Zeller, H. D. 1952. Nitrogen and phosphorus concentrations in fertilized and unfertilized farm ponds in central Missouri. Trans. Amer. Fish. Soc. 82:281-288.
Zicker, E. L., K. C. Berger, and A. D. Hasler. 1956. Phosphorus release from bog lake muds. Limnol. Oceanogr. 1:296-303.

ACCUMULATION OF DDT FROM FOOD AND FROM WATER BY GOLDEN SHINER MINNOWS, Notemigonus Crysoleucas

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INTRODUCTION

This report deals with one aspect of an overall study to determine the behavior and activity of pesticides in the aquatic environment. The specific objective of the study was to determine in the laboratory the dynamics of pesticide movement in aquatic environments with particular emphasis on the mode of accumulation of these materials by organisms. Bioaccumulation of carbon-14 labelled DDT from food and water was followed in golden shiner minnows, *Notemigonus crysoleucas.*

DDT is very insoluble in water hence only small amounts can be carried by water. A much higher burden can accumulate in the food of fish especially if the food contains lipids. Carbon-14 labelled DDT was chosen both for the ease and accuracy with which it can be detected and because it could be separated from the DDT burden present in all available fish. Although not attempted here, it would also be possible to add unlabelled DDT to the water and simultaneously feed the fish food contaminated with labelled DDT. The importance of each type of contamination could then be measured in the presence of the other. The following report is a result of research to determine the rate of accumulation of DDT by golden shiners from a constant aqueous concentration and from food at a constant daily dosage.

REVIEW OF LITERATURE

Serious water pollution by pesticides began after World War II when the organic insecticides were first marketed. DDT was one of the first and most widely used insecticides (Nicholson, 1967). It belongs to the chlorinated hydrocarbon class of insecticides that degrade slowly in the environment and are the most toxic to fish (Johnson, 1968).

Several investigators have exposed fish to aqueous concentrations of DDT. Premda and Anderson (1963) found that salmon killed by six hours exposure to 1 part per million DDT-C^{14} had accumulated about 3.7 parts per million. Goldfish exposed to a sublethal 30 parts per billion DDT-C^{14} for five hours accumulated 5 parts per million (Gakstatter and Weiss, 1967). Cope (1965) exposed fish to an initial 20 parts per billion DDT and found that after two weeks the fish had accumulated 1 part per million although the aqueous DDT concentration fell greatly during this time. Holden (1962) also noticed that the concentration of DDT-C^{14} in exposure tanks fell rapidly, and he suggested that a system of maintaining a constant concentration of toxicant be used. Butler (1966) found that pinfish soon reached a maximum accumulation of aqueous DDT and suggested that uptake was balanced by metabolic losses.

Most of the work dealing with pesticides and fishes has been summarized by Johnson (1968) in an excellent literature review. He noted that a gap existed in understanding the relative importance of the digestive tract and the gills as locations for pesticide absorption. Butler (1966) believed that the dynamic maximum accumulation of aqueous DDT in pinfish was increased by adding DDT to their food source.

Cherrington et al. (1969) found that in salmon p,p' DDT was degraded by the intestinal microflora to the less toxic p,p' TDE, and they suggested that this may increase the chances of survival of fish that ingest DDT-contaminated prey.

MATERIALS AND METHODS

Fifty microcuries (6.5 mg) of ring labelled DDT-C-^{14}, $(ClC_6{}^{14}H_4)_2CHCCl_3$, were dissolved in 1 liter of Nanograde ®[1] acetone to give a stock solution of 6.5 parts per million DDT. Analysis by gas chromatography indicated that this was a mixture of isomers—70% p,p'-DDT and 30% o,p-DDT.

Small golden shiners (*Notemigonus crysoleucas*) with a mean dried weight of 0.3 g were used in this study. All weights given are freeze-dried weights; the conversion factor to fresh weight is 6.2. The background content of unlabelled insecticide was determined by gas chromatography to be 0.5 parts per million DDE (freeze-dried weight). The conversion factor for concentration in terms of fresh weight is 0.16.

All radioassays were made on a Nuclear Chicago liquid scintillation system using the channels ratio method of quench correction.

