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ORGANIC HALOGEN COMPOUNDS, EOX, IN MUSSELS FROM A CLEAN LAKE AND A PULP MILL RECIPIENT

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Abstract

The extractable organic halogen (EOX) residue of duck mussels, *Anodonta anatina*, from the unpolluted Lake Höytiäinen, Finland, was found to be 690 µg/g of lipids. The residue was characterized by analyzing chlorinated phenols, hydrocarbons and fatty acids. These compound classes accounted only for 1.9% of the EOX.

Mussels from the same lake were incubated in a lake receiving pulp and paper mill effluents for 12 months to study the bioaccumulation of organochlorine compounds. The EOX of these mussels was 2045 µg/g lipids and only 1.1% of the residue was explained by the low molecular weight compounds. Thus, the majority of EOX remains undefined. However, potential differences in composition are suggested by the relative proportion of defined compounds and the differences among the measured compound classes. These halogen compounds may be of natural origin or reaction products of anthropogenic compounds with humic substances. The presence of such compounds should be taken into account when evaluating the accumulation of pulp mill effluent-related EOX in mussels.

No difference was found in the EOX between male and female mussels caged close to a pulp mill, but, on dry weight basis, mussels infected by a digenean parasite had higher EOX concentration than healthy females.

Keywords: Extractable organic halogen, EOX, chlorophenols, chlorinated hydrocarbons, chlorinated fatty acids, *Anodonta anatina*, *Pseudanodonta complanata*, pulp mill effluent, parasites

Introduction

The presence of anthropogenic organic halogen compounds in the nature is widely considered as one of the major environmental problems of our time. The effects of DDT and its metabolites on populations of predator birds is probably the best known example of the effects of organohalogen com-

pounds on wildlife.

At present, the recognized anthropogenic sources of organohalogen compounds include the manufacture and use of pesticides, solvents and polymers, combustion of waste materials and bleaching of pulp with chlorine chemicals. In Finland, pulp bleaching has been considered the most important (Jokela et al. 1992).

Pulp mill effluents contain hundreds of organic chlorine compounds and most have not yet been identified (Suntio et al. 1988, Kringstad and Lindström, 1984). Because of the large number of compounds present, a new concept, Extractable Organic Halogen (EOX), was introduced to simplify the quantitation of organochlorine residues in environmental samples. EOX is a group parameter that describes the total concentration of lipophilic halogen compounds in the sample. The use of EOX has been criticized because its relation to toxicity has not been shown. Further, there is no standard method for EOX determination, so the resultant quantitation may depend on the method chosen. However, the strength of using EOX is our current inability to determine all the organochlorine compounds as individual chemicals.

EOX is a useful descriptive parameter for the exposure of the aquatic environment to mixtures of organic halogen compounds (Carlberg et al. 1987, Martinsen et al. 1988, Pellinen et al. 1993). EOX levels of mussels increased 2-2.5 fold when the animals were held in cages in the recipient lake of a pulp and paper mill (Pellinen et al. 1993). Even mussels from an unpolluted lake used for a reference contained high levels of EOX. Therefore, a study on the origin and characteristics of the EOX was necessary to evaluate the role of anthropogenic compounds in relation to natural organochlorine compounds.

Recent work has indicated that a large portion of EOX in mussels and fish may be due to the presence of chlorinated fatty acids (Wesén et al. 1992, Wesén et al. 1994). Therefore, the mussels were analyzed for 9,10-dichlorostearic acid and 9,10,12,13-tetrachlorostearic acid. Mussels were also analyzed for chlorinated hydrocarbons and phenols to study the composition of EOX. The animals were collected from an uncontaminated lake (Lake Höytiäinen) and a part of them was incubated in a lake receiving pulp mill effluent (Lake Saimaa). The EOX concentration of two mussel species was compared and the EOX was determined at different times of the year. Parasite infection affects fat and glycogen metabolism of duck mussels (Jokela et al. 1993) and, therefore, the effect of parasites on the accumulation of chloroorganics was assessed.

