

Occurrence of polybrominated diphenyl ethers (PBDEs) in brown trout bile and liver from Swiss rivers

Paul C. Hartmann^a, Patricia Burkhardt-Holm^b, Walter Giger^{a,*}

^a *Eawag, Swiss Federal Institute of Aquatic Science and Technology, Ueberlandstrasse 133, P.O. Box 611, 8600 Duebendorf, Switzerland*

^b *Department of Environmental Science, University of Basel, Vesalgasse 1, 4051 Basel, Switzerland*

Received 22 June 2005; received in revised form 11 June 2006; accepted 15 June 2006

PBDEs with the most abundant BDE-47 were determined in brown trout bile and liver from Swiss rivers.

Abstract

The ranges of total polybrominated diphenyl ethers (PBDEs) in fish from four Swiss rivers were 0.8–240 ng/g in the bile and 16–7400 ng/g lipid in the liver. PBDE concentrations varied within each river and among the various rivers. Female fish tended to have higher concentrations in the liver, while the male fish had higher concentrations in the bile. From the resulting PBDE concentrations in fish it could not be inferred that these contaminants contribute to the causes of the observed fish catch decline in Swiss rivers.

© 2006 Elsevier Ltd. All rights reserved.

Keywords: Trout; Brominated flame retardants

1. Introduction

Polybrominated diphenyl ethers (PBDEs) are a class of brominated flame retardants that are receiving a lot of attention due to their similarity in structures to PCBs and reports of their wide spread and increasing concentrations in the environment (Alaee et al., 2003). They are used as additives in a myriad of products such as textiles, polyurethane foams, circuit boards and plastics. The global market demand of PBDEs was about 67,100 t in 1999 and is still increasing at ~4%/year, although the penta-PBDE formulation was banned in the EU in 2004. The penta formulation is composed primarily of BDE-47 and BDE-99 with smaller amounts of BDE-100, BDE-85, BDE-153 and BDE-154. No other congeners comprise more than 1% of the penta mixture. Because they are not chemically bound in a product, they leach through

volatilization or abrasion over the lifetime of these products and enter the environment.

PBDEs have been widely found in various environmental compartments with many studies finding increasing concentrations over time (Zegers et al., 2003; Kierkegaard et al., 2004; Zhu and Hites, 2004; Law et al., 2006). Zegers et al. (2003) found PBDEs starting around 1973 and increasing until 1999 with a congener distribution resembling the commercial product in sediment cores from northern Europe. Archived fish and fish extracts from Sweden also indicated an increasing trend from the late 1960s to the mid 1980s followed by a low decrease (Kierkegaard et al., 2004). Studies from the Great Lakes on archived lake trout have shown a doubling of PBDE concentrations every 3–4 years which is still continuing (Zhu and Hites, 2004). At this time there is very little data on PBDEs in the environment in Switzerland with one study on PBDE in whitefish in lakes and farmed rainbow trout (Zennegg et al., 2003).

A considerable fish catch decline and reduced fish health, mainly of brown trout, was observed in Swiss rivers over the past two decades (Burkhardt-Holm et al., 2002). In the frame

* Corresponding author. Tel.: +41 44 342 5526; fax: +41 44 342 2517.

E-mail addresses: patricia.holm@unibas.ch (P. Burkhardt-Holm), giger@eawag.ch (W. Giger).

of a Swiss-wide project called Fischnetz, which studied the causes and explored possible solutions to this problem, four river basins were chosen to represent a range of conditions in Switzerland. They were selected because they all show a considerable decline (>40%) in brown trout catch over the last 10–20 years, extensive historical data exist and they are typical in that three of them exhibit a multitude of potential causal factors for this decline. Only for the river Necker, no obvious potential causes for the decline were identified in advance. There were 12 hypotheses as to the cause of the decline (Burkhardt-Holm et al., 2002, 2005), one of which was chemical contaminant contributing to reduced recruitment or reproductive failure of the population. In this context, the potential contribution of PBDE pollution was studied. PBDEs, due to their structural similarity to other chlorinated and brominated aromatic compounds, may affect fish reproductive and health processes mediated through the arylhydrocarbon receptor (AhR) or the estrogen receptor (ER) (Legler and Brouwer, 2003). In parallel investigations, indicators of estrogenic activity were investigated. A good indicator for AhR mediated responses is the induction of cytochrome P4501A (Cyp1A), which could be measured as EROD activity (Zimmerli et al., *in press*). The induction of vitellogenin (VTG) was investigated as an estrogen receptor mediated process (Körner et al., *in press*). There is evidence of impaired thyroid function in fish from areas known as PBDE-contaminated, such as the Great Lakes (Leatherland, 1993; Luross et al., 2002), but clear cause–effect relationships are not established. This is in contrast to thyroid disruptive effects in birds and mammals.

