CASWELL FILE

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

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MEMORANDUM

SUBJECT: 773-EUP-E, Imazalil (Clinafarm 15% EC)
Caswell No. 497AB (Accession No. 258175

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

FROM: Carlos A. Rodriguez CAR 11/14/85
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Review Section No. 6
Toxicology Branch
Hazard Evaluation Division (TS-769C) 11/20/85

Registrant: Pittman-Moore, Inc.
P.O. Box 344
Washington Crossing, NJ 08560

Pittman-Moore, Inc. has submitted an application for an EUP to use the fungicide Imazalil (enilconazole) 1-[2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl]-1H-imidazole in poultry houses for the control of Aspergillus fumigatus contamination in hatcher cabinets and in poultry house litter when birds are not present.

Recommendation(s):

1. TOX Branch defers to RCB concerning possible secondary residues in poultry as a result of the proposed usage pattern which might involve the hens eating the treated litter. We are also concern about residues in RAC's grow in land which has been covered with the treated litter from poultry houses.

2. The eye irritation study submitted on this product formulation indicates that Clinafarm 15 percent concentration produces mild to moderate corneal opacity which was not reversible during the 14-day observation period, therefore, the label precautionary statements must be revised as follows:

   a. Change the signal word from "Warning" to "Danger."

b. Revise the statement "Causes substantial, but temporary, eye injury" to read "Causes eye damage."

2. The acute oral LD_{50} (rat and mouse), the acute dermal LD_{50} (rabbit), the 21-day dermal toxicity (rabbit), and primary dermal irritation (rabbit) studies submitted with this application are adequate and designated Core-Minimum studies.

3. Submit acute inhalation LC_{50} and sensitization studies on this product as formulated to determine the safety to humans that are likely to experience inhalation and dermal exposure after application.

4. Toxicology Branch recommends against the experimental use permit for reasons cited in 1a, 1b, and 3. These deficiencies must be resolved for further consideration on this application.

5. The following is a summary of the Category of Toxicity and Core Classification for the toxicity studies submitted with this application of Clinafarm (15% ai imazalil):

Study Type	Category of Toxicity	Core-Classification
a. Acute oral LD_{50} (ml/mouse): male mice = 0.056 (0.043-0.073) ml/mouse. female mice = 0.091 (0.049-0.0170) ml/mouse.	III	
The corresponding doses of formulation in mg/kg: male mice u 2600 mg/kg. female mice u 4200 mg/kg.		Minimum
b. Acute oral LD_{50} (ml/rat) - male rat = 0.52 (0.40-0.68) ml/rat. female rat = 0.31 (0.17-0.57) ml/rat.	III	
The corresponding doses of formulation in mg/kg: male rat = 2100 mg/kg. female rat = 1300 mg/kg		Minimum

c.	Acute dermal LD_{50} (rabbit) male and female > 6 ml/kg	III	Minimum
d.	Primary eye irritation (rabbit)	I	Minimum
e.	Primary dermal irritation (rabbit)	IV	Minimum
f.	21-Day repeated dermal (rabbit) NOEL 1/ml/kg		Minimum

6. Additional toxicity data considered in setting this action:

a. Teratology (rat) - Teratogenic NOEL > 40 mg/kg/day (HDT). Maternal NOEL = 10 mg/kg. Maternal LEL = 40 mg/kg (decreased food consumption, increased mortality). Fetotoxic NOEL > 40 mg/kg.

b. 3-Generation Reproduction (rat) - Reproduction NOEL > 40 mg/kg/day (HDT). Systemic NOEL > 40 mg/kg/day (HDT). Levels tested 2.5, 10, and 40 mg/kg.

c. Fertility (rat) - Fertility NOEL > 40 mg/kg/day (HDT). Levels tested 2.5, 10 and 40 mg/kg

d. 2-Year Feeding (dog) - NOEL = 1.25 mg/kg. LEL = 5 mg/kg (decreased body weight). Levels tested 1.25 and 5 mg/kg.

e. 2-Year Chronic Feeding/Oncogenic (rat) - Oncogenic NOEL > 40 mg/kg/day. Systemic LEL = 40 mg/kg/(rel. liver and kidney wt. increase in females). systemic NOEL = 10 mg/kg. Dosage Levels Tested: 2.5 10, and 40 mg/kg.

f. 18-Month mouse oncogenic NOEL > 40 mg/kg (HDT). Doses tested 0, 2.5, 10, 40 mg/kg.

g. Metabolism (rat) - More than 80 percent of the dose excreted in urine and feces within 48 hours. Accumulation in fatty tissues did not occur.

h. Mutagenic Micronucleus test (rat) - No structural chromosomal aberrations induced at 160 mg/kg (highest level tested).

i. Mutagenic - dominant lethal (mice) - Not a mutagen at 160 mg/kg (HDT).

A. Composition of Clinafarm 15% EC:

Active Ingredient:

Enilconazole: 1-(2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl)-1H-imidazole.	13.76%
Inert Ingredients:.	86.24%
Total	100.00%

B. Use in Hatcheries and Poultry Houses:

For the control of Aspergillus fumigatus contamination in hatcher cabinets and in poultry house litter.

