# Chlorohydrocarbons in Marquette Fish Hatchery Lake Trout (*Salvelinus namaycush*)

Ronald Parejko and Chii-ling Jeang Wu
*Department of Biology, Northern Michigan University*
*Marquette, Mich. 49855*

Chlorinated hydrocarbons have been identified in Lake Superior lake trout (Salvelinus namaycush) (PAREJKO et al. 1975). These fish, in a high tropic echelon of the lake's ecosystem have likely acquired these compounds through predation on native forage species.

As some data are presently available on the chlorohydrocarbons (PCBs and insecticides) content of Lake Superior lake trout, the present study was made to determine if similar concentrations of like compounds are detectable in lake trout not directly exposed to the waters of Lake Superior but yet which live within this lake's watershed. If a difference in chlorohydrocarbon content of these fish can be detected it would be important to identify potential contributing sources.

## Materials and Methods

Material: Eleven sexually mature females, two immature females and two immature unsexed lake trout were removed from pools at the Michigan Department of Natural Resources, Marquette Fish Hatchery in early 1973 (most removed in March). This hatchery uses impounded waters of Cherry Creek, for its fish propagation pools and is located some 2½ miles inland from the shores of Lake Superior. Cherry Creek flows through predominantly nonagricultural, forested areas interspersed with occasional clearings. The fish were killed, wrapped in aluminum foil and stored frozen (-10°C) until analyzed.

Ventral abdominal tissue, 20-30g (muscle, connective and adipose) was taken from an area between the pelvic and pectoral fins. Fish feed samples manufactured by Zeigler Bros. Inc. (PR-6 Dry Pellet) and by Bioproducts Inc. (Oregon Moist Pellet) used for feeding adult and trout fry respectively were also analyzed for chlorohydrocarbon content.

Methods: Fish tissue was macerated with excess sodium sulfate and extracted three times with petroleum ether in pre-washed 50 ml. nylon centrifuge tubes.

The dry (PR-6) fish feed pellets were extracted according to the standard methods of extracting dry products using 35% water/acetonitrile (UNITED STATES DEPARTMENT OF HEALTH, EDUCATION AND



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WELFARE, 1971). The Oregon moist pellets were extracted by using the same procedure used in extracting fish tissue.

Acetonitrile partitioning and Florisil column chromatography were used to clean extracted samples (PAREJKO et al. 1975) and the PCBs were separated from the chlorohydrocarbon pesticides by 3% water deactivated silicic acid-Celite 545 column chromatography (ARMOUR AND BURKE 1970). Samples were concentrated, stored and analyzed for chlorohydrocarbon compounds by gas-liquid chromatography (PAREJKO et al. 1975).

Reagent blanks passed through the entire analytical procedure produced no gas chromatographic peaks identified as Arochlor (PCBs) or as chlorohydrocarbon pesticides. Recoveries of p,p'-DDT and Arochlor 1254 from salted (spiked) fish tissue samples were 99% and 65% respectively.

Statistical analyses utilized standard t tests at the 95% confidence interval.

## Results and Discussion

The physical characteristics of the fish used for analysis are listed in Tables 1 and 2. There is a statistically significant difference in fat contents of lake trout in these two age groups with the older fish having a higher fat content. Also, the fat contents of lake trout taken from Lake Superior were determined statistically to be significantly greater than the fat contents of Marquette hatchery lake trout (PAREJKO et al. 1975).

TABLE 1

Physical characteristics of 4 (3-5 yr.)
Marquette Fish Hatchery lake trout analyzed

| Characteristic | Measurement |
|---|---|
| Length | Range: 11.4" (29.0 cm) to 16.7" (42.4 cm)<br>Mean: 14.8" (37.7 cm) |
| Weight | Range: 0.38 lb (172.5 g) to 1.36 lb (617.4 g)<br>Mean: .99 lb (450.6 g) |
| Percent fish-oil extracted | Range: 2.5 to 10.8<br>Mean: 6.8<br>95% Confidence Interval: 6.8 ± 4.9 |