This study was undertaken in order to (i) investigate the body burden of PBDEs in wild fish of typical middle European rivers and (ii) assess whether or not the occurrence of PBDEs are worthwhile investigating in a parallel study as a contributing factor to declining brown trout populations. In order to address these questions, brown trout of the four test rivers were examined for their content of PBDEs in liver and bile. To approach the second goal, results were interpreted in the context of population, health and reproductive data of the same fish population raised in other parallel studies (Burkhardt-Holm et al., *in press*; Körner et al., *in press*; Zimmerli et al., *in press*; Schager et al., *in preparation*). Most areas in Switzerland where we experienced a considerable fish catch decline are anthropogenically strongly influenced. As a consequence, pristine reference rivers are difficult to find. This is why we applied a gradient approach (Downes et al., 2002), studying fish populations at an upstream site (headwater site = HW), midstream (downstream site 1 = D1) and downstream site (downstream site 2 = D2) in each river. While the HW sites are mostly upstream to effluents of sewage treatment plants (WWTPs), and serves as a reference site, the both downstream sites D1 and D2 are influenced by WWTP discharges. In each basin, the brown trout population was studied at three sites. These sites were either separated by barriers or the distance between the sites was great enough that migration was considered to be of minor importance.

It is difficult to explain the mentioned fish catch decline observed during the last 10 years. No single factor can be

identified to be responsible for the wide spread catch decline, but rather a combination of stressors contribute to the observed negative effects (Burkhardt-Holm et al., 2002, 2005). One possible contributing cause could be the impact of chemical contaminants which have increased inputs during the last 10 years. European and worldwide investigations have shown that environmental PBDE increased up to about 10-fold (Rayne et al., 2003; Hites, 2004; Law et al., 2006). There is also evidence from Swiss sewage sludges and a lake sediment core that decabromo-diphenyl-ethers were increasingly discharged into the Swiss aquatic environment (Kohler et al., 2005a,b). Consequently, PBDEs were chosen as analytes for the study reported here.

2. Materials and methods

2.1. Study areas

The catchment area of the river Emme is characterized by a steep pre-alpine region at the headwaters (1400 m) with seasonally fluctuating flow due to snow melt in spring and in the midland reach where several tributaries and storm events influence the Emme river's flow. A history of flooding led to intensive river management activities in the 19th and 20th centuries with many barriers placed along the river, resulting in separation of tributaries and poor gravel transport. Additionally, water is removed for irrigation purposes. Consequently, the Emme basin exhibits particularly low flow in summer resulting in low depth and a decreased number of adequate habitats for brown trout. Natural trout habitats are mostly found in the upper portion. Two large wastewater treatment plants (WWTP) discharge into the downstream Emme, while a number of smaller WWTPs discharge into the rivers tributaries. The catch of brown trout was found to have declined by 60% between 1989 and 1999.

The Liechtensteiner Binnenkanal (LBK) in the Swiss midlands is a channel constructed in the 1930s for flood control and land usage with a rather constant flow. The only prominent barrier at the mouth of the channel was removed and the stretch was restored in 2000 allowing free migration between the LBK and the River Rhine. Restrictions of natural habitats are mainly due to longitudinal constrictions leading to poor variability in width and to regulated flow resulting in high levels of fines and sediment clogging. Only one small (4500 inhabitants) WWTP discharges into the LBK. The catch of brown trout was found to have declined by 94% between 1981 and 2002.

The Necker has its source at 1300 m above sea level. It is a pre-alpine river with natural, seasonally fluctuating flow. River morphology is hardly disturbed, providing a variety of habitats for all life stages of brown trout. A small amount of wastewater is discharged into the Necker (input of four small WWTPs, with treated wastewater of fewer than 10,000 people). The catch of brown trout was found to have declined by 58% between 1988 and 2002.

The river Venoge is located in the western midlands of Switzerland and flows into Lake Geneva. The flow is influenced by snow melt in the spring and increased rain fall at the end of the year. Many vertical barriers were constructed in the last century, which pose, together with the natural hindrances, migration barriers. The fish habitats are poor in the lower portion but sufficient in the upper portion. The area of the basin is 231 km² and land use includes 47% agriculture and 34% forest. Eighteen mostly small (2: 10,000–50,000 people, others ≤10,000 people) WWTPs discharge to the Venoge or its tributaries. The catch of brown trout was found to have declined by 40% between 1987 and 2001.

2.2. Fish sampling

Brown trout were captured by electro-fishing techniques at two or three sites each in the Venoge, Emme and Necker rivers in Switzerland and the Liechtensteiner Binnenkanal in the Principality of Liechtenstein, in spring 2002 (Table 1). The sites are labeled headwater site (HW), downstream site 1 (D1) and downstream site 2 (D2). Ten male and female fish were caught

Table 1
Fish sampling summary

River (site)	Fishing date in 2002	No. of fish (male/female)	Average length (cm)	Average weight (g)
Venoge (D2)	12 April	1/3	30.6	301
Venoge (D1)	12 April	18/8	32.2	366
Venoge (HW)	10 April	8/21	25.5	168
Emme (D2)	30 April	13/7	27.9	236
Emme (HW)	04 May	10/10	23.6	123
LBK (D2)	24 April	12/7	24.0	167
LBK (D1)	24 April	5/14	24.2	163
LBK (HW)	25 April	6/13	23.6	152
Necker (D2)	03 May	8/11	29.6	282
Necker (D1)	06 May	10/11	29.4	280
Necker (HW)	06 May	7/14	21.8	93

The sites are listed downstream to upstream. D2, most downstream site; D1, middle stream site; HW, headwater site.

per site where possible, although at most sites we were not able to catch the desired number of fish or fish all of the same age (Table 1). At most sites more than the desired number of fish of one sex were caught in trying to get the desired number of the other sex. Fish were euthanized with an overdose of tricaine methanesulfonate (MS 222 100 mg/L), weighed and measured. The liver and gall bladder were removed and weighed, wrapped in aluminum foil and returned to the laboratory on ice, where they were stored at -22°C until analyzed.