Aqueous Studies

The system used to provide a constant aqueous concentration of DDT is illustrated in Figure 1. Two jars with a combined capacity of 30 liters were connected by Siphon 1 causing them to act as one jar. The air traps in the siphons were necessary to prevent bubbles from blocking the system. The tygon air tube connecting the two jars equalized air pressures. After the jars were filled, the air tube was clamped shut, and

1 Nanograde ® is a registered trademark of the Mallinckrodt Chemical Works.

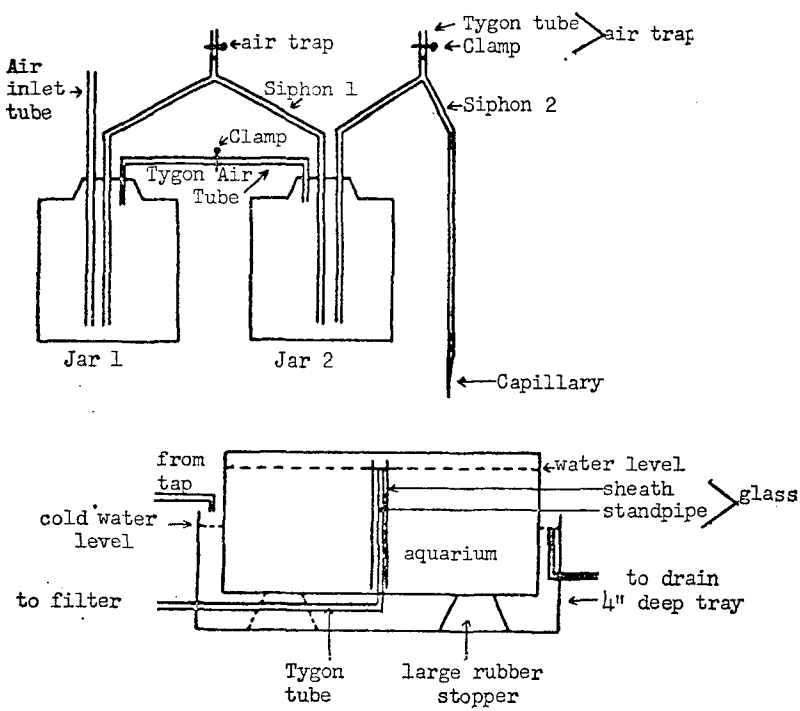

FIGURE 1. Continuous Flow System

air was forced through the air inlet tube. This forced a column of water through the siphon connecting the two jars. The clamp was then released to equalize air pressure. Water could then flow through the siphon in the direction necessary to equalize water levels.

Siphon 2 ended in a capillary tube that emptied into a 17-liter aquarium. After the siphon between the two jars was established and the air tube clamp released, continued air pressure from the inlet tube started siphon 2. While pressure was still in the system, the clamps on the air traps were carefully opened and closed, allowing a 5-cm column of water to rise in the trap. Any air bubbles forming in the system would displace water from this column rather than clog the siphon.

The desired rate of flow was roughly attained by drawing the capillary to the proper size. Fine adjustment was obtained by moving the air inlet tube up or down as necessary. The difference in level between the bottom of the air inlet tube and the opening of the capillary was equivalent to the difference between the level of liquid and the mouth of an ordinary siphon system. This system was adjusted to deliver 1000 ± 10 ml/hr of water.

The aquarium was equipped with a standpipe to maintain a constant volume of 17 liters. A larger tube ensheathing the standpipe caused drainage to occur from the bottom. The effluent was passed through a charcoal filter to remove the DDT. The aquarium was placed in a larger tank of flowing tap water that maintained the temperature in the aquarium at $23 \pm 1°$ C.

At the start of the experiment the system was filled with water dechlorinated by aeration for 24 hours. Then 0.1 ml/liter stock DDT-C^{14} solution was added to both jars and the aquarium. Each day thereafter the jars were refilled with dechlorinated water containing 0.1 ml/liter DDT.

Initially and daily a 100 ml water sample from the aquarium was extracted in a separatory funnel with two 10 ml portions of scintillating fluid (4.0 g/liter PPO and 0.1 g/liter POPOP in Nanograde toluene). The extracts were combined and a 15-ml portion pipetted into a 20-ml glass scintillation vial for analysis.

