Screening of bisphenol A in fish from Swedish waters

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REPORT

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Summary

Bisphenol A (BPA) is a high volume chemical with potential to interact with hormone systems in humans and wildlife. Previous studies in both Sweden and elsewhere have demonstrated the presence of BPA in freshwaters as well as in the marine environment, mostly in surface waters and sediments. In order to strengthen the knowledge on the environmental occurrence of BPA in Sweden, a study was undertaken where BPA was analysed in fish from background lakes, urban lakes, coastal sites and the marine environment. In total 23 samples were analysed.

Bisphenol A was detected in most samples in the range of <0.24 to 4.7 ng/g ww in muscle. Liver from cod caught outside Gotland displayed concentrations in the range <0.24-1.77 ng/g ww. There were no pronounced differences in concentrations between different species or when urban sites were compared to background sites. With respect to possible risks for toxicological effects on fish or their consumers (e.g. humans), the concentrations are regarded as low.

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1. Introduction

Bisphenol A (BPA, CAS-no 80-05-7) is a large volume chemical that is mainly used for synthesis of epoxy resins and other polymers, but also as an stabilising additive in various plastic products. The environmental occurrence of BPA is of possible concern because the substance has oestrogenic properties, and may affect biota as well as humans if exposure is sufficiently high.

A Swedish screening study of BPA was performed in 2003-2004 (WSP, 2004). This screening showed that BPA was detectable in a number of matrices. For instance, BPA was ubiquitously occurring in sludge from municipal sewage treatment plants, with levels generally less than 1 mg/kg dw. It was occasionally found in surface sediments. Perch from 49 sites were analysed but only four samples contained detectable levels (i.e. > 20 ng/g ww). Particulate BPA was also detected in air at the ng/m^3 level at several urban sites.

Studies in other countries have commonly found BPA in surface waters (e.g., Kolpin et al., 2002; Belfroid et al., 2002). Very few data exists on the occurrence of BPA in biota, but levels around 1 ng/g ww in fish have been documented from Norway and the Netherlands (Fjeld et al., 2004; Belfroid et al., 2002).

As cited above, the limited environmental data available suggests a moderate diffuse release as well as a few point source releases. ECB (2003) state that bisphenol A is rapidly degraded by photolysis why long-range atmospheric transport should be insignificant. However, low levels of BPA have been measured at background sites (Berkner et al., 2004). A certain atmospheric transport of BPA can thus be expected but whether this transport is sufficiently extensive to affect background areas is not known.

BPA has a low to moderate tendency for bioaccumulation in fish, with BCF values in the range 5-200 (see WSP 2004). In fish BPA is conjugated mainly with glucorinide, and the conjugates are excreted rapidly. Nevertheless, BPA has been detected in field collected fish (Belfroid et al., 2002; WSP, 2004; NILU, 2004). This suggests that monitoring BPA in fish may be used to improve our knowledge on the environmental occurrence and release patterns of BPA.

The purpose of this study is to extend our knowledge on the bioaccumulation and occurrence of BPA in field-collected fish. The specific goals are:

1. To study whether bisphenol A occurs at detectable levels in wild fish
2. To investigate whether the levels are correlated to the fat content of various individuals and species
3. To determine if levels are elevated at urban sites
4. To determine the distribution between liver and muscle (in cod).

The study is performed as a screening study, i.e. the number of samples is fairly low. Therefore, the conclusions should be regarded as indicative.

2. Analysis of BPA in biological samples

10 g of biological tissue homogenises with sodium sulphate. Internal standard $^{13}C_{12}$-BPA is added and the sample is ultrasound extracted with MTBE. The supernatant solution is decanted and remaining particles are removed by centrifugation. BPA is liquid-liquid extracted with 1 M NaOH-solution. This aqueous BPA-solution is acidified to pH 3 with 1M HCl solution and applied to a SPE-cartridge (HLB plus adsorbent). After rinsing the HLB cartridge 3 times with water the sample is eluted with acetone/methanol (5/1). After a final evaporation step the sample is analysed by LC/MS-TOF in negative ESI mode with 50 mDa mass resolution. Conjugated BPA species are not detected by the analytical method.

A microbial metabolite of BPA is p-OH-benzoic acid (Suzuki et al., 2004) with a weak estrogenic acitivity. The intention was to also analyse the fish samples for this metabolite. However, there were strongly interferring signals on the chromatogram why the peak could not be resolved.

