TOXAPHENE IN GREAT LAKES FISH: A TEMPORAL, SPATIAL AND TROPHIC STUDY

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ABSTRACT

We report here on the trophic and temporal distribution of toxaphene, a complex mixture of hexa- to decachlorinated norbornanes and norbornenes, in Great Lakes' lake trout and smelt sampled in 1982 and 1992. As shown in Figure 1, the lake trout in the three largest lakes (Superior, Michigan and Huron) had higher toxaphene concentrations than the smelt, while in Lakes Ontario and Erie, these two trophic levels had about the same concentrations. In both species, the 1992 concentrations were lower than their 1982 counterparts, except in Lake Superior, where there was no statistical difference. Except for the Lake Superior samples, these trends were expected. The ban on toxaphene in 1982 eliminated new inputs; thus, the 1992 fish received their load of toxaphene from sources already present in the environment, such as resuspended sediments. The fact that the Lake Superior samples did not show lower concentrations over time suggests that there may be some lake specific source that is continuing to produce toxaphene or that old toxaphene is not being removed from Lake Superior's ecosystem as quickly as the other Great Lakes.

INTRODUCTION

In the late 1940's, the Hercules Company first introduced toxaphene in the United States (US) as an insecticide. Hercules extracted crude α-pinene from pine stumps, using methyl isobutylketone, heat, and pressure. Isomerization of the α-pinene produced camphene, bornylene and α-terpineol. The camphene was subsequently chlorinated to produce toxaphene (*1*). Since chlorination of camphene can take place to varying levels and on various sites, at least 670 congeners exist (*1*).

The primary use of toxaphene, estimated at 67 to 90% of its total consumption (*1*), was in the southeastern US to kill insects such as tobacco budworms, boll worms, and boll weevils (*2*). The remainder was used throughout the US as an insecticide and a herbicide on soybeans and peanuts, as well as to treat scabies on livestock, and as a piscicide to remove rough fish from lakes (*1, 3, 4*). Toxaphene's use was encouraged after the US Environmental Protection Agency (EPA) banned DDT in 1972 (*5*).

As the uses of toxaphene increased, so did concerns about its environmental behavior. In the early 1970's, scientists began to realize that toxaphene was being atmospherically transported away from its application sites to remote locations, where it entered lakes and streams and accumulated in fish

(*1, 6*). In 1977, the EPA issued a rebuttable presumption against toxaphene's registration due to its oncogenicity and tendency to poison non-target species (*5*). The EPA canceled the registrations for most of toxaphene's uses in 1982, but allowed the existing stocks to be used in limited circumstances until 1986 (*7*).

Little is known about global use of toxaphene today. In addition to the US and Canada, its use has been banned or restricted in England, Sweden, Finland, Denmark, France, Switzerland, Hungary, Italy, Egypt, and Algeria. Toxaphene (or structurally similar compounds) may still be used in Mexico, Romania, Germany, Poland, the former USSR, and India (*1*). Estimates place the Mexican consumption at an average of 1,600 tonnes per year between 1975 and 1983 (*8*).

Toxaphene accumulates in biota and biomagnifies in the food web. Evans *et al.* (*9*) have shown that toxaphene concentrations increase 5 times between plankton and fish. The analysis of beluga whale blubber by Stern *et al.*(*10*) suggests that some congeners accumulate more easily than others, thus resulting in selective biomagnification. These authors isolated two congeners (designated T2 and T12) from the blubber samples.

The fact that toxaphene can bioaccumulate has several implications: First, the theory of atmospheric transport can be supported by high concentrations of toxaphene in fish taken from remote areas (*4, 11*). Second, biota can serve as environmental sinks, degrading toxaphene by metabolism (*1*). Third, the consumption of contaminated fish is a direct pathway for toxaphene exposure in humans, regardless of proximity to the original site of application.

