Final Draft
of the original manuscript:

Suehring, R.; Moeller, A.; Freese, M.; Pohlmann, J.-D.; Wolschke, H.;
Sturm, R.; Xie, Z.; Hanel, R.; Ebinghaus, R.:
Brominated flame retardants and dechloranes in eels from
German Rivers
In: Chemosphere (2012) Elsevier

DOI: 10.1016/j.chemosphere.2012.08.016
Brominated Flame Retardants and Dechloranes in Eels from German Rivers

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Abstract

The levels of PBDEs, alternate BFRs and Dechloranes in European Eel (Anguilla anguilla) samples (elvers, yellow and silver eels) were investigated to compare the contamination of eels from the rivers Elbe and Rhine and to estimate the BFR contamination throughout the eel’s life cycle.

PBDEs were the dominating flame retardants (FRs) in muscle tissues of yellow and silver eels, while the alternate BFR 2,3-dibromopropyl-2,4,6-tribromophenyl ether (DPTE) and the Dechlorane 602 were the dominating FRs in elvers (juvenile eels).

Concentrations of FRs in silver eels from river Rhine were generally higher than concentrations in other eels analysed with up to 46 ng g⁻¹ wet weight (ww) \( \sum \)PBDEs.

The concentrations in yellow and silver eels from river Elbe were similar with an average of 9.0 ± 5.1 ng g⁻¹ ww and 8.1 ± 3.7 ng g⁻¹ ww respectively. PBDE concentrations in elvers were comparably low (0.02 (BDE-100) to 0.1 (BDE-183) ng g⁻¹ ww), which lead to the conclusion that these contaminants were mostly ingested within the rivers.

Among the alternate BFRs and Dechloranes, DPTE as well as the Dechlorane 602 and Dechlorane Plus (DP) were found in all life cycle stages and rivers with concentrations between 0.01 ng g⁻¹ ww and 0.7 ng g⁻¹ ww. Dechlorane 603 could only be detected in silver eels from river Rhine. Pentabromoethylbenzene (PBEB) was only found in yellow and silver eels and bis(2-ethylhexyl)tetrabromophthalate (BEHTBP) could only be detected in elvers.

These are the first reports of Dec-602 and 603 in aquatic organisms from Europe.

The results of this study show the lasting relevance of PBDEs as contaminants in rivers and river-dwelling species but also the growing relevance of emerging contaminants such as alternate BFRs and dechloranes.

Keywords

European Eel, Brominated Flame Retardants, PBDEs, alternate BFRs, Dechloranes
1. Introduction

The European Eel (*Anguilla anguilla*) is a catadromous, carnivorous fish. It is widely distributed over Europe and has a high economic value for the fishing industry. Its overall population has been declining rapidly since the 1980s and has by now dropped to 1% of the average population during the 1970s (ICES 2008), (Fisheries Forum 2003). Therefore the European Eel was added to the UN CITES Appendix II list, implying trading restrictions, as well as to the Red List of species by the International Union for Conservation of Nature (IUCN), rating it as "critically endangered". Several natural as well as anthropogenic causes, such as overfishing, destruction of habitats, parasites, hydropower plants, predation and chemical pollution have been discussed (Dekker 2004). Chemical pollution has become one of the main focuses as eels are predestined to take up large quantities of lipophilic organic pollutants due to their high lipid contents (Palstra et al. 2006), (Belpaire & Goemans 2007), (Robinet and Feunteun 2002). This is especially problematic as eels are a possible way of human exposure to hazardous chemicals.

One group of organic pollutants possibly threatening to the European Eel are halogenated flame retardants (HFRs) and especially brominated flame retardants (BFRs). For several decades polybrominated diphenyl ethers (PBDEs) have been applied as BFRs. Some PBDEs are known to be bioaccumulative, persistent and to undergo long-range transport (LRT) (Darnerud 2003), (Wania and Dugani, 2003). Many of them are toxic for aquatic organisms, some induce endocrine effects or are carcinogenic (de Wit, 2002). Due to these adverse effects to the environment and human health PBDEs have been banned for production and usage in the European Union (EU) (European Court of Justice 2008). As a further banishment step congeners used in the technical penta- and octa- BDE mixtures have been officially classified as Persistent Organic Pollutants (POPs) under the Stockholm Convention (SCOP 2009).
Due to the restriction of PBDEs and the increasing demand of flame retardants (FRs) the usage of alternate (non-PBDE) BFRs have increased. There is little knowledge concerning POP potential of these substitutes for PBDEs yet many alternate BFRs are suspected to at least partially fulfil the criteria (Harju et al. 2009).

