# Fate of $^{36}$Cl-Toxaphene in Rats

by LARRY A. CROWDER and EDWARD F. DINDAL

*Department of Entomology*
*University of Arizona*
*Tucson, Ariz. 85721*

## INTRODUCTION

Toxaphene (chlorinated camphene containing 67-69% chlorine) is widely used in the control of cotton insects. However, the gaps in our knowledge concerning tissue accumulation, metabolism, and excretion of toxaphene in non-target species might jeopardize its continued usage.

In a study concerning toxaphene storage in the animal body, sheep and steers fed 10 ppm daily in the diet showed no accumulation in the fat after 30 days of exposure (BATEMAN et al. 1953). However, with repeated dosage, storage in the fat of these animals as well as of dairy cows was reported by CONLEY (1952) and DIEPHUIS (1949). The former investigator also noted that a slow detoxification of toxaphene in the liver was indicated by the excretion of ethereal sulfate and glucuronate.

Toxaphene was found in milk of dairy cows given 20-140 ppm in the feed (CLAYBORN 1963). At lower concentrations, ZWEIG et al. (1963) reported that the amount of toxaphene occurring in milk was less than 0.03 ppm; uncontaminated milk was observed within 2 weeks after the animals were removed from the toxaphene diets.

Due to the paucity of information concerning the fate of toxaphene in the mammalian system, the following study was undertaken. Reported herein are routes and rates of excretion as well as the amount and loci of accumulation in various tissues of male rats administered an oral dose of $^{36}$Cl-toxaphene.

## METHODS AND MATERIALS

Experimental Design: Thirty-day-old albino rats (Holtzmann Co.), weighing an average of 114 gm, were deprived of food 24 hrs prior to dosage. A dose of 20 mg/kg of technical grade $^{36}$Cl-toxaphene (42 $\mu$Ci/gm; Hercules, Inc.) in 0.5 ml of a peanut oil-gum acacia solution was orally administered to the rats via a stomach tube. Control rats were dosed with 0.5 ml of the peanut oil-gum acacia solution. In each of two experiments, 3 treated and



Bulletin of Environmental Contamination & Toxicology.
Vol. 12, No. 3 © 1974 by Springer-Verlag New York Inc.

3 control rats were placed into glass metabolism chambers, which provided for separate collection of urine and feces (HALLADAY 1973). Another group of 27 treated and 9 control animals was held for organ and tissue sampling at various time intervals; 3 treated and 1 control were used at each interval. All holding and metabolism cages were maintained in an air-conditioned environment (22-25°C; 50% RH; L:D - 11:13). The animals were given Purina Laboratory Chow and water ad libitum.

In order to study the manner in which $^{36}Cl$-toxaphene is excreted under the influence of a previous body burden, the following experiment was conducted. Three treated and 3 control rats were given an additional dose of 20 mg/kg on the ninth day; these animals were referred to as "redosed".

Urine and feces were collected daily, weighed, and stored at 0°C to await further analysis. At schedule time intervals, rats were sacrificed and their organs and tissues excised, weighed, and stored at 0°C. Additionally, the animals employed in the excretion experiment were sacrificed for organs and tissue samples at the end of 9 and 20 days. Blood obtained from heart punctures was immediately centrifuged at 3,000 rpm for 5 minutes in a refrigerated superspeed Sorvall centrifuge, model RC2-B, to precipitate cellular matter.

Analytical Procedures: Feces were thawed, air-dried, ground to a powder, and 1 gm samples extracted with 25 ml each of hexane and water. Urine samples were treated in a similar manner but extracted with only 5 ml each of hexane and water. Aliquots of all extracts were then digested and solubolized in NCS® (Amersham/Searle Corp.) and Triton® X-100, using heat to aid digestion. Tissue samples were thawed, minced with scissors, and homogenized with NCS® over heat.

Determination of $^{36}$chloride in urine and feces water extracts was accomplished with acidification (3-4 drops of 1 M $HNO_3$) followed by precipitation (several drops of 0.5 M $AgNO_3$). After centrifugation, the precipitant was discarded and the procedure repeated until $AgNO_3$ saturation was attained. The supernatants were sampled to determine non-ionic $^{36}Cl$; the ionic $^{36}Cl$ was then calculated by subtracting non-ionic $^{36}Cl$ from total $^{36}Cl$.

