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Uptake of Pentachlorophenol in Fish of Acidified and Nonacidified Lakes

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The atmospheric transport of acidifying substances (e.g., SO_2 and NO_2) and the effects of low pH on biota in lakes of northern Europe and North of America are well-known. There are a variety of organic compounds, such as chlorinated phenols, chlorobenzenes, PCBs and polychlorinated dioxins and furans, that are released from industrialized areas in connection with the emission of acidifying substances. These persistent pollutants are globally distributed in the atmosphere (Bidleman and Christensen 1979; Tanabe et al. 1983; Bunce and Nakal 1989). Most of these pollutants are neutral, i.e. they are not affected by pH when dispersed into aquatic environments. One exception to this is chlorophenols as they exhibit weak acidic properties in water. This means that chlorophenols dissociate in alkaline waters but are undissociated in waters with low pH (e.g., Kaiser and Valdmanis 1982; Saarikoski et al. 1986).

In Sweden, the most serious acidification of lakes is found in the southwestern part of the country, due to a combination of high industrial exposure and the non-buffering capacity of the soil (igneous rock, Cronberg et al. 1988). Are the negative effects on biota of these lakes only an effect of acidification or do alternative or additive explanations exist? One such explanation could be the effect of organic pollutants that are potentially undissociated/dissociated in the lake water.

Pentachlorophenol (PCP) has been detected in the atmosphere over industrial (Ingram et al. 1986) as well as remote areas (Crosby 1981; Bunce and Nakal 1989), in sediment of lakes receiving industrial effluents (Xie 1983) and in lakes wich are not subjected to direct discharges (Salkinoja-Salonen et al. 1984) and in aquatic biota (Paasivirta et al. 1985; Carey et al. 1988). PCP has a pK_a of 5.2 (Renberg 1981), which means that this pollutant is mainly ionized in natural waters not subjected to acidification. In acidified waters, however, PCP is undissociated and thus considerably more available for uptake in gill-breathing organisms. It has been shown in laboratory studies that the accumulation of PCP by fish is inversely related to pH (Saarikoski and Viluksela 1982, Saarikoski et al 1986), which also is the case for toxicity (Exon 1984; Steiert and Crawford 1985).

The objectives of this study were to examine the uptake of PCP in fish (northern pike, *Esox lucius* L.) in one acidified and two non-acidified lakes. The lakes are situated near each other, and to our knowledge are only exposed to persistent pollutants from the atmosphere. Because the lakes are of similar size and depth and situated in the same fall-out area, atmospheric input of PCP should be similar.

MATERIALS AND METHODS

The three lakes are situated in southern Sweden within 30 km of each other. The lakes are small, <0.5 km^2 and with a mean depth of 2-3 m. Lake Baen, the acidified lake, has a median pH of 5.82 during the year with a minimum of 5.17 in the spring and a maximum value of 6.30 in the autumn. The lake is oligotrophic, with a conductivity of 7.5 mS/m_{25^oC}, alkalinity around 0.03 mekv/L, and total phosphorus around 10 ug/L. Lake Lyng has a median pH of 8.13 with a minimim of 7.97. The lake has a conductivity around 10 mS/m, alkalinity around 0.3 mekv/L and total phosporus of 20 ug/L. Lake Sie has a median pH of 8.0 and a minimum value of 7.99. The lake has a conductivity around 35 mS/m, alkalinity around 2.4 and total phosporus of 25 ug/L.

Northern pike were caught by electrofishing (10 in each lake) in the autumn of 1990. The fish were weighed (nearest g), length measured (nearest mm) and sex was determined. The pike weight ranged from 60 to 600 g, length from 220 to 480 mm and sex ratio was 50:50.

