The Persistence of Toxaphene in Lake Water and Its Uptake by Aquatic Plants and Animals

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Water, aquatic plants, aquatic invertebrates, and fish from two mountain lakes treated with toxaphene in a fish-eradication program were analyzed for toxaphene residues during a 3-year period. The initial residues declined sharply to less than 2 p.p.b. and remained at or near this level for approximately 5 years in a deep, biologically sparse lake, and approximately 1 year in a shallow lake, rich in aquatic life. Trout could not be restocked in the deeper lake for 6 years. Residues in aquatic plants from this lake reached levels as high as 17 p.p.m. There was evidence that trout placed in live boxes obtained toxic quantities of toxaphene directly from the water and that the amount of toxaphene accumulating in fish tissues reached a plateau in about 2 weeks. Trout could be restocked in the shallow lake within one year after treatment, when toxaphene levels were at approximately 1 p.p.b. The trout grew rapidly in spite of the accumulation of whole body concentrations of toxaphene up to 14 p.p.m. There was some evidence that toxaphene was partially metabolized by the plant and animal organisms.

TOXAPHENE was tested as early as 1951 (*1*) in the rehabilitation of fresh water fishing lakes and reservoirs. It was employed in several states and in Canada (*3, 4, 6*) for this purpose. A report of an investigation of the persistence of toxaphene after a fish rehabilitation project (*4*) showed that toxaphene residues in the treated water were reduced rapidly, from over 28 p.p.b. a few days after treatment to less than 1 p.p.b. in 6 months. Analyses of trout placed in the lake 6 months after treatment revealed toxaphene deposits on a whole body basis of from 0.8 to 3.5 p.p.m. A limited number of analyses of aquatic plants soon after the toxaphene treatment showed a rapid buildup of toxaphene, up to 18 p.p.m. after 9 days.

Several hundred samples of water, bottom mud, fish, aquatic invertebrates, aquatic plants, and miscellaneous vertebrates have been examined in a similar but more extensive study of two lakes treated with toxaphene for fish and lamprey eradication. Although the two lakes were in the same area of the Oregon Cascades, they differed considerably in physical and biological properties. Miller Lake, treated in 1958 at an estimated rate of 40 p.p.b., has a surface area of 565 acres, an estimated volume of 50,265 acre-feet, and a maximum depth of 147 feet. It is sparse in biological life in comparison to other Central Oregon lakes. Davis Lake, treated in 1961 at an estimated rate of 88 p.p.b. (in two applications), has a surface area of 3207 acres, an estimated volume of 15,830 acre-feet, and a maximum depth of 20 feet. It is rich in biological life. The effect of these biological and physical properties on the persistence of toxaphene in the two lakes is of interest.

Analytical Methods

Twelve hundred-milliliter water samples were extracted in separatory funnels by shaking for 10 minutes with 480 ml. of redistilled hexane (Skellysolve B). The hexane extract was dried with anhydrous sodium sulfate before concentration for gas chromatography.

Aquatic plants, without drying, were passed through a food chopper and then tumbled end-over-end for 1 hour with hexane–isopropyl alcohol (1-to-1), 2 ml. of solvent per gram of tissue. The solvent was removed by decantation, washed free of alcohol, and dried with anhydrous sodium sulfate.

Samples of aquatic invertebrates or fish were macerated in a Waring Blendor in the presence of anhydrous sodium sulfate, 1 gram per gram of tissue. This mixture was then further macerated for 5 minutes with a 9-to-1 mixture of hexane and ethyl ether, 4 ml. of solvent per gram of tissue. The extract was dried with anhydrous sodium sulfate.

Extracts of aquatic plants, equivalent to 25 grams of plant material, were passed through a $^3/_4$-inch column filled with 10 grams of a 1-to-1 mixture of magnesium oxide (Sea Sorb 43) and Celite 454. The columns were eluted with 125 ml. of 94-to-6 petroleum ether–ethyl ether.