Toluene-based fluor (5 gm PPO and 0.06 gm POPOP/liter toluene) was added to all samples and radioassayed on a dual channel (Nuclear-Chicago Model 6822) liquid scintillation spectrophotometer. Quench was corrected using the external standard method.

## RESULTS AND DISCUSSION

The average excretion of radioactivity derived from $^{36}Cl$-toxaphene, represented as percent of the administered dose, is reported in Table 1. During the 9 days, 52.6% of a single dose was excreted. About one-half of this occurred the first day. Approximately 30% was excreted in the urine while 70% appeared in the feces. Excretion on an accumulative basis is presented in Fig. 1; feces plateaued between 2-3 days while urine excretion continued an upward trend. Feces have also been reported as the major excretion route for dieldrin (MATTHEWS et al. 1971) and mirex (MEHENDALE et al. 1972 and GIBSON et al. 1972) in rats. However, the amount of toxaphene excretion in urine demonstrated herein was greater than observed with either dieldrin or mirex.

Following a second dose, urine played a greater role in excreting $^{36}Cl$ than with the single dose (Table 1). The peak of excretion in feces appeared delayed about 1 day longer than in the single dose; here it took 2 days for 50% of the excretion to occur. Again the same trends were noted--excretion in feces plateaued early while urine continued an upward trend (Fig. 1). Based upon total recovery expressed as 100%, urine increased while feces decreased (Fig. 2). On the third day, relative percents of urine and feces were equal. This importance of urine excretion during the later dats was evident in both single-dosed and redosed animals. It was observed that less of the toxaphene dose was excreted in redosed animals than single-dosed. In contrast to this, GIBSON et al. (1972) reported that redosed rats eliminated 25% of an administered dose of mirex, as opposed to 18% for single-dosed.

In both the single-dose and redose experiments, 90% of the $^{36}Cl$ was recovered in the water fractions of feces (Table 2). Likewise in the urine, only a small amount of radioactivity was observed in the hexane fractions.

Because the radioactivity was found in the water fractions, it appeared that a considerable amount of toxaphene metabolism had resulted; therefore, the water and hexane fractions were analyzed for ionic-$^{36}$chloride (Table 2). Of the radioactivity excreted by single-dosed rats via feces, 68.2% existed as ionic-$^{36}Cl$; the amount in redosed animals was somewhat less. In urine, the ionic-$^{36}Cl$ increased from 76.2% in single-dosed to 90.2% in redosed rats; this resulted from a decrease of non-ionic chloride found in the urine-water fractions of redosed animals. Total ionic-$^{36}Cl$ excretion in combined feces and urine was less in redosed rats. By comparing the individual feces and urine accumulative ionic-$^{36}Cl$ excretion (Fig. 3), it appears that this lower amount in redosed rats resulted from a smaller excretion by feces. This analysis emphasizes the considerable degree of toxaphene metabolism occurring in rats following an oral dose.

TABLE 1

Excretion of radioactivity in urine and feces of rats following a 20 mg/kg dose of $^{36}$Cl-toxaphene. Average % administered dose (N=6).

| SINGLE DOSE | | | | REDOSE[a] | | | |
|---|---|---|---|---|---|---|---|
| DAY | URINE | FECES | TOTAL | DAY | URINE | FECES | TOTAL |
| 1 | 1.46 | 23.95 | 25.4 | 10 | 1.81 | 6.00 | 7.8 |
| 2 | 3.20 | 7.45 | 10.6 | 11 | 3.55 | 11.60 | 15.2 |
| 3 | 2.89 | 1.25 | 4.1 | 12 | 2.26 | 1.40 | 3.7 |
| 4 | 2.35 | 1.10 | 3.5 | 13 | 3.08 | 1.10 | 4.2 |
| 5 | 1.82 | 1.06 | 2.9 | 14 | 3.09 | 1.20 | 4.3 |
| 6 | 1.19 | 1.23 | 2.4 | 15 | 1.77 | .60 | 2.4 |
| 7 | 1.15 | .69 | 1.8 | 16 | 1.07 | .40 | 1.5 |
| 8 | .54 | .27 | .8 | 17 | .97 | .40 | 1.4 |
| 9 | .72 | .31 | 1.0 | 18 | 1.31 | .20 | 1.5 |
| | | | | 19 | .73 | 0 | .7 |
| | | | | 20 | .42 | 0 | .4 |
| Total[b] | 15.3 | 37.3 | 52.6 | | 20.1 | 22.9 | 43.0 |
| Total[c] | 29.1% | 70.9% | 100% | | 46.7% | 53.3% | 100% |

[a] The single-dosed animals were redosed with 20 mg/kg on the 9th day.