C. Application:

Dilute Clinafarm 1:100 in water to provide an antifungal concentration of 0.15 percent active ingredient for spray application for cabinets and litter in the absence of birds.

D. Review of Toxicity Data Submitted:

Acute Studies:

1. Acute Oral LD_{50} Study of Enilconazole 15% EC (R23979) in Mice (Dept. of Pharmacology, Janssen Pharmaceutica, Study Report #R23979/43 (V4860), September 1983).

Twenty-five male and twenty-five female adult albino mice weighing between 20 g and 23 g for the males and between 20 g and 24 g for the females were used in this study. The animals were distributed into five groups, each group composed of five male and five female mice. The sample was administered by gavage at dose levels of 0.025, 0.05, 0.10, and 0.2 ml per mouse. A control group

received 0.2 ml of solvent article used in the study. Before oral administration, the mice were fasted overnight, individually caged and observed for 14 days for behavioral effects and number of deaths. All animals in the study were subject to gross necropsy.

Results:

Acute oral LD_{50} (ml/mouse)

male mice = 0.056 (0.043 - 0.073) ml/mouse
female mice = 0.091 (0.049 - 0.170) ml/mouse

The corresponding doses of formulation in mg/kg:

male mice ≅2600 mg/kg
female mice ≅4200 mg/kg

Dosage Levels	Clinical Observations
0.05 ml/mouse	hypotonia and hypothermia
0.10 ml/mouse	hypotonia, hypothermia, tremors, diarrhea and loss of righting reflex.
0.20 ml/mouse	loss of righting reflex, exophthalmos and sedation.

Necropsy Observations:

White deposits in stomach, intestinal bleeding, slight gastric lesions.

Study Classification:

Core-Minimum

Toxicity Category:

III

2. Acute Oral LD_{50} Study of Enilconazole 15% EC (R23979) in Rats, (Dept. of Pharmacology, Janssen Pharmaceutica, Belgium, Report # R2399/42 (V4859), September 1983).

Thirty male adult Wistar rats weighing between 230 to 275 g and 30 female adult Wistar rats weighing between 221 to 262 g were used in this study and distributed into 6 groups, each group composed of 5 male and 5 female rats. The test material was administered as a single oral dose on a milliliter per rat basis. Dosage levels

3. Primary Dermal Irritation with Clinafarm-R 23979-15% EC in Rabbits, (Dept. of Toxicology, Janssen Pharmaceutica, Belgium, Experiment # 1353, October 30, 1983).

A dose of 0.5 ml of the undiluted test material was applied to six mature albino rabbits of the New Zealand White strain weighing between 2 and 3 kg. Twenty-four hours before dosing, the fur was removed from the dorsal area of the trunk of the animals. A control group of three animals received 0.5 ml of placebo. Each test site was occluded with a gauze patch and held in place with a non-reactive tape. Four hours after dosing, the wrapping was removed and any residual test solution was removed using water. The skin reactions were evaluated and the reactions were scored at 30 - 60 minutes, 1, 2, 3, 7 and 14 days. The Draize method of scoring was used.

Results:

Very slight erythema (Grade 1) in one male rabbit and in two female rabbits persisted through the 3rd day reading. Control placebo produced similar results.

Classification:

Core-Minimum

Toxicity Category:

IV, mild dermal irritation

4. Adult Dermal LD_{50} Study with Clinafarm-R 23979-15% EC in Rabbits, (Dept. of Toxicology, Janssen Pharmaceutica, Belgium, Experiment # 1355, January 10, 1984).

Ten male and ten female adult New Zealand White rabbits weighing between 2.160 to 3.090 g for males and 1.720 to 2.600 g for the females and whose initial age varies between 8 to 12 weeks were used in this study. Twenty-four hours before dosing, all fur was removed from the dorsal area of the trunk of each animal and subsequently abraded. Five males and five females were treated with the test material at a dose of 6 ml/kg (900 mg/kg ai). The other five males and five females served as a control group and were treated with placebo. The test sites were covered with a porous gauze dressing and non-irritating tape throughout a 24-hour exposure

period. The test sites were further wrapped in rubberized cloth to avoid evaporation of the test material. The dressings were removed 24 hours after exposure and the residual of the test material removed using water or an appropriate solvent. The rabbits were observed daily during a 14-day period for toxic signs and mortality. Body weights were recorded on day 0, 7 and 14. Gross necropsy were performed on all dead and surviving animals at the termination of the study.

Results:

Acute Dermal LD_{50} > 6 ml/Kg

Mortality - no mortality occurred due to the exposure of the test material.

Autopsy - no gross pathological lesions were observed.

Body Weights - body weights of all animals (control placebo and test material) were comparable throughout the study. All animals gained weight.

Clinical Observations - very slight erythema up to 4 days after exposure.