Materials and methods

The study sites

Mussels (*Anodonta anatina* L. [=*A. piscinalis* Nilsson] and *Pseudanodonta complanata* Rossm.) were collected from Lake Höytiäinen, an unpolluted lake with no industrial discharges close to Joensuu in Finland (62°51'N, 29°47'E). Mussels from the same lake were incubated for 12 months in 1990-1991 in Southern Lake Saimaa (61°30'N, 28°15'E) which receives effluents from an integrated pulp and paper mill in Lappeenranta (Kaukas Inc.; for more details see Pellinen et al. 1993). Mussels for the study on the effect of parasites were collected from River Siikakoski in Central Finland (62°37'N,

26°25'E) where no industrial discharges exist. *A. anatina* from River Siikakoski are infected with *Rhipidocotyle fennica* (Jokela et al. 1993). These mussels were incubated for 9 weeks in July to September 1991 close to the pulp mill of Metsä-Serla Inc. in Äänekoski (Kuhnamonjärvi, 7 km from the mill; 62°32'N, 25°49'E). The animals were frozen (–20 °C) in polyethylene bags until analyzed.

Chemical analyses

Extractions

The thawed mussels were weighed and opened. The shell was removed and weighed. Care was taken to keep the haemolympha in the soft tissue sample, but the water within the shell was discarded. Soft tissue was weighed and, in some cases, digestive gland and gills were removed and weighed for separate analyses. The EOX method was adopted from the literature (Martinsen et al. 1988) and modified slightly. The internal standards (250 ng of 2,3,6-trichlorophenol) and 250 ng of 2,3,6-trichlorobiphenyl (PCB 30; both from Ehrenstorfer, Augsburg, Germany) were added before the extraction. The mussel tissue was homogenized by sonicating for 5 min with an ultrasonic rod (Sonic Vibra cell, 375W). Ten grams of the homogenate was extracted first with 25 ml of isopropanol (Merck, pa) for 15 min. Then, 25 ml of cyclohexane (Merck, pa) was added and the extraction was continued for 10 min in an ultrasonic bath and subsequently on a shaking board for 2 h. The extract was centrifuged (5000 g, 15 min) and the organic phase separated. The tissue homogenate was then extracted for a second time with 25 ml of cyclohexane and the extracts were combined. The combined extract was washed 3 times with acidic water (pH 2, H_2SO_4) to remove inorganic halides (Wesén, 1988) and dried with anhydrous Na_2SO_4. Then, the solvent was evaporated with a rotary evaporator at 40°C and the residual lipids weighed. In the preliminary experiments we found that all the lipids were not extracted using this method, but an additional 19% of total lipids was obtained in a subsequent extraction using a chloroform-methanol mixture (1:1).

EOX determination

EOX was determined using a Dohrmann DX-20B organic halide analyzer (Dohrmann, Santa Clara, CA) with an EOX quartz boat filled with quartz wool. The extracted lipid was dissolved in 500-1000 µl of cyclohexane and 100 µl of that was used for the EOX determination while the rest was used for the other analyses. From 2 to 6 µl of the solution was pipetted on the quartz wool. The combustion and titration of the sample are automatic processes and the instrument integrates the microcoulometric titration signal to give output as ng of halogen. The analyzer was calibrated with a 2,4,5-trichlorophenol standard solution and the results were disregarded if the recovery of halogen was less than 90%. The concentrations were corrected for recovery percentage. The EOX added to the samples as internal standards increased the EOX by up to 3% which was not subtracted from the results. Solvent blank indicated negligible background. All samples were run at least 3 times. The coefficient of variation in the final analysis was less than 3% (n= 30).

Determination of fatty acids

The extracted lipids were analyzed for fatty acids following an acid-catalyzed transesterification procedure (Wesén et al. 1992, Wesén et al. 1991). The lipid solution from EOX determination was diluted to 2 ml with cyclohexane half of which was used for the determination of fatty acids. One ml of the solution was treated with 2 ml of 1.5% H_2SO_4-methanol solution overnight at 60°C. One ml of cyclohexane and two ml of water were added to cooled samples which were shaken and centrifuged. The cyclohexane layer was collected and the aqueous phase was washed twice with cyclohexane. The combined cyclohexane solution was washed twice with water, dried with Na_2SO_4 and evaporated to 1 ml.

The fatty acid standard solution used included two chlorinated fatty acids: 9,10-dichlorostearic acid and 9,10,12,13-tetrachlorostearic acid (Helix Biotechnology, Inc., Vancouver, BC). The acids were methylated with BF_3-methanol as follows: about 10 mg of the fatty acid was dissolved in 3 ml of BF_3-methanol (14%, Sigma B-1252) in a screw cap test tube which was sealed and placed in an oven for 9 min at 60°C. After cooling, the sample was transferred with 20 ml of hexane to a separation funnel and shaken twice with 10 ml of saturated NaCl solution. The organic layer was collected, dried with Na_2SO_4, evaporated with N_2 to 1 ml and analyzed with a Hewlett-Packard 5890 GC/MSD with a NB-54 column (HNU Nordion, Finland) using splitless injection and SIM technique with electron impact ionization. The weak but detectable ~~molecular~~ 294 peaks of the two chlorinated fatty acid methyl esters were used to confirm their identification. External standard method was used for quantitation using the base peak of the spectrum (m/e=74). The detection limit of the two chlorinated fatty acids was 40 µg/g lipids and the recovery of dichlorostearic acid in a spiking experiment was 68.5%.