Extracts of fish and invertebrates (equivalent to 5 grams of tissue) and bottom mud (equivalent to 25 grams) were purified by passage through a sulfuric acid–Celite column. The $^3/_4$-inch chromatograph columns were prepared by adding 10 grams of Celite mixed with 6 ml. of 3-to-1 fuming concentrated sulfuric acid to a bed of 3 grams of dry Celite 454. The columns were eluted with 100 ml. of purified hexane.

All samples were concentrated to 5 ml. on a steam bath and then transferred to blood sedimentation tubes. The concentration was continued at room temperature under an air jet to 0.10 ml. Suitable aliquots of this concentrated solution were introduced into the gas chromatograph for analysis.

In gas chromatography with the usual 4- to 6-foot columns, toxaphene is resolved into its several components. With a microcoulometric gas chromatograph, this results in chromatograms with 4 to 6 peaks during a 6- to 8-minute period. The chromatograms are characteristic of toxaphene and, hence, not easily mistaken, but the method suffers from a lack of sensitivity. A solution to this problem has been suggested by Witt (*7*), who describes the use of a short (18 inch) column in the microcoulometric gas chromatograph. When such a column is used, toxaphene emerges as a single peak. The method is useful only when other halogenated pesticides are absent or are present in insignificant amounts.

All samples were analyzed gas chromatographically with the short column, and selected extracts of fish, aquatic invertebrates, and aquatic plants were analyzed with the long column. The appearance of a typical multipeak chromatogram confirmed the presence of toxaphene in the samples.

The short columns were of 18 × $^1/_4$ inch aluminum packed with 20% sili-

cone Hi Vac grease on Chromosorb P and were operated at 250° C. The longer columns were 4 or 6 feet × 1/4 inch aluminum, packed with 5% silicone SE-30 on Chromosorb W, and were operated at 210° C.

Controls for the samples from treated lakes were obtained from similar bodies of water nearby. The apparent toxaphene content of the water controls, using the short columns, ranged from 0.2 to 0.6 p.p.b. (average, 0.38 p.p.b.). Apparent toxaphene levels in control fish ranged from 0.3 to 1.0 p.p.m. (average 0.55 p.p.m.).

The reliability of the extraction procedures and of the gas chromatographic methods was confirmed by frequent analyses of fortified samples. Water and plant or animal slurries or extracts of these were fortified with known amounts of toxaphene and carried through the entire procedure. Recoveries were always better than 80%. The gas chromatograph was standardized with toxaphene by injections before and after every three or four unknowns.

Results and Discussion

Tables I and II summarize the analytical data for the two lakes. During the analytical program the water concentration of toxaphene declined slowly, less than 1 p.p.b. per year. Although not shown here, frequent analyses during the spring and summer revealed yearly fluctuation in the water residue levels of Miller Lake, with the concentrations higher in early spring than in late summer. This is thought to be due to the spring turnover which brings up bottom sediment, thus resuspending adsorbed toxaphene.

In agreement with the results described by Kallman (*4*), aquatic plants were found to accumulate appreciable residues of toxaphene. Plants absorbed up to 15.5 p.p.m. when growing in water containing approximately 2 p.p.b. Although the analyses listed in Table II are for entire plants, separate analyses of foliar and root portions showed that the roots usually contained considerably more toxaphene. For example, in the 1963 analyses of aquatic plants from Miller lake, foliage averaged 1.55 p.p.m. while roots averaged 3.12 p.p.m.

Although individual analyses vary considerably, the concentration factor, from water to plant, water to invertebrate, water to fish, is very large. In Davis Lake, the concentration factor is approximately 500 for aquatic plants, 1000 to 2000 for aquatic animals other than fish, and 10,000 to 20,000 for rainbow trout.