[b] % administered dose.

[c] % recovered dose expressed as 100%.

TABLE 2

Partitioning characteristics of radioactivity in excretion after a single-dose and redose of $^{36}$Cl-toxaphene. Percent recovered dose expressed as 100%.

| FRACTION / EXCRETION | HEXANE | IONIC WATER | NON-IONIC WATER |
|---|---|---|---|
| Single-Dose Feces | 10.5 | 68.2 | 21.3 |
| Redose Feces | 8.2 | 64.5 | 27.3 |
| Single-Dose Urine | .2 | 76.2 | 23.3 |
| Redose Urine | .5 | 90.2 | 9.3 |

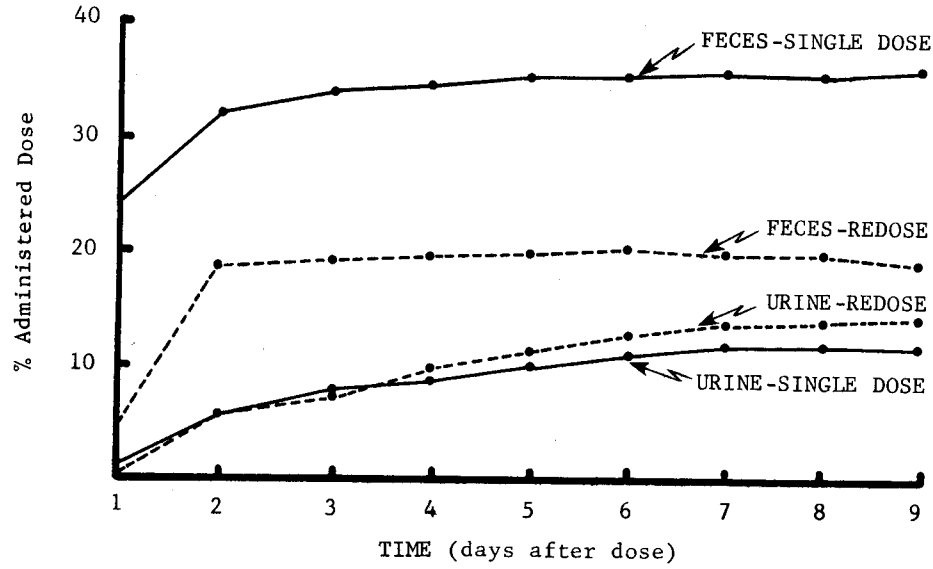


Fig. 1 Accumulative excretion of radioactivity in rats administered a single-dose and redose of $^{36}Cl$-toxaphene. The single-dosed animals received an additional 20 mg/kg on the ninth day for the redose.

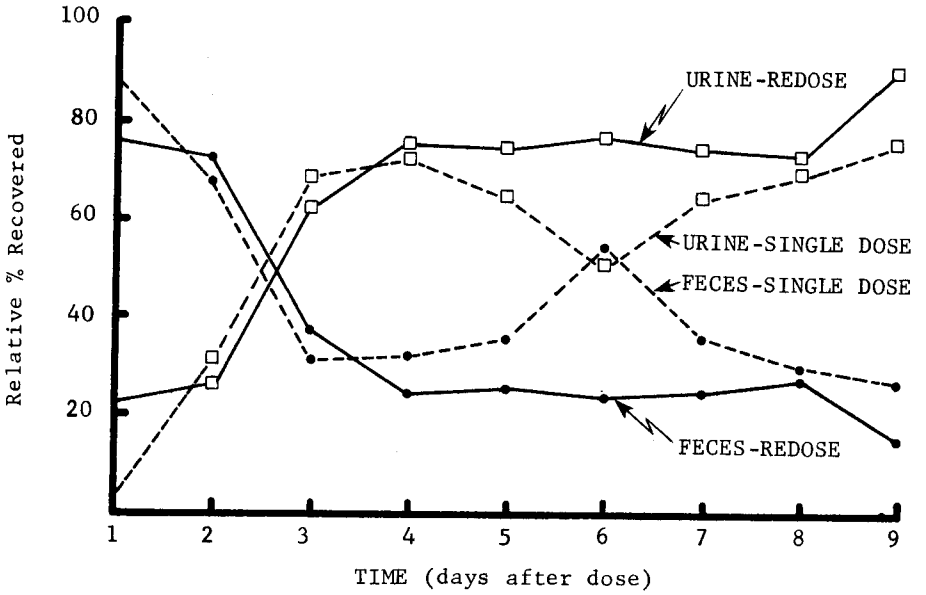


Fig. 2 Relative percent recovery of $^{36}Cl$ in excretion of rats following $^{36}Cl$-toxaphene. The percent of urine and feces related to total excretion is based on the total recovery expressed as 100%.

