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

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MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

DATE: November 9, 1981

SUBJECT: Review of Data Submitted in Accordance with the Registration Standard for Terrazole EPA Registration No. 1258-812. Caswell No. 428

FROM: Roger Gardner, Toxicologist Roger Gardner
Toxicology Branch, HED (TS-769)

TO: Henry Jacoby, Product Manager
Registration Division (TS-767) WSJ

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Action Requested: Review of a subchronic 21-day dermal toxicity study and a teratogenicity study.

Recommendations:

1. The submitted dermal toxicity data should be accepted to satisfy requirements stated in the Registration Standard for Terrazole.

2. The rat teratology study should be accepted to partially satisfy the teratogenicity data requirements. There is still a need for a teratogenicity study in a second species which is to be conducted (See Background below).

Conclusions

The 21-day dermal toxicity study was re-submitted with a more detailed histopathology report (See Background below) which demonstrates a no-observed-effect level of 94 mg/kg/day and a lowest-effect-level of 188 mg/kg/day.

The teratogenicity study in rats demonstrates that 10 mg/kg/day has no effect on fetuses in treated pregnant rats under the conditions of the study (See the discussion of the Teratology study below). This dose was the highest level tested.

Background

In September, 1980, the Agency published a pesticide Registration Standard for Terrazole. Data gaps for subchronic 21-day dermal toxicity and a teratology study in a second species were identified. A letter dated November 18, 1980 was received from the Olin Corporation (the Registrant). The letter contained comments on the Standard which were discussed in a meeting held on December 16, 1980.

Olin contended that there was no need to repeat the existing 21-day dermal study. The Toxicology Branch requested that the registrant provide a detailed pathology report before the contention could be addressed. The Registrant also stated that the teratogenicity data gap should not be identified until the audit of an existing study conducted by IBT was completed. Subsequent to the December 16 meeting, an agreement was made between the Registrant and the Agency (Memorandum dated January 2, 1981, from Marcia Williams, Director, SPRD. To: Edwin Johnson, DAA, OPP. Subject: Meeting with Olin Corporation discussing Terrazole Registration Standard) to repeat the teratology study.

Review of Percutaneous Toxicity Study in Rabbits.

Citation: Larson, P. S., and J. F. Borzelica. 1965. The percutaneous toxicity to rabbits (abraded skin) of Olin Compound 2424 (Olin 2424-9 Technical). Unpublished study conducted in the Department of Pharmacology, Medical College of Virginia. Received February 28, 1981. EPA Accession Number 244688.

Materials and Methods: Test substance. Technical grade Terrazole (Olin 2424-9). No other description provided.

Animals Groups of 6 male and six female albino rabbits were used for each dosage group and the control group. They were housed individually, hair was clipped from the trunk of each animal once each week during the study beginning with the first day of treatment. Exposed skin was also abraded at the beginning of the experiment. Abrasion did not penetrate the stratum corneum as indicated by the lack of bleeding.

Dosages and application of the test substance. The test substance was applied as furnished to the abraded skin of the rabbits at dosages of 0, 94, 188, 375, or 750 mg/kg each day 5 days each week for three weeks. The test substance was spread over the abraded skin with a glass rod. Control rabbits were sham treated.

Observations. Rabbits were weighed daily 5 days each week for 3 weeks. Blood was taken from all survivors at the end of the three week treatment period. The marginal ear vein was used to obtain these samples which were used to determine hematocrit, hemoglobin, as well as total and differential white cell counts. Bladder urine was obtained at necropsy and analyzed for pH, reducing substances and protein.

The day after the last treatment two thirds of the rabbits in each group were sacrificed. Brain, thyroid, heart, lung, liver, gall bladder, spleen, adrenal, kidney, gonad, urinary bladder, stomach, small intestine, large intestine, femur, sternum and skin (from exposure site) tissues were preserved. Only the thyroid, heart, lung, liver, adrenal, kidney, testes skin, and bone marrow were examined microscopically. The remaining third of the animals were sacrificed and examined two weeks after the last dose was administered.

Results After the first or second application of Terrazole, erythema was observed. The treated skin hardened after 3 to 5 applications. The crusts that formed during treatment or two weeks following treatment did not separate, and treated skin still had to be clipped once each week. The central nervous system appeared to be depressed prior to death, but no overt signs of toxicity were noted in survivors during the treatment period.

All of the rabbits treated with the 750 mg/kg dose for three days died, while no deaths occurred in the 94 mg/kg treated group during the treatment period. In the group receiving the 375 mg/kg dose, all of the rabbits died after four to six applications. Two males treated at the 188 mg/kg dose died after 5 applications, while one female died after 13 applications of the same dose were made.

Body weight gain was seen in both the control and 94 mg/kg dose group. The mean body weight for the 188 mg/kg/day group was 79.5% or 76.6% of that for the control group at the end of the treatment period for males and females, respectively. Body weights of the lowest dose were 81.6% of the control body weights for males, and females had body weights that were 10.5% greater than controls.

