UTILITY OF MUSCLE AND LIVER SAMPLES OF PERCH (*PERCA FLUVIATILIS*) AS AN INDICATOR OF ORGANOTIN CONTAMINATION IN FRESH AND BRACKISH WATERS

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Introduction

Trisubstituted organotin compounds (OTCs), tributyltin (TBT), and triphenyltin (TPT) have been used in antifouling paints of boats and ships due to their strong biocidal activity. TBT and TPT are highly toxic to many aquatic species, and the most sensitive effect, imposex, occurs in some molluscs already at 1 ng/l levels of TBT [1, 2]. Especially TPT has also the potential for trophic magnification [3]. For humans, fish is the main source of OTCs [4] and a scientific panel of the European Food Safety Authority (EFSA) has set a Tolerable Daily Intake of 250 ng/kg body weight for the sum of TBT, dibutyltin (DBT), TPT, and dioctyltin (DOT) based on the immunotoxicity of OTCs [5]. Due to these detrimental effects, the use of organotin compounds in antifouling paints has been banned in EU since the beginning of 2008 and levels are expected to decrease in the future [6].

According to EU Directive 2008/105/EC Member States shall arrange the long-term trend analysis of concentrations of those priority substances, including OTCs, that tend to accumulate in sediment and/or biota [7]. In this context, Common Implementation Strategy for the Water Framework Directive (WFD) states that biota is the preferred matrix for OTC monitoring. Perch (*Perca fluviatilis)* is a fairly stationary fish species up to a size of approximately 20 cm and it accumulates environmental contaminants in a given area and is thus a good marker for environmental quality. As it has European wide occurrence in fresh waters, it is the recommended species for monitoring in lakes and can also be used in the Baltic Sea [8].

The choice of appropriate tissues for monitoring is critical. Muscle tissues provide sufficient sample amounts for analyses and it reflects the portion consumed by humans. However, accumulation in muscle can be a long-term process and it does not necessarily reveal recent temporal variations and total direct bioaccumulation. Liver is an important target organ for many chemicals reflecting their current bioaccumulation, but for some species it may not provide sufficient amount of tissue for analysis unless pooled samples can be prepared [8]. Muscle would be a more convenient tissue especially if it could be used at the same time for both ecosystem and human exposure monitoring. In an ideal monitoring species, the proportion of TBT and TPT in muscle and liver should be high compared to their degradation products, reflecting the high bioaccumulation potential and limited ability of the species to eliminate OTCs through degradation. Also the liver/muscle ratio of TBT and TPT should be relatively invariable across individuals and sampling locations so that the total bioaccumulation could be well estimated from muscle concentrations.

The aim of this study was to compare OTC concentrations and profiles in muscle and liver of perch and selected other fish species to evaluate whether perch muscle is suitable for ecosystem monitoring and also to get a local upperbound estimate of human exposure to OTCs from any fish. To do this, we utilized fish samples from different areas of the Finnish coast of the Baltic Sea and selected lake areas. These fish were part of a larger survey that studied levels of POPs in the Finnish coast of the Baltic Sea, Finnish lakes and rivers.

Materials and methods

Fish species, sampling sites, collection and preparation of fish samples

Fish were collected from three Baltic Sea areas: 1) The Bothnian Bay, 2) Archipelago Sea and 3) Eastern Gulf of Finland (Figure 1). Species collected were perch, salmon (*Salmo salar*), pike-perch (*Sander lucioperca*) and burbot (*Lota lota*). The selection of perch was based on the above recommendation, and the other species were selected as they are popular catch species of both recreational and professional fishing. From Lake Päijänne and

Lake Saimaa, only perch was collected. Two fish pools of different length range containing five individual fish/pool were collected for each species from each sampling site. Before homogenization and pooling, fish samples were halved along the dorsal fin and edible parts were removed. The muscle of all fish and the liver of salmon and burbot were pooled according to the mass equal to weighted mass of the smallest sample. The whole liver tissue of each individual perch and pike-perch were pooled due to their small size. Muscle and liver material were homogenized and freeze-dried before analysis.

Chemical analysis of fish samples and quality control

The compounds analysed were monobutyltin (MBT), DBT, TBT, monophenyltin (MPT), diphenyltin (DPT), and TPT. All weights and concentrations of OTCs are expressed as organotin cations. Perdeuterated analogues of MBT, DBT, TBT, MPT, DPT, and TPT were used as internal standards for the respective native 1H-compounds. The pretreatment of freeze dried fish samples (0.25 g) was performed by solvent extraction, ethylation with sodium tetraethylborate and GC-MS analysis as described previously [9]. Limits of quantification (LOQ) varied from 0.1 to 0.5 ng/g fw depending on the compound.