TABLE 2

Physical characteristics of 11 (13-17 yr.) Marquette Fish Hatchery lake trout analyzed

| Characteristic | Measurement |
|---|---|
| Length | Range: 23.3" (59.2 cm) to 28.8" (73.2 cm)<br>Mean: 25.9" (66.0 cm) |
| Weight | Range: 3.06 lb (1389 g) to 6.80 lb (3087 g)<br>Mean: 5.17 lb (2233 g) |
| Percent fish-oil extracted | Range: 6.6 to 21.0<br>Mean: 14.2<br>95% Confidence Interval: 14.2 ± 3.3 |

The components of the fish feed pellets used to feed the hatchery lake trout are listed in Tables 3 and 4. Oregon moist pellet is used to feed lake trout fry and the PR-6 is used to feed larger fish. The chlorinated hydrocarbons detected in these pellets and their concentrations are given in Table 5.

TABLE 3

Percent composition of Oregon Moist Pellet* (dry weight)

| Component | Content (%) |
|---|---|
| Cotton seed meal | 22 |
| Fish meal (herring, Peruvian anchovey, hake, menhaden) | 22 |
| Crab or shrimp meal | 4 |
| Wheat germ meal | 3 |
| Distiller's dried corn solubles | 3 |
| Kelp meal | 2 |
| Vitamin premix | 1.5 |
| Tuna viscera or Pasteurized Salmon viscera or Turbot or Dogfish | 40 |
| Soybean oil | 1.8 |
| Choline chloride | 0.6 |
| Antioxidant | 0.05 |

*Manufactured by BioProducts Inc., Warrenton, Oregon 97146.

TABLE 4

Percent composition of PR-6 Dry Pellet* (dry weight)

| Component | Content (%) |
|---|---|
| Canadian or Alaskan herring meal | 33-35 |
| Corn gluten meal | 6 |
| Wheat standard middlings | 13-15 |
| Corn ferment extract | 8 |
| Soybean oil meal | 10 |
| Dried Brewers yeast | 5 |
| Delactosed whey | 10 |
| Dehydrated alfalfa meal | 3 |
| Trace mineral salts (Fe, Cu, Co, $I_2$, Zn, Mn) | 2 |
| Vitamin premix #25 | 4 |
| Soybean oil | 4 |

*Manufactured by Zeigler Brothers Inc., Box 95, Gardners, Pennsylvania 17324

TABLE 5

Chlorinated hydrocarbons in lake trout feed used at the Marquette Fish Hatchery

| Chlorinated Hydrocarbon | PR-6 Dry Pellet (Dry Weight) | Oregon Moist Pellet (moist weight) | Oregon Moist Pellet (extracted-oil weight) |
|---|---|---|---|
| 1-hydroxychlordene | 0.139 | 0.055 | 0.95 |
| Heptachlor | 0.021 | 0.015 | 0.26 |
| Total heptachlor analogs | 0.160 | 0.070 | 1.21 |
| δ-hexachlorocyclohexane | 0.023 | 0.009 | 0.15 |
| Methoxychlor derivative | 57.7 | 20.5 | 359 |
| p,p'-DDE | 0.26 | 0.20 | 3.4 |
| Aroclor | | | |
| 1248 | -- | 0.356 | 6.14 |
| 1254 | 0.118 | 0.103 | 1.78 |
| 1260 | -- | 0.180 | 3.11 |
| Total Aroclor | 0.118 | 0.639 | 11.03 |

Concentrations expressed, in this and subsequent tables, in ppm based on wet-tissue and on extracted fish-oil weights.

Of the chlorohydrocarbons detected in the fish feed 1-hydroxychlordene was detected in all (100%) of the lake trout analyzed and heptachlor was detected in all but one (93%) of the samples (Table 6). The compound 1-hydroxychlordene is a hydrolytic metabolite of heptachlor. Total heptachlor analogs concentrations in these fish did not exceed the FDA action limit for heptachlor of 0.3 ppm.

