LEVELS OF TRICLOSAN AND METHYL TRICLOSAN IN THE PLASMA OF FISH FROM THE DETROIT RIVER

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Introduction:

Triclosan (2,4,4' trichloro-2'-hydroxy diphenyl ether) is a broad-spectrum antibacterial and antifungal agent[1], which inhibits the enzyme enoyl-acyl carrier protein reductase (ENR) blocking lipid biosynthesis.[2] Over the past thirty years Triclosan has been used in a wide range of products such as toothpaste, cosmetics, detergents, plastic cutting boards, textiles, toys etc. Triclosan is a persistent and lipophilic (log K_{ow}=4.48) compound that is acutely toxic to biota. EC_{50} of Triclosan ranges from 0.35 mg/L to 0.0015 mg/L for rainbow trout and algae, respectively. Orvos *et al.*[3] recently reported triclosan toxicities in freshwater invertebrates, fish and algae. The biotransformation product methyl Triclosan (4-chloro-2- (2,4-dichlorophenoxy) anisole) is more persistent[4] and hydrophobic with potential for biomagnification in the food web[5].

Even though Triclosan was detected in the effluent of sewage treatment plants in the late 1970's[6], there is only limited information available on the occurrence of Triclosan in biota and abiotic samples. For example, Triclosan has been detected in breast milk (5-300 ng/g lipid weight) from women in Stockholm, Sweden[7], in the bile of rainbow trout (*Oncorhynchus mykiss*) exposed to municipal wastewater, and in wild fish (i.e., roach (*Rutilus rutilus*), eelpout (*Zoarces viviparus*) and perch (*Perca fluviatilis*)) from the receiving waters of three wastewater treatment plants.[4] Triclosan at levels from 0.07-14 000 µg/L have been measured in wastewater treatment systems in the United States[8], Switzerland [9] and recently in Windsor, Ontario, Canada (Letcher, unpublished results). Triclosan has also been reported in surface waters (50-2300 ng/L) in Switzerland.[4,9] Reports on the occurrence of the methyl derivative of Triclosan in biota is limited to fish from the Tama River in Japan[10] (>1- 38 ppb). The only known report of methyl Triclosan in surface and wastewater is one report from Switzerland.[5]

In the present study, we report on the presence of Triclosan and methyl Triclosan in the blood plasma of 13 species of benthic- and pelagic-feeding fish from the Detroit River, which were collected in the summer of 2001.

Materials and Methods:

Blood-plasma samples from 13 benthic-feeding and pelagic species of fish from the Detroit River, and collected in the summer of 2001, and analyzed for Triclosan and methyl Triclosan. Li *et al.*[11]

provided detailed description of the sample extraction and cleanup. In short, plasma from 3 to 4 individual fish for each species was pooled. Four grams of plasma was acidified with 1 mL of 6N HCl and 3 mL of 2-propanol was added. The denatured plasma was extracted 3 times with 1:1 MTBE:hexane. The organic extracts were partitioned with 1M KOH in 50% ethanol to isolate a neutral fraction containing methyl Triclosan, and a phenolic fraction containing Triclosan. The isolated phenolic fraction was back-extracted in MTBE:hexane after acidification of the alkaline extract, which was subsequently dried with sodium sulfate and methylated with diazomethane. The methyl-derivatized fraction was further fractionated using silica/sulfuric acid (22%) columns. The hexane fraction containing methyl Triclosan was cleaned up and fractionated with 8 g of 1.2% deactivated Florisil. GC/MS identification and quantification of methyl Triclosan was performed on a Micromass Ultima HRGC/HRMS, equipped with a HP 6890 gas chromatograph and CTC A200 auto-sampler. Gas chromatographic separation prior to MS was achieved using a 60 m X 0.25 mm X 0.25 μm Restek Rt_x5MS capillary column. The mass spectrometer was operated in the electron ionization (EI) mode, under selected ion recording (SIR) conditions. Source temperature was 250^{o}C and the resolving power of the analyzer was 10 000.

Results and Discussion:

Similar to other phenolic compounds, Triclosan can easily be derivatized with diazomethane or acetic anhydride. Derivatized Triclosan (2,4,4' trichloro-2'-methoxy diphenyl ether) is amenable to analysis by GC; therefore, various GC based techniques such as GC/ECD and GC/MS can be applied to the analysis of phenolic compounds. In EI mode the spectra for Triclosan is dominated by M+, and [M-50]+ indicating the loss of the $ClCH_3$. Analysis of Triclosan in plasma was accomplished by monitoring M+ and [M+2]+ ions.