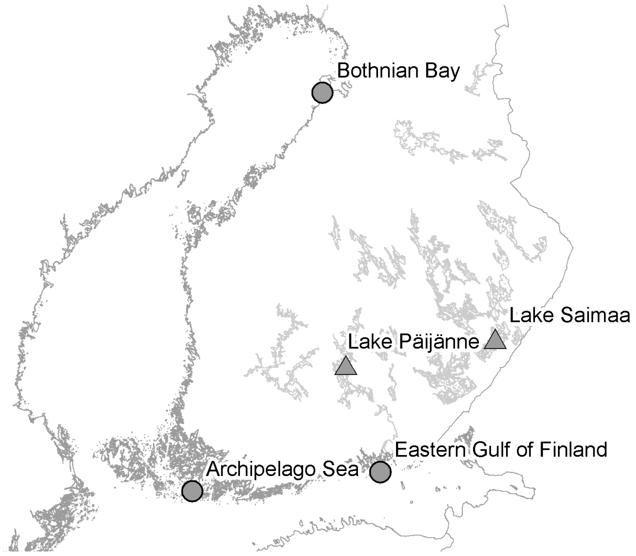

Figure 1. Baltic Sea and lake sampling sites.

In each series of samples, one laboratory reagent blank sample was treated and analysed with the same method as the actual samples as well as certified mussel tissue CRM 477 that has certified concentrations for MBT, DBT, and TBT, and indicative concentrations for MPT, DPT, and TPT [10]. Method was accredited.

Results and discussion

MBT and MPT were detected only in a limited number of samples and results of those compounds are not discussed in this paper. In perch and pike-perch, concentrations of phenyltins in muscle and liver were quite similar at each Baltic Sea site, but pike-perch tends to accumulate more butyltins than perch as shown in Figure 2. Bars represent average concentrations of two pools of different length range.

Concentrations of OTCs in muscle and liver of burbot were lower than in perch and pike-perch (Figure 2). Profiles of butyltins in burbot were not consistent in the eastern Gulf of Finland as compared to Bothnian Bay and Archipelago Sea. In salmon, concentrations were very low in all samples. However, in contrast to burbot, profiles of different OTCs in salmon muscle and liver at different sampling sites were highly consistent.

In pike-perch, the liver/muscle concentration ratios of TBT and TPT were slightly more variable than respective ratios in perch (Table). In burbot, liver/muscle concentration ratio of TBT was even more variable and as compared to other species, very strong accumulation of TPT to large liver of burbot as compared to muscle was evident. In salmon, liver/muscle ratios for both TBT and TPT were highly stable across sampling sites. In addition, also the liver/muscle ratios of DBT (19.2-23.0), and DPT (3.9-4.7) were very stable in salmon (data not shown).

Concentrations of butyltins in perch, the only fish collected from lakes, were very low (mainly < LOQ). TPT was the only OTC detected in every muscle and liver sample (data not shown). However, the liver/muscle ratio of TPT in lakes was very similar to those in the Baltic Sea (3.7 in both lakes) even though the TPT concentration in liver in Lake Saimaa was as low as 0.77 ng/g fw, i.e. only 1.2% of that in eastern Gulf of Finland.