Due to toxaphene's complex nature and the consequent analytical difficulties, there have been few comprehensive studies of its environmental fate. Furthermore, culling information from the studies that do exist is often difficult. Differences in the analytical methods and in reporting protocols have hindered comparability and have made analysis of concentration trends difficult. This study was an attempt to learn more about the trophic and temporal distribution of toxaphene in the Great Lakes. The original experimental design was simple: The Great Lakes Fish Contaminate Monitoring Program (a cooperative effort of the EPA, the National Biological Survey, the US Food and Drug Administration, and the eight Great Lakes states) was to provide five lake trout (*Salvelinus namaycush)* and five smelt (*Osmerus mordax)* composite samples from each of the five Great Lakes for each of two time periods (1982 and 1992). These sites were chosen since they are located at offshore fishing grounds that are not impacted by local sources. Unfortunately, some substitutions had to be made: First, lake trout were rare in Lake Erie, so walleye (*Stizostedion vitreum vitreum*) were substituted; these fish occupy a trophic level equivalent to that of the trout. Second, smelt samples were problematic. There were no smelt collected in 1992, and when the National Biological Survey returned to the sites in 1994, smelt samples could only be collected in Lakes Ontario, Michigan, and Superior. Thus, gaps remain in the experimental design for smelt from Lakes Huron and Erie.

EXPERIMENTAL

Sample collection and preparation. At least 50 fish of a given species were captured in gill nets in the Fall (to allow maximum contaminant uptake prior to spawning), and composited into 10 samples of five fish that were twice ground together, placed in solvent washed glass jars and stored at less than -30 °C. Due to the difficulties associated with determining the age of fish in the field, and a desire to sacrifice as few fish as possible, the composite samples were composed of five fish

of similar length. Since the age-length relationship is very strong within a given lake, by keeping the size range small, the ages of the fish in a given composite sample remain very close to one another. However, it should be noted that due to the differences between lakes, the age-length correlation will not necessarily be maintained between lakes. Scales from each of the fish, as well as any tagging device that might have been present were preserved to allow retrospective determination of ages. The composite samples were stored in freezers at the National Biological Survey office in Ann Arbor, Michigan until analysis.

The analytical method employed for toxaphene was based on that of Swackhamer *et al.* (*12*). A ten gram portion of the ground fish tissue was blended with 80 grams of sodium sulfate to remove water present in the sample. The mixture was spiked with the internal standard, either $^{37}Cl_6$-*trans*-nonachlor (EPA Repository, Research Triangle Park, NC), or polychlorinated biphenyl 204 (Ultra Scientific, North Kingston, RI) and Soxhlet extracted for 24 hours with 50% acetone in hexane. Previous work has shown that values quantitated using PCB 204 and $^{37}Cl_6$-*trans*-nonachlor are comparable, as long as interferences are sufficiently resolved. With every batch of five to six tissue samples, a procedural blank, consisting of sodium sulfate spiked with the internal standard was prepared and similarly extracted. To ensure adequate recovery, a procedural blank spike, consisting of glass wool spiked with a known amount of toxaphene (Ultra Scientific, North Kingston RI), was extracted with every other batch. All of these control experiments gave acceptable results.

Samples were reduced in volume under a gentle stream of nitrogen (Pierce 18780 Reacti-vap Evaporation Unit, Rockford, IL) or diluted with GPC solvent until a maximum lipid concentration of 100 mg/ mL solvent was achieved. The majority of the lipids were removed using a gel permeation chromatography system consisting of a glass column (2.5 x 1000 cm, YMC Inc., Wilmington NC) packed with porous styrene divinylbenzene copolymer beads ("Bio-Beads", SX-3 or SX-8, Bio-Rad Laboratories, Hercules CA). The solvent, 60% cyclohexane in dichloromethane, was set to flow at a rate of 10 mL/min. Two fractions were collected. The first, which contained only lipids, was archived. The second, which contained toxaphene, was solvent exchanged into hexane, and subjected to further chromatographic clean up on 1% deactivated silica (100-200 mesh grade, Davidson Chemical, Baltimore MD) in a 1.5 x 30 cm column (Indiana University Glass Shop). Four solvent fractions were collected: hexane, 10% dichloromethane in hexane, dichloromethane, and methanol. The first three fractions were combined, solvent exchanged into hexane, and reduced to 200 µL under a steady stream of nitrogen, in preparation for analysis by electron capture gas chromatographic mass spectrometry (GC/MS).