Mean hematocrit and hemoglobin values were lower in treated animals than controls, but no significance was placed on the results and ranges were not provided. These result are summarized as follows:

	Males		Females	
Dosage (mg/kg/day)	Hematocrit	Hemoglobin (gm/100 ml)	Hematocrit	Hemoglobin (gm/100 ml)
0	46	14.0	42	12.9
94	41	11.9	35	10.7
188	41	11.5	36	10.4
Two weeks after treatment ended				
0	43	13.1	41	11.1
94	40	12.7	37	11.3
188	36	10.0	44	10.4

The mean total white blood cell counts were also lower in treated animals. However, no ranges or discussion of their significance was provided. These results are as follows:

Dose (mg/kg/day)	WBC count X10^3 Males	Females
0	14.2	17.4
94	11.6	9.4
188	12.1	13.7
Two weeks after treatment ended.		
0	13.5	15.1
94	18.8	11.7
188	13.7	10.8

Urinary values were within normal ranges in treated animals.

Lesions of the liver, kidney, myocardium, and skin appeared to be compound related. Calcification was observed in the myocardium, kidneys, liver, lungs, strap muscle, and skin of rabbits in the 188 mg/kg/day group with the extent of involvement varying from animal to animal. Calcification was not observed in rabbits receiving the 94 mg/kg daily application. The histopathology in treated animals was otherwise characterized by focal necrosis and inflammatory changes. No other compound related changes were noted in the organs examined.

Discussion and conclusion This study suggests that a no-effect level of 94 mg/kg/day applied to abraded skin for three weeks is likely. The body weights for males treated with the lowest dose were approximately 80% of control weights. Female rabbits treated the same way had mean body weights 10% more than that of control females. In view of the weight gain noted in the low dose males, it is unlikely that compound related toxicity occurred at the lowest dose. The lowest effect level is 188 mg/kg/day.

Core Classification: Minimum

Review of a Teratogenicity Study in rats.

Citation: Matsutani, T., M. Tamaru, M. Nagayoshi, and Y. Sakamoto. 1975. Studies of the teratogenicity of F 2424 in rats. Report Number 3668. School of Medicine, Nagoya Hoken Elsei University. Unpublished data data submitted by Olin Corporation, Standard, Ct. EPA Accession Number 245423.

Materials and Methods: Test substnce: The test substance was described only as F 2424. Animals: Wistar Imamichi strain rats were used. Four pregnant rats were housed in each cage until day 21 of gestation. At that time those dams permitted to nurse their litters were housed individually. Day 0, of gestation was determined as the day sperm were found in the animals vaginas. Animals were kept in a room with a temperature of 24±1° C and 60±5% relative humidity. Food (MF, Oriental Kono Co.) and water were provided ad libitum.

Experimental procedure. Groups of 16 pregnant rats were given daily doses of 0, 0.4, 2, or 10 mg Terrazole per kg body weight on days 7 through 16 of gestation. Dosages were suspended in 0.5% tragacantl solution and given by stomach tube. The rats were sacrificed under ether anesthesia on the 21st day of gestation. Laporatomies were performed and the numbers of implantations, live fetuses, resorbed embryos and dead fetuses were noted. The sex, body weight and length, tail length and general appearance of live fetuses was also recorded. Organs were removed from these fetuses, and the carcuses were fixed in absolute ethanol. The Dawson method for staining bone was used, and the skeletons were examined for anomalies. Body weight and food consumption for the dams was measured daily on days 7 through 21 of gestation.

A second experiment in which the 0, 0.4, 2, or 10 mg/kg/day doses were given to groups of 5, 7, 7, or 6 pregnant rats, respectively, the dams were allowed to give birth to their litters and nurse the offspring for 21 days. Daily doses were given on days 7 through 16 of gestation. In addition to the measurements of maternal body weight and food consumption on days 7-21 of gestation, these parameters were observed within 24 hours and on days 4, 7, 14, and 21 after birth. The sex, body weight, and general appearance of newborn pups was noted immediately after birth. The time of eye opening and behavior during the first 21 days after birth were observed. The animals were sacrificed by exsanguination 21 days after nursing and their organs were examined for anomalies.

Reported Results: There were no compound related effects on body weight or food consumption in treated rats from the two experiments. No effects on fetuses or newborn pups were attributable to the test substance.

Conclusions: No fetoxicity or teratogenic effects were found in rats treated with 10 mg Terrazole per kg body weight daily for 10 days during gestation. The 10 mg/kg/day dose was the highest dose tested.

The authors states that observation of 10 fetuses from dams given the highest dosage had 14th ribs. They described the incidence as similar to that usually seen in untreated rats, but not data are included in the report to confirm such a statement.

There is also no indication that dosage selection was based on a range finding study. There are no-effects on body weight or mortality to indicate that a maximum tolerated dose was tested.

The submitted report is apparently a translation made by an unidentified individual or organization. Also, the photographs of anomalies and variations which were noted in the text did not reproduce well enough to be useful. The material tested was also not clearly identified (F 2424 is not the same designation for technical grade Terrazole as that of Olin 2424-9-0 in the dermal toxicity study).

Core Classification: Minimum