To allow the pesticide concentration to equilibrate, the system was started 24 hrs before fish were introduced. Thirty-one fish were initially added to the system. Samples of three fish were taken at 1, 2, 6, 9, and 12 days. At 15 days the two surviving fish were sampled. During the course of the experiment fourteen fish died, apparently of a fungus infection, and were discarded. At no time did any fish show any symptoms of DDT poisoning, and the aqueous DDT concentration remained well below the lethal level established by bioassay.

Each fish sample was immediately rinsed in 20-ml of scintillation fluid to remove any absorbed DDT. A 15-ml portion was pipetted into scintillation vial. The fish were then freeze-dried for 24 hrs, weighed, ground with anhydrous Na_2SO_4 as an abrasive and disiccant, and extracted by shaking for 4 hrs with 10 ml of Nanograde hexane. The extract was pipetted through a column of florisil. The sediment in the vial was rinsed twice by shaking for 5 min with 10 ml of hexane, the rinse pipetted through the column, and the column quantitatively rinsed with hexane. The eluate was collected in a 50-ml beaker, evaporated to approximately 7-ml under an air current, and quantitatively transferred with hexane to a scintillation vial. The extract was then evaporated to dryness under an air current with gentle heating (50°C). Then 15-ml of scintillation fluid added for radioassay.

Food Studies

Eighteen 4-liter glass jars were filled with 3 liters of dechlorinated water, and one fish was placed in each jar. A nylon net packet containing 1 g. of charcoal was placed in each jar to absorb any DDT released into the water. The fish were accustomed to 10-mg feedings of pelletized rat food daily for three days. Then each fish was fed twice daily a 10-mg portion onto which 12-ng of DDT-C^{14} in 1 ul of acetone was applied with a 10-ul syringe. The total dose of DDT-C^{14} for each fish

was calculated from individual records of food actually consumed. A sample of three fish was taken each day for 6 days. Each sample was rinsed and extracted as described above. At the end of the 6-day period a 100-ml water sample was taken from the remaining three jars, combined, extracted overnight with 20-ml of scintillating fluid, and a 15-ml portion of the extract pipetted into a scintillation vial for radioassay.

RESULTS AND DISCUSSION

During the 15-day aqueous study the mean aqueous DDT concentration was 265 parts per trillion with a standard deviation of 81 parts per trillion. An 8-day period of initial stability was followed by a steady increase to nearly twice the initial concentration (Table 1). The drop in concentration on the sixth day was caused by flow failure during the night. The increase after 8 days is probably a result of three factors. Initially the rate of flow in the system was not fast enough to overcome the removal of DDT by fish and physical factors such as codistillation and adsorption. Later, adsorption may have approached a saturation point. Finally, as the fish were removed, their decrease in number from 31 to 2 would be accompanied by a decrease in the total rate of DDT removal by the fish.

TABLE 1. Bioaccumulation by golden shiners, *Notemigonus crysoleucas*, of DDT-C^{14} from water.

Time (days)	Aqueous DDT	Weight of Fish	Absorbed DDT	Absorbed DDT
	(ppt)[1]	(mg)	(ppb)	(ppb)
Initial	0.21	...	....	.....
1	0.20	495	11.0	630
2	0.19	...	....	.....
2.6	....	628	15.6	750
3	0.19	...	....	.....
4	0.23	...	....	.....
5	....	....	....	.....
6	0.16	716	53.7	5,740
7	0.23	...	....	.....
8	0.23	...	....	.....
9	0.25	539	105.9	10,640
10	0.25	...	....	.....
11	0.31	...	....	.....
12	0.35	830	88.2	19,370
13	0.36	...	....	.....
14	0.39	...	....	.....
15[2]	0.42	471	180.1	22,530

1 ppt—parts per trillion.
2 all samples an average of three fish except day 15 which is 2 fish.

Limitations in the volume of the jars and the flow through the filter did not permit a faster rate of flow. The flow system described by Burke and Ferguson (1968) would have been ideal if a filter capable of handling the greater effluent had been available.

The absorption and adsorption of DDT by fish followed similar trends but at different magnitudes (Table 1). Since the weight of the skin and mucous layer on which DDT was adsorbed was not determined, adsorption is expressed in terms of whole body weight. Thus, for the entire fish the concentration of adsorbed DDT is about 1% of the absorbed, but at the site of adsorption it is much higher than this.