Analysis by electron capture GC/MS. A Hewlett Packard 5989A mass spectrometer was used to analyze the samples. The samples were injected into a Hewlett Packard 5890 Series II gas chromatograph containing a 30 m DB-5MS™ column (film thickness 0.25 µm, 250 µm i.d., J&W Scientific, Folsom, Ca). Helium was used as the carrier gas at a linear velocity of 25 cm/s. The 1 µL injections were made in the splitless mode, with a vent time of 1.9 min. The injection port temperature was maintained at 285 °C to ensure complete volatilization of the sample. The temperature program for the column began with a 1 minute hold at 40 °C, followed by a 10 °/min ramp up to 200 °C, a 1.5 °/min ramp up to 230 °C and a 10 °/min ramp to 300 °C, which was held for 5 min. After eluting from the column, the samples were carried through a 300 °C transfer line into the ion source of the mass spectrometer, which was held at 125 °C. Methane was used as the reagent gas in the ion source; its pressure was maintained at 0.43 Torr.

The electron capture GC/MS analysis procedure, using selected ion monitoring, was developed by Swackhamer *et al.* (*12*). The only notable difference is that quantitation was based on the M^- ions (quantitation ion *m/z* = 344, confirmation ion *m/z* = 342) for the hexachlorinated homologues; as in the earlier paper, the $(M\text{-}Cl)^-$ ions of the hepta- to decachlorinated norbornanes and norbornenes were monitored. Four time windows, each monitoring a subset of the ions, were used to increase sensitivity relative to monitoring all of the ions all of the time. At the beginning of each day of analysis, and after every four to five sample injections, a relative response factor standard was injected into the GC/MS system. This standard, with its known concentration of $^{37}Cl_6$-*trans*-nonachlor and toxaphene allowed accurate quantitation of the toxaphene present in the sample. With every other batch, a 50 pg/μL instrument detection limit standard was analyzed to ensure consistent performance.

RESULTS AND DISCUSSION

The lipid normalized, total toxaphene concentrations in fish measured in this study are shown in Figure 1 as their means and 90% confidence limits. Note that the smelt samples for Lakes Huron and Erie are missing. Several trends are apparent.

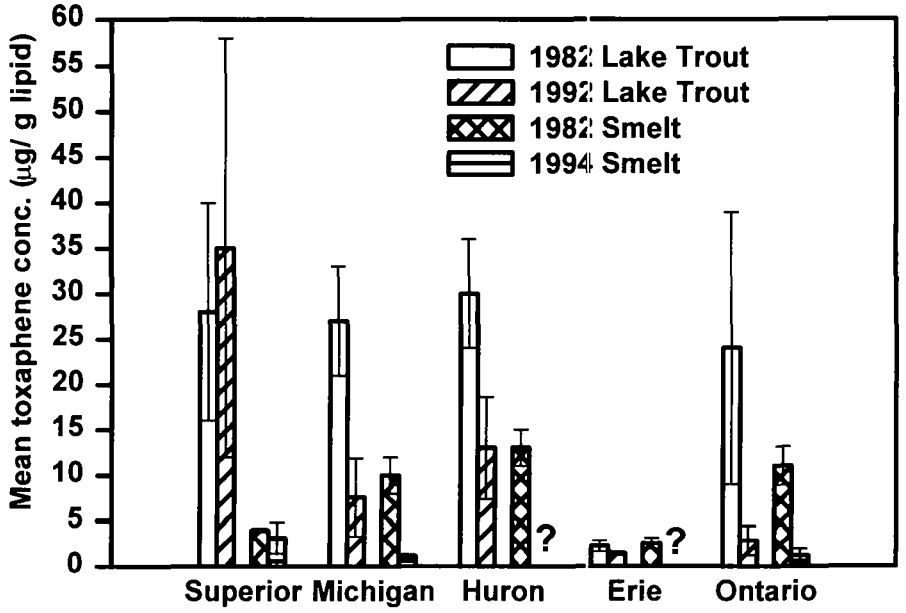

Figure 1. Average concentrations of toxaphene in lake trout and smelt in the Great Lakes in 1982 and 1992. Error bars represent the 90% confidence level of the average.