2.3. Chemicals

Analytical standards of 2,4,4'-dibromodiphenyl ether (BDE-28), 3,4,4'-tribromodiphenyl ether (BDE-37), 2,2',4,4'-tetrabromodiphenyl ether (BDE-47), 2,3',4,4'-tetrabromodiphenyl ether (BDE-66), 3,3',4,4'-tetrabromodiphenyl ether (BDE-77), 2,2',4,4',5-pentabromodiphenyl ether (BDE-99), 2,2',4,4',6-pentabromodiphenyl ether (BDE-100), 2,2',3,4,4',5'-hexabromodiphenyl ether (BDE-138), 2,2',4,4',5,5'-hexabromodiphenyl ether (BDE-153), 2,2',4,4',5,6'-hexabromodiphenyl ether (BDE-154), 2,2',3,4,4',5',6'-heptabromodiphenyl ether (BDE-183) and 2,3,3',4,4',5,6'-heptabromodiphenyl ether (BDE-190) were purchased from Cambridge Isotope Laboratories, Andover, MA, USA. All solvents and silica gel (Kieselgel 60, 70–230 mesh) were purchased from Fluka Chemie GmbH, Buchs, Switzerland. Sulfuric acid (98% w/w) was obtained from Merck, Darmstadt, Germany.

2.4. Extraction and clean-up

The extraction methods were developed for fish bile and liver. The bile extraction was a liquid/liquid extraction while the liver was done by accelerated solvent extraction (ASE). 9.9 ng of BDE-77 and BDE-138 were added as internal standards.

For extraction of bile, composite samples were extracted in 60 ml centrifuge tube with 5 ml dichloromethane extracted Milli-Q water and 10 ml 60:40 mixture of hexane and dichloromethane.

For extraction of liver, liver samples were homogenized with sodium sulfate (1:1). Quartz was then mixed in 2:1 ratio and placed in a 33 ml ASE extraction cell. ASE conditions: pre-heat 5 min; static 10 min; Temperature 100°C ; pressure 100 bar; solvent 60:40 hexane/acetone; flush 110%; purge 60 s; cycles 3.

The extracts, both of liver and bile, were transferred to 15-ml conical shaped glass centrifuge tubes containing 2 ml of concentrated sulfuric acid. The flask was rinsed with a small amount (~ 0.5 ml) of hexane and added to centrifuge tube. The tube was capped so there were no leaks and shaken with a vortex mixer until well mixed (about 30 s). They were then centrifuged to separate the layers. The hexane layer was removed with a Pasteur pipette and transfer to a second 15-ml centrifuge tube. An additional 2 ml of hexane was put on the acid without shaking and then transferred into the second centrifuge tube. This procedure was repeated with another 2 ml of hexane. Five

milliliters of pre-extracted de-ionized water were added to the combined hexane extracts (~ 5 ml) in the 15-ml tube, shaken well and centrifuged to separate the phases. The bottom aqueous phase was removed and discarded. Another 5 ml of pre-extracted de-ionized water was added to the hexane extract in the 12-ml tube, shaken well and allowed to stand until the phases separated. The hexane was transferred by Pasteur pipette to a 25ml pear-shaped flask, being careful not to transfer any water with it. Two milliliters of hexane were added to the aqueous phase, shaken well, centrifuged and the hexane added to the 25-ml pear-shaped flask. The samples were then purified using silica gel chromatography eluting 5 ml of hexane waste followed by 10 ml hexane/dichloromethane (60:40) containing the analytes. After evaporation to ~ 100 μl , the sample was transferred to an injection vial and 20 μl of BDE-190 added as a recovery standard. The injection vial was then heated at 50°C until the volume was <100 μl .

2.5. Gas chromatography/mass spectroscopy

Two milliliter aliquots were injected to the GC/MSD under the following conditions: Column 30 m DB-5 MS, 0.25 mm ID; injection port 270°C ; oven: 100°C for 2 min; $10^{\circ}\text{C}/\text{min}$ to 300°C ; hold 5 min; $10^{\circ}\text{C}/\text{min}$. to 320°C ; hold 5 min; carrier gas helium at 1 ml/min or 33 cm/s; transfer line 300°C ; acquisition mode SIM quantifying and confirmed with two ions as follows: BDE-28 (m/z 405.8, 247.9), BDE-37 (m/z 405.8, 407.8), BDE-47 and BDE-66 (m/z 325.8, 323.9), BDE-77 (m/z 487.6, 485.6), BDE-99 and BDE-100 (m/z 403.7, 401.8), BDE-138, BDE-153 and BDE-154 (m/z 483.6, 481.7), BDE-183 and BDE-190 (m/z 563.6, 721.5). The PBDEs analyzed were chosen because they are the most prominent congeners in the commercial PBDE formulations.