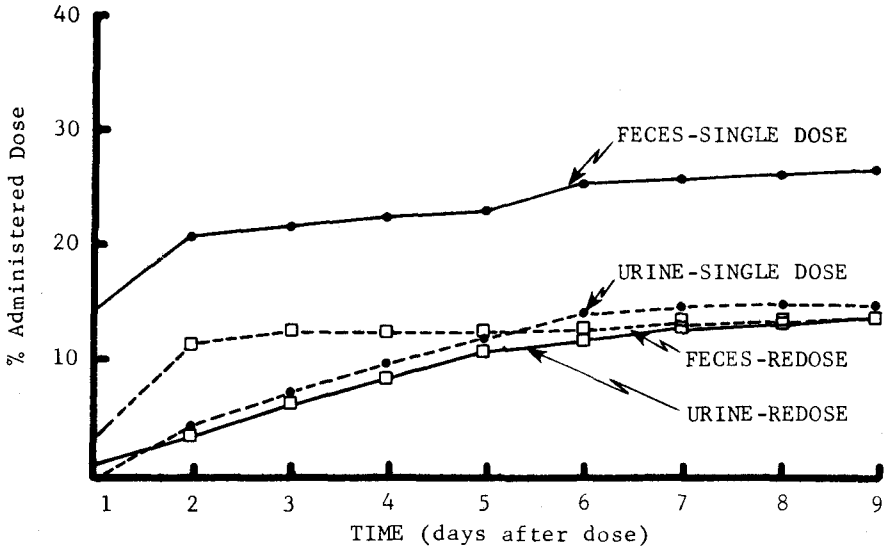


Fig. 3 Ionic-$^{36}Cl$ excretion in water fractions of the rat feces and urine following $^{36}Cl$-toxaphene. The single-dosed animals were redosed with 20 mg/kg on the 9th day.

The uptake of radioactivity in various tissues over a period from 3 hours to 20 days is in Table 3. In almost all cases, the greatest concentration was found at 12 hours followed by a rapid decrease which is in close agreement to the 6 hour peak reported by LAMB et al. (1970) for dieldrin in pheasant tissues. Blood cells exhibited a peak at 3 days. Even though a total concentration of 90% of the administered dose occurred, less than 10% remained after day 1. By contrast, 34% of a dose of Mirex was retained in the tissues and organs of rats after 7 days (MEHENDALE et al. 1972). Most of the large concentration up to day 1 could be accounted for by the amount in the stomach. With respect to the nervous system, brain tissue did not concentrate an extraordinary amount of radioactivity. Fat storage appeared nonsignificant as is also reported by BATEMAN et al. (1953) for sheep and steers.

The passage of $^{36}Cl$ through the intestinal tract required about 1 day. The peak in the small intestine occurred at 6 hours: no differences were noted between the first 2 cm and last 2 cm segments. At 12 hours, or an additional 6 hours, the large intestine contained the bulk of $^{36}Cl$. The amount passing into feces increased rapidly and peaked at 2-3 days.

TABLE 3

Uptake of radioactivity in various rat tissues and organs following a single-dose of $^{36}Cl$-toxaphene (24 mg/kg). Expressed as % administered dose.