3. Samples

Two sets of samples were analysed: 1) background sites; 2) an urban gradient. The samples from the background sites were supplied from the Environmental Specimen Bank at the Swedish Museum of Natural History. These samples were sampled in the framework of the national environmental monitoring programme. The urban gradient samples were supplied from ITM at the Stockholm University. The latter samples were collected in 2000/2001 as part of a larger study on toxicological effects on fish in the urban environment (e.g. Hanson et al., 2006).

An overview of the sampling strategy is listed in Table 1 and all sample details are displayed in the appendix. The background sites cover both freshwaters and marine/brackish environment. In addition lakes that are influenced by urban activities (Stockholm) and industrial/urban (Vättern) are included.

The samples of cod liver and muscle represent individual specimen, whereas all other samples are pooled from (generally) five individuals.

Table 1. Sample overview. The numbers refers to the number of sites.

Character	Herring	Perch	Salmon	Cod
Marine / brackish background	2			1[A]
Brackish coast		2		
Background lakes		4		
Urban background lakes		1		
Urban lake		2		
Lake Vänern (large lake with some industrial impact)		1	1	

A. At this site, liver and muscle were analysed from five individuals.

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4. Results and discussion

4.1. Levels

Bisphenol A was detected in most of the samples and all results are displayed in appendix 1. The levels in muscle were ranging from <0.24 to 4.7 ng/g ww. The concentrations in muscle are shown in Figure 1, where data is grouped according to site category. The dataset contains four different fish species, partly with different trophic position. However, BPA is not suspected to biomagnify in the food-chain and it is therefore reasonable to compare data across species.

In the Baltic Sea, one pooled sample of herring and five individual samples of cod range from 1.4 to 4.7 ng/g ww, whereas the herring from the North Sea (Väderöarna) is lower in BPA. Most other pollutants are also lower in herring from the North Sea as compared to the Baltic Sea (e.g. Bignert et al., 2004). Perch from the two coastal sites show similar levels as in the open Baltic.

Perch in the background lakes show similar levels as fish from the Baltic Sea. The samples in Stockholm were slightly elevated compared to the urban background site. However, fish from the urban sites in Stockholm and from Lake Vättern (which is influenced by both urban areas and industrial activities) actually displays slightly lower concentrations than in the background lakes. This was somewhat unexpected; urban sites were expected to display at least as high concentrations as the background sites. All these samples represent adult perch, why different trophic positions leave no explanation. Whether this concentration pattern actually reflects the ambient concentrations in water, or whether the urban fish have a different metabolic capacity for BPA cannot be determined.

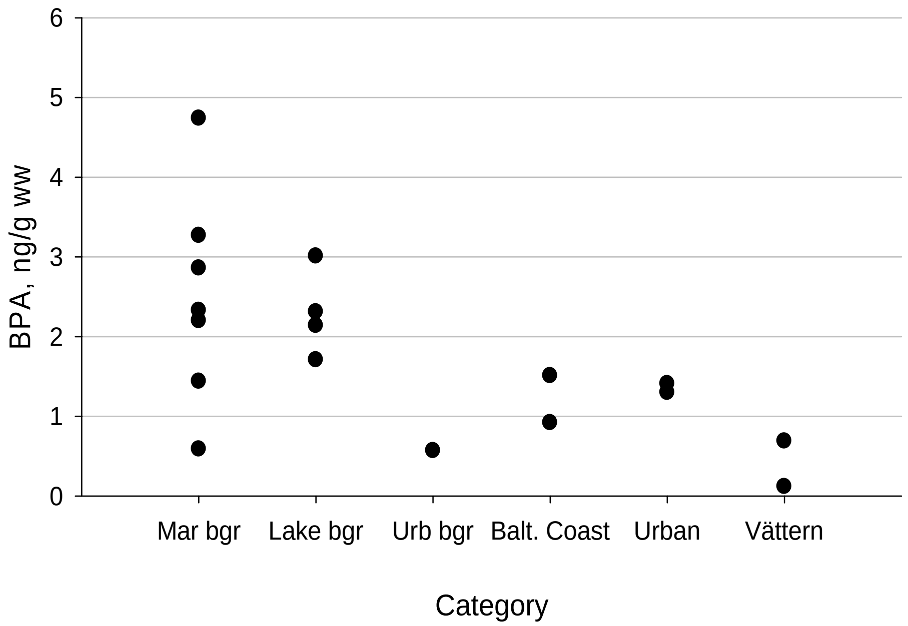

Figure 1. Concentrations of bisphenol A in muscle, in fish from different site categories. Mar: marine; bgr: background; Urb: urban.