Twenty-three parts per million DDT were absorbed after 15 days (Table 1). This represents nearly 100,000 times the mean aqueous con-

centration. No significant plateau was reached that would correspond to that found by Butler (1966), but 2 weeks may not have been time enough to establish a dynamic equilibrium. The rate of both adsorption and absorption was greatest at the end of the experimental period. This may be a result of the increase in aqueous concentration of DDT, which would also tend to mask any early trend toward Butler's dynamic equilibrium. No attempt was made to determine metabolites; all were reported as DDT.

It is to be expected that not all the DDT introduced would be recovered. The differences between column 2 and column 4 are accounted for in part by: (1) inefficiency in extraction and clean-up, (2) non-ingestion of DDT by fish, and (3) non-assimilation of DDT by fish—the feces were never assayed. No DDT was detected in the water sample, and none was found to be externally adsorbed. Therefore, any DDT excreted in the feces was either tightly bound and not released into the water, or quickly absorbed by the charcoal. Also the fish did not appear to secrete DDT into the mucous layer, and the process of extracting adsorbed DDT did not remove any absorbed DDT. The samples taken on days 5 and 6 included several fish that ate only part of the food portion.

Future research involving very long term accumulations from food, water, or both would be fruitful. Fish could be exposed to aqueous DDT-C^{14} and fed DDT-Cl^{36} at the same time, and, using modern scintillation assays, the two sources could be followed simultaneously in the same fish. Also, the metabolites should be followed, for in the light of findings by Cherrington et al. (1969) differences in the metabolite proportions may be found in the assimilation of DDT from food and water.

TABLE 2. Bioaccumulation by golden shiners, *Notemigonus crysoleucas*, of DDT-C^{14} from food.

Time (Days)	DDT fed	Weight of fish	Absorbed DDT	Absorbed DDT
	(ng)	(mg)	(ng)	(ppb)
1	72	831	16.4	19
2	144	1146	52.2	45
3	214	846	85.4	100
4	279	815	98.8	121
5	309	998	143.5	143
6	...	851	132.1	155

LITERATURE CITED

Burke, W. David, and Denzel E. Ferguson. 1968. A simplified flow-through apparatus for maintaining fixed concentrations of toxicants in water. Trans. Amer. Fish. Soc. 97:498-501.

Butler, Philip A. 1966. Fixation of DDT in estuaries. Thirty-first N. Amer. Wildl. Conf. Trans. 184-187.

Cherrington, A. C., U. Paim, and O. T. Page. 1969. In vitro degradation of DDT by intestinal contents of Atlantic Salmon (*Salmo salar*). J. Fish. Res. Bd. Canada 26:47-54.

Cope, Oliver B. 1965. Research in Pesticides. Academic Press, New York, p. 115.

Gakstatter, Jack H., and Charles M. Weiss. 1967. The elimination of DDT-C^{14}, dieldrin-C^{14} and lindane-C^{14} from fish following a single sublethal exposure in aquaria. Trans. Amer. Fish. Soc. 96:301-307.

Holden, A. V. 1962. A study of the absorption of ^{14}C-labelled DDT from water by fish. Ann. Appl. Biol. 50:467-477.

Johnson, Donald W. 1968. Pesticides and fishes—a review of selected literature. Trans. Amer. Fish Soc. 97:398-424.

Nicholson, H. P. 1967. Pesticide pollution control. Science 158:87-876.
Premda, F. H., and J. M. Anderson. 1963. The uptake and detoxification of C^{14}-labelled DDT in Atlantic Salmon, *Salmo salar*. J. Fish. Res. Bd. Canada 20:827-837.

EVALUATION OF THE EFFECTS OF CHANNELIZATION ON FISH POPULATIONS IN NORTH CAROLINA'S COASTAL PLAIN STREAMS

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ABSTRACT

This research study was designed to determine the degree of damage, if any, to fish populations resulting from channelization, and to determine the rate of recovery, if the damage was significant.

This study points out the detrimental effects stream channelization has on fish populations and on the flora and bottom fauna of streams. The study also indicates that following channelization, and with no channel maintenance, nature can ultimately restore a coastal plain stream and its fish population to a stage reasonably near its natural condition, provided no further alterations of the stream bed, banks, forest canopy, or aquatic vegetation occur.

INTRODUCTION

Ecologists have thought for some time that channelization projects are detrimental to fish and wildlife in project areas. Channelization, a type of stream alteration often employed under Public Law 566 and