Another HFR used and recommended by the EU as substitute for Deca- BDE is the highly chlorinated Dechlorane Plus (DP) (Pakalin et al. 2007). It was originally developed as a substitute for the banned pesticide Mirex but has mostly been applied as FR (Hoh et al. 2006). Even though it has been produced and used for more than 40 years there is little data available on behaviour and possible adverse effects in the environment. Since its first detection in 2006 (Hoh et al. 2006) reports on DP in the environment have increased rapidly and it has even been reported from remote areas such as the Arctic and Antarctic (Möller et al. 2010). For other used dechloranes, namely Dec-602, Dec-603 and Dec-604 there are even less data available even though they are suspected to be bioaccumulative and have been reported in biota far away from production sites (Sverko et al. 2011).

This paper presents the analysis of PBDEs, alternate BFRs and Decs in elvers (juvenile eels) from river Vidå, yellow eels (stationary, river dwelling adult eels) from six sampling sides along the river Elbe and silver eels (adult eels migrating back to the spawning grounds in the Sargasso Sea) from the rivers Elbe and Rhine. The aim of this research project was to compare the contamination level of silver eels from Elbe and Rhine as well as estimate the BFR and Dec contamination during the eel’s freshwater phase.

2. Material and Methods

2.1. Samples

All adult eels were caught as part of the EU Data Collection Regulation (DCR) (Stransky et al. 2008). All eels were taken in the German part of the rivers. 30 elvers with a mean length of 12 cm were taken from the river Vidå and combined into ten
samples of three fish each. From six sampling sites along the river Elbe five yellow
eels per sampling site were taken. All yellow eels used were between eight and
twelve years old and in the silverying stages II or III (growth phase) (Durif 2005). Ten
silver eels were taken each from the estuary mouth of the river Elbe and the upper
river Rhine. All silver eels were in the silverying stage V (migrating phase) (Durif
2005). Contact with materials containing brominated flame retardants was avoided
at all sampling sites. Muscle tissue was excised from the skeletal muscle behind the
level of the anus from yellow and silver eels and as much muscle tissue as possible
from elvers. A detailed list of the analysed samples can be found in Table S1.

2.2. Extraction and clean-up

The frozen yellow and silver eel samples were homogenised with anhydrous Na₂SO₄
(Merck) (2:1; w/w) for approximately 20 min. using a stainless steel/glass 1 L
laboratory blender (neoLab Rotorblender). For each extraction 11 mL stainless steel
extraction cells were filled with 3g Na₂SO₄ and 3g of the Na₂SO₄-eel-mixture (equal
to 1g eel tissue) or one of the pooled elver samples. The samples were spiked with
mass labelled (internal) standards (IS) $^{13}$C-HBB, $^{13}$C-BDE-77, $^{13}$C-BDE-138 and $^{13}$C-
synDP. The remaining volume was filled with anhydrous Na₂SO₄.

The samples were extracted via accelerated solvent extraction (Dionex ASE-200)
using dichloromethane (DCM) at 100°C and 120 bar. The lipid content of the samples
was determined gravimetrically from separate sample aliquots.

After extraction the samples were reduced in volume to approx. 2mL using rotary
evaporators. Gel permeation chromatography (GPC) was used as a first clean up
step, using a glass column (height: 500 mm, i.d.: 30mm) filled with 35 g Bio-Beads S-
X3 (pre swollen with 200 mL DCM:hexane (1:1 v/v) for 12h) (Bio-Rad Laboratories).
Analytes were eluted with 110 mL DCM:hexane (1:1; v/v).
The eluates were again reduced to about 2 mL and the solvent changed to hexane. The samples were further purified by 10% deactivated silica gel (2.5 g, 0.063-0.200 mm) (Merck) and eluted with 20 mL hexane. The eluates were reduced to 150 µL under a gentle stream of nitrogen and transferred to measurement vials. Finally, 50µL PCB-207 (10 ng mL⁻¹) were added as an injection standard. For further specifications regarding the used method and standards see Tables S2, S3.