Within a year after being treated with toxaphene, Davis Lake was restocked with rainbow trout, Atlantic salmon, and kokanee salmon. Although the records and the identification of a given sample of fish do not allow precise measurement of the time the fish were in the lake before their capture and analysis, a good estimate can be made. It was considered of interest to compare these two parameters—probable time in the lake and whole body toxaphene level—as was done in Table III. The data

Table I. Toxaphene Residues in Plants and Animals from Davis Lake[a]

Sample	Toxaphene Residues, Average[b] and Range		
	1962	1963	1964
	Parts per Billion		
Water	0.63 (0.5–0.9)	0.41 (0.3–0.6)	<0.2
	Parts per Million		
Aq. plants	0.39 (0.2–0.6)	0.21 (0.1–0.9)	...
Aq. invertebrates	1.43 (0.1–2.6)	0.47 (0.2–0.5)	...
Rainbow trout	5.7 (1.2–12.0)	7.72 (2.75–13.7)	3.5 (3.2–3.8)
Atlantic salmon	2.75 (2.6–2.9)[c]	3.24 (1.11–5.50)	1.8 (1.5–2.1)[c]
Bottom mud	0.65 (0.1–1.0)[c]	0.8 (0.1–3.1)	...

[a] Toxaphene applied in the fall, 1961, at a calculated rate of 88 p.p.b.
[b] Except as noted, all averages represent six or more analyses.
[c] Atlantic salmon, average of two analyses; bottom mud, average of four analyses.

Table II. Toxaphene Residues in Plants and Animals from Miller Lake[a]

Sample	Toxaphene Residues, Average[b] and Range		
	1962	1963	1964
	Parts per Billion		
Water	2.10 (0.7–3.1)	1.20 (0.7–1.6)	0.84 (0.7–1.1)
	Parts per Million		
Aq. plants	4.59 (0.3–15.5)	2.78 (0.39–7.50)	5.77 (1.87–13.1)
Aq. Invertebrates	2.67 (0.69–5.36)	3.00 (0.18–8.23)	...
Brook trout[c]	...	...	12.46 (8.30–24.80)
Bottom mud	0.75 (0.39–1.43)	3.91 (0.33–13.80)	...

[a] Toxaphene applied in the fall, 1958, at a calculated rate of 40 p.p.b.
[b] All averages represent five or more analyses.
[c] Time of entry into lake unknown.

Table III. Toxaphene Residues in Trout and Salmon after Different Periods in Davis Lake

Toxaphene level in water, 0.4–0.6 p.p.b.

Days in Lake, Estimated	Rainbow Trout, P.P.M.	Atlantic Salmon, P.P.M.
38	4.20	...
46	8.35	...
55	...	2.60
84	...	2.90
90+	12.00	...
90+	7.20	...
165	...	2.60
165+	8.10	...
195	12.80	5.50
205	...	4.20
205+	8.28	...
255	12.60	3.40, 5.50
280	13.70	...

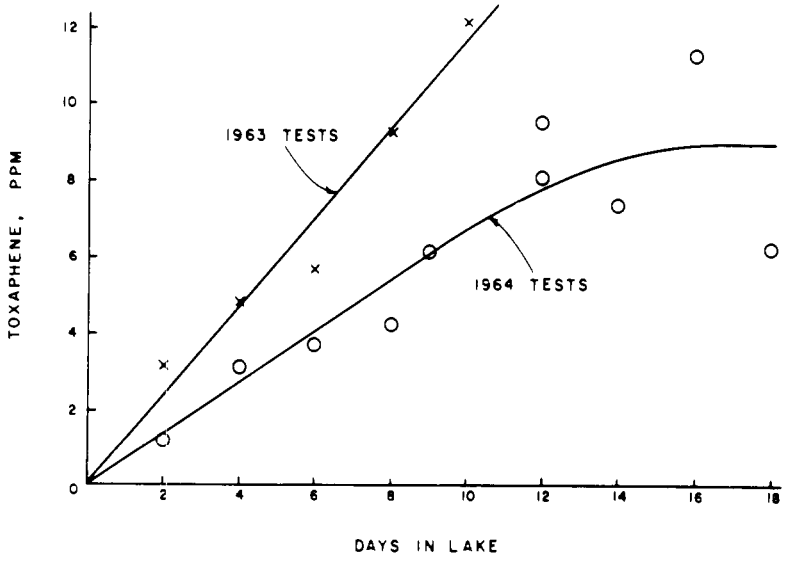

Figure 1. The rate of uptake of whole body levels of toxaphene by rainbow trout in live boxes in Miller Lake