Determination of chlorophenols

Chlorophenols were analyzed from the other portion of the lipid extract. The cyclohexane solution of lipids (1 ml) was shaken with 4 ml of 1 M K_2CO_3 and centrifuged. The organic phase was set aside and the aqueous phase was washed twice with cyclohexane. The cyclohexane washes were combined and used later for the determination of chlorinated hydrocarbons. One ml of acetic anhydride (Merck, pa.) was added to the aqueous phase and shaken carefully. Then, the formed acetates were extracted with hexane, dried with anhydrous Na_2SO_4 and concentrated with a stream of nitrogen for GC analysis. The acetylation was carried out in test tubes so the samples could be centrifuged during the work-up because of the formation of emulsions.

The phenol acetates were analyzed with a Hewlett-Packard 5890 gas chromatograph and 7673 Autosampler using splitless injections on two parallel columns (J&W DB-1701 and DB-1) and two EC detectors. The compounds were identified and quantitated on the basis of their retention times and response factors obtained by using a calibration mixture (HNU Nordion, Helsinki, Finland): 2,4-

Dichlorophenol (24-DCP), 2,4,6-Trichlorophenol (246-TCP), 2,4,5-Trichlorophenol (245-TCP), 2,3,6-Trichlorophenol (internal standard; 236-TCP), 2,3,5,6-Tetrachlorophenol (2356-TeCP), 2,3,4,6-Tetrachlorophenol (2346-TeCP), 4,5-Dichloroguaiacol (45-DCG), 4,5-Dichlorocatechol (45-DCC), 3,4,5-Trichloroguaiacol (345-TCG), Pentachlorophenol (PCP), 3,4,5-Trichlorocatechol (345-TCC), and Tetrachloroguaiacol (TeCG). Internal standard method was used for quantitation.

A positive identification required the detection of the compound on both channels, except for pentachlorophenol and 3,4,5-trichlorocatechol which coeluted on the DB-1 column and were determined using the DB-1701 column only.

The procedure was verified by spiking chlorophenols to fish oil triglycerides (Larodan 56-1130). The recovery varied from 86.4 to 109.1 % depending on the phenol. The blank samples indicated an interference on both channels at the retention time for tetrachlorocatechol which was therefore not determined. The detection limit was 3-36 ng/g lipids for dichlorinated, 4-45 ng/g for trichlorinated, 2-6 ng/g for tetrachlorinated and 2 ng/g for pentachlorinated compounds.

Determination of chlorinated hydrocarbons

Chlorinated hydrocarbons were determined from the cyclohexane solution from phenol analysis described above. The solution was treated with concentrated sulfuric acid and centrifuged to separate the organic phase. The sulfuric acid phase was washed twice with cyclohexane, and the combined cyclohexane phases were washed three times with concentrated H_2SO_4 to remove lipids. The cyclohexane solution was evaporated to near dryness with nitrogen and dissolved in 200 µl of hexane. The chlorohydrocarbons were analyzed with the same chromatograph and columns as the chlorophenols. The method was verified with spikes (trichlorobiphenyl) which yielded a recovery of 74.7% (sd=12.4%, n=12).

The compounds analyzed were: 2,3,6-Trichlorocymene (236-TCCym ; T. Kuokkanen, Univ. of Jyväskylä), 2,3,5,6-Tetrachlorocymene (2356-TeCCym; T. Kuokkanen), Hexachlorobenzene (HCBz; J. Jokela, Univ. of Helsinki), 2,3,6-Trichlorobiphenyl (TCB; internals standard, Ehrenstorfer), γ-Hexachlorocyclohexane (Lindane; Fluka), DDE and DDT (Ehrenstorfer). The detection limits were deter-

Table 1. Comparison of EOX residue of two mussel species: *Anodonta anatina* and *Pseudanodonta complanata* (n=3 for all).