2.6. Quality assurance and quality control

Quantification limits ranged from 0.98 to 4.2 ng/sample. Five spiked blanks of fish tissue were run at concentrations of 9.90 ng and 14.9 ng per sample. The values were corrected for the values in the tissue without spiking. The method recovery of the analytes to the internal standard ranged from 75% to 111%. Recovery of internal standards in the bile samples averaged 58% for BDE77 and 66% for BDE-138 while in the liver samples the recoveries were 37% and 60%, respectively. The lower recoveries in the liver samples were likely due to leaks during the ASE extraction.

3. Results and discussion

3.1. Site comparisons

PBDE concentrations of individual congeners ranged from not detected to 92.8 ng/g in the bile samples and from not detected to 4370 ng/g lipid in the liver samples (Figs. 1 and 2). Total PBDE concentrations ranged from 0.8 ng/g to 240 ng/g in the bile and from 16 to 7400 ng/g lipid in the liver. The inability to quantify was often the result of too small sample sizes. Pooling all the bile from males and females from each site resulted in samples from 0.05 g to 1.75 g with a median of 0.74 g. The liver samples after pooling were also small ranging from 0.04 to 0.45 g lipid. The tetra-brominated BDE-47 was the only congener detected and quantifiable in most samples, thus interpretation of the data is mostly based on this congener. Overall, the fish from the Venoge River and Liechtensteiner Binnenkanal had relatively high concentrations of PBDEs while those caught in Emme and Necker rivers had lower concentrations.

The average of the male and female for each site show that the concentration in the bile of BDE-47 is quite low with only

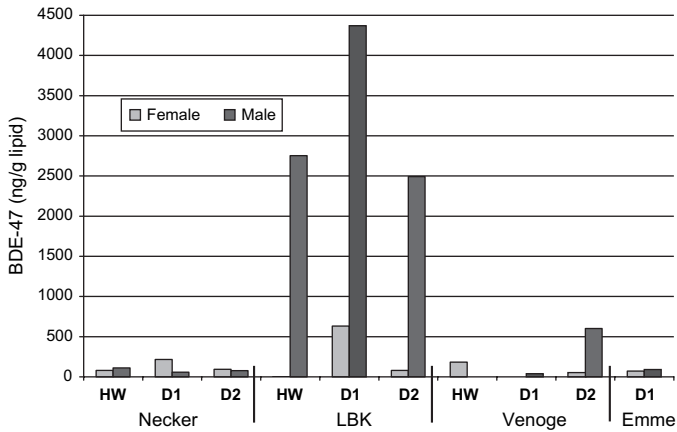


Fig. 1. BDE-47 concentrations in bile from male and female brown trout.

three sites above 10 ng/g. Two of those sites are in the Venoge (D2 and HW) and the other in the LBK (D1). The slightly higher concentration might be expected in these two rivers because of their proximity to more populated areas relative to the Necker. The Emme D1 site is relatively low even though it is close to a population center. When comparing the concentrations in the liver samples, the pattern is a little different with the three highest concentrations, over 1000 ng/g lipid, all in the LBK. These were ~ 1 order of magnitude higher than in fish from the other rivers. The higher concentration in the LBK trout is also notable because the fish at these sites are smaller (younger) as compared to the Venoge D1 and D2 in which the fish were larger (older). The younger fish therefore accumulated a larger body burden over a shorter time period, possibly indicating a higher pollution at these sites. The Venoge D2 site was also somewhat elevated compared to the rest of the samples, but not as proportionately as high as in the bile sample. In both the bile and liver samples, the D1 site has the lowest concentration of BDE-47 of the sites in the Venoge. The comparatively high concentration in brown trout caught at HW may be explained at this site as runoff from a nearby airfield provides a possible point source.

Comparing our data to other fish data shows that contamination of Swiss brown trout is, in general, in the same range as

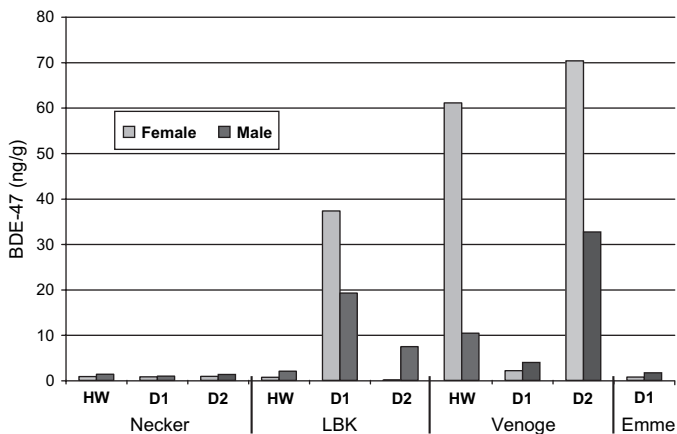


Fig. 2. BDE-47 concentrations in liver from male and female brown trout.

