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Chlorothalonil Equivalents in the Blood and Urine of Rats Following Oral, Endotracheal, and Dermal Administration of ^{14}C-Chlorothalonil

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Chlorothalonil is a highly effective fungicide which is active against a wide variety of commercially important plant diseases. Chlorothalonil may have the potential to enter the body directly through the skin or by inhalation and/or ingestion.

To evaluate what the potential human exposure to chlorothalonil might be, it is necessary to know what quantity of chlorothalonil and/or its metabolites exist in the blood and urine following various avenues of exposure. Therefore, the oral, endotracheal, and dermal administration of chlorothalonil to an animal model was undertaken to better understand its potential for absorption in a mammalian system.

MATERIALS AND METHODS

The ^{14}C-label was uniformly distributed throughout the six aromatic-ring carbons of chlorothalonil. Specific activity was 4.6 mCi/ mmole. Purity of the ^{14}C-chlorothalonil was 99.5% as evaluated by thin layer chromatographic development on silica gel plates in a hexane: acetone:ethyl acetate, 50:30:20 (v/v/v), solvent system to a distance of 14.5 cm from the point of application. A single component (>99% pure) was detected by the combined methods of visualizing under short (254 nm) and long (380 nm) ultraviolet light and scanning of the silica gel strip with a scanner.

Male Sprague-Dawley rats weighing 300 g from Camm Research Institute were used. Food and water were available ad libitum before and after the administration of the test substance. For a period of at least 1 week following receipt of animals, all rats were individually housed in cages and observed for any abnormal signs. After dosing, the rats were maintained in stainless steel metabolism cages for 48 h.

Dosing samples were prepared as follows: Toluene (0.04 mL) was added to a tube containing 160 uCi ^{14}C-chlorothalonil. To this tube 2 mL of 1% Tween 80 in isotonic saline was added. The tube was vortexed and a 1 mL aliquot of the vortexed suspension was used to dose the

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rats dermally at 0.05 mL/rat. Another 1 mL aliquot was diluted to 6 mL with 1% Tween 80 in isotonic saline. This dilution was again vortexed and used to dose the rats orally and endotracheally at 0.3 mL/rat. All rats received about 5 uCi of ^{14}C-chlorothalonil.

Oral Dosing. A 1.0-mL tuberculin syringe was fitted with a 3 inch, 19 gauge curved animal feeding needle for each intubation. Approximately 0.3 mL of the ^{14}C-chlorothalonil suspension was drawn into the syringe, weighed on an analytical balance and administered to each rat by gavage.

Endotracheal Dosing. A 1.0-mL tuberculin syringe was fitted with a 3.75-inch, 16 gauge Teflon® needle for each intubation. Approximately 0.3 mL of the ^{14}C-chlorothalonil suspension was drawn into the syringe, weighed on an analytical balance and administered to each rat by direct intubation into the trachea via the mouth, by means of a lighted oral speculum, after anesthetization with methoxyflurane vapor.

Dermal Dosing. A dermal patch (2 x 2 cm) on the back of each animal was prepared by using a #40 head clipper. A 1.0-mL tuberculin syringe was fitted with a 22 gauge needle for each application. Approximately 0.05 mL of the ^{14}C-chlorothalonil suspension was drawn into the syringe, weighed on an analytical balance and applied to the 2 x 2 cm shaved section on the back of each rat. All the empty syringes were reweighed to determine the exact quantity administered to each rat.

Blood was collected from each animal by puncture of the orbital sinus at 2.5, 5, 24, and 48-h and analyzed immediately, or refrigerated and maintained at 4°C until analysis. Urine was collected from each animal at 24 and 48-h and analyzed immediately or frozen and maintained at -22°C until analysis. After collection of the 48-h urine sample, the floor and urine collection cup of each cage were rinsed with 10 mL water followed by 10 mL methanol. The samples were analyzed immediately.

