



CASWL FILE

497AB

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

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AUG 10 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: Imazalil: Review of 21 day Dermal Toxicity Study
in the New Zealand White Rabbit.

BARCODE D171325
Submission: S407030
MRID # 42085201
Tox Chem NO. 497 AB
HED Project : 2-0524
PC Code 111901

TO: Kathryn Davis,
PM # 51
Reregistration Branch
SRR Division (H7508W)

FROM: Henry Spencer, Ph.D. *Heds 7/30/93*
Review Section 3
Toxicology Branch 1
Health Effects Division (H7509C)

THRU: Karen Hamernik, Ph. D. *K. Hamernik 7/30/93*
Section Head
Review Section 3
Toxicology Branch 1
Health Effects Division (H7509C) *KB 8/2/93*

ACTION: Review the 3 week dermal toxicity study in
N.Z. rabbits with Imazalil.

CONCLUSIONS AND RECOMMENDATIONS:

1. The dermal toxicity study in rabbits MRID #
42085201, has been reviewed and has a NOEL at the
HDT, 160 mg/kg of Imazalil applied to the backs of
test animals. An LEL was not established in the
study.

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2. The study is classified as Core: Supplementary based on the apparent lack of an LEL in the study. The HDT was not at a limit dose of 1000 mg/kg. Since the dosing regimen for the present submission was chosen from a range-finding study (stated in report), this range-finding study data will be needed in order to establish if the present dosing levels are appropriate for the study.

3. Provided that the dose-selection can be supported by the range-finding study data, the study may be upgraded.

4. The present study does not fulfill the Guidelines requirements ,82-2.

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FINAL

DATA EVALUATION REPORT

Imazalil

Study Type:
Twenty-one Day Repeated-Dose Dermal Toxicity Study

Study Title:
Evaluation of the Subchronic Dermal Toxicity of Imazalil -- (technical grade)
- R 23979 in New Zealand White (NZW) rabbits

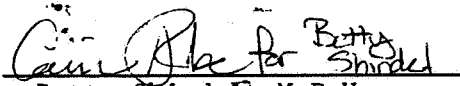
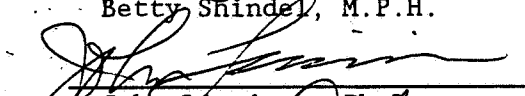
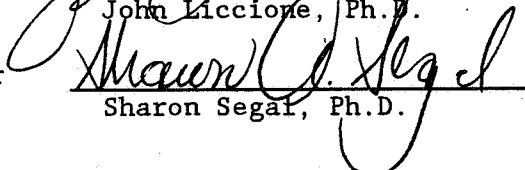
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January 29, 1993

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Contract Number: 68D10085
Work Assignment Number: 1-52.1
Clement Number: 91-256
Project Officer: James Scott

KLH 7/28/93
EPA Section Head: Karen L. Hamernik, Ph.D.
Toxicology Branch I, Review Section III
Insecticides/Rodenticides, (HED)

EPA reviewer: DR. Henry Spencer
Signature: *[Signature]*
Date: 7/30/93

DATA EVALUATION REPORT

STUDY TYPE: Twenty-one day repeated-dose dermal toxicity study

TEST MATERIAL: Imazalil technical

CASWELL NUMBER: 497-AB

SYNONYMS: Fungaflor technical; R 23979

MRID NUMBER: 42085201

STUDY NUMBER: 2418

REG. NUMBER: 43813-2

P.C. NUMBER: 111101

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

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

TITLE OF REPORT: Evaluation of the Subchronic Dermal Toxicity of Imazalil -- (technical grade) - R 23979 in New Zealand White (NZW) Rabbits

AUTHORS: G. Teuns, J VanDenberghe, A. Lampo, W. Coussement, and H. VanCauteren

REPORT ISSUED: August 22, 1991

CONCLUSIONS: NOEL (systemic) = >160 mg/kg/day for males and females
LOEL (systemic) was not established

CORE CLASSIFICATION: Core Supplementary. This study is classified as Core Supplementary because toxicity was not observed at any of the doses tested in males or females. Dose levels used in this study were selected on the basis of the results of a range-finding study in rabbits. The range finding data will be required to complete the evaluation for acceptability of this study. Other study deficiencies include the absence of information on the size of the test site, details regarding test site preparation, temperature and humidity conditions, and dose volume. In light of the skin stippling observed, the sponsor should justify the use of sesame oil as the vehicle.