in other countries. In trout from the U.S. Great Lakes, whole fish average concentrations ranged from 70 to 1340 ng/g lipid of BDE-47 in lakes Erie and Michigan, respectively (Luross et al., 2002). In samples from this study, the average concentration was 12 ng/g in the bile and 557 ng/g lipid in the livers. A study on the Des Plaines river in Illinois, USA which at the time of year sampled contained $\sim 75\%$ treated waste water, found an average of 281 ng/g lipid weight total PBDEs downstream of a WWTF (Rice et al., 2002) which appears considerably lower than the test sites in this study. It is important to note that based on a study by Burreau et al. (2000), PBDEs partition more strongly into the liver than fish as a whole or the muscle tissue. In several studies near known point sources, values from 2400 to 47,900 ng PBDE/g lipid have been found (Andersson and Blomkvist, 1981; Hale et al., 2001; Dodder et al., 2002). The three samples from male fish from the LBK in this study had concentration from 3600 to 7400 ng/g lipid, indicating the possibility of a nearby point source. WWTP have been shown to discharge from 0.1 to 2.2 ng/L (Metzger and Kuch, 2002) in their effluent as most of the PBDEs partition to the sewage sludge. The Venoge sites have more WWTP nearby than the LBK sites, yet lower overall concentrations of PBDEs. This may indicate that WWTP are not the primary source to these rivers. The application and subsequent run-off of sewage sludge applied to farm fields is a more plausible explanation. The LBK sites in particular had farm fields adjacent to the river. Since PBDE are semi-volatile, they undergo air-surface exchange and atmospheric transport and are subject to deposition and degradation. As a consequence, concentrations at a given site may be influenced by local and regional advection and/or surface exchange. Atmospheric deposition was also suggested to be the most prominent input pathway in Swiss lakes (Zennegg et al., 2003). Furthermore, the low correlation between different pollutants found by Covaci et al. (2005) supports the assumption that measured concentrations are due separate local pollution sources. In our study, as local sources, production facilities (upholstery, electronic devices) in the vicinity of LBK and combustion by households are most probable. The obviously high importance of local inputs for PBDE occurrences in fish disqualifies the gradient approach as a useful approach for the situation we were confronted with: the measured concentrations are obviously independent of the gradient along the river flow. This is in contrast to other pollution parameters, such as wastewater percentage, nitrate and nitrite (Burkhardt-Holm et al., 2007).

3.2. PBDE congener distribution

BDE-47 is generally the congener found in the highest concentration in environmental samples (Table 2). In our study, BDE-47 was the only congener detected in quantifiable amounts in most samples. This supports findings that BDE-47 is rapidly assimilated compared to other congeners studied in carp (Stapleton et al., 2004). In addition, this congener is probably produced by debromination of higher brominated compounds (Stapleton et al., 2004). Burreau et al. (2000)

Table 2
BDE-47 concentrations (in ng/g lipid weight) in tissues of wild fish of different origins

Origin	Fish species	Muscle	Liver	Reference
Swiss rivers	Brown trout	n.m.	5–7000	This study
Swiss lakes	Whitefish	19–96	n.m.	Zennegg et al., 2003
Swiss fish farms	Rainbow trout	8–16	n.m.	Zennegg et al., 2003
Swedish rivers	Pike	40–2000	n.m.	Sellström et al., 1998
North Sea	Herring	23–47	19–52	Boon et al., 2002
North Sea	Cod	26–74	63–307	Boon et al., 2002
North Sea	Whiting	7.1–40	7.6–132	Boon et al., 2002

n.m., not measured.

showed that in pike, 90% of BDE-47 was taken up from the gastrointestinal tract while only 62% and 40% of BDE-99 and -153 were taken up, respectively. Boon et al. (2002a) attribute this as a function of the smaller molecular cross-section of BDE-47 and -99 allowing them to bioaccumulate more easily. This is also reflected in this data set in which BDE-47 was by far the most abundant congener found. Sjodin et al. (1998) showed that a typical penta-BDE formulation contained 37% BDE-47, 35% BDE-99 and 6.8% BDE-100. The distribution of BDEs in the fish from the LBK is similar to a typical penta-BDE formulation when the relative bioaccumulation potential of BDE-47 and -99 are taken into account relative to BDE-99. The congener distribution in the Emme indicates there may be a small source of the higher molecular weight formulation as BDE-183 was found in small quantities in a number of samples.