4.2. Comparison of tissues

Five individual specimen of cod from outside Gotland were analysed for BPA in liver and muscle. One liver sample displays an anomalously high level (39 ng/g ww) whereas the remaining four livers contained less BPA (<0.24-1.77 ng/g ww) than the corresponding muscle samples. In contrast, experimental studies on BPA kinetics in rainbow trout found higher levels in liver than in muscle (Bjerregaard et al., 2007). Similar to our results, Belfroid et al. (2002) also found generally lower concentrations in liver than in muscle but with one liver sample showing very high levels. The authors speculate that the liver/muscle concentration ratio may reflect time since exposure (Belfroid et al., 2002). Such a hypothesis would also explain the higher liver/muscle concentration ratio in laboratory exposed fish compared to field collected fish.

4.3. Relation to lipid content

Based on the assumption that lipophilic contaminants are stored in lipids, data are commonly reported on a lipid weight basis in order to reduce variability. The validity of this assumption for BPA can be checked on the cod data, since these results represent specimen taken at one site and one time. External factors should thus have a low influence on the levels. The levels of BPA do not correlate with the lipid content, and the variability actually increases upon lipid normalisation.

Furthermore, both the lean perch and the fat salmon were studied in lake Vättern (samples 22 & 23). Levels were higher in the less fat perch than in the salmon.

In support of these observations, BPA has two phenolic groups and more specific binding than only passive partitioning to lipids can be expected in the cells. Therefore, it was suggested not to present and discuss data on a lipid weight basis.

4.4. Comparison to other studies

Only a few studies have previously reported data on bisphenol A in fish. Freshwater fish from southern Norway contained 1-14 ng/g ww, whereas cod liver displayed highly varying levels ranging between <1.7-62 ng/g ww (Fjeld et al., 2004). In the Netherlands, a few samples of fish from both lakes and the marine coast displayed concentrations of 0.24-2.6 ng/g ww in muscle, and 1.5-26 ng/g ww in liver (Belfroid et al., 2002). In the preceding Swedish screening, most fish samples did not contain detectable levels. However, the detection limit in that study was 20 ng/g ww (WSP, 2004).

In summary, earlier studies have detected BPA in both freshwater and marine coastal fish, with levels mostly between 0.5 and 10 ng/g ww.

4.5. Bisphenol A compared to other pollutants

The perch from Stockholm have previously been analysed for organotin compounds (WSP, 2006), DDTs and PCBs (Hansson et al., 2006; Linderoth et al., 2006). Tributyltin and triphenyltin concentrations were ca 70 and 120 ng/g ww, respectively, whereas ΣDDTs ca 20 ng/g ww and ΣPCBs ca 100 ng/g ww. Thus, BPA in urban perch was about two orders of magnitude less abundant than either ΣPCBs and TBT+TPT.

At background sites, however, BPA occurs at similar levels as sum-PCB (e.g., Bignert et al., 2004; Bignert, 2005) when compared on a fresh weight basis.

In agreement, the Norwegian study (Fjeld et al., 2004) included data on many other pollutants and BPA was among the more abundant, occurring at similar levels as e.g. PBDE.

4.6. Local or regional impact

Bisphenol A was detected at all background sites, in remote lakes as well as in the open Baltic Sea. A previous study concluded that fish in Swedish background sites mainly contains persistent and slowly metabolising chemicals, whereas most non-halogenated compounds were undetectable (Sternbeck et al., 2004).

Considering the wide range in general anthropogenic impact between the sites, the concentration range must be considered very moderate. Furthermore, there is a tendency to higher concentrations at background sites. This may possibly be interpreted as a result of a large scale atmospheric transport. Theoretical assessments conclude that BPA has low potential for large-scale atmospheric transport (Cousins et al., 2002; ECB, 2003), but concentrations in the ng/m^3 range have actually been measured in remote areas (Berkner et al., 2004). Another study also detected BPA in fish from certain marine sites in the Netherlands, although it was not present in surface waters (Belfroid et al., 2002).

An alternative explanation for the presence in background areas would be that BPA is formed in the environment from precursors that are more prone to large-scale transport. BPA can be produced by microbial transformation of tetrabromobisphenol A, a common flame retardant (Voordeckers et al., 2002). However, the low levels of TBBPA in the environment (e.g. Asplund et al., 2003) makes this hypothesis unlikely.

With regard to a possible urban influence, sample 1 is a local background to the urban samples 3 and 4. Although levels in the urban samples are 2-3 times enriched compared to this reference site, other background lakes (samples 16-19) show even higher levels. Therefore, these results do not show any evidence for a significant urban impact.