A. MATERIALS, METHODS, AND RESULTS

1. Test Article Description

Name: Imazalil technical

Physical description: Slightly yellow to brown crystalline mass (can occur as an oily liquid)

Lot number: ZR023979

Manufacturing date: January 23, 1989

Source: Janssen Research Foundation

Stability: Test article solutions stable at approximately 14 days (determined in this study)

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Vehicle/lot number: Sesame oil/25951
Storage: Room temperature, closed containers
Purity: 98.1% (sponsor analysis)
UV absorption: Standard spectrum

2. Test Article Analyses for Purity, Stability, and Concentration

The concentration and stability of the test article solutions were determined from pretest samples. The target concentrations of the test article solutions were 0.5, 2, and 8% w/w. The actual concentrations of the test article solutions were found to be 0.490, 1.950 and 7.77 % w/w, representing 98%, 97.5%, and 97.1% of target, respectively. The number of measurements at each concentration level was not specified. The actual concentrations of the test article solutions approximately 2 weeks later were 0.481, 1.940, and 7.85% w/w, representing 96.2, 97.0, and 98.1% of target (unspecified number of measurements), respectively. The concentrations of the test article solutions approximately 2 weeks later were between 98-101% of the initial concentrations, demonstrating that the test article was stable in solution over a period of approximately 14 days.

3. Test Animals

Species: Rabbit

Strain: New Zealand White

Source: Established stock-farm; detailed information not provided

Sex and numbers: Males, 20; females, 20

Age at study initiation: Not reported

Weight range at study initiation: 2.180-2.800 kg

Rabbits were housed individually in sequentially numbered cages. Information on the temperature and humidity of the animal room was not provided; the room was air-conditioned. Animals were identified by an ear tattoo and a cage label which identified the animal by R-number, experiment, animal number, sex and dosage group, start and end date. The rabbits were acclimated to laboratory conditions for 1 week prior to dosing. Feed (Huybrechts® pelleted rabbit food, administered in self-raising hoppers) and tap water (administered via automatic drinking nipples) were provided ad libitum throughout the study. Rabbits were assigned into groups according to initial body weight by a stratified sequenced randomization procedure. Rabbits were not subjected to any previous experiments.

Dose administration: Groups of 5 rabbits of each sex were dermally administered imazalil technical dissolved in sesame oil, 6 hours a day, 5 days a week, for 3 weeks at doses of 10, 40, and 160 mg of imazalil technical/kg/day (At the high dose, 2 g test article solution/kg/day was administered). Five rabbits of each sex served as a vehicle control group and were administered 2 g of sesame

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oil/kg/day. Imāzalil was dermally applied to a shaved area (dimensions unspecified) on each animal's back. The application site was covered with a porous gauze dressing held in place with a plastic nonirritating tape. The study report did not indicate if shaving was performed approximately 24 hours before the test as recommended by guidelines. In order to prevent ingestion of the test article, the animals were placed in restraint boxes during the 6-hour exposure period. After the exposure period, residual test article was removed from the application site with lukewarm water and the application site was dried with soft cloths. The rabbits remained in the restraint boxes for 1 hour after the end of the exposure period before they were returned to their individual cages.

The rationale for the selection of dose levels was based on a previous range-finding study which was not available for review. In the range-finding study, rabbits were exposed to 63, 250 and 1,000 mg/kg/day of test article for 4 consecutive days. Use of a vehicle was not specified. No further details were provided concerning the administration of the test article. At dose levels of 250 and 1,000 mg/kg/day, slight erythema was observed and after 4 days skin lesions occurred. No dermal irritation was observed at the 63-mg/kg/day dose. At dose levels of 250 and 1,000 mg/kg/day, slight-to-moderate hepatotoxicity was observed upon necropsy. No further details were provided on the results of the range-finding study.

4. Statistical Methods

The Mann-Whitney U test for pairwise comparison with control according to Siegel (Siegel S. 1956. Nonparametric statistics. McGraw-Hill, New York, 1956) was used for statistical analysis of body weight, food consumption, hematology and blood clinical chemistry parameters, urinalysis, organ weights, and histopathology data. The Chi-square test for pairwise comparison with control according to Siegel was used for statistical analysis of mortality and gross pathology data.

5. General Observations

(a) Mortality/moribundity/survival

Each animal was observed once a day for mortality and moribundity. No mortalities were reported for any of the treated or vehicle control animals during the study period.