3.3. Toxicity

There are only a few studies that have investigated the toxicity of PBDEs to fish. One study in which the spawning fish were fed Bromokal 70-5DE, a penta mixture, showed a spawning success 25% of the control group. The low-dose fish were then found to have an average concentration of 861,000 ng/g lipid (Holm et al., 1993) which is 2 orders of magnitude higher than the highest sample in this study. Although that study does clearly show the potential for lowering reproductive success, it was not performed at environmentally relevant concentrations. Rainbow trout fed on ~0.9 mg/kg/day BDE-47 for 6 or 22 days indicated a strong influence on the cytochrome P-450 ethoxyresorufin-*o*-deethylase activity (EROD) dependent activity for the tetra BDEs (Tjarnlund et al., 1998). The body burden was not measured in the fish, but assuming only 10% goes to the body burden and the fish contain 5% lipids, that would result in a concentration of 9500 ng/g lipid. This is higher than the highest levels seen in this study. This experiment also used doses that for the most part are not environmentally relevant. However, results on effects of BDE in fish to EROD are contradictory, which might be due to differential metabolic capacities in different species (Johnson and Olson, 2001; Boon et al., 2002b) and due to toxic effects at

higher concentrations. The latter can be suggested since in vitro studies showed that flame retardants induced EROD activity at low test concentrations but started to inhibit activity at higher concentrations (Nakari and Pessala, 2005). In our study, EROD was measured in the fish (Zimmerli et al., in press) and compared to the observed PBDE concentrations. EROD values higher than 50 pmol/mg/min may indicate an exposure to dioxin-like chemicals (Whyte et al., 2000). Such elevated values were measured at only three sites, the two downstream sites of LBK and the most downstream site of Venoge. As a consequence, the elevated burdens of PBDE in liver and bile may be responsible for the EROD induction at LBK and at Venoge, although not statistically significant. Interestingly, brown trout from the two downstream sites in LBK also showed an increased prevalence of hepatic changes, as examined by a standardized histopathological procedure, particularly mitoses, nuclear alterations and single cell necrosis (Zimmerli et al., 2007). Vitellogenin induction was noted in single fish at some of the sites, but not to a statistically significant degree when samples from the different sites were compared (Körner et al., 2007). Evidence for estrogenicity of BDE-47 and other PBDEs was measured as vitellogenin induction in primary rainbow trout hepatocyte culture, however, at much higher concentration than required for natural 17-beta-estradiol (Nakari and Pessala, 2005).

In addition, intersex was observed at both the Venoge (20%) and LBK (25%) (Körner et al., 2005), which are the two rivers with the highest observed PBDE concentrations in brown trout. This does not specifically indicate that PBDEs are responsible as neither PBDEs nor other endocrine disrupting compounds, or other causes besides chemicals that may be responsible for this phenomenon, were studied in experimental settings.

3.4. Comparison of male to female fish

Comparing the bile of male and female fish from the same site, there appeared to be strong differences at some sites and little differences at others, although this could not be tested statistically (Fig. 1). The three sites with the highest concentrations were all higher for the female fish while the three in which the male had the higher concentration were all down near the detection limit. The differences in the concentration between the male and female fish at the higher concentrations in the female fish is likely real while at the lower concentrations it reflects a higher uncertainty in the data. In contrast to the bile samples, in the liver samples the males at the three LBK sites and the Venoge D2 site all had higher concentrations (Fig. 2). These are also the four sites with the highest concentrations. The other sites in which there is no difference or the female fish have a higher concentration are all close to the detection limit. Linear regression of both the total PBDEs and BDE-47 between the liver and bile indicates there is no correlation in the concentration. Based on comparing the differences between the bile and liver concentrations in male and female fish, it appears there may be either a mechanism that causes a difference in partitioning between them or differences

in the metabolism or elimination. The food conversion efficiency was suggested to be lower in males than females, which means that males must take more energy in, i.e. eat more, than females to produce the same amount of energy (Burreau et al., 2004). This would result in higher intake of contaminants as well, reflected in higher concentrations of PBDE in the liver. It has been suggested for other species and other hydrophobic organic compounds that females of reproductive age often have lower body burdens due to egg production, passing on some of the body burden to the fetus or eggs (Larsson et al., 1993; Law et al., 2002; Burreau et al., 2004).

Because the samples had to be pooled, each data point represents one analysis and therefore we cannot statistically assess whether or not there is a difference between sites. However, where there is a large difference between the male and female samples, it would take a very large variance for the difference not to be real. Therefore the data suggest there is a difference at some sites although this cannot be stated definitively.

4. Conclusions

The PBDE concentrations in fish from the four test rivers were similar to what has been found in other fish from European rivers and lakes, with isolated sites having higher concentrations. Because the samples were all pooled, individual fish at some sites could have concentrations that approach levels that have been found to be toxic in laboratory studies. Based on the observed concentrations of PBDEs and the available laboratory data on levels that are inducing toxic effects, it is unlikely that the PBDE contaminations are significantly contributing to the causes of population decline of brown trout in Swiss rivers.

Acknowledgements

This work was supported by the Swiss National Science Foundation (Project PHEBRO, 4050-066566) within the framework of the National Research Program NRP 50 on “Endocrine Disruptors: Relevance to Humans, Animals and Ecosystems”. Additional funding came from the Project Fishnet (Burkhardt-Holm et al., 2002, 2005). The authors thank Simone Zimmerli, Maria Roos, Eva Schager and Martin Kohler for scientific discussions. The fishery authorities of the cantons of Berne, Thurgau, Waadt and of the principality of Liechtenstein are acknowledged for their support during the field work.