Fish were ground whole, and a subsample of 20 g was taken for extraction. 2,6-dibromophenol (650 ng) was added as an internal standard. The sample was mixed with 20 g of anhydrous Na_2SO_4 and left for 3 hr to dry. The dried sample was extracted with acetone/n-hexane (60 mL, 1:1) in a Soxhlet apparatus for 2 hr. The acetone/hexane mixture was transferred to a flask and acid water (H_2SO_4 to pH 2) was added. The hexane fraction was separated, and the remaining water washed with 2 x 2 mL of hexane. The combined hexane extract was evaporated to dryness in a vacuum-centrifuge, and the fat content determined on a balance. The fat was dissolved in 3 mL of cyclohexane. Fat and the halogenated phenols were separated on a gel permeation column (method modified after Servizi et al. 1988, 40 cm long, ID 10 mm, Bio-Beads SX-2, mesh 200-400) coupled to a Varian HPLC. The sample was eluated with cyclohexane (according to Stalling et al. 1972, 0.5 mL/min). The first 44 mL was discarded (containing the fat) and the remaining 45-70 mL was collected (containing the halogenated phenols). To determine the PCP and fat elution volumes, a PCP standard was added to the fish fat. The elution was followed with a UV-detector and verification of the PCP fraction was performed by GC/ECD (see further below).

The halogenated phenols were derivatized by acetylation according to Xie (1983). The cyclohexane was shaken for 20 min in 5 mL 0.1 M K_2CO_3 (pH 11). The basic water

phase with the chlorinated phenols was separated and 1 mL isooctane containing 237 ng PCB 53 was added. PCB 53 (2,2',5,6'-tetrachlorobiphenyl) was used as a chromatographic standard. Acetic anhydride (180 uL) was added and the aliquot was immediately, vigorously shaken for 3 min. The extract (1 uL) was used for GC-analysis.

The extract was analysed for PCP, 2,6-dibromophenol and PCB 53 on a Varian 3700 GC/ECD, equipped with quartz capillary column according to Okla and Wesen 1984. PCP was identified and quantified with a pure PCP standard (Fluka, puriss). Detection limit for PCP in fish was 0.5 ng/g fresh weight.

RESULTS AND DISCUSSION

Concentrations of PCP were significantly higher in pike from the acidified lake than from the non-acidified lakes (Fig. 1, Mann-Whitney U-test, p=0.02). Levels in pike from lake Baen varied from 2.05 to 8.72 ng/g fresh weight (geometrical mean 3.95, or 201 to 799 ng/g extractable fat) while levels in the non-acidified lakes varied from 1.50 to 3.21 ng/g fresh weight (geometrical mean 2.19, or 114 to 346 ng/g extractable fat). As the lakes are situated near to each other, and have no source of contamination other than the atmosphere, the different lakes should receive similar atmospheric fallout of PCP. The difference between PCP uptake in fishes from the two lake types could then be a result of differences in the lake pH. As pH decreases a larger fraction of PCP becomes undissociated and thus more available for uptake by fish (Saarikoski et al. 1986). The pH of Lake Baen is as low as pH 5.2 and the pK_a for PCP is around 5 (depending on author e.g. Renberg 1981; Renner 1990). The pH of the other two lakes is around 8. The results suggest that fish in acidified lakes are subjected to higher PCP exposure and uptake than fish from non-acidified lakes. The results should be regarded as tentative, as PCP was not measured in water of the lakes.

The octanol/water partitioning coefficient (Log P) for chlorophenols are pH-dependent (Kaiser and Valdmanis 1981) and log P for PCP is about 4 at pH 4 while it is 0 at pH 8 (Renberg 1981). PCP is the strongest acid of the chlorophenols (comparable in acid strength to acetic acid), and its acidic properties decrease with decreasing chlorine substituents. Consequently, PCP is the chlorophenol that should vary most along a pH gradient in lakes (pH 4.5 to 8).