Average toxaphene in water, 1963 = 1.20 p.p.b.; average in 1964 = 0.84 p.p.b.

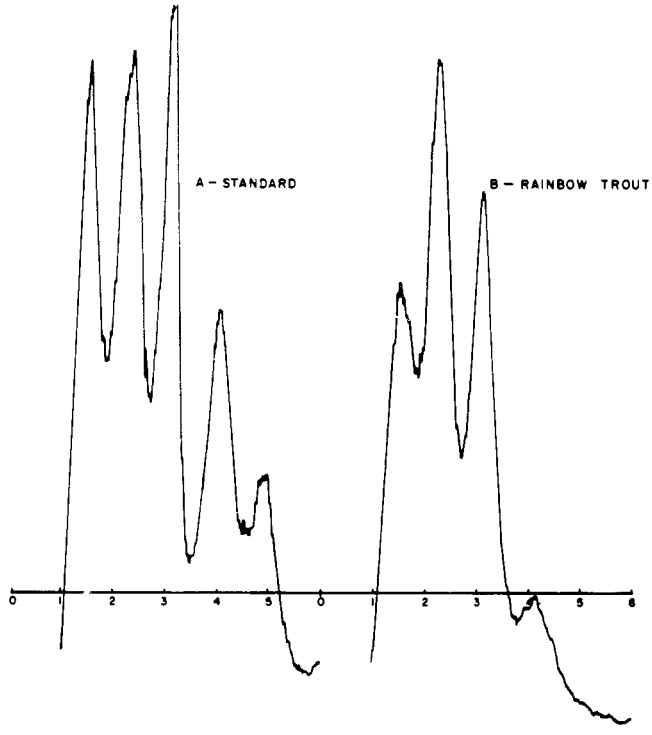

Figure 2. Chromatograms of standard toxaphene, *A*, and extracts of rainbow trout, *B*, from Davis Lake

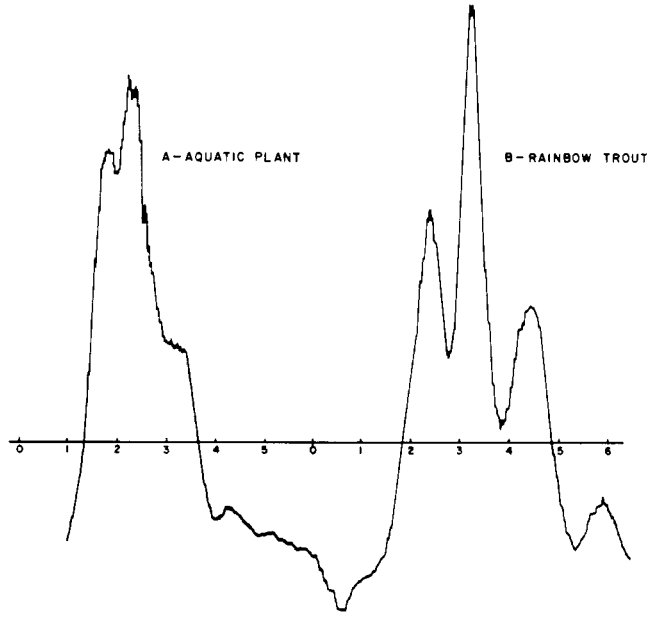

Figure 3. Chromatograms of extracts of aquatic plants, *A*, and rainbow trout, *B*, from Miller Lake

indicate that toxaphene deposits reach a plateau in 30 to 50 days, and that Atlantic salmon show less tendency to accumulate toxaphene.