References

Alaee, M., Arias, P., Sjödin, A., Bergman, A., 2003. An overview on commercially used brominated flame retardants, their use patterns in different countries/regions and possible modes of release. *Environ. Int.* 29, 683–689.

Andersson, O., Blomkvist, G., 1981. Polybrominated aromatic pollutants found in fish in Sweden. *Chemosphere* 10, 1051–1060.

Boon, J.P., Lewis, W.E., Tjoen-A-Choy, M.R., Allchin, C.R., Law, R.J., Boer, J.D., Zegers, C.N., 2002a. Level of polybrominated diphenyl ether

(PBDE) flame retardants in animals representing different trophic levels of the North Sea food web. *Environ. Sci. Technol.* 36, 4025–4032.

Boon, J.P., van Zanden, J.J., Lewis, W.E., Zegers, B.N., Goksoy, A., Arukwe, A., 2002b. The expression of CYP1A, vitellogenin and zona radiata proteins in Atlantic salmon (*Salmo salar*) after oral dosing with two commercial PBDE flame retardant mixtures: absence of short-term responses. *Marine Environ. Res.* 54, 719–724.

Burkhardt-Holm, P., Peter, A., Segner, H., 2002. Decline of fish catch in Switzerland - Project Fishnet: A balance between analysis and synthesis. *Aquat. Sci.* 64, 36–54.

Burkhardt-Holm, P., Giger, W., Güttinger, H., Peter, A., Scheurer, K., Suter, M.J.-F., Ochsenbein, U., Segner, H., Staub, E., 2005. Where have all the fish gone? The reasons why fish catches in Swiss rivers are declining. *Environ. Sci. Technol.* 39, 425A–448A.

Burkhardt-Holm, P., Borsuk, M.E., Scheurer, K., 2007. Application of the weight-of-evidence approach to assess the decline of brown trout (*Salmo trutta*) in Swiss rivers. *Aquat. Sci.*, 67.

Burreau, S., Broman, D., Orn, U., 2000. Tissue distribution of 2,2',4,4'-tetrabromo[¹⁴C]diphenyl ether ([¹⁴C]-PBDE-47) in pike (*Esox lucius*) after dietary exposure—a time series study using whole body autoradiography. *Chemosphere* 40, 977–985.

Burreau, S., Zebuhr, Y., Broman, D., Ishaq, R., 2004. Biomagnification of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) studied in pike (*Esox lucius*), perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) from the Baltic Sea. *Chemosphere* 55, 1043–1052.

Covaci, A., Bervoets, L., Hoff, P., Voorspoels, S., Voets, J., Van Campenhout, K., Blust, R., Schepens, P., 2005. Polybrominated diphenyl ethers (PBDEs) in freshwater mussels and fish from Flanders, Belgium. *J. Environ. Monit.* 7, 132–136.

Dodder, N.G., Strandberg, B., Hites, R.A., 2002. Concentrations and spatial variations of polybrominated diphenyl ethers and several organochlorine compounds in fishes from the northeastern United States. *Environ. Sci. Technol.* 36, 146–151.

Downes, B.J., Barmuta, L.A., Fairweather, P.G., Faith, D.P., Keough, M.J., Lake, P.S., Mapstone, B.D., Quinn, G.P., 2002. *Monitoring Ecological Impacts*. Cambridge University Press, Cambridge.

Hale, R.C., Guardia, M.J.L., Harvey, E.P., Mainor, T.M., Duff, W.H., Gaylor, M.O., 2001. Polybrominated diphenyl ether flame retardants in Virginia freshwater fishes (USA). *Environ. Sci. Technol.* 35, 4585–4591.

Hites, R.A., 2004. Polybrominated diphenyl ethers in the Environment and in people: a meta-analysis of concentrations. *Environ. Sci. Technol.* 38, 945–956.

Holm, G., Norrgren, L., Andersson, T., Thuren, A., 1993. Effects of exposure to food contaminated with PBDE, PCN or PCB on reproduction, liver morphology and cytochrome P450 activity in the three-spined stickleback, *Gasterosteus aculeatus*. *Aquat. Toxicol.* 27, 33–50.

Johnson, A., Olson, N., 2001. Analysis and occurrence of polybrominated diphenyl ethers in Washington state freshwater fish. *Arch. Environ. Contam. Toxicol.* 41, 339–344.

Kierkegaard, A., Bignert, A., Sellström, U., Olsson, M., Asplund, L., Jansson, B., de Wit, C.A., 2004. Polybrominated diphenyl ethers (PBDEs) and their methoxylated derivatives in pike from Swedish waters with emphasis on temporal trends, 1967–2000. *Environ. Pollut.* 130, 187–198.

Kohler, M., Zennegg, M., Gerecke, A.C., Schmid, P., Heeb, N.V., 2005a. Increasing concentrations of decabromodiphenyl ether in Swiss sewage sludge since 1993. *Organohalogen Comp.* 61, 123–126.