(b) Clinical observations

Each animal was observed once a day for clinical signs of toxicity. No changes in behavior were observed following treatment. Clinical observations were minimal and consisted of transient diarrhea in 1 male at the 40-mg/kg/day dose during the last week of treatment, and an occasional slight waste of food in 1 female from each of the 10-, 40-, and 160-mg/kg/day dose groups. Red stippling of the skin was reported at all dose levels and in the vehicle controls. The incidence (10/10) and severity (slight-to-severe) were greatest in the vehicle controls. With increasing

dose of imazalil technical the incidence and severity decreased. At the low and mid doses, incidences were 9/10 and 7/10 respectively and the severity was mild-to-severe. At the high dose, the incidence was 2/10 and the severity was slight. In the vehicle controls stippling was first observed at day 7, whereas in the high dose group the stippling was not observed until day 14. Therefore, the stippling appeared to be related to the sesame oil vehicle and not the test article.

(c) Skin irritation

Dermal irritation was scored using the Draize method once daily, 1 hour postexposure. The study report included determination of the Primary Irritation Index (P.I.I.) by averaging all Draize scores for erythema and edema per dosage group.

Evaluation of Primary Irritation Index

<u>P.I.I. Index</u>	<u>Evaluation</u>
0.00	No irritation
>0 and ≤ 1	Irritation barely perceptible
>1 and ≤ 2	Slight irritation
>2 and ≤ 3	Moderate irritation
>3 and ≤ 4	Severe irritation

At dose levels of 10, 40 and 160 mg/kg/day, skin irritation was classified as "barely perceptible" based on P.I.I.s of 0.01, 0.02 and 0.05, respectively. There was no incidence of fissures, necrosis or edema for any of the rabbits. No erythema or edema (P.I.I. = 0.00) were noted for any of the vehicle control animals during the 21-day study; however, slight scaling was noted in 2 vehicle-control females on day 5. At 10 mg/kg/day on day 5 or days 5-7, very slight erythema (Draize score 1) was observed in 1 male and 1 female, and 3 males had slight scaling of the skin. At 40 mg/kg/day, very slight erythema and slight scaling of the skin was observed in 4-5 females as early as day 4 and was resolved no later than days 7-8. At 160 mg/kg/day, very slight-to-well-defined erythema (Draize score 1-2) was observed in 2 females and 4 males on days 4 to 7, and in 1 animal on day 14; slight-to-moderate scaling was observed in 5 females and 4 males on days 4 to 7.

(d) Body weight/body weight gain/food consumption

Body weight was determined prior to study initiation, and on days 7, 14, and 21. Food consumption was measured on days 7, 14, and 21. There were no statistically significant differences in body weight or body weight gain between the vehicle control and treated animals. There were no statistically significant differences in food consumption between the vehicle control and treated animals, except for a dose-related and slight decrease ($p \leq 0.05$) in males at 160 mg/kg/day on day 14 of the study. However, this decrease was transient over time; on days 7 and 21 the mean food consumption

values in males at 160 mg/kg/day were higher than the male vehicle control group. The study authors considered this decrease in food consumption to be insignificant because of its transient nature.

6. Clinical Pathology

Hematology and clinical chemistry parameters were examined in all animals at the end of the study. Blood samples were drawn from the orbital venous plexus. The study report did not indicate if animals were fasted overnight prior to blood collection. The checked (X) parameters were examined.

(a) Hematology

X Hematocrit (HCT)*	X Leukocyte differential count*
X Hemoglobin (HGB)*	X Mean corpuscular hemoglobin (MCH)
X Leukocyte count (WBC)*	X Mean corpuscular HGB concentration (MCHC)
X Erythrocyte count (RBC)*	X Mean corpuscular volume (MCV)
X Platelet count*	

* = Recommended by Subdivision F (November 1984) Guidelines

All hematology parameters were comparable between treated and vehicle control animals except for a statistically significant dose-related decrease ($p \leq 0.05$) in white blood cell count (WBC) in males at 160 mg/kg/day as compared to the male vehicle control group. In males, the WBC counts for the vehicle control, 10-, 40-, and 160-mg/kg/day dose groups were 16.2, 13.4, 11.4, and 10.4 $\times 10^3$ cells/mm³, respectively. A dose-related decrease in WBC was not observed in females. Although the dose-related decrease in WBC in males was statistically significant, the study authors stated that the WBC was within the normal range and did not consider the decrease to be biologically significant. The normal range values for WBC for this species of rabbit was not provided.