TABLE 6

Heptachlor analogs concentrations [1] in Marquette Fish Hatchery lake trout

| Heptachlor Analog | Base | Conc. Range Detected | Mean | *S.D. | **%T.R. | ***%F.H.R. |
|---|---|---|---|---|---|---|
| Heptachlor | wet | 0.015-0.054 | 0.027 | 0.010 | 22.1 | 93.3 |
| | fish-oil | 0.17-1.01 | 0.28 | 0.21 | 21.8 | 93.3 |
| 1-hydroxy-chlordene | wet | 0.021-0.208 | 0.089 | 0.041 | 78.1 | 100 |
| | fish-oil | 0.19-3.85 | 0.97 | 0.86 | 78.2 | 100 |
| Total Heptachlor Analogs | wet | 0.040-0.228 | 0.114 | 0.044 | 100 | 100 |
| | fish-oil | 0.36-4.22 | 1.24 | 0.96 | 100 | 100 |

[1]Concentration range, in this and subsequent tables, includes only those fish in which the residue was detected.

*S.D. = Standard Deviation in this and subsequent tables.
**%T.R.= % Total Residues in this and subsequent tables.
***% F.H.R. = % Fish Having Residue in this and subsequent tables.

Delta-hexachlorocyclohexane is one conformational isomer of hexachlorocyclohexane (BHC) used as insecticide. This isomer was detected in hatchery lake trout (Table 7). Also detected was a compound tentatively identified as a methoxychlor derivative (Table 7). This tentatively identified compound was detected in hatchery lake trout in a wide range of concentrations and was found at significantly high levels averaging 70 ppm. Mature lake trout in the hatchery are given a diet of PR-6 pellets which have a concentration of 57.5 ppm (dry wt) of the chlorohydrocarbon tentatively identified as a methoxychlor derivative. The fish fry are also given a feed (Oregon moist pellet) which contains this compound at 20.5 ppm (moist wt).

TABLE 7

Delta-hexachlorocyclohexane and methoxychlor derivative concentrations in Marquette Fish Hatchery lake trout

| Chlorohydrocarbon | Base | Conc. Range Detected | Mean | S.D. | % F.H.R. |
|---|---|---|---|---|---|
| δ-Hexachloro-cyclohexane | wet | 0.011-0.106 | 0.040 | 0.022 | 100 |
| | fish-oil | 0.10-1.96 | 0.45 | 0.45 | 100 |
| Methoxychlor derivative | wet | 5.2-268.3 | 70.0 | 66.9 | 100 |
| | fish-oil | 29-4975 | 813 | 1175 | 100 |

All of the hatchery lake trout were found to contain p,p'-DDE within the concentration range of 0.21-1.46 ppm (wet wt) with a mean of 0.54 ppm (wet wt). This residue which was also found in the feed pellets at concentrations of 0.26 ppm (dry wt) and 0.20 ppm (moist wt) in the PR-6 dry pellets and in the Oregon moist pellets respectively, represented some 80% of the total DDT analogs detected in the hatchery fish. The residues p,p'-DDD and p,p'-DDT were detected in 40% of the fish analyzed and represented about 14% and 6% of the total DDT residues found respectively. The residues p,p'-DDD and p,p'-DDT were not identified in detectable levels in either of the fish feed pellets.

Total DDT analog concentrations detected identified only two mature fish (13.3%) as having concentrations higher than 1 ppm. The mean concentrations detected in these trout was considerably less than the FDA action limit of 5.0 ppm (wet wt) for DDT and its analogs (Table 8). There is a statistically significant difference of total DDT concentrations between immature and mature hatchery lake trout, with the older fish having more total DDT. The total DDT analogs concentrations of Marquette Fish Hatchery lake trout were statistically compared to the total DDT and analogs concentrations of Lake Superior lake trout and it was determined that a statistically significant difference exists with the Lake Superior fish having a higher concentration.

Polychlorinated biphenyls (Aroclor) were identified in these fish (Table 9). Statistical analyses of the means of Aroclor contents of the two age groups of fish indicates that there is a statistically significant difference in PCBs concentrations with the mature fish having a higher concentration of total PCBs. This result agrees with Bache who detected an accumulation in PCBs in Cayuga Lake lake trout with age (BACHE 1972). A statistical comparison of PCBs concentration of Lake Superior lake trout with those of Marquette Fish Hatchery lake trout indicates a statistically significant difference with the Lake Superior fish having more PCBs than the hatchery fish (PAREJKO et al. 1975).

