Uptake of ^{36}Cl-Toxaphene in Mosquito Fish, *Gambusia affinis*

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INTRODUCTION

The mode of action of toxaphene (chlorinated camphene containing 67-69% chlorine) is poorly understood. The high susceptibility of fish and discovery of resistant strains have encouraged research in this area. Although there is information on toxicity and residues, there has been little work done to quantify actual uptake of toxaphene from the aquatic medium.

Uptake and excretion of related insecticides have been investigated in mosquito fish. FERGUSON et al. (1966) demonstrated the existence of processes of uptake and excretion for endrin, but did not quantify these processes. The major source of endrin uptake was contaminated water rather than accumulation through the food chain. Endrin was also released into the water by contaminated fish which indicated some type of excretion mechanism.

WELLS and YARBROUGH (1972, 1973) and YARBROUGH and WELLS (1971) studied retention of DDT, aldrin, dieldrin, and endrin in resistant and susceptible mosquito fish. Using radioactive tagging, they demonstrated that cell membranes of resistant fish bind more insecticide than membranes of susceptible fish. These results suggested that resistance is in part the result of a membrane barrier in resistant fish.

Quantification of the amount of toxicant actually absorbed is important for investigations into the mode of action of these insecticides. The present research was undertaken to quantify the uptake of toxaphene by mosquito fish and relate these data to the toxicity syndrome.

METHODS AND MATERIALS

Experimental Animals: Gambusia affinis (Baird and Girard) were obtained from a sewage oxidation pond located on Oracle Road, 0.6 miles N. of River Road, Tucson, Pima County, Arizona. Fish were stored in 115 l poly-trash cans and maintained in the laboratory in aged tap water for 24 hours prior to testing. Mean weight ($\pm$ S.D) of the fish was 1.082 $\pm$ 0.627 gms. An LC_{50}

Bulletin of Environmental Contamination & Toxicology,
Vol. 15, No. 5 © 1976 by Springer-Verlag New York Inc.

at 24 hours of 860 ppb was determined for the population. At 2 ppm, the LT_{50} was 12 hours; however, 8 hours was selected as the optimum period for uptake and excretion experiments since mortality increased rapidly after 8 hours at this concentration making excretion experiments difficult.

Experimental Design: Two 5 l aquaria were set up with aged tap water. To each was added ^{36}Cl-toxaphene (42 µCi/gm; Hercules, Inc.) in 2.44 cc acetone to produce suspensions containing 2 ppm of toxaphene. A solvent control test at this rate of acetone resulted in 0% mortality after 48 hours. Fifty G. affinis were introduced to each aquarium at 0800 and sampled hourly for 8 hours. Each sample consisted of 10 fish which were rinsed with fresh tap water and frozen individually in glass vials for subsequent extraction. The experiment was duplicated. At the time of sampling, the toxicity stage exhibited by each fish was noted. The toxicity syndrome had been determined earlier and divided into 5 characteristic stages in fish exposed to a lethal concentration.

In the manner above, the same number of fish were exposed for 8 hours to 2 ppm ^{36}Cl-toxaphene. Following exposure, all fish were transferred to 5 l aquaria containing untreated, aged tap water. At the time of transfer, dead fish were removed, rinsed, and frozen; a sample of 10 fish were also collected and frozen at transfer. Samples were taken every hour until all fish were removed. Toxicity stages were recorded at the time of sampling. This experiment was duplicated.

To correlate the amount of toxaphene residue with toxicity symptoms, G. affinis were placed in aquaria containing 2 ppm ^{36}Cl-toxaphene. Samples were taken during the next 10 hours so that 10 fish were sampled at each of the 5 toxicity stages. The samples were rinsed in tap water and frozen.

Analytical Procedures: To extract the sorbed radio-labelled toxaphene and toxaphene related (TR) residues, fish were ground in 40 ml redistilled acetone. The extract was filtered, evaporated to dryness, redissolved in 0.25 ml distilled water and 0.25 ml hexane, and scintillation fluor added. Employing an internal standard, extraction of fish resulted in a recovery rate of 96.01 $\pm$ 5.28%; 96% recovery was used in the calculations.

To partially characterize the composition of radioactive fish extract, 34 treated fish were ground in 400 ml acetone. Following evaporation to dryness, the extract was partitioned in 100 ml distilled water and 100 ml hexane. Both fractions were divided into 10 ml aliquots and these were evaporated to dryness, redissolved in 0.25 ml distilled water and 0.25 ml hexane, and prepared for radioassay.