2.3. Instrumental Analysis

For instrumental analysis a method developed and published by Möller et al. (2010) (Möller et al. 2010) was used. Briefly, analyses were done by a GC/MS-system (6890 GC/5973 MSD) in negative chemical ionisation mode (NCI) with methane as ionization gas fitted with a HP-5MS column (30m x 0.25mm i.d. x 0.25µm film thickness, J&W Scientific). The instrument was operated in selected ion monitoring mode. Samples were analysed for nine PBDEs, 11 alternate (non-PBDE) BFRs, DP, the one- and two-fold dechlorinated DP species (aCl₁₁DP [-1Cl+1H], aCl₁₀DP [-2Cl+2H]), DPMA and Dechlorane 602, 603 and 604 (see Table S4 for chemical structures and properties).

2.4. QA/QC

Extraction and clean-up were conducted in a clean lab (class 10000). BFR containing material was avoided during preparation and analysis. Recovery rates of IS were determined for every sample (for a detailed list see Table S5). Mean IS recoveries ranged from 45 ± 19% for ¹³C-HBB to 86 ± 19% for ¹³C-DP in elvers; 68 ± 24% to ¹³C-BDE-138 and 82 ± 20% for ¹³C-BDE-77 in yellow eels and 66 ± 31% to ¹³C-BDE-138 and 83 ± 24% for ¹³C-BDE-77 in silver eels. All concentrations were recovery corrected.
Relative recoveries of the analytes (corrected by recovery rates of the IS) were determined during method development and ranged from 67% for BDE-66 to 159% for DPTE. The recovery for BEHTBP was low (5%). Results for BEHTBP were therefore treated as semi-quantitative.

A blank test, using Na$_2$SO$_4$ treated similar to real samples, was conducted with every extraction batch (eleven samples). DPTE and Dec-602 could each be detected in one blank sample with absolute concentrations in the two- to low three- digit pg range. BDE-183 was found in five of eleven blank samples in absolute concentrations in the three- digit pg range. The blank concentrations were considered in the calculation of the sample concentrations of the appropriate batch.

For a detailed list of the measured blanks see Table S6.

The limit of detection (LOD) was calculated from a signal to noise ratio of three, the limit of quantification from a signal to noise ratio of ten. The LOD ranged from 0.004 ng g$^{-1}$ wet weight ( ww) for Dec-602 to 0.073 ng g$^{-1}$ ww for BDE-183 in elvers; 0.008 ng g$^{-1}$ ww for Dec-602 to 0.14 ng g$^{-1}$ ww for BDE-183 in yellow eels and 0.004 ng g$^{-1}$ ww for Dec-603 to 0.14 ng g$^{-1}$ ww for BDE-100 for silver eels. The LOQ ranged from 0.013 ng g$^{-1}$ ww for Dec-602 to 0.24 ng g$^{-1}$ ww for BDE-183 in elvers; 0.026 ng g$^{-1}$ ww for Dec-602 to 0.46 ng g$^{-1}$ ww for BDE-183 in yellow eels and 0.014 ng g$^{-1}$ ww for Dec-603 to 0.46 ng g$^{-1}$ ww for BDE-100 in silver eels. For a detailed list of LODs, LOQs see Tables S7 and S8.

A twofold measurement was done for every sample. The standard deviation between measurements of five aliquots of one eel sample was 12%.

3. Results and Discussion

3.1. BFRs and Dechloranes throughout the Eels Lifecycle

The average results for PBDEs, alternate BFRs and Dechloranes from this study in comparison to recent studies are displayed in Table 1.

For a complete list of the results of this study see Tables S9 and S10.
3.1.1. PBDEs

The elvers analysed in this study have been in fresh water between a few months and one year. Their journey from the Sargasso Sea to Europe has taken up to three years (Tesch et al. 1990, Bonhommeau et al. 2010). It is therefore likely that most of the contaminations found were ingested during their stay in the ocean and estuary or passed on by spawners.

Elvers had low PBDE concentrations compared to the PBDE levels in eels from other life cycle stages and the contribution of PBDEs to the sum contamination in elvers was similar or lower than the contribution of alternate BFRs and Dechloranes. Three of the nine analysed PBDE congener could be detected in elvers, with concentrations ranging from 0.02 (BDE-100) to 0.1 (BDE-183) ng g\(^{-1}\) ww. In all other eels analysed PBDEs were the major group of contaminants. Six and seven different congeners could be detected in yellow eels from river Elbe and silver eels from river Rhine, respectively. \(\Sigma\)PBDEs concentrations ranged from 9.0 ± 5.1 ng g\(^{-1}\) ww in yellow eels from river Elbe to 21.3 ± 13.8 ng g\(^{-1}\) ww in silver eels from river Rhine.

