# Elimination Rate and Tissue Residues of Chloropropylate and Chlorobenzilate in Rats[1]

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Although many of the chlorinated hydrocarbons have been extremely effective as pesticides, most have the distinct disadvantage of being only slowly degraded in the environment. Recently a new series of chlorine containing compounds consisting of various esters of chlorobenzilic acid have been developed by Geigy Chemical Company for use as miticides. The ethyl ester, better known as "chlorobenzilate" has been in use for some time on oranges. The isopropyl ester, "chloropropylate" has been found to be effective for mite control on apples.

Isopropyl 4,4' - dichlorobenzilate (chloropropylate) has an acute oral $LD_{50}$ in rats of 5,000 mg/kg (1). Dosages of 500 mg/kg per day administered over a period of 4 weeks resulted in no toxic symptoms. Ethyl 4,4' - dichlorobenzilate (chlorobenzilate) had an acute oral $LD_{50}$ in rats of about 1500 mg/kg (2). Both compounds are practically insoluble in water but soluble in most organic solvents. This report describes the elimination and tissue residues of these acaricides when single, relatively small, doses are intubated into young, male rats.

## Methods

Chloropropylate and chlorobenzilate labeled with carbon-14 at the acetate moiety were obtained from the Geigy Chemical Company, Ardsley, New York. They were

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demonstrated to be chromatographically pure by thin layer and gas chromatography. The animals used in the experiments were 200-gram male Holtzman rats which had been maintained on the following basal diet: casein, 25%; dextrine, 18.5%; dextrose, 18.5%; sucrose, 18.4%; minerals, 4.0%; non-nutrient fiber, 4.0%; vitamin mix, 1.0%; corn oil, 10.0%. The rats were allowed to adapt to the modified Roth metabolism cage for 24 hours before pesticide administration (3). At the end of the conditioning period, each rat was intubated via a stomach tube with 0.5 ml of corn oil containing .14 mg (1μc) of either chloropropylate or chlorobenzilate. Three animals were dosed with each pesticide. Carbon dioxide-free air was passed through the cages at 50 cc/min and the expired $CO_2$ was trapped in 0.1 N NaOH. Respired carbon dioxide, urine, and fecal samples, if present, were collected every four hours for the duration of the 48-hour period.

Respiratory carbon dioxide was assayed using a modified Grignard carbonation apparatus to transfer the $^{14}CO_2$ to a .1 N Hyamine solution. An aliquot of the Hyamine was then counted in a toluene scintillation cocktail (4 grams POP, 0.5 grams POPOP per liter of toluene). Urine volumes were measured and an aliquot counted in a dioxane cocktail (4 grams POP, 0.5 grams POPOP, 100 grams naphthalene per liter dioxane). Fecal samples were extracted with petroleum ether: isopropanol (4:1, v/v) and the extract counted in the toluene cocktail. The extracted fecal residue was digested and counted (4).

At the termination of the experiment, the animals were sacrificed, dissected, and the tissues to be assayed for radio-activity were freeze-dried. The dried samples were then ground to pass a 20-mesh sieve. A 30 mg portion was digested and counted in the same manner as the fecal residues.

All radio-assay was done in a Nuclear Chicago Mark I scintillation counter using external standardization. Efficiencies were checked periodically using an internal standard.

## Results and Discussion

Adult male rats intubated with a single dose of either carbon-14 labeled chloropropylate or chlorobenzilate eliminated only a small percentage of the ingested radioactivity as respiratory $CO_2$ as seen in Table 1. A slightly larger amount was evolved from chlorobenzilate but, in both cases, the activity was under 1% of the total recovery. Figure 1 shows the time-course recovery of respired $CO_2$ from either substrate over the 48-hour period. Maximum radioactive recovery of $^{14}CO_2$

from both compounds occurred at about 28 hours after dosing; however, a higher rate was evolved with chlorobenzilate.