Temporal trends. In the 10 years between the sample collection in 1982 and in 1992, toxaphene was banned by the EPA. Presumably this action should have lowered the concentrations of the pesticide in the environment. To test this supposition, we examined the differences in concentrations over time for each species. As shown in Figure 1, the lake trout and smelt from Lake Michigan and Ontario and the lake trout from Lake Huron and the walleye from Lake Erie show obvious

toxaphene concentration decreases during the decade; all had statistically significant differences between the two time periods.

Lake Superior did not follow this trend; neither the trout nor the smelt showed a significant decline in toxaphene concentrations. These Lake Superior samples indicate that inputs of toxaphene into the Great Lakes did not end with the EPA ban. Since Mexico allowed the use of this pesticide after the EPA ban, and since there is atmospheric transport from that country to the Great Lakes basin, it is possible that Lake Superior could get an influx of this pesticide from this source, but it is unlikely that it would be the only lake affected. This suggests that there are additional, lake specific sources which continue to release toxaphene into the environment. Determining the origin of these new inputs will require more investigation.

In summary, the cancellation of toxaphene's registration in 1982 has had a big effect on the concentrations found in fish tissue in the Great Lakes. When comparing the samples taken after the legislative action with those taken before, a decrease in the overall toxaphene concentration by about a factor of 4-5 was noted for all of the Great Lakes *except* Superior. The Lake Superior discrepancy can probably be attributed to either new toxaphene sources in the basin or to comparatively slower removal processes.

Trophic level trends. Examining the differences in concentrations over trophic levels is one way to investigate the bioaccumulation (uptake from the ambient surroundings) and biomagnification (increased concentrations from one link of the food chain to another) of toxaphene. In 1982, Lakes Michigan, Huron, and Superior showed differences between the concentrations of the lake trout and smelt while Lakes Erie and Ontario showed no statistical distinction between trophic levels; see Figure 1.

Unfortunately, the lack of 1992 smelt samples interferes with a comprehensive analysis of the 1992 data, but there were significant differences between the trophic levels for Lake Superior and Lake Michigan. As in 1982, the toxaphene concentrations in the 1992 Lake Ontario samples were statistically the same between the species.

The astute reader will no doubt have noticed that the higher trophic level fish that we analyzed from Lake Erie were, in fact, not lake trout at all, rather they were walleye. We were concerned that lake trout and walleye might accumulate toxaphene differently, possibly due to variances in absorption or metabolism and that these differences would result in uneven "trophic steps" up from smelt. To assuage this concern, we obtained and analyzed four filets from Lake Erie lake trout taken in 1993 and compared these results to our 1992 walleye samples. While the nature of these lake trout samples is markedly different from the others that were analyzed (filets from one fish versus composites of five whole fish), the lipid content of the filet samples was comparable to the whole lake trout samples. The average lipid normalized toxaphene concentration of the four analyzed lake trout filets was 1.1 ppm, a concentration which agrees very well with the 1.4 ppm average concentration of the five walleye composites. Thus, while it seems that lake trout filets are indeed similar to walleye, the lack of 1992 or 1994 smelt samples makes it impossible to determine if and how much biomagnification is occurring in Lake Erie in the later time period.

The inferences that can be drawn from these results are somewhat conflicting. The samples from Lakes Superior, Michigan, and Huron indicate that toxaphene does biomagnify in lake trout relative to smelt, but in Lakes Erie and Ontario biomagnification is not detected. The sizes and shapes of

Lakes Ontario and Erie may also (at least partially) explain why biomagnification is not seen in these lakes to the extent that it is in the other lakes. Lakes Ontario and Erie are the shallowest of the Great Lakes and thus they have the shortest water retention times. These conditions may increase the flux of toxaphene to the sediment. This would lower ambient water concentrations, thus minimizing possible exposure to toxaphene and decreasing bioaccumulation of toxaphene and biomagnification in these two lakes.

In addition to the physical features of the lakes, the nature of the food chains within each lake may play a role in toxaphene accumulation. With each link in a food chain, bioconcentration of contaminants, both due to bioaccumulation and biomagnification, occurs. The greater the number of intermediate links between two species, there is greater potential for increased uptake by the higher trophic level species, relative to the lower species. The lack of biomagnification in Lakes Ontario and Erie may simply indicates that the lake trout food chain is shorter in these lakes, relative to the other three lakes.

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