Duplicate weighed aliquots (0.1 mL) of urine samples were evaluated directly for radioactivity by liquid scintillation counter. Duplicate weighed aliquots (0.05 mL) of blood samples and 1 mL aliquots of cage washings were similarly evaluated by liquid scintillation counter.

All samples were corrected for sample size, background radioactivity, and counting efficiency. Total blood volume of each rat was estimated by multiplying 64.1 mL blood per kg body weight times the kg body weight of each rat (ALTMAN & DITTNER 1974).

RESULTS AND DISCUSSION

Using male Sprague-Dawley rats as a model, three possible means of exposure, i.e., oral, respiratory, and dermal to the fungicide chlorothalonil, was evaluated. This study was confined to the investigation of the absorption into the blood and excretion in the urine of ^{14}C-chlorothalonil administered at a dose of 1 mg/kg body weight by an oral, endotracheal, or dermal routes. All routes of administration were examined in five rats per group. The ^{14}C-chlorothalonil equivalents found in the blood and urine as a percent of the dose administered are presented in TABLES 1 and 2.

TABLE 1. Percent of ^{14}C-Chlorothalonil Equivalents Found in the Blood of the Rats Following Endotracheal, Oral, and Dermal Administration of ^{14}C-Chlorothalonil

Time of Sampling (h)	Endotracheal*	Oral*	Dermal*
2.5	5.2 ± 0.7	0.35 ± 0.24	0.03 ± 0.02
5	3.9 ± 0.7	0.11 ± 0.03	0.06 ± 0.02
24	4.1 ± 0.1	0.15 ± 0.04	0.11 ± 0.03
48	3.7 ± 0.4	0.23 ± 0.03	0.27 ± 0.21

*Mean ± SD

TABLE 2. Percent of ^{14}C-Chlorothalonil Equivalents Found in the Urine of the Rats Following Endotracheal, Oral, and Dermal Administration of ^{14}C-Chlorothalonil

Time of Sampling (h)	Endotracheal*	Oral*	Dermal*
24	5.7 ± 4.1	2.9 ± 1.3	0.9 ± 0.5
48	0.7 ± 0.2	0.7 ± 0.2	0.8 ± 0.5

*Mean ± SD

Using Duncan's New Multiple Range Test, the percent of the dose found in the blood and urine for the 3 routes of administration versus sample time was compared. At

each time period a significantly higher percent of the dose (4 to 5%) was found in the blood of the endotracheal group as compared to the oral (0.1-0.3%) or dermal (0.03 to 0.3%) groups. This would indicate that the greatest absorption occurs following endotracheal exposure. The low percent of the dose found in the blood following dermal exposure suggests that chlorothalonil is not readily absorbed through the skin of the rat. In the urine, a significant amount of the dose was found in the 24-h urine of the endotracheal (5%) and oral (3%) groups but not in the dermal (0.9%) group. This pattern suggests a rapid absorption into the blood and excretion in the urine following exposure by the lungs and oral routes versus the dermal route.

Looking at individual treatment groups versus blood sample times, there is no significant difference in the results from the oral group between the 2.5, 5, 24, and 48-h samples. In the endotracheal group, the 2.5-h sample contained significantly higher levels of ^{14}C-chlorothalonil equivalents than the 5, 24, and 48-h samples. In the dermal group, the 48-h sample contained significantly more ^{14}C-chlorothalonil equivalents than the 2.5, 5, and 24-h blood samples, indicating a much slower rate of absorption of the ^{14}C-chlorothalonil by the dermal route of administration. In the oral group, the percent of the dose excreted in the 48-h urine was significantly lower than that in the 24-h urine indicating a rapid elimination. The percent of the dose found in the urine at 24-h was not significantly different from that at 48-h in the endotracheal group or in the dermal group due to the large standard deviation. However, because all of the recovered radioactivity represented such a small percentage of the total dose, tissue deposition and/or fecal excretion probably accounts for the remainder of the dose administered in endotracheal and oral groups.

This study demonstrated that the absorption rate of chlorothalonil in the rat, in the order of decreasing importance, would be via respiratory, oral and dermal exposure.

REFERENCES

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