Since Triclosan is used in many products extra care should be taken to avoid contamination during sample processing. Triclosan was detected in blood-plasma samples from all 13 fish species that were analyzed, which indicates the presence of Triclosan in biota from the Detroit River. Levels of Triclosan ranged between 0.61 ng/g-wet weight for brown bullhead and 10.4 ng/g-wet weight for white bass (arithmetic mean =3.4 and geometric mean =2.8). With the exception of white sucker Triclosan levels were higher than another major halogenated phenolic compound (HPC), pentachloro phenol (PCP; 0.06 to 3.42 ng/g wet weight) determined in these same samples.[11] In fact the level of Triclosan was 40 times that of PCP in Lake Sturgeon. Triclosan levels on average were about 1/3 of the level of the major HPC class, the hydroxy PCBs (between 0.02 and 1.5 fold) that is the generally the sum of 12 congeners[11]. In addition, Triclosan averaged twice the concentration of CB-153 (between 0.5-6.6 fold) [11].

Methyl Triclosan was also detected in the neutral fraction from all 13 fish plasma that were analyzed, ranging between 0.0004 ng/g-wet weight for common Carp and 0.0132 ng/g-wet weight for largemouth bass (arithmetic and geometric means were 0.0037 and 0.0027 respectively). Methyl Triclosan is about 1000 times lower than Triclosan in fish plasma, this might be due the fact that methyl Triclosan is more lipophilic and is absorbed into the fatty tissue. It is not known whether the methylation of Triclosan occurred prior to or following the uptake. Levels of methyl Triclosan in fish plasma from Detroit River are significantly low that the levels observed in fish

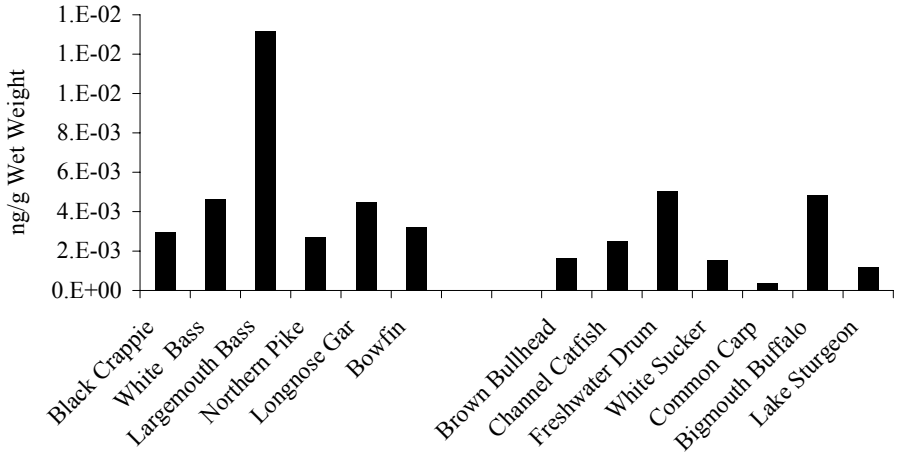

Figure 1. Concentration of Triclosan in the plasma of fish from the Detroit River

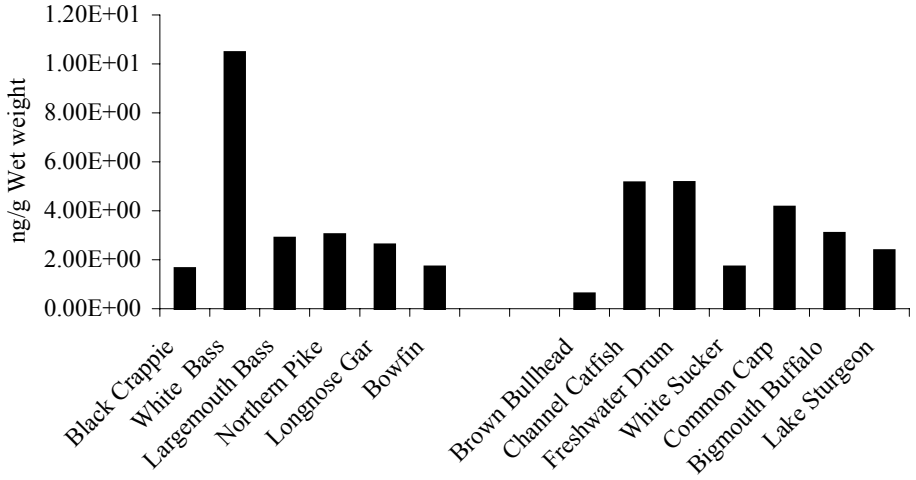

Figure 2. Concentration of methyl Triclosan in the plasma of the fish from the Detroit River

from Tama River [9]. Lipophic compounds such as methyl Triclosan partition favorably in the fatty tissue hence the concentration of methyl Triclosan should be higher in the whole body than in plasma. Whittle *et al*.[12] observed about 10-fold increase in the levels of PCBs and other organochlorine contaminants between plasma and whole fish from the Great lakes. Methyl Triclosan might have higher tendency to partition into fatty tissue hence higher concentration in the whole body.

To our knowledge this is the first evidence of the biological uptake of Triclosan and methyl Triclosan in fish plasma from the Great Lakes area. Further investigation into the uptake and methylation of Triclosan in biota from the Great Lakes is needed.

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