| TISSUE \ DAY | 3/24 | 6/23 | 12/24 | 1 | 2 | 3 | 5 | 7 | 9 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|
| Kidney | .05 | .13 | .43 | .10 | .03 | .03 | .01 | 0 | .03 | 0 |
| Muscle | .93 | 1.6 | 5.3 | 1.3 | 0 | .65 | 2.4 | .40 | .14 | .81 |
| Fat | .14 | .15 | .86 | .57 | .31 | .18 | .18 | .02 | 3.65 | .03 |
| Testes | .02 | .08 | .28 | .06 | .04 | .03 | .03 | .02 | .06 | 0 |
| Brain | .03 | .06 | .23 | .05 | .04 | 0 | 0 | 0 | .01 | 0 |
| Blood Cells | 3.1 | 0 | 0 | .06 | .90 | 2.6 | 0 | 0 | 1.10 | 1.17 |
| Blood Supernatant | .64 | 1.20 | 2.35 | 1.30 | .60 | .36 | 0 | .18 | .09 | .06 |
| Liver | .33 | 1.10 | 2.33 | .50 | .31 | 0 | .01 | 0 | .48 | 0 |
| First 2 cm Small Intestine | .06 | .34 | .34 | .05 | .01 | 0 | 0 | 0 | .84 | .09 |
| Last 2 cm Small Intestine | .10 | .34 | .28 | .13 | .01 | .15 | 0 | 0 | 0 | 0 |
| Large Intestine | .19 | .60 | 1.20 | .19 | .08 | .02 | .03 | .04 | 0 | 0 |
| Esophagus | .04 | 0 | .04 | .03 | .01 | .01 | .02 | 0 | .03 | 0 |
| Spleen | .04 | .06 | .08 | .05 | .02 | 0 | .03 | .24 | .06 | 0 |
| Stomach | 3.70 | 18.6 | 77.20 | 2.00 | .63 | .61 | .39 | .16 | .12 | 0 |
| TOTAL | 9.37 | 24.26 | 90.90 | 6.39 | 2.99 | 4.64 | 3.10 | 1.06 | 6.57 | 2.16 |

Tissues and organs of rats, following a redose of $^{36}Cl$-toxaphene, retained 6.0%. The redosed tissues at the end of 20 days were corrected for the $^{36}Cl$ remaining after the single dose to arrive at 3.87% of the second administered dose being retained. However, the rats actually had 2 doses and, thus, the redosed tissues might be expected to possess 2 times the single-dosed, or 4.32%. This points to rats retaining less in the tissues when they contain a previous body burden. By comparing only the last 9 days of the redosed rats to the first 9 days of single-dosed, the manner in which the first dose was concentrated compared to the second was determined. Here it was demonstrated that redosed rats contained 0.55% less in the selected tissues; this is equivalent to almost 20% less dose retention than in the single-treated animals.

## SUMMARY

In the rat, 52.6% of an oral dose of $^{36}Cl$-toxaphene was excreted within 9 days. Approximately 37% was found in the feces and 15% in the urine. Upon extraction, most of the radioactivity occurred in the water fractions of urine and feces as ionic chloride. Animals given a second dose on the 9th day excreted toxaphene in a similar manner except $^{36}Cl$ excretion in feces was reduced. Less than 10% of the dose was found in selected tissues and organs 1 day following the treatment.

## ACKNOWLEDGEMENTS

The authors thank Hercules, Inc. for supplying the $^{36}Cl$-toxaphene. This project has been financed in part with Federal funds from the Environmental Protection Agency under grant number R800384. The contents do not necessarily reflect the views and policies of the Environmental Protection Agency, nor does mention of trade names or commercial products, constitute endorsement or recommendation for use. University of Arizona Agricultural Experiment Station, Journal Paper No.

## REFERENCES

BATEMAN, G. Q., C. BIDDULPH, J. R. HARRIS, D. A. GREENWOOD, and L. E. HARRIS: J. Agr. Food Chem. 1, 322 (1953).

CLAYBORN, H. V., H. D. MANN, M. C. IVEY, R. D. RADELEFF, and G. T. WOODARD: J. Agr. Food Chem. 11, 286 (1963).

CONLEY, B. E.: J. Amer. Med. Assoc. 149, 1135 (1952).

DIEPHUIS, F., and C. L. DUNN: Montana Experimental Station Bulletin. 461 (1949).

GIBSON, J. R., G. W. IVIE, and H. W. DOROUGH: J. Agr. Food Chem. 20, 1246 (1972).

HALLADAY, S. C.: Bull. Environ. Contam. Toxicol. 10, 155 (1973).

LAMB, D. W., Y. A. GREICHUS, and R. L. LINDER: J. Agr. Food Chem. 18, 168 (1970).

MATTHEWS, H. B., J. D. McKINNEY, and G. W. LUCIER: J. Agr. Food Chem. 19, 1244 (1971).

MEHENDALE, H. M., L. FISHBEIN, M. FIELDS and H. B. MATTHEWS: Bull. Environ. Contam. and Toxicol. 8, 200 (1972).

ZWEIG, G., E. L. PYE, R. SIFANI, and S. A. PEOPLES: J. Agr. Food Chem. 11, 70 (1963).