TABLE 1

Recovery of Radioactivity from Adult Male Rats Dosed with Chloropropylate-$^{14}C$ or Chlorobenzilate-$^{14}C$

| Tissue Residue or Excreting Product | Percent of Total Recovery | |
|---|---|---|
| | Chloropropylate | Chlorobenzilate |
| Brain | 0.03 ± 0.01[1] | 0.03 ± 0.01 |
| Heart | 0.02 ± 0.00 | 0.10 ± 0.00 |
| Spleen | 0.35 ± 0.05 | 0.08 ± 0.01 |
| Kidney | 0.49 ± 0.17 | 0.63 ± 0.05 |
| Liver | 8.86 ± 1.93 | 3.31 ± 0.41 |
| G.I. Tract[2] | 8.35 ± 0.84 | 15.47 ± 2.32 |
| Respiratory $CO_2$ | 0.14 ± 0.01 | 0.67 ± 0.06 |
| Urine | 5.08 ± 1.05 | 25.63 ± 2.96 |
| Feces | 64.47 ± 4.50 | 42.78 ± 3.90 |

[1] Mean ± standard error

[2] Cecum, intestine, stomach tissues. The stomach contents contained an additional 11-12% radioactivity of the recovered dose.

The recovery of administered radioactivity in the urine is shown in Figure 2. $^{14}C$-chlorobenzilate and/or metabolites from chlorobenzilate was excreted more rapidly and in larger quantities than from $^{14}C$- chloropropylate. Only five percent of the total recovered radioactivity of chloropropylate was excreted via the urine, compared to 25 percent of the urine from the rats dosed with chlorobenzilate (Table 1).

Fecal elimination of parent compound and also possible metabolites (Figure 3) resulted in a more rapid and larger excretion with $^{14}C$-chloropropylate dosage than $^{14}C$-chlorobenzilate dosed in the rat. The former represented approximately 65 percent of the total recovered while recovery of the latter was only 43 percent. In both cases, after an initial lag, the rate of elimination continued to rise. Radioactivity began to appear in the feces of chloropropylate-treated rats between 4 and 12 hours, while in chlorobenzilate-treated rats, its appearance was delayed to between 12 and 16 hours. Although the rate of elimination was somewhat lower initially in chlorobenzilate-treated

animals, it increased very rapidly after about 34 hours from dosing.

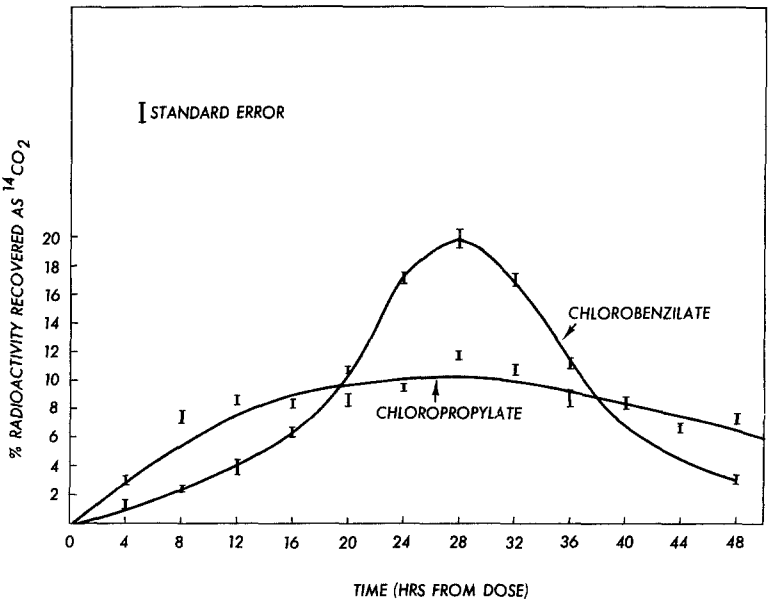


Figure 1. Time course recovery of radioactive $CO_2$ from rats dosed with chlorobenzilate-$^{14}C$ or chloropropylate-$^{14}C$.

