is used should be considered. It is generally assumed that membranes are virtually impermeable to sulfate ions by diffusion. This idea is supported by studies on sulfate permeation in erythrocyte ghosts, in which only a carrier-mediated transport of sulfate has been observed. In this experimental model the kinetics of sulfate transport have extensively been studied; the question remains, of course, whether the conclusions from these studies can be extended to other cell types.

In brief, the results indicate the involvement of a carrier protein embedded in the cell membrane, that catalyzes the self-exchange of sulfate, and its exchange with chloride.[118-128] Many other anions, for instance chloride, compete for the same transport process. The rate of chloride self-exchange, however, was about 20,000 times larger than sulfate self-exchange.[126] Differences in properties and affinities of both chloride and sulfate transport have been found for the inner and outer membrane surface in erythrocyte ghosts, suggesting asymmetry of the transport system.[128,130]

The sulfate transport system can be saturated with sulfate, and the rate of transport is pH-dependent: the flux decreases when the pH of the medium is increased from 6.5 to 8.5.[122] Several sulfonic acids irreversibly inhibit sulfate self-exchange in erythrocytes. Zaki et al.[119] analyzed proteins of the red cell membrane after solubilization by SDS polyacrylamide gel electrophoresis after these had been labeled with radioactive dinitrofluorobenzene (DNFB). When they incubated the cells with DNFB in the

presence of 4,4'-diacetamide-stilbene-2,2'-disulfonic acid, they prevented labeling with DNFB of only one protein band. At the same time, the irreversible inhibition of sulfate transport by DNFB was prevented. Thus it was postulated that this protein band might be associated with the sulfate carrier. This carrier protein is only digested by trypsin when this protease is included internally in erythrocyte ghosts,[120] or when inside-out vesicles were prepared.[121] Its amino acid sequence is under investigation.[122] Even when the carrier protein is split into two major peptides, sulfate exchange is not completely lost.[120] Erythrocytes from every species studied were sensitive to the action of the protease pronase, and lost their sulfate transport activity.[123]

By treatment of erythrocyte ghosts with the detergent Triton® X-100, smaller vesicles can be prepared which still contain the carrier protein and can accumulate anions.[121] The carrier protein has been purified and incorporated into vesicles prepared from egg phosphatidylcholine; when erythrocyte lipid, cholesterol, and glycophorin were added, the reconstituted band 3-protein catalyzed sulfate transport into these vesicles. This transport was sensitive to pyridoxal phosphate-$NaBH_4$, a potent inhibitor of anion transport in red blood cells.[123] For further characterization of this carrier and its properties, a series of sulfonic acid derivatives of some isocyanates has been used with human red cells. These compounds competitively inhibited sulfate exchange initially, whereas upon incubation for a longer period of time, irreversible inhibition occurred.[125] Depending on the sulfonic acid used, from approximately 4 to 34% of the sulfate efflux was resistent to this inactivation for some as yet unknown reason. The mechanism of this irreversible inhibition is supposedly covalent binding of the sulfonic acid derivatives to the sulfate carrier system or to some protein in its immediate environment. The presence of a "transport site" and a "modifier site" has been suggested to explain the competitive and noncompetitive inhibition characteristics by a number of compounds. Recently, reversible inhibition of anion transport in human erythrocytes by tetrathionate has been reported; this inhibitor was only effective from the outside.[126] The authors suggest that it is bound to the "modifier site." This inhibitor is of interest because it is not an amphiphilic compound as the other sulfonic acid inhibitors are. Recently, Grinstein et al.[124] presented some evidence that the band-3 carrier protein might contain a mobile transport element that moves from the extracellular surface to the cytoplasmic face.

The sulfate exchange is strongly inhibited by relatively low intracellular calcium concentration,[127] whereas extracellular calcium does not affect the transport rate. Magnesium weakly activated sulfate exchange, but did not interfere with the calcium binding site. The K_i for intracellular calcium was only about 6 μM, whereas the apparent dissociation constant for magnesium was 4 μM. Since the free calcium concentration in erythrocytes is only of the order of 0.2 to 0.7 μM, the inhibition by calcium may be of doubtful physiological significance, and may be only relevant under some pathological conditions.[127] A number of anions inhibit sulfate self-exchange in red cells;[122] the order of potency is $NO_3^- > Cl^- >$ acetate and oxalate $> HPO_4^{2-}$. A recent review on the properties of the anion transport system of red blood cells is available.[119]