Toluene-based fluor (5 gm PPO and 0.06 gm POPOP/liter toluene) and Bray's solution (60 gm naphthalene, 10 ml methanol,

20 ml ethylene glycol, 4 gm PPO and 0.2 gm POPOP in 1 l dioxane) were employed in the radioassays. Samples were counted on a dual channel (Nuclear-Chicago Model 6822) liquid scintillation spectrophotometer. Quench was corrected using the external standard method. Radioactivity was interpreted in terms of the specific activity of toxaphene; however, this may give somewhat misleading values since not only toxaphene but probably metabolites of toxaphene containing ^{36}Cl were present in the extracts.

RESULTS AND DISCUSSION

Uptake of ^{36}Cl-toxaphene and TR residues was a linear function and directly proportional to the length of exposure (Fig. 1). Since fish were processed whole in these experiments, it was impossible to determine what portion of these residues had been absorbed into particular organs and what portion was simply adsorbed on the scales and fins.

In the excretion experiments, analysis of data resulted in statistically nonsignificant excretion equations. Calculation of confidence intervals about individual points indicated that at the 0.05 level, there was no significant difference in body load over time. Therefore, there was no indication of excretion of toxaphene and TR residues during the first 6 hours following exposure.

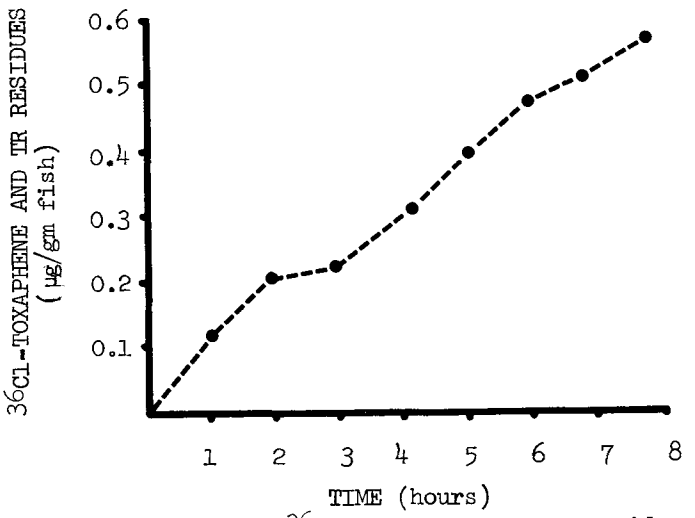

Figure 1. Uptake of ^{36}Cl-toxaphene and TR residues in G. affinis exposed to 2 ppm ^{36}Cl-toxaphene. Regression analysis resulted in a linear uptake equation $y = 0.00068 + 0.00058(x)$ which was significant at the 0.01 level.

Partitioning of fish extracts revealed that 88.7% of ^{36}Cl was recovered from the nonpolar fraction. This represented 0.586 μgm ^{36}Cl-toxaphene and TR residues per gm of fish. Recovery rates, however, for the procedure using extracts of fish and a ^{36}Cl-toxaphene standard, averaged 87.6%. Thus, there was apparently minimal metabolic alteration in the toxicant during the 8 hour period. Dehydrochlorination has been reported as a method of toxaphene metabolism in rats (OHSAWA et al. 1975). If this were the case in fish, ^{36}Cl would be expected to appear in the water fraction. One might also expect fish to excrete chloride. The excretion data show no evidence of excretion within 6 hours which corresponds with the lack of toxaphene metabolism during this period. FERGUSON et al. (1966) found no evidence of matabolic or chemical alteration of endrin in G. affinis.

Observation of the toxicity syndrome and characterization of a graded series into 5 stages was a subjective process. Interpretation of toxicity symptoms without a knowldege of the mode of action of toxaphene is impossible although certain behavior may suggest physiological correlates. The first stage of the toxicity syndrome was recorded as apparently healthy fish. The second stage, and the most difficult to assess, was when fish began to swim at the surface perpendicularly to the side of test containers. Swimming at the surface is normal in water with low oxygen content and is also the normal feeding position for G. affinis. Normal fish, however, retreated from the surface when the aquarium was approached whereas poisoned fish remained at the surface. Subsequent toxicity stages were all marked by rapid gill ventilation which further suggested respiratory involvement. The third stage was characterized by fish swimming parallel to the side of aquaria, but with a loss of equilibrium as evidenced by sinking of the posterior end so that fish attempted to swim up towards the surface. Finally, fish lost their ability to maintain their normal dorso-ventral orientation and rolled to the side. At stage 4, fish sank to the bottom and were prostrate with rapidly ventilating gills. At this stage, there was occasional darting behavior. Death (stage 5) was identified by the cessation of gill movements.