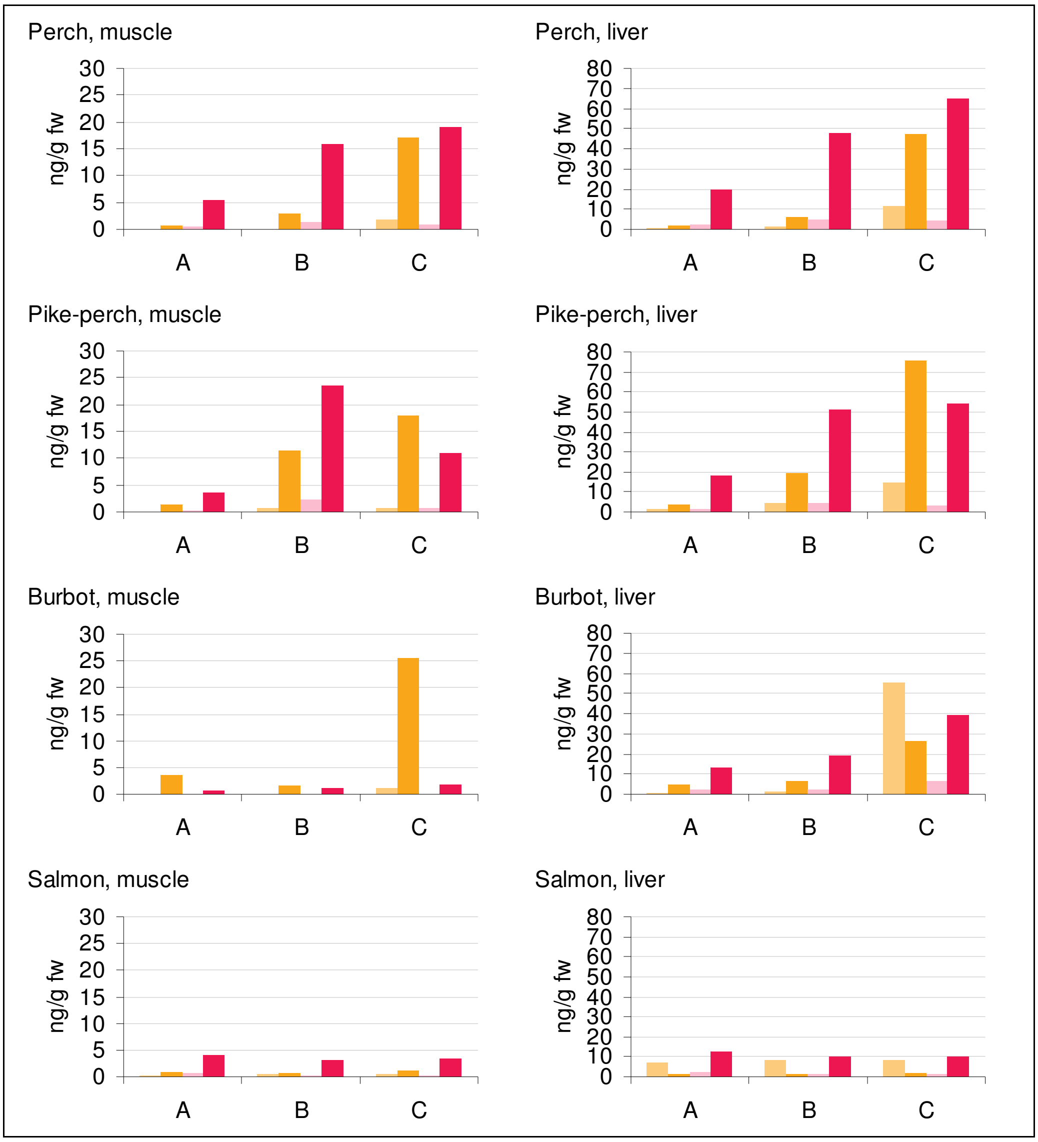

Figure 2. Concentrations of OTCs in the liver and muscle of perch, pike-perch, burbot, and salmon. A: Bothnian Bay; B: Archipelago Sea; C: Eastern Gulf of Finland. DBT TBT DPT TPT

Table. Range of liver/muscle ratio of TBT and TPT (a), and range compound ratios in liver and muscle (b) in different fish species from the three Baltic Sea sampling sites.

	a		b			
	Liver/muscle	Liver/muscle	Liver		Muscle	
Species	ratio of TBT	ratio of TPT	TBT/DBT	TPT/DPT	TBT/DBT	TPT/DPT
Perch	1.9-2.8	3.0-3.6	3.6-5.4	8.9-16.3	9.0	11.1-22.5
Pike-perch	1.7-4.2	2.2-4.9	3.9-5.2	12.6-16.9	18.9-27.7	10.4-16.3
Burbot	1.0-4.3	18.9-21.3	0.5-6.2	4.9-8.8	-	-
Salmon	1.4-1.6	3.0-3.4	0.13-0.19	4.8-7.8	1.6-2.6	6.1-10.8

Perch TBT/DBT ratios in liver were very similar to respective ratios in pike-perch and also perch TPT/DPT ratios in liver and muscle were similar to respective ratios in pike-perch (Table). Comparison of TBT/DBT ratios in perch and pike-perch muscle was difficult due to large proportion of samples <LOQ for DBT in perch, but it is likely that TBT/DBT ratio in perch muscle was lower than in pike-perch.

In muscle tissue of burbot, TBT/DBT and TPT/DPT ratio comparisons cannot be made because concentrations of disubstituted tins were less that the LOQ. For liver tissue, TBT/DBT concentration ratios were highly variable, but TPT/DPT ratios were more stable. In eastern Gulf of Finland, there may be some site specific special feature that results in the high relative proportion of DBT as compared to TBT in burbot liver sample. However, TBT/DBT profiles in Bothnian Bay and Archipelago Sea are comparable for burbot. An interesting feature with the salmon is the exceptionally low liver TBT/DBT ratio at all sites that demonstrate its capability to metabolize TBT to DBT. TBT/DBT ratios in muscle were also low reflecting the effective total clearance of TBT from salmon. The elimination of TPT from salmon was not as efficient as that of TBT, because TPT/DPT ratios in liver and muscle were more like in other species.

Of the studied fish species, pike-perch was the most effective to accumulate OTCs, and perch almost as good. Burbot and salmon, possibly due to their effective metabolism of OTCs, and for salmon also because of migrating behavior, were much less effective. Tissue and compound ratios show that in perch and pike-perch TBT and TPT are not strongly concentrated to liver as compared to muscle. Perch and pike-perch are also similar in the sense that TBT and TPT are not strongly degraded to their metabolites in either tissue.

In conclusion, pooled muscle samples of both perch and pike-perch are well suited for local monitoring of OTC contamination, and to get a local upperbound estimate of human OTC exposure from fish in lakes and in the Baltic Sea area.

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