TABLE 8

DDT analogs concentrations in Marquette Fish Hatchery lake trout

| DDT Analog | Base | Conc. Range Detected | Mean | S.D. | % T.R. | % F.H.R. |
|---|---|---|---|---|---|---|
| p,p'-DDE | wet | 0.21-1.46 | 0.54 | 0.32 | 79.4 | 100 |
| | fish-oil | 2.4-12.2 | 4.8 | 2.3 | 82.8 | 100 |
| p,p'-DDD | wet | 0.13-0.45 | 0.25 | 0.11 | 14.7 | 40 |
| | fish-oil | 1.5-2.1 | 1.8 | 0.2 | 12.4 | 40 |
| p,p'-DDT | wet | 0.07-0.23 | 0.11 | 0,05 | 6.5 | 40 |
| | fish-oil | 0.4-1.1 | 0.9 | 0.3 | 6.2 | 40 |
| Total DDT Analogs | wet | 0.21-1.91 | 0.68 | 0.41 | 100 | 100 |
| | fish-oil | 2.4-12.2 | 5.8 | 2.3 | 100 | 100 |

TABLE 9

Aroclor (PCB) concentrations in Marquette Fish Hatchery lake trout

| Aroclor | Base | Conc. Range Detected | Mean | S.D. | % T.R. | % F.H.R. |
|---|---|---|---|---|---|---|
| 1248 | wet | 0.360-1.117 | 0.635 | 0.251 | 62.4 | 86.6 |
| | fish-oil | 3.98-10.05 | 5.31 | 1.50 | 58.9 | 86.6 |
| 1254 | wet | 0.087-0.361 | 0.227 | 0.093 | 25.7 | 100 |
| | fish-oil | 0.74-4.33 | 2.14 | 0.93 | 27.4 | 100 |
| 1260 | wet | 0.189-0.313 | 0.261 | 0.050 | 11.8 | 40 |
| | fish-oil | 2.28-3.38 | 2.66 | 0.38 | 13.6 | 40 |
| Total Aroclors | wet | 0.107-1.467 | 0.882 | 0.415 | 100 | 100 |
| | fish-oil | 0.74-12.77 | 7.81 | 3.20 | 100 | 100 |

Concentration ratios of the chlorohydrocarbons detected in Marquette Fish Hatchery lake trout are given in Table 10. Some four times as much 1-hydroxychlordene was found as its parental compound heptachlor. About 5 times as much DDE was detected than was DDT and DDD was detected at about ½ the concentration of DDE. Total PCBs concentrations were detected to be present at about twice the concentration of DDE and at about 1.5 times the concentration of total DDT analogs.

TABLE 10

Ratios of Chlorohydrocarbons
Detected in Marquette Fish Hatchery Lake Trout

| Ratio | N | Range | Mean | S.D. |
|---|---|---|---|---|
| 1-Hydroxychlordene/heptachlor | 14 | 0.91-10.40 | 3.70 | 2.37 |
| DDE/DDT | 6 | 3.00-7.85 | 5.35 | 1.71 |
| DDD/DDE | 6 | 0.30-0.58 | 0.44 | 0.10 |
| Total PCB/DDE | 15 | 0.19-3.76 | 1.73 | 0.96 |
| Total PCB/Total DDT and Analogs | 15 | 0.17-2.82 | 1.49 | 0.70 |

Based on the occurrence and concentrations of chlorohydrocarbons detected in lake trout confined to a fish hatchery as compared to those compounds detected in the standard diet of these fish, it appears consistent that these fish consume and absorb chlorohydrocarbons from their diet. Differences in kind and amount of chlorohydrocarbons detected in lake trout taken from Lake Superior (PAREJKO et al 1975) and in lake trout taken from a fish hatchery within that lake's watershed have been identified.

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