	EOX µg/g lipids	SD	EOX µg/g DW	SD	EOX µg/g WW	SD	Dry wt % (WW)	SD	Lipids % (DW)	SD
Soft tissue:										
P. complanata	538	155	49	23	1.8	0.8	3.8	0.8	9.4	4.9
A. anatina	615	75	47	12	2.2	0.4	4.9	0.5	7.5	1.8
Digestive gland:										
P. complanata	366	16	105	3	6.7	0.3	6.3	0.5	29.2	0.8
A. anatina	334	53	105	14	10.2	1.5	9.6	0.3	32.4	7.4

Underlined figures differ significantly.

mined to be 5-10 ng/g lipids for chlorinated cymenes, 7-385 ng/g for PCB congeners, and 5-10 ng/g for the other chlorohydrocarbons.

Statistics and quality control

Analysis of variance followed by Scheffe´s test at $p<0.05$ were used to compare different groups. The calculations were performed using Statview for Macintosh (version 4.0).

The quality of analyses was followed by analyzing spiked samples and reagent and process blanks. Detection limits were determined as described in the literature (3σ method; Keith 1992).

Results

There was no statistically significant difference in the EOX residues on a lipid normalized basis between *A. anatina* and *P. complanata* from Lake Höytiäinen (Table 1) although the residue of *A. anatina* was 14% higher than that of *P. complanata*. However, on wet weight basis, the digestive gland of *A.anatina* contained significantly higher EOX concentration. The two species are difficult to distinguish by an untrained person. Therefore, this finding may be of importance because *A. anatina* has been used for monitoring organic halogen compounds in Finnish water courses and confusion of the two species at collection could have led to a significant increase in variation when concentrations are compared on a wet weight basis.

The data (Table 2) suggests that there is a seasonal trend in lipid normalized EOX concentrations that can be described by EOX (µg/g lipid)=111+1.31*(Julian Day). The correlation coefficient was low ($r2=0.36$, $n=15$), possibly because of measurement errors and the small sample size. Differences among sampling dates were significant on both a wet and dry weight basis. However, the significance was removed with lipid normalization.

The mussels of Lake Höytiäinen have relatively few parasites, but those of River Siikakoski in Central Finland are often infected by sporocysts of *Rhipidocotyle fennica*. The mussels from River Siikakoski were incubated in Lake Kuhnamonjärvi which receives effluents from the pulp mill of Metsä-Serla Inc. in Äänekoski to find out if the infection or sex affect the bioaccumulation of chlorine compounds.

Table 2. EOX, dry weight and lipid weight of *A. anatina* at different times of the season (n=3 for all samples).

	Julian Day	EOX µg/g lipids	SD	EOX µg/g DW	SD	EOX µg/g WW	SD	Dry wt % (WW)	SD	Lipids % (DW)	SD
Date:											
Apr 10	100	233	87	16.8	6.1	1.0	0.4	6.2	1.0	6.9	0.6
May 26	146	345	28	32.5	2.9	1.9	0.2	5.9	0.5	9.5	0.9
July 7	188	324	56	17.6	10.9	1.0	0.6	6.0	0.5	7.5	2.5
Aug 8	220	413	130	35.8	6.3	2.5	0.3	7.1	0.5	9.0	1.8
Oct 1	283	529	291	34.6	13.8	1.4	0.3	4.2	0.9	6.9	0.9

Infected mussels had a significantly higher EOX content on dry weight basis than females which may be related to lower dry weight percentage of the infested mussels (Table 3). Sex or parasites did not have any significant effect on the EOX residue on lipid basis.

Only a few chlorine compounds were detected in mussel lipids. The only chlorinated hydrocarbons found in mussels from Lake Höytiäinen were DDE and DDT (Table 4). No pattern indicating the presence of PCB's (e.g. Arochlor 1242) was found although these compounds are very wide-spread. Considerably higher chlorohydrocarbon residues were found in mussels incubated in Lake Saimaa. The concentration of the effluent-related 2,3,6-trichlorocymene was particularly high. The concentration of dichlorostearic acid accounted for the greatest portion of the identified chlorinated compounds in both areas. Tetrachlorostearic acid was not detected in any of the samples. Overall, the chemical analyses explained up to 1.9% of the total halogen containing compounds (Table 5).

A preliminary experiment was made to characterize the EOX in mussels indicated that 85% of EOX was in the neutral fraction, less than 15% in the phenol fraction and only less than 0.3% in the free acid fraction. These results also differ from those for *Mytilus edulis* where the neutral fraction contained only 22% of EOCl (Extractable Organic Chlorine; usually most of EOX is EOCl; Wesén et al. 1994). The reason for the differences may be related to different mussel species or water (brackish water vs. fresh water) or to the fact mentioned by Wesén et al. (1994) that mussel triglycerids hydrolyze upon storing to free fatty acids.