The pH-dependent uptake of chlorophenols in fish has been shown in several laboratory studies. Saarakoski et al. (1986) showed that the uptake rate of PCP in guppy (*Poecilia reticulata*) was below 10% at pH 8 compared to pH 5. The effect of pH was also significant for the uptake of 2,4-dichlorophenol, 2,4,5- and 2,4,6-trichlorophenol, in guppy. However, the uptake of phenol was not affected by pH in this interval, nor was the uptake of neutral, organic pollutants. In another laboratory experiment (Stehly and Hayton 1990), using ^{14}C-PCP, the authors showed that the bioconcentration factor increased from 52 at

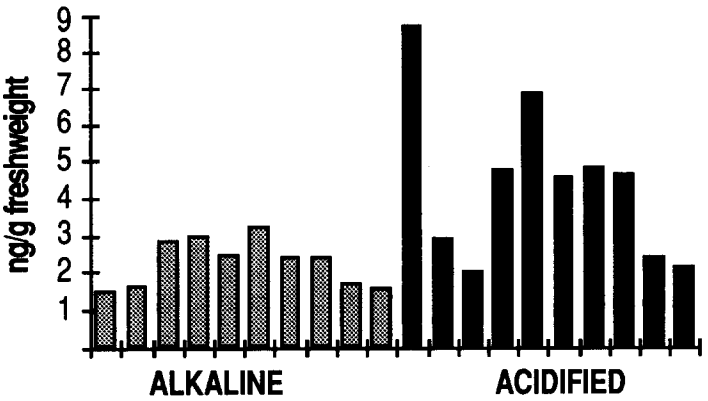

Figure 1. Pentachlorophenol in pike from two alkaline and one acidified lakes in southern Sweden

pH 9 to 607 at pH 7 in goldfish (*Carassius auratus*). Similar results were found by Saarikoski and Viluksela (1982). Consequently, pH-dependent uptake of chlorophenols in fish and the mechanisms behind the chlorophenol behavior, have been shown in several laboratory studies, but the conclusions have not been verified in the field.

Levels of PCP in freshwater fishes have been recorded in Finland (Paasivirta et al. 1985). On a fresh weight basis the levels of PCP were below 1 ng/g in pike and roach (*Leuciscus rutilus*), around 3 in burbot (*Lota lota*) and around 7 in ide (*Leuciscus idus*). In salmon (*Salmo salar*) caught in rivers mean levels were 2.7 ng/g fresh weight. Levels of PCP in different freshwater fishes of the Fraser River, Canada, varied between 2.7 to 40.3 ng/g fresh weight. It was concluded in this study that the Fraser River was contaminated by chlorophenols produced by the forest industry (Carey et al. 1988).

The toxicity of chlorinated phenols increases with increasing chlorine substituents (Exon 1984). Toxicity also increases as pH decreases, especially for chlorinated phenols with increasing acid strength (Saarakoski and Viluksela 1981). The PCP 96-hr LC50 of PCP for guppy was 0.16 umol/L at pH 5, 0.44 at pH 6, 1.66 at pH 7 and 3.42 at pH 8. The pH values used in this laboratory study correspond to values measured in acidified and non-acidified lakes. In general, the toxicity of chlorophenols to fish is high compared to other chlorinated pollutants (Davies and Hoos 1975).

As numerous lakes of northern Europe and North America are subjected to acidification, chlorophenol uptake and toxicity could add to the indirect pH-stress on biota. Because chlorophenols are used industrially or formed when plastic products are burned (Eklund et al. 1986), the atmospheric sources for SO_2 and chlorophenols could very well be situated

in the same area. The atmospheric fallout of H_2SO_4 and chlorophenols may thus interact, increasing stress on biota in lakes.

REFERENCES

Bidleman TF, Christensen EJ (1979) Atmospheric removal processes for high molecular weight organics. J Geophys Res 84: 7857-7862

Bunce NJ, Nakal JS (1989) Atmospheric chemistry of chlorinated phenols J Appl Poll Contr Ass 39: 820-823

Carey JH, Fox ME, Hart JH (1988) Identity and distribution of chlorophenols in the north arm of the Fraser River estuary. Water Poll Res Can: 23 31-44.