There was no visible mortality of the planted fish in Davis Lake after any of the 1962 and 1963 plantings. Biologists in charge of the program pronounced the growth of the fish unusually good. Many of the trout brought in for analysis within a year after restocking were over 2 pounds in weight and during the second year after planting, one sample fish weighed 6 pounds. Trout up to 8 pounds have been reported by fishermen. As the data show, whole body toxaphene levels of 5 to 10 p.p.m. were common in these apparently healthy fish.

Although treated earlier and at a lower rate, Miller Lake detoxified more slowly than Davis Lake, remaining toxic to fish for at least 5 years. There are several possible explanations for the slow recovery of Miller Lake—thermal stratification due to depth, a slow rate of dilution from small tributaries, and markedly less plant and animal life. In 1963 and 1964, 6- to 8-inch trout were held in the lake in live boxes for varying periods up to 18 days. Two living fish were removed from the boxes every two days and analyzed for toxaphene. The results of the analyses are shown graphically in Figure 1. In the 1964 tests, as with the fish of Davis Lake, a plateau or threshold level of toxaphene appears to be reached rather quickly. In the 1963 tests, trout could not be maintained in the live boxes longer than 10 days. Other live box tests conducted during the 1963 season showed that the lake was still too toxic for restocking.

Since the fish in the live boxes could have eaten nothing but microorganisms and small insects which could penetrate the screening of the boxes, it must be assumed that, except for this minor source of toxaphene, accumulated residues came from the water. A concentration factor of about 10,000 is indicated.

As the fish analysis data of this study show, trout and salmon, living in water containing low levels of toxaphene, tend to achieve a threshold level of the toxicant in a relatively short time. This threshold may occur as a result of selection wherein the weaker individuals are eliminated or the attainment of a balance between intake and metabolic processes including growth.

The long-column chromatograms obtained during the GLC analyses of fish, plants, and invertebrates collected in the study indicate that some alteration of the toxicant has occurred (Figures 2 and 3). An effort was made to determine whether this change had altered the toxicity of the pesticide. Houseflies were used to compare extracts of two fish, containing 15 and 24 p.p.m. of toxaphene on a whole body basis, with extracts of control fish fortified at these levels with standard toxaphene. The insects were given 24-hour exposure to deposits of the extracts and their mortalities recorded. No significant difference in toxicity was seen. Additional tests are needed to determine if the altered toxaphene represents a detoxication as far as fish themselves are concerned

The Davis Lake study shows that a toxaphene content of less than 1 p.p.b. need not be toxic to trout or salmon. Water concentrations above this figure, however, as shown in the Miller Lake study, may be hazardous to fish. In either case, there are modifying circumstances which must be considered.

Trout may be unaffected by low levels of toxaphene in their surroundings but may accumulate residues in excess of the 7 p.p.m. tolerance established for a number of commodities. Hemphill (*7*) has speculated that toxaphene present in fish tissue would be destroyed by cooking. This idea has been tested in the present instance with trout subjected

to the customary boiling and frying methods. After 10 minutes in an open pan held at 375° F. or 15 minutes in boiling water, no significant reduction of toxaphene levels was observed.

The data gathered here may have some value in estimating the hazard of other chlorinated organic pesticides to fresh water fish. Various studies of the relative toxicity of pesticides to seven species of fish (*2*, *5*) have shown that toxaphene is approximately 1/10th as toxic as endrin, three times as toxic as dieldrin, and five times as toxic as DDT. On this basis it might be expected that fresh water lakes and streams would be nontoxic if they contained less than 100 parts per trillion of endrin, 3 p.p.b. of dieldrin, and 5 p.p.b. of DDT.