Study Classification:

Core-Minimum

Toxicity Category:

III

5. 21-Day Repeated Dose Dermal Study with Clinafarm 15% EC, (Dept. of Toxicology, Janssen Pharmaceutica, Belgium, Experiment #1386, June 5, 1984).

Thirty adult New Zealand White rabbits (15 males and 15 females) weighing between 1.85 and 3.27 kg and whose initial age varies between 8 to 12 weeks were used in this study. Twenty-four hours before dosing all fur was removed from the dorsal area of the trunk of each test animal and subsequently abraded. The animals were divided into three groups consisting of five males and five females each. Each rabbit received a total of 15 dermal applications of the test material over a 3-week period. Control animals received dermal applications of physiological saline and placebo.

Each group was treated as follows:

Groups	Treated Level
1. Control (physiological saline)	1 ml/kg
2. Control (placebo)	1 ml/kg
3. Clinafarm 15%	1 ml/kg

Animals were weighed on day 0, 7, 14 and 21 and observed for systemic effects and mortality throughout the study.

At the completion of the 21-day study and prior to sacrifice a complete haematological analysis including haematocrit, haemoglobin, thrombocyte, red and white blood cell count and differential count were performed.

Blood chemistry analysis including sodium, potassium, chloride, total protein, albumin calcium, alkaline phosphatase, total bilirubin, urea nitrogen, glucose, aspartic amino-transferase, inorganic phosphorus, haptoglobin, cholesterol, creatinine, alanine aminotransferase, globulin, albumin/globulin and BUN/creatinine were performed at termination of the study.

Animals were sacrificed at termination of the study and all organs were examined macroscopically at autopsy with special examination of the following major organs: kidneys, liver, adrenals, testes, ovaries, and treated and untreated skin.

Results:

Mortality - no mortality occurred in the control groups or in the treated groups.

Body Weight - The average body weight gain was considered normal in all the groups throughout the study.

Toxic Effects:

No irritation was observed in the physiological saline control group. Minimal irritation consisting of mild erythema was observed in both the control placebo and the group receiving the test substance. The irritation was more pronounced in the test group.

Hematology:

Hematological determination at study completion (21-day study) showed parameters tested in treated rabbits to be comparable to control groups.

Clincial Chemistry:

Blood chemistries determination at study completion (21-day study) showed parameters investigated in treated rabbits to be comparable to control groups.

Gross Pathology Observations:

Control Group (Physiological saline)

lung - a few very small hemorrhagic spots in two rabbits.

liver - a few nodules in two rabbits.

Control Group (Placebo)

lung - focal pneumonia in three rabbits.

liver - yellow spots in four rabbits, white-yellow spots in one rabbit, a few nodules in one rabbit.

Test Group (Clinafarm 15%)

lung - local pneumonia in one rabbit.

liver - white spots in one rabbit, yellow spots in three rabbits, white-yellow spots in two rabbits, local pneumonia in one rabbit, a few nodules in three rabbits, diarrhea in one rabbit.

Data extracted from pages R-10 and R-11 of laboratory results.

Histology (liver):

Results of the histological examination of the liver indicates the present of coccidiosis in the various groups. This finding was more pronounced in the Clinafarm 15% group. Other findings failed to reveal abnormalities of any type that could be related to adverse compound effects.

Data extracted from pages R 12 and R 13 of laboratory results.

Conclusions:

A systemic NOEL of 1 ml/kg in this study from the one dose level selected.

This study can only be classified as Minimum.

6. Primary Eye Irritation Study in the Rabbit with Clinafarm 15% EC, (Dept. of Toxicology, Janssen Pharmaceutica, Belgium, Experiment No. 1291, June 30, 1983).

Nine young adult New Zealand strain, six males and three females weighing approximately 2 kg were used in this study. One-tenth (0.1) ml of the undiluted test material was instilled into the lower conjunctival sac of the left eye of each rabbit. The lids were held closed for one second. The right eye of each rabbit was untreated and served as control. The three female rabbits had their eyes flushed 30 seconds after treatment and rinsed with 30 ml of lukewarm water. The six male rabbit eyes were not rinsed. Ocular reactions were scored according to Draize Scoring Method at 1 hour, and 1, 2, 3, 4, 7, 10, and 14 days after instillation.

Results:

Nonirrigated eyes - mild to moderate corneal opacity persisted in 6:6 rabbits throughout the 14-day observation period. Mild iritis persisted in 1:6 rabbits through the 14-day observation period. Mild to moderate conjunctivitis in 3:6 rabbits through the 14-day observation period.

Irrigated eyes - mild to moderate corneal opacity persisted in 3:3 rabbits through the 14-day observation period. Mild iritis persisted in 1:3 rabbits through the 14-day observation period.

Study Classification:

Core Minimum

Toxicity Category:

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85635:Rodriguez:C.Disk:KENCO:10/23/85:TAR:VO
Revised:Rodriguez:C.Disk:KENCO:10/30/85:EK:PS
85519:Rodriguez:C.Disk:KENCO:11/26/85:EK:JH