Cronberg G, Lindmark G, Björk S (1988) Mass development of the flagellate Gonyostomum semen (Raphidophyta) in Swedish forest lakes - an effect of acidification? Hydrobiol 161: 217-236.

Crosby T (1981) Environmental chemistry of pentachlorophenol. Pure Appl Chem 53: 1051-1080.

Davies JC, Hoos RAW (1975) Use of sodium pentachlorophenate and dehydroabietic acid as reference toxicants for salmonid bioassays. J Fish Res Board Can 32: 411-416.

Eklund G, Pedersen J, Strömberg B (1986) Phenol and hydrochloric acid at 550 ^{o}C yield a large variety of chlorinated toxic compounds. Nature 320: 155-156.

Exon J H (1984) A reveiw of chlorinated phenols. Vet Human Toxicol 26 508-520.

Ingram LL, McGinnis GD, Gjovik LR (1986) Studies on the vaporization of pentachlorophenol from treated wood. Arch Environ Contam Toxicol 15: 669-676.

Kaiser KLE, Valdmanis I (1982) Apparent octanol/water partitioning coefficients of pentachlorophenol as a function of pH. Can J Chem 60: 2104-2106.

Okla L, Wesén C (1984) A simple on-column injector for capillary gas chromatography. J Chromatogr 299: 420-23.

Paasivirta J, Heinola K, Humppi T, Karjalainen A, Knuutinen J, Mäntykoski K, Paukku R, Piilola T, Surma-Aho K, Tarhanen J, Welling L, Vihonen H (1985) Polychlorinated phenols guaiacols and catechols in the environment. Chemosphere 14: 469-491.

Renberg L (1981) Gas chromatographic determination of chlorophenols in environmental samples. National Swedish Environment Protection Board Report 1410,135 pp.

Renner G (1990) Gas chromatographic studies of chlorinated phenols, chlorinated anisoles, and chlorinated phenylacetates. Toxicol Environ Chem 27: 217-224.

Saarikoski J, Viluksela M (1981) Influence of PH on the toxicity of substituted phenols to fish. Arch Environ Contam Toxicol 10: 747-753.

Saarikoski J, Viluksela M (1982) Relation between physiochemical properties of phenols and their toxicity and accumulation in fish. Ecotox Environ Saf 6: 501-512.

Saarikoski J, Lindström R, Tyynelää M, Viluksela M (1986) Factors affecting the absorption of phenolics and carboxylic acids in the guppy (*Poecilia reticulata*). Ecotox Environ Saf 11: 158-173.

Salkinoja-Salonen MS, Valo R, Apajalahti J, Hakulinen R, Silakoski L, Jaakkola T (1984) Biodegradation of chlorophenolic compounds in wastes from wood-processing industry. In: Klug, MJ Reddy CA (ed) Current Perspectives in Microbial Ecology. Amer Soc Microbiol, Washington, DC, pp 668-676

Servizi J, Gordon RW, Carey JH (1988) Bioconcentration of chlorophenols by early life stages of Fraser River Pink and Chinok salmon. Water Poll Res Can 23: 88-99.

Stalling DL, Tindle RC, Johnson JL (1972) Clean-up of pesticide and polychlorinated biphenyl residues in fish. Extracts by gel permeation chromatography. J Assoc Off Anal Chem 55: 32-38.

Stehly GR, Hayton WL (1990) Effect of pH on the accumulation kinetics of pentachlorophenol in goldfish. Arch Environ Contam Toxicol 19: 464-470.

Steiert JG, Crawford RL (1985) Microbial degradation of chlorinated phenols. Trends Biotechnol 3: 300-305.

Tanabe S, Hidaka H, Tatsukawa R (1983) PCBs and chlorinated hydrocarbon pesticides in antarctic atmosphere and hydrosphere. Chemosphere:12 277-288.

Xie T-M (1983) Determination of trace amounts of chlorophenols and chloroguaiacols in sediment. Chemosphere 12: 1183-1191.

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