The congener distribution of the PBDEs differed in elver samples and samples from other life cycle stages. In elvers BDE-183 was the main congener, indicating a contamination through the technical octa-BDE mixture. In yellow and silver eels BDE-47 was the main congener with concentrations between 6.2 ± 3.6 ng g\(^{-1}\) ww in yellow eels from river Elbe and 14.3 ± 9.05 ng g\(^{-1}\) ww in silver eels from river Rhine. The congener distribution in adult eels matched the distribution reported in other studies analysing PBDEs in eels (Belpaire 2008) with BDE-47 > BDE-100 > BDE-153 > BDE-99 > BDE-154 > BDE-183.

The low concentrations in elver samples indicated that PBDEs have mostly been ingested in the rivers. The strong contribution of lower brominated PBDEs yellow and silver eels suggests the technical penta-BDE mixture as main source of the contamination. The high contribution of BDE-47 is typical for all fish due to the higher
uptake rate and biomagnifications of BDE-47 within the aquatic food web (Eljarrat et al. 2011). BDE-47 has also been proven to be formed via enzymatic debromination of higher brominated diphenyl ethers during the metabolism in fish (Eljarrat et al. 2011).

3.1.2. Alternate BFRs

DPTE could be detected in eels of all life cycle stages analysed with mean concentrations between 0.2 ± 0.1 ng g⁻¹ ww in elvers, 0.22 ± 0.35 ng g⁻¹ ww in yellow eels from river Elbe and 0.89 ± 0.64 ng g⁻¹ ww in silver eels from river Rhine. The detection of DPTE within the elver samples could be an indication that the eels ingested DPTE during their time in the ocean or estuary as well as the river. There are no data on current DPTE production, however, DPTE has frequently been detected in various matrices most recently by Möller et al. who detected DPTE in water samples from the North Sea, river Elbe and river Weser (Möller et al. 2012). DPTE is suspected to be persistent in sediments making them a possible source of DPTE contamination (Fisk 2003).

BEHTBP could only be detected in elvers, with a medium concentration of about 0.1 ng g⁻¹ ww and does therefore seem to not be ingested within the rivers. In recent studies BEHTBP has as well mostly been detected in ocean dwelling species such as dolphins and porpoise (Lam et al. 2009) while it could not be detected in sources typically discharging into fresh water such as sewage sludge (Moskeland 2010). The concentrations found in this study were higher than the average PBDE concentration in elvers which again indicated, that the main contamination with PBDEs occurred within the rivers.

The second alternate BFR detected in yellow and silver eels was PBEB. The detected concentrations were similar for all adult eels analysed with 0.025 ± 0.007 ng g⁻¹ ww in yellow eels from river Elbe, 0.027 ± 0.009 ng g⁻¹ ww in silver eels from river Elbe and 0.027 ± 0.015 ng g⁻¹ ww in silver eels from river Rhine. It could not be detected in elver samples and has therefore probably only been ingested in the
rivers. These results accorded with results from recent studies that reported PBEB in
samples from industrialised areas rather than oceanic samples (Harju et al. 2009).
Recently the German Environment Agency also detected low amounts of PBEB in
bream samples from German rivers such as Elbe and Mulde (Sawal et al. 2011).

3.1.3. Dechloranes
Dechlorane Plus and Dec-602 could be detected in all life cycle stages analysed with
up to 0.67 ng g\(^{-1}\) ww (in elvers). Dec-603 could only be detected in silver eels from
river Rhine with concentrations between <LOD (0.0042 ng/g ww) and 0.076 ng g\(^{-1}\)
ww.