Relatively small amounts of recovered activity were observed in the brain, heart, spleen, and kidneys of rats treated with either analog of dichlorobenzilate. The liver and gastrointestinal tract contained the highest amounts of chloropropylate and chlorobenzilate radioactivity. Recovery of radioactivity in the GI tract (intestine, stomach, and cecum) tissues was greater when chlorobenzilate was administered.

It appears that the substitution of various alcohol moieties to chlorobenzilic acid markedly alters the way in which the analogs are eliminated from the rat. The occurrence of considerably more total radioactivity in the urine of the ethyl ester-treated animals with a more rapid rate of elimination of $^{14}CO_2$, together with the lowered 48 hour fecal excretion, suggests that this compound is degraded relatively faster to polar, water soluble compounds as compared to the isopropyl ester-treated rats. The identification of these metabolic products are unknown and await further investigations.

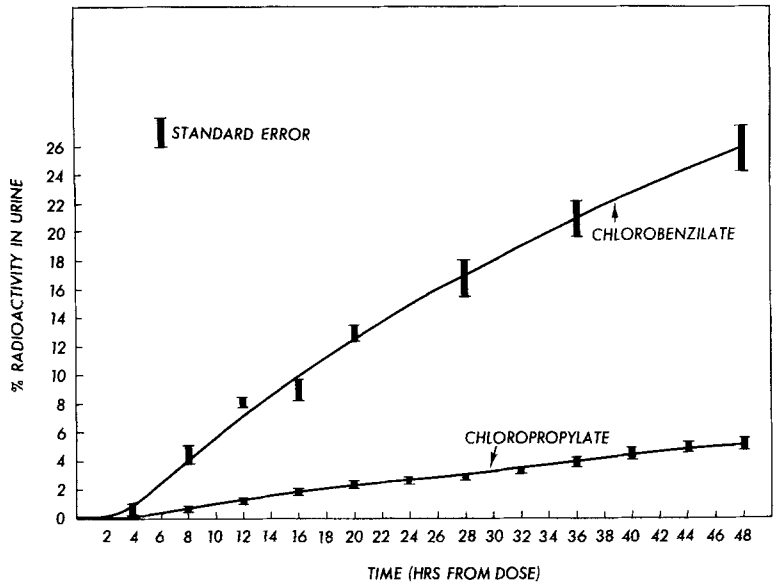


Figure 2. Percent of radioactivity of the administered dose of chloropropylate-$^{14}$C or chlorobenzilate-$^{14}$C excreted in rats urine.

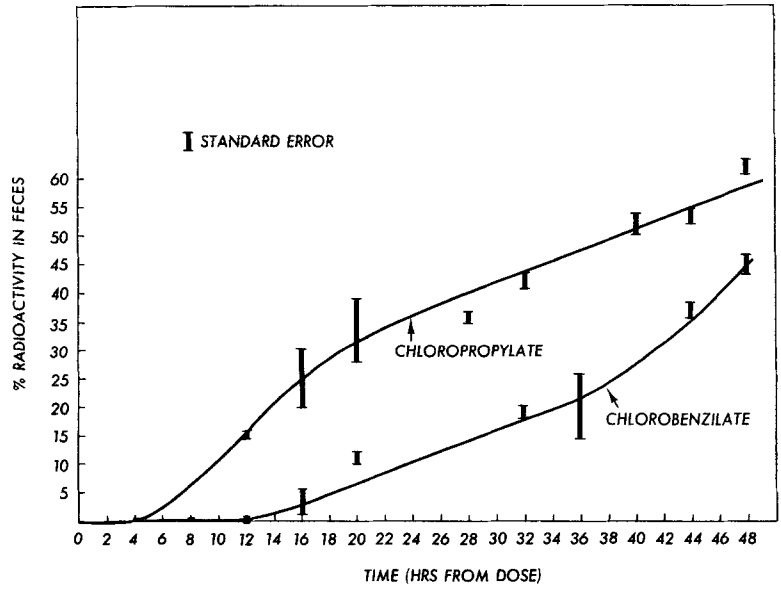


Figure 3. Percent of radioactivity of the administered dose of chloropropylate-$^{14}$C or chlorobenzilate-$^{14}$C excreted in rats feces.

## References


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