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SOIL FUMIGANT HYDROLYSIS

Hydrolysis of *cis*- and *trans*-1,3-Dichloropropene in Wet Soil

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***cis*- and *trans*-1,3-dichloropropene are hydrolyzed in wet soil to *cis*- and *trans*-3-chloroallyl alcohol, respectively. Solvolysis rates in the presence of massive amounts of soil are enhanced at most threefold as compared to the rates of solvolysis in water.**

Alkyl halides are widely employed as soil fumigants to combat the destructive action of plant parasitic nematodes and other soil organisms. Lack of knowledge of the fate of these substances in a soil matrix precludes serious considerations of their toxicity, mode of action, and residue analysis. As a part of our studies of the cleavage of carbon-halogen bonds by soil and soil organisms (*2*), we report the nonbiological hydrolysis of *cis*- and *trans*-1,3-dichloropropene in wet soil.

The disappearance of these dihalides from soil has recently been noted; however, no conversion products could be detected (*3*). The present findings portray the first step of what may be a series of biological and nonbiological transformations of these substances.

Experimental

Materials. Pure *cis* - (b.p. 101° C.) and *trans*-1,3-dichloropropene (b.p. 111° C.) were obtained by careful repeated fractionation of the commercial nematocide mixture, Telone, through a helices-packed column. The isomers were shown to be pure by gas chromatography on a 6-foot Dow Corning −710 column at 90°. The corresponding *cis*- and *trans*-3-chloroallyl alcohols were obtained from the pure chlorides by refluxing in 10% Na_2CO_3 (*4*). The *cis*- and *trans*-3-chloroallyl alcohols had boiling points of 143–44° (735 mm.) and 153–55° C. (760 mm.), respectively, upon fractionation through a spinning band column. Isomer purity was shown by gas chromatographic assay on a Carbowax 20M column at 190° C.

Soil from a lemon grove was employed in the present work because it had been found to contain organisms capable of dehalogenating other substrates. The results of this study, however, should be independent of the organismic content of a soil.

Methods. Chloride was determined by direct potentiometry employing a Ag–AgCl electrode and a calomel reference electrode (*6*). The working electrode was prepared by anodizing a clean spiral of silver wire in a solution of 1*M* KCl and 0.1*M* HCl with a current of 10^{-3} ampere for 12 hours. Direct pCl measurements were made with a Leeds & Northrup pH meter after the solution or suspension to be analyzed (usually 2 ml.) was diluted with an equal volume of a solution of 1.75*M* KNO_3 and 0.25*M* HNO_3 in order to maintain a high constant ionic strength (1.0) and proton concentrations. It was later found that this precaution was not necessary and samples were analyzed directly. The potentiometer was standardized daily at

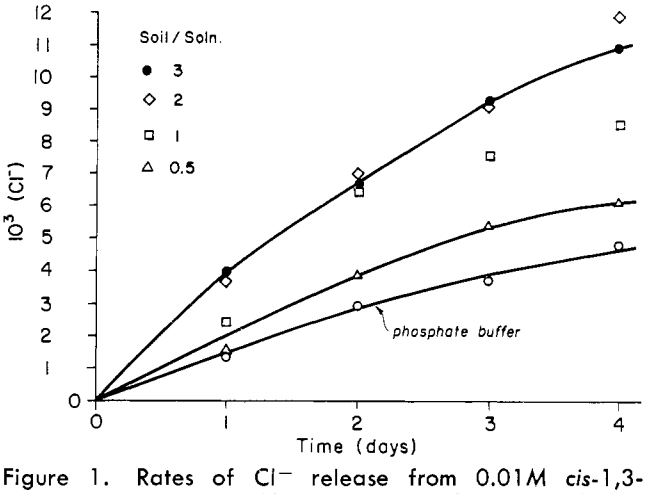

Figure 1. Rates of Cl^- release from 0.01*M* *cis*-1,3-dichloropropene in 0.1*M* phosphate buffer at pH 6.9 or 7.5 and rates in soil-solution ratios of 0.5, 1, 2, and 3