In elvers, yellow eels and silver eels from river Rhine the syn-isomer of the two
technical stereoisomers syn- and antiDP could be detected in slightly higher
concentrations and more individual samples. The synDP/\(\Sigma\)DP ratio (\(f_{syn}\)) was highest
in yellow eels with an average of 0.96 ± 0.12, followed by \(f_{syn}\) in elvers with an
average of 0.80 ± 0.14. In silver eels from river Rhine syn- and antiDP concentrations
were almost equal (0.040 ± 0.030 ng g\(^{-1}\) ww and 0.033 ± 0.022 ng g\(^{-1}\) ww
respectively, \(f_{syn} = 0.52 ± 0.084\)), yet synDP could be detected in more individual
samples. In silver eels from river Elbe the detected synDP and antiDP concentrations
were similar as well (n.d. - 0.030 ng g\(^{-1}\) ww and n.d. - 0.021 ng g\(^{-1}\) ww respectively)
yet antiDP could be detected in 70 % of the samples, while synDP was detectable in
only 30% of the samples. The resulting \(f_{syn}\) was therefore low with only 0.24 ± 0.30.
The significant change in the isomer ratio from the technical mixture (75% antiDP) to
the isomer ratio found in the eel samples (between 50% and 90% synDP) matched
observations from previous studies indicating that synDP bioaccumulates and
biomagnifies stronger than antiDP in fish (Shen et al. 2011), (Jiang-Ping Wu et al.
2010). For the eels analysed in this study the isomer ratio of syn- and antiDP seems
to have mostly been driven by uptake rate and/or metabolism and not by location, as
the significant changes were between life cycle stages (yellow and silver eels) and not between rivers (silver eels from Elbe and Rhine).

Dec-602 has not yet been reported in aquatic organisms in Europe. It has however been found in sea bird eggs from Spain and various matrices from the US and Canada (Guerra et al. 2011). The detection in eels from all life cycle stages was surprising as there is no reported producer or importer within the EU. Dec-602 has however been reported to have a high bioaccumulation potential (the biota- sediment accumulation factor (BSAF) is about 500 times higher than the BSAF of DP) and to be very bio available (Shen et al. 2011).

There is no reported source for Dec-603 in Europe yet it has as well been detected in sea bird eggs from Spain (Guerra et al. 2011). Dec-603 has also been detected in the banned organochlorine pesticides formulations of aldrin and dieldrin (Shen et al. 2011a). As the reported half-life for Dec-603 in sediments is 11 years (Sverko et al. 2011) residues of these pesticides leaking from sediments could be a possible source. The fact that it could only be detected in silver eels from the river Rhine indicates that it, so far, mainly occurs in highly industrialised areas (in this case the Rhine-Ruhr metropolitan region) close to sources. Both Dec-602 and Dec-603 could also enter the EU incorporated in products. Dec-602 for example is used in fibreglass- reinforced nylon (Shen et al. 2011) which is a common component in consumer products.

In the group of dechloranes Dec-602 was the main contaminant in yellow eels while in silver eels from river Rhine $\Sigma$DP and Dec-602 had similar concentrations and $\Sigma$DP concentrations in silver eels from river Elbe slightly exceeded Dec-602 concentrations (see figure: 1). This change of the contamination pattern could indicate that Dec-602 is easier metabolised and/or eliminated than DP. An increase of the DP/Dec-602 ratio could not have been caused by a change of diet, as silver eels stop feeding. The increase is therefore likely to have been caused by different metabolism strategies or different ways of uptake between Dec-602 and DP, such as
a higher uptake of DP via gills or skin. Another reason could be that the highly migratory silver eels ingested the high DP concentration at a different part of the river and have not ingested any new contaminants as silver eels stop feeding due to their physiological changes from yellow to silver eels.

3.2. Concentration profile of BFRs and dechloranes in eels along the Elbe

PBDEs showed increasing concentrations (significance: 99.9% confidence level; Neumann-test) towards inland sampling sites, again supporting the thesis that eels were primarily exposed to these contaminants in the rivers. The trend was mainly driven by the BDE-47 congener but most PBDEs measured apart from BDE-183 and BDE-154 showed a similar trend. Highest PBDE concentrations were measured in eels from the Dessau sampling site (km 261) (12.6 ± 5.7 ng g⁻¹ ww) close to where the river Mulde flows into the Elbe. The Mulde is known to be contaminated by a variety of chemicals (e.g. hexachlorocyclohexane) due to leakage of landfills containing chemical waste from the former German Democratic Republic (Ministerium für Landwirtschaft und Umwelt, 2005). A study done by the German Federal Environmental Agency, analysing PBDEs as well as some alternate BFRs in bream from rivers Mulde and Elbe also reported higher concentrations in the Mulde than in any of the samples from river Elbe (Sawal et al. 2011).