In summary, these data do not show evidence of any pronounced spatial patterns.

4.7. Significance for health and the environment

The possible risks to effects on health or the environment due to the occurrence of BPA in fish is briefly discussed below. The data presented in this study only represents 23 samples, and the conclusions regarding risks should be interpreted with this in mind.

Regarding health risks, the European Food Safety Authority derived a TDI of 50 µg /(kg bw)/d (EFSA, 2007). Assuming that the concentrations measured in fish muscle are representative for the fish that is consumed by humans, tens of kg fish per kg bw can be eaten daily. Clearly, BPA in fish poses a very low risk to humans. This agrees with the conclusion in the European risk assessment of BPA (ECB, 2003).

BPA is a chemical that is rapidly metabolised in fish. The levels in muscle or liver do therefore not adequately reflect the dose, which is a better measure of exposure and thus of risk. The available standards for ecotoxicity are expressed either as water concentrations or as dose. We can, however, conclude that the levels measured in muscle are about two orders of magnitude lower than those associated with vitellogenin production in rainbow trout (Bjerregaard et al., 2007).

5. Conclusions

Bisphenol A is present in fish muscle at roughly equal levels in lacustrine and marine background sites as well as in fish caught in urban areas. This pattern does not agree with the expected exposure from water. The levels do not suggest that BPA in fish poses any risks to the environment or to human health. Previous studies have showed low bioaccumulation of BPA in fish. Taken together, these results indicate that biomonitoring may not be the most efficient means to obtain knowledge on the environmental occurrence and trends of bisphenol A.

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Appendix 1. List of samples and concentrations.

Sample	Site	Site character	Species and tissue	Age of specimen	Sampling date	ng/g fw BPA	lipid %
1	Adelsö, lake Mälaren	Urban background	Perch muscle	adult	2000/2001	0.57	1.5
2	Kummelsnäs	Baltic coast	Perch muscle	adult	2000/2001	1.51	1.23
3	Slussen, Stockholm	Urban	Perch muscle	adult	2000/2001	1.30	1.1
4	Riddarfjärden, Stock-holm	Urban	Perch muscle	adult	2000/2001	1.41	1.15
5	Kvädöfjärden	Baltic coast	Perch muscle	4 yrs	2005-11-24	0.92	0.13
6	Baltic Sea, Gotland	Marine background	Cod muscle	3-4 yrs	2005-09-26	2.33	0.28
7	Baltic Sea, Gotland	Marine background	Cod muscle	3-4 yrs	2005-09-26	4.74	0.19
8	Baltic Sea, Gotland	Marine background	Cod muscle	3-4 yrs	2005-09-26	2.20	0.11
9	Baltic Sea, Gotland	Marine background	Cod muscle	3-4 yrs	2005-09-26	2.86	0.26
10	Baltic Sea, Gotland	Marine background	Cod muscle	3-4 yrs	2005-09-26	1.44	0.29
11	Baltic Sea, Gotland	Marine background	Cod liver	3-4 yrs	2005-09-26	<0.24	54.6
12	Baltic Sea, Gotland	Marine background	Cod liver	3-4 yrs	2005-09-26	1.77	55.8
13	Baltic Sea, Gotland	Marine background	Cod liver	3-4 yrs	2005-09-26	<0.24	48.2
14	Baltic Sea, Gotland	Marine background	Cod liver	3-4 yrs	2005-09-26	39.00	49.5
15	Baltic Sea, Gotland	Marine background	Cod liver	3-4 yrs	2005-09-26	1.20	50.55
16	Lake Fiolen	Lake background	Perch muscle	3-5 yrs	2004-08-24	2.14	0.3
17	Lake Stensjön	Lake background	Perch muscle	6-8 yrs	2005-11-25	1.71	0.3
18	Lake Remmarsjön	Lake background	Perch muscle	7-8 yrs	2005-11-22	3.01	0.68
19	Lake Krageholmssjön	Lake background	Perch muscle	3 yrs	2005-11-25	2.31	0.34
20	Landsort	Marine background	Baltic herring muscle	5-6 yrs	2005-11-28	3.27	1.45
21	Väderöarna	Marine background	Herring muscle	3 yrs	2005-11-28	0.59	9.45
22	NV Stora Röknen, Lake Vättern	Industrially influenced lake	Perch muscle	5-8 yrs	2005-11-28	0.69	0.52
23	S. Vättern, Visingsö	Industrially influenced lake	Salmon muscle	2-3 yrs	2005-11-28	<0.24	7.1