Discussion

Mussels are commonly used for environmental monitoring of contaminants in Finland (Herve et al. 1988). However, high variation in results due to variation in species composition and physiological condition, e.g. lipid and dry weight, can complicate interpretation of the data. Simple misidentification of species, *P. complanata* versus *A. anatina*, may result in variation in concentration of EOX for organisms collected at the same time (Table 1). In addition to variation resulting from species identification, collection of samples at different seasons is also a component of variation. Many organisms show seasonal changes in condition, e.g. reproductive state and accumulation of lipids for over wintering. These changes in condition can lead to substantial changes in the accumulation of contaminants (Table 2). Unlike the variation

Table 3. EOX residues of mussels incubated close to a pulp mill in Äänekoski, Central Finland. Mussels infected by parasites, females and males were analyzed separately.

	EOX µg/g lipids	SD	EOX µg/g DW	SD	EOX µg/g WW	SD	Dry wt % (WW)	SD	Lipids % (DW)	SD
Group (n):										
Infected (10)	2410	550	155	49	12	4	7.6	0.2	6.5	0.9
Females (8)	1940	240	109	12	10	1	8.8	0.4	5.7	0.2
Males (10)	2250	300	142	19	12	1	8.3	0.2	6.4	0.7

Table 4. Chlorohydrocarbons, chlorophenols and chlorinated fatty acids in mussel lipids from a clean lake (Lake Höytiäinen) and from a lake receiving pulp mill effluents (Lake Saimaa).

	Lake Höytiäinen ng/g lipids	SD	n	Lake Saimaa ng/g lipids	SD *	n
Chlorohydrocarbons:						
TriCCym	nd	-	-	3790	220	2
TeCCym	nd	-	-	32	-	1
g-HCH	nd	-	-	20	-	1
HCBz	nd	-	-	81	43	2
DDE	150	72	8	751	54	2
DDT	670	270	8	1820	624	2
Total	820			6490		
Chlorophenols:						
245-TCP	360	360	7	#		
246-TCP	380	56	2	2180	-	2
2346-TeCP	nd			330	-	2
45-DCC	nd			190	-	2
45-DCG	460	350	4	nd		
345-TCG	nd			2380	-	2
TeCG	80	130	4	210	-	2
Total	1280			5290		
Dichlorostearic acid	62000	78000	9	90000	81000	7

n is the number of samples that contained the corresponding chemical. * SD calculated from the average of two CG channels. # Not determined, nd not detected.

in species, these seasonal changes are not removed by lipid normalization. In fact, there appears to be a seasonal trend in lipid normalized EOX concentrations (Table 2). This trend in lipid normalized concentration over time likely indicates a change in lipid composition and capacity for EOX. Variation in lipid composition is well recognized as a factor that can contribute to changes in lipid normalized concentrations (Gardner et al. 1990). However, lipid composition was not measured in this study. Therefore additional studies are necessary to demonstrate whether lipid composition or other mechanisms are responsible for the observed trend.

In a recent study (Wesén et al. 1994), the highest EOX concentration (790 µg/g lipids) was observed for blue mussels (*Mytilus edulis*) in the Finnish archipelago. The authors suggested that the EOX concentration was related to the wide-spread discharges of chloroorganic compounds by pulp mills. However, the highest values found in the blue mussel are comparable to those found in mussels from our reference lake (Table 1). According to the literature (Wesén 1988), interference by chloride in the EOX determination cannot be excluded in the case of mussels which have a low lipid content.

The mussels caged in the area receiving pulp mill effluents contained significantly higher EOX residues that the reference mussels. Thus, pulp mills contribute to the residues in our monitoring organisms. Freshwater mussels, *Dreissena polymorpha*, which were infected with the digenean para-

Table 5. The proportion of EOX accounted for by the determined low molecular mass compounds?