The Mulde as main source for PBDEs in the Elbe would explain the decrease in the concentration upstream the Dessau sampling site as well as the gradually decreasing trend towards the estuary mouth as the contamination is bound to decrease with distance to the source. As yellow eels are relatively residential the decreasing trend of contamination along the river can be expected to be reflected in the contamination of the eels at different sampling sites. The concentrations of alternate BFRs were relatively constant throughout the Elbe with two exceptions for DPTE. One exception was the low concentrations at the
Hohengöhren sampling site (km 378). The second exception was one very high contaminated eel from Jork sampling site (km 643). The lack of a trend in the contamination indicated continuous contamination throughout the river via e.g. diffuse emission and/or deposition. Remobilisation from contaminated sediments could also be a possible reason for this lack of a clear contamination pattern. The high DPTE concentration at Jork sampling site (km 643) however indicated that this specific eel was exposed to a large dose of DPTE probably by a point source. PBEB concentrations were found in low concentrations in samples from most sampling sites again indicating diffuse emissions and/or immission via deposition or discharge from contaminated sediments.

At Gorleben sampling site (km 492) highest individual Dec-602 concentrations were measured (0.25 ± 0.24 ng g⁻¹ ww). Towards the estuary mouth Dec-602 could however be detected in more individual samples. Upstream Gorleben some fish still had high Dec-602 concentrations (at Hohengöhren (km 378)) yet overall synDP was the main contaminant of the dechloranes. The high concentrations of Dec-602 at Gorleben sampling site might indicate a point source in that area. The contamination found in fish from Hohengöhren sampling site could be due to the movement of the fish along the river even though yellow eels are supposed to be relatively stationary.

The overall DP concentration was highest at the Dessau sampling site (km 261) (0.038 ± 0.013 ng g⁻¹ ww) and gradually decreased towards the estuary mouth (significance: 99.9% confidence level; Neumann-test) apart from one high contaminated sample from Jork sampling site (km 643). The trend indicated that the primary DP source was near the Dessau sampling site and therefore probably influenced by the river Mulde. The high contaminated sample from Jork was the same sample that also showed alternate BFR concentrations above average, again indicating a contamination of this individual fish by a point source.

3.3. Comparison of Silver Eels from Elbe and Rhine
The concentrations of PBDEs and dechloranes in silver eels from river Rhine were up to three times higher than the concentrations found in silver eels from river Elbe. This was to be expected as the samples from river Rhine were taken in a highly industrialised area (close to potential sources) and fish from river Rhine are known to be contaminated with up to several 100 ng g\(^{-1}\) lw PBDEs (Sawal et al. 2011). The congener distribution of the PBDEs in samples from Elbe and Rhine were similar, yet in addition to the PBDEs found in silver eels from river Elbe BDE-66 could be detected in silver eels from river Rhine.

The contribution of the individual dechloranes to the sum dechlorane contamination differed for silver eels from Rhine and Elbe. Again there were more individual substances detectable in the river Rhine (DP, Dec-602, Dec-603). The concentrations of alternate BFRs found in silver eels from river Rhine and Elbe were similar, indicating a contamination through diffuse sources.

The comparably high concentrations of FRs and detection of additional components like Dec-603 and BDE-66 display the overall higher contamination of the river Rhine in comparison to river Elbe and might be an indication for sources in this area.

4. Conclusions

The results of this study show the lasting relevance of PBDEs as contaminants in rivers and river-dwelling species but also the growing relevance of emerging contaminants such as alternate BFRs and dechloranes. There are in many cases not enough data to evaluate the risk of the emerging contaminants yet many BFRs are expected to be toxic for aquatic organisms and are therefore likely to affect the eel’s health and ability to reach its spawning ground.

Further tests concerning adverse effects and properties of the analysed substances and their metabolites should be conducted. Sources and ways of environmental release and distribution, especially for substances without a known source such as DPTE and the dechloranes have to be identified and monitored.
Supporting Information

Tables on the samples, method, used standards, recovery rates, blank values, detection and quantification limits as well as a detailed list of the results is available in the supporting information.

Acknowledgements

I’d like to thank my research group at the Helmholtz Centre for their support and suggestions throughout my work on this paper.

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Hydrobiologie und Hydrographie 75 (6): 866-866.