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(b) Blood (clinical) chemistry

Electrolytes

X Calcium*
X Chloride*
X Sodium*
X Phosphorus*
X Potassium*

Other

X Albumin*
X Glucose*
X Blood creatinine*
X Blood urea nitrogen* (BUN)
X Total bilirubin*
X Direct bilirubin
X Total protein*
X Globulin
X Cholesterol
X Haptoglobin
X Triglycerides
X Phospholipids

Enzymes

X Alkaline phosphatase (ALP)
X Serum alanine aminotransferase (SGPT)*
X Serum aspartate aminotransferase (SGOT)*
X Gamma glutamyltransferase (GGT)
X Creatinine phosphokinase (CPK)
X Lactic dehydrogenase (LDH)
X Cholinesterase

* = Recommended by Subdivision F (November 1984) Guidelines

All clinical chemistry parameters were comparable between treated and vehicle control animals except for a statistically significant decrease in the mean sodium value for males at 160 mg/kg/day ($p \leq 0.05$) and females at 40 mg/kg/day ($p \leq 0.01$), as compared to their respective vehicle control. Although the decreases in sodium were statistically significant, they were not biologically significant because the study authors stated that the sodium values were within the normal range; the normal range for sodium was not provided. The decreases in sodium were extremely slight and did not exhibit a dose-response relationship. The sodium levels for males for the vehicle control, 10-, 40-, and 160-mg/kg/day dose groups were 144, 144, 142, and 142 mEq/L, respectively. The sodium values for females for the vehicle control, 10-, 40-, and 160-mg/kg/day dose groups were 143, 142, 140, and 142 mEq/L, respectively.

(c) Urinalysis

Urinalysis was performed on each rabbit at the end of the study period. The study report did not indicate how the urine samples were collected (i.e. voided or collected directly from the bladder). The checked (X) parameters were examined.

X pH	X Protein	X Glucose
X Bilirubin	X Urobilinogen	X Acetone bodies
X Creatinine	X Specific gravity	X Sediment (microscopic)
X Occult blood		

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Table 1 summarizes selected urinary parameters. Statistically significant decreases in creatinine levels ($p \leq 0.01$), specific gravity ($p \leq 0.01$), and urobilinogen levels ($p \leq 0.05$) were observed in males in the 160-mg/kg/day dose group as compared to the male vehicle control group and were considered by the study authors to be possibly treatment related. The only statistically significant difference between urinary parameters in treated females as compared to the female vehicle control group was a statistically significant decrease ($p \leq 0.05$) in urobilinogen in the 40-mg/kg/day dose group.

7. Sacrifice and Pathology

A gross necropsy was performed on all rabbits at the end of the study. Tissues marked with an (X) were collected from rabbits in the vehicle control and high-dose group, preserved in 10% buffered formalin, and histologically examined. Those tissues marked with (w) were also weighed.

<u>Digestive</u>	<u>Respiratory</u>	<u>Urogenital</u>
X Liver ^w Pancreas ^w	X Lungs ^w	X Kidneys ^w Gonads ^w
<u>Glandular</u>	<u>Neurologic</u>	<u>Cardiovascular/Hematologic</u>
X Adrenals ^w Thyroid ^w	X Brain ^w	Heart ^w Spleen ^w Thymus ^w
<u>Other</u>		
X Normal and treated skin*		

* = Recommended by Subdivision F (November 1984) Guidelines

(a) Macroscopic pathology

The study authors stated that gross pathology findings were not related to treatment and were common findings in a normal rabbit population. Dark or red foci on the skin, generally measuring 1-3 mm, and frequently swollen were observed with the greatest incidence in the vehicle controls (8/10) and with decreasing frequency with increasing doses of imazalil technical. The incidences were 7/10, 6/10 and 2/10 at the low, mid, and high doses. One control female and one high-dose male had dark foci in the lungs. Pale and swollen kidney and pronounced lobulation of the liver were observed in 1/5 females at 160 mg/kg/day. One male receiving 40 mg/kg/day had a small thymus.

(b) Organ weights and organ/body weight ratios

Statistically significant differences in organ weights of treated animals as compared to vehicle control animals were observed for the pancreas, brain and heart. A statistically significant

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decrease ($p \leq 0.05$) in relative heart weight was observed in females at 40 mg/kg/day, as compared to the female vehicle control group. A statistically significant decrease ($p \leq 0.05$) in absolute pancreas weight was observed in males at 40 mg/kg/day, as compared to the male vehicle control group. Statistically significant increases in absolute brain weights were observed in males at 40 mg/kg/day ($p \leq 0.01$) and 160 mg/kg/day ($p \leq 0.05$), as compared to the male vehicle control group. No dose-response relationship was observed for the differences in organ weights between treated and vehicle control animals. The study authors stated that the changes in organ weights were insignificant because the differences were minimal and the values remained within the normal physiological range.