	EOX µg/g lipid	Cl-hydrocarbons %	Cl-phenols %	Cl-fatty acids %	Total %
L. Höytiäinen	690±38 n=9	0.05	0.09	1.80	1.94
L. Saimaa	2045±560 n=5	0.10	0.12	0.83	1.05

site, *Phyllodistomum* sp., contained higher heavy metal concentrations than healthy individuals (Kraak et al. 1990). Bucephalid digeneans are common parasites of duck mussels in Finland (Haukioja and Hakala 1978, Taskinen et al. 1991, Jokela et al. 1993). Since *R. fennica* has been found to affect fat and glycogen metabolism of *A. anatina* (Jokela et al. 1993), infection might affect also the accumulation of chlorinated organic compounds. Sporocysts of *R. fennica* reside mainly in the gonads of the mussel where they can cause castration of the host if present in large numbers (Taskinen et al. 1991). Infected mussels are found throughout the year (Taskinen et al. 1994) and they probably carry the parasites for the rest of their life. The difference between EOX concentration of infected and healthy mussels was, however, small, except for dry weight based EOX between infected and female mussels (Table 3). This may be related also to the lower dry weight of infected mussels compared to healthy mussels.

Infected mussels were treated as one group since the sex can not be determined reliably. Also the degree of infection varied within this group. In further studies, mussels with a low, moderate or high intensity of infection should be studied separately. Yet, the results suggest that parasitisation of mussels by digeneans may increase the bioaccumulation of chlorine compounds in the host. At least to some degree the accumulation rate is a function of the filtration rate of a mussel. On average, 9,500 cercaria larvae of *R. fennica* escape from an individual *A. anatina* per day (Taskinen et al., 1991). It can be expected that the mussels respond to the parasitism by increased filtration activity to compensate the parasite-induced loss of energy and material. This, on the other hand, could result in a higher consentration of EOX in the infected specimens if the accumulation is greater in the mussel tissues than in the parasite tissues or cercarial larvae.

The questions of interest are what is the source and composition of the EOX and what effect does EOX have on the environment? Because substantial contributions of EOX were found in our unpolluted reference lake, Lake Höytiäinen, where no point sources are known, the only other sources are atmospheric transport of anthropogenic halogen compounds or the presence of natural halogen compounds in the lake or its drainage area (Asplund et al. 1989). The anthropogenic source may contribute to EOX directly or be a reaction product formed from a halogenated compound and humic substances (De Jong et al. 1994; Lassen et al. 1994).

Our attempts to determine the composition of the EOX left the bulk of the material unidenti-

fied, so no conclusions about the source can be made. The contribution by pulp mill effluents on the residues of identified compounds was marked, but the portion of EOX accounted for by these compounds was less than that in the reference lake (Tables 4 and 5). The total concentration of chlorophenols in the reference mussels from Lake Höytiäinen was close to that reported in the literature (Herve et al. 1988) for reference mussels incubated upstream the Äänekoski pulp mill. Wesén et al. (1994) reported that from 3 to 17% of the EOX in *M. edulis* from the Baltic Sea was made up by chlorinated fatty acids. The larger contribution of chlorinated fatty acids in EOX in their study is at least partly due to the fact that their analyses included also dichloromyristic and dichloropalmitic acids.

The hypothesis of atmospheric transport is supported by the observation that AOX (Adsorbable Organic Halogen) concentration of rain water in Finland was 7 µg/l corresponding to an annual precipitation of 4.8 mg/m^2 (Jokela et al. 1992). Thus, the main source of EOX could well be atmospheric deposition coupled with water shed runoff of anthropogenic material as the AOX concentration of lake water was 11 µg/l.

However, the concentrations of AOX are much higher in many non-anthropogenically contaminated lakes in Sweden (up to 160 µg/l; Asplund et al. 1989) and in Finland (up to 30 µg/l; Jokela et al. 1992). Also the concentrations of organic halogen in soil, peat and humic substances are as great as 1400 µg/g dw (Asplund et al. 1989). These data suggest an additional source for EOX beyond atmospheric deposition. Many microorganisms, notably fungi and marine algae, are known to synthesize halogenated compounds (Asplund et al. 1991, Gribble 1992, Gribble 1994, De Jong et al. 1994). These reactions have been proposed to occur via fungal chloroperoxidase to produce chlorinated humic substances (Asplund et al. 1991, Carlsen and Lassen 1992, Lassen et al. 1994). How important such reactions are to the contribution of EOX in the environment remains to be determined.

In conclusion, normalization of EOX concentration to lipid content reduces most of the variance associated with organism condition, e.g. lipid content, species, sex, disease state, for Finnish fresh water mussel populations. However, it is important to know that the EOX residue varies during the growing season as the lipid normalization does not remove that variation.

The composition of the EOX in mussels remains largely undefined with chlorinated fatty acids contributing the greatest concentration of the known compound classes. While it is clear that the pulp mill discharges contain higher concentrations of EOX than reference sites, a combination of atmospheric deposition and biogenic synthesis may well be an additional source.

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