Much less work has been done with other cell types. Some data, however, are available on ascites tumor cells.[116-119] The findings with these cells are very similar to those with red cells: a carrier system catalyzes the sulfate exchange with a K_m of 2 mM for sulfate. In incubations with [^{35}S]-labeled sulfate at the outside of the cells, and unlabeled sulfate inside, the uptake of [^{35}S]-sulfate is enhanced when the ascites cells are preloaded with high sulfate concentrations, presumably because of a high exchange rate. High chloride concentrations inside the cells also stimulate sulfate uptake, probably through sulfate-chloride exchange. The Cl^-/SO_4^{2-} exchange has a stoichiometry of 1:1.[118] If chloride is present at the same surface as sulfate, it inhibits sulfate

uptake by the cells, and sulfate can inhibit Cl^- self-exchange. This carrier is apparently relatively unimportant for chloride transport, since 94% of chloride uptake is provided by sulfate-independent transport; later findings, however, were interpreted by Levinson such that Cl^- and SO_4^{2-} share a common transport mechanism which is separately and differently influenced for Cl^- and SO_4^{2-} respectively.[139] The same pH-dependence of sulfate exchange was observed as was found with red cells: the flux decreased at higher pH values. Moreover, the pH seemed to determine the steady state ratio between intracellular and extracellular sulfate concentrations in the ascites cells: at pH 8 this ratio was about 0.5, whereas at pH 6 this ratio was about 0.85. The extracellular chloride concentration also affected this ratio with higher values at low chloride (30 to 35 m*M*) than at high chloride (130 to 150 m*M*): differences were 20 to 40% between these extremes. In studies on pH and chloride dependence of sulfate exchange, these effects should be kept in mind because they influence the steady-state intracellular sulfate concentration, and thereby, the sulfate exchange rate indirectly.

Sulfate transport by vesicles isolated from the brush-border membrane of rat-kidney cortex has been studied by Lücke et al.[140] They conclude that an electroneutral Na^+/SO_4^{2-} cotransport is catalyzed by these vesicles. Sodium ion was the only monovalent ion that catalyzed the sulfate transport. This carrier transport evidently is much different from that in erythrocytes. It is not inhibited by chloride, or known inhibitors of the erythrocyte anion transport system. Low concentrations of $HgCl_2$ almost completely inhibited this transport.

It is quite clear that intravenously or intraperitoneally injected inorganic sulfate (for which [^{35}S]-labeled sulfate conveniently has been used) is taken up by many tissues since it is incorporated in many tissues into cellular macromolecules or small molecules. However, usually the rate of uptake and the extent of uptake in these tissues has not been determined. Only in the case of the liver are some data available. Mulder and Scholtens[141] determined the rate of incorporation of intravenously injected [^{35}S]-labeled sodium sulfate into harmol sulfate, a low molecular-weight sulfate ester that is mainly synthesized in the liver and excreted in bile and urine in the rat. No lag-phase for the incorporation of [^{35}S]-sulfate was found, suggesting that sulfate in blood is immediately (i.e., within 1 min) available for sulfation in the liver. Moreover, their data also suggest that the specific radioactivity with which sulfate is incorporated into harmol sulfate follows the specific radioactivity of sulfate in blood. This confirms that there is (almost) no barrier for uptake of inorganic sulfate by the hepatocytes, as suggested by earlier results of Herbai[142] in mice where a rapid equilibration of sulfate in blood with the hepatic pool was found.

Often more detailed data on uptake of sulfate by a tissue are lacking because these data are best obtained using isolated cells, which cannot be obtained from every tisuse as easily as from liver (although even with isolated hepatocytes this type of study has not yet been performed). An alternative preparation in which rates of uptake can be determined is the isolated perfused organ. When the isolated perfused rat liver is provided with various concentrations of inorganic sulfate in the perfusion medium,[107] there is no first pass effect for sulfate. It seems that sulfate rapidly equilibrates with the hepatic intracellular sulfate pool. Even at extremely low concentration (about 0.03 m*M*) it is only slowly eliminated from the perfusion medium by the liver through excretion in bile and presumably, incorporation into compounds in the liver. Mulder and Keulemans[107] calculated from their data that the sulfate concentration in the liver (of the sulfate pool available for sulfation of drugs like harmol) was of the same order of magnitude as the plasma concentration, i.e., about 0.8 m*M*. In this calculation it has been assumed that the sulfate space in the liver was 50% of its weight. If the value determined by Barratt and Walser[108] is correct, namely 36% of liver weight, the concentration in that compartment would be slightly higher than in serum.

In a study in the dog, an extracorporeal system has been used to perfuse the cerebral circulation *in situ*,[143] and the unidirectional flux of sulfate and various other substances into brain was measured. Only slow uptake of sulfate was found, which confirmed the finding by Dziewiatkowski.[7] This author determined that only after 20 hr the concentrations of radioactivity in plasma and brain tissue in the rat were about the same. Ovine brain synaptosomes take up sulfate by a saturable mechanism with a K_m of 4.4 m*M* that is inhibited by *p*-hydroxymercuribenzoate.[144]

The placenta offers no barrier to sulfate passage, which is not surprising since sulfate is required for the synthesis of many substances needed for growth and regulation of the fetus. Several investigations have shown that [^{35}S]-sulfate is rapidly passed to the fetus, and is incorporated into various tissues, such as cartilage.[23,145-148]

Intracellular sulfate may be taken up by subcellular organelles. Rat-liver mitochondria rapidly take up inorganic sulfate, catalyzed by the dicarboxylate carrier;[149,150] similar findings were reported for corn mitochondria.[151] As yet it is not clear whether some organelle(s) may accumulate inorganic sulfate.

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