(c) Microscopic pathology

A scoring system was used to grade the degree of histological change: score 0 = absent, scores 1 to 5 = slight-to-severe histological change or small-to-large quantity of a histological entity. The mean scores for microscopic findings did not show statistically significant differences for the kidneys, liver, lungs, and skin of high-dose groups as compared to their respective vehicle control groups. Therefore, none of the microscopic findings were considered to be treatment related. The low and mid-dose groups were not examined histopathologically.

The reviewers have no other comments regarding the materials and methods sections.

A signed Good Laboratory Practice Compliance Statement and a signed Quality Assurance Statement were included.

B. DISCUSSION

Toxicity was not observed in males or females ~~or females~~ at any of the doses tested; therefore this study provides only supplementary information on the systemic toxicity of imazalil technical following dermal exposure to imazalil technical for 21 days. Dose levels used in this study may not have been appropriately selected on the basis of the results of a range-finding study in rabbits, and the age of the animals. In the dose range-finding study, imazalil technical at dose levels of 63, 250, and 1,000 mg/kg/day was administered to rabbits for 4 consecutive days. Information regarding the duration for which the test material was in contact with the skin in the range-finding study was not available. At dose levels of 250 and 1,000 mg/kg/day, slight erythema and skin lesions (after day 4) were observed; slight-to-moderate hepatotoxicity was observed upon necropsy. Based on these range-finding results, dose levels of 10, 40 and 160 mg/kg/day were chosen for use in the 21-day repeated dose dermal toxicity study. However, no effects of toxicological significance were seen at the high dose of 160 mg/kg/day. Therefore, dose levels >160 mg/kg/day could have been tested.

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Several important details concerning the conduct of this study were not mentioned. The size of the test site was not specified; therefore, the reviewers cannot determine with certainty that not less than 10% of the animal's body surface area should be clear for test substance application according to the Guideline requirement. The animals' ages were not reported; guidelines specify the use of adult animals. The study report did not indicate if shaving of the test site was performed approximately 24 hours before the test as recommended by guidelines. The study report did not indicate if animals were fasted overnight prior to blood collection.

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The only results that were considered to be possibly treatment related by the study authors were statistically significant decreases in the urinalysis parameters of creatinine, specific gravity, and urobilinogen in high-dose males as compared to the male vehicle control group. Statistically significantly decreased urobilinogen was also noted in females in the 40-mg/kg/day dose group. However, since these changes in urinary parameters were not accompanied by histopathological findings or changes in relevant **clinical chemistry** parameters, the toxicological significance of these changes is not clear. The results of urinalyses testings are variable, especially when evaluating only 5 animals per sex, per group. Statistical analyses of pairwise comparisons for organ weight, body weight, food consumption, hematology and clinical chemistry parameters, urinalysis and histopathology data were performed by use of the nonparametric Mann-Whitney test. Because the data were probably normally distributed, a more powerful parametric test could have been used. However, it is unlikely that a more powerful test would have changed the conclusions of this study.

Dermal irritation resulting from administration of imazalil technical was classified as "barely perceptible" based on a calculated Primary Irritation Index (P.I.I.) of 0.01, 0.02, and 0.05 at doses of 10, 40 and 160 mg/kg/day, respectively.

In summary, the NOEL for systemic toxicity in males and females was >160 mg/kg/day. A LOEL for systemic toxicity was not established in males or females.

Table 1. Selected Urinary Parameters from Rabbits Dermally Administered Imazalil Technical for 21 Days^a

Dose Group (mg/kg)	Urinary Parameters		
	Creatinine (mg%)	Specific Gravity	Urobilinogen (Ehrlich U)
<u>Males</u>			
0 ^b	116	1.045	1.20
10	95	1.039	1.00
40	87	1.026	0.74
160	60**	1.022**	0.52*
<u>Females</u>			
0 ^b	125	1.044	1.10
10	136	1.042	0.80
40	67	1.026	0.34*
160	112	1.035	0.72

^aData extracted from Study Report no. 2418, p.45.

^bVehicle control (sesame oil)

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

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

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