Average body load of ^{36}Cl-toxaphene and TR residues at each toxicity symptom is shown in Fig. 2. By the time fish exhibited the first toxicity response to toxaphene (stage 2), they had already sorbed 90.3% of the average fatal residue. Fish which were characterized as normal (stage 1) had accumulated 35% of the fatal residue.

Average residue per gm of fish and toxicity symptoms exhibited at each hour are shown in Table 1. Fish progressed through toxicity symptoms at approximately the same rate until the eighth hour. This is where mortality increased rapidly and it was possible to examine differences in body load of fish with identical exposure times, but which exhibited different toxicity symptoms.

TABLE 1

^{36}Cl-Toxaphene and TR residues (μg/gm fish) and toxicity symptoms exhibited at each hour in G. affinis. Exposure to toxaphene occurred between hours 1-8. Following exposure, fish were transferred to untreated aged tap water and sampled between hours 9-14. Each value represents 10 fish.

Toxicity Stage	Hours													
	-----Exposure to 2ppm ^{36}Cl-toxaphene----------													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
5								0.68						
4												0.75		
3								0.61			0.66	0.71	0.53	0.52
2					0.42	0.43	0.49	0.54	0.61	0.57	0.63	0.65		
1	0.11	0.20	0.25											

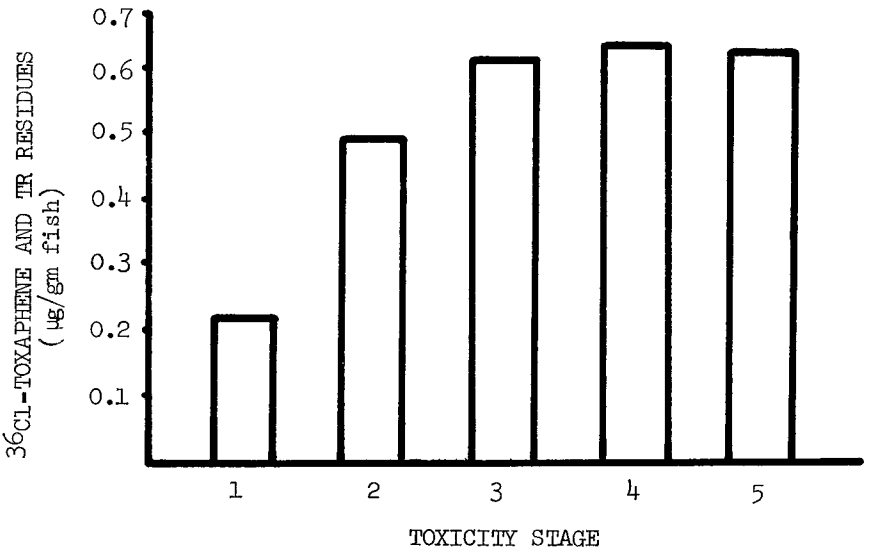

Figure 2. Mean body burden of ^{36}Cl-toxaphene and TR residues in G. affinis at each stage of the toxicity syndrome. Toxicity stage 1 is apparently healthy fish; 2, 3, and 4 represent progressively greater toxicity response. Stage 5 is death.

Where more than one toxicity symptom was exhibited, the mean body load was different for fish showing different symptoms, and the amount of residue present was consistent with the severity of the symptom. Since exposure time was equal for all fish, different body loads at various toxicity stages appear to reflect differences in rates of uptake.

Data presented here show that the onset of toxicity symptoms varies within any given population, and that the onset of particular toxicity symptoms is directly proportional to body load. Thus within this population, differences in toxicity response appeared to be due to different body loads rather than to different tolerances of a particular toxicant level.

SUMMARY

The toxicity syndrome of toxaphene to G. affinis was divided into 5 stages, and the residue level at each stage was determined. By the time fish were exhibiting the first toxicity response (stage 2), 90.3% of the mean fatal residue level had been sorbed. Regression analysis indicated that sorption of toxaphene is a linear function with respect to time. Excretion was not observed following an 8 hour exposure to 2ppm

toxaphene. Metabolic alteration of toxaphene during the experiments appeared to be minimal. Differences in individual mortality appeared to be due to differences in uptake rather than differences in ability to tolerate particular body loads of toxaphene.

ACKNOWLEDGEMENTS

The authors thank Hercules, Inc. for providing 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. 2516.

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