Kohler, M., Zennegg, M., Hartmann, P.C., Sturm, M., Gujer, P., Schmid, P., Gerecke, A.C., Heeb, N.V., Kohler H.-P.E., Giger, W., 2005b. The historical record of brominated flame retardants and other persistent pollutants in a Swiss lake sediment core. Abstract volume of the 15th European SETAC Meeting in Lille, May 22–26. p. 213.

Körner, O., Vermeirssen, E.L.M., Burkhardt-Holm, P., 2005. Intersex in feral brown trout from Swiss midland rivers. *J. Fish Biol.* 67, 1734–1740.

Körner, O., Vermeirssen, E.L.M., Burkhardt-Holm, P., 2007. Reproductive health of brown trout inhabiting Swiss rivers with declining fish catch. *Aquat. Sci.*, 69.

Larsson, P., Okla, L., Collvin, L., 1993. Reproductive status and lipid content as factors in PCB, DDT and HCH contamination of a population of pike (*Esox lucius* L. *Environ. Toxicol. Chem.* 12, 855–861.

- Law, R.J., Allchin, C.R., Bennett, M.E., Morris, S., Rogan, E., 2002. Polybrominated diphenyl ethers in two species of marine top predators from England and Wales. *Chemosphere* 46, 673–681.
- Law, R.J., Allchin, C.R., de Boer, J., Covaci, A., Herzke, D., Lepom, P., Morris, S., Troczynski, J., de Wit, C.A., 2006. Levels and trends of brominated flame retardants in the European environment. *Chemosphere* 64, 187–208.
- Leatherland, J.F., 1993. Field observations on reproductive and developmental dysfunction in introduced and native salmonids from the great lakes. *J. Great Lakes Res.* 19, 737–751.
- Legler, J., Brouwer, A., 2003. Are brominated flame retardants endocrine disruptors? *Environ. Intern.* 29, 879–885.
- Luross, J.M., Alae, M., Sergeant, D.B., Cannon, C.M., Whittle, D.M., Solomon, K.R., Muir, D.C.G., 2002. Spatial distribution of polybrominated diphenyl ethers and polybrominated biphenyls in lake trout from the Laurentian Great Lakes. *Chemosphere* 46, 665–672.
- Metzger, J.W., Kuch, B., 2002. Organic flame retardants in wastewater treatment plants. *Chimia* 57, 24–26.
- Nakari, T., Pessala, P., 2005. In vitro estrogenicity of polybrominated flame retardants. *Aquat. Toxicol.* 74, 272–279.
- Rayne, S., Ikonou, M.G., Antcliffe, B., 2003. Rapidly increasing polybrominated diphenyl ether concentrations in the Columbia River system from 1992 to 2000. *Environ. Sci. Technol.* 37, 2847–2854.
- Rice, C.P., Chernyak, S.M., Begnoche, L., Quintal, R., Hickey, J., 2002. Comparisons of PBDE composition and concentration in fish collected from the Detroit River, MI and Des Plaines River, IL. *Chemosphere* 49, 731–737.
- Sellström, U., Kierkegaard, A., de Wit, C., Jansson, B., 1998. Polybrominated diphenyl ethers and hexabromocyclodecane in sediment and fish from a Swedish River. *Environ. Toxicol. Chem.* 17 (6), 1065–1072.
- Stapleton, H.M., Letcher, R.J., Li, J., Baker, J.E., 2004. Dietary accumulation and metabolism of polybrominated diphenyl ethers by juvenile carp (*Cyprinus carpio*). *Environ. Toxicol. Chem.* 23 (8), 1939–1946.
- Tjarnlund, U., Ericson, G., Orn, U., de Wit, C., Balk, L., 1998. Effects of two polybrominated diphenyl ethers on rainbow trout (*Oncorhynchus mykiss*) exposed via food. *Marine Environ. Res.* 46, 107–112.
- Whyte, J.J., Jung, R.E., Schmitt, C.J., Tillit, D.E., 2000. Ethoxyresorufin-O-deethylase (EROD) activity in fish as a biomarker of chemical exposure. *Crit. Rev. Toxicol.* 30, 347–570.
- Zegers, B.N., Lewis, W.E., Booij, K., Smittenberg, R.H., Boer, W., de Boer, J., Boon, J.P., 2003. Levels of polybrominated diphenyl ether flame retardants in sediment cores from Western Europe. *Environ. Sci. Technol.* 37, 3803–3807.
- Zennegg, M., Kohler, M., Gerecke, A.C., Schmid, P., 2003. Polybrominated diphenyl ethers in whitefish from Swiss lakes and farmed rainbow trout. *Chemosphere* 51, 545–553.
- Zhu, L.Y., Hites, R.A., 2004. Temporal trends and spatial distribution of brominated flame retardants in archived fish from the Great Lakes. *Environ. Sci. Technol.* 38, 2779–2784.
- Zimmerli, S., Bernet, D., Burkhardt-Holm, P., Schmidt-Posthaus, H., Vonlanthen, P., Wahli, T., Segner, H., 2007. Assessment of fish health status in four Swiss rivers with decline of brown trout catch. *Aquat. Sci.* 69.