Halogenated flame retardants in biota samples from the German North- and Baltic Sea

Dreyer A[1], Neugebauer F[2], Rüdel H[3], Paulus M[4], Lohmann N[2], Rauert C[5], Koschorreck J[5]

[1] Eurofins GfA GmbH, Air Monitoring, Hamburg, Germany, 21107, annekatrindreyer@eurofins.de, [2] Eurofins GfA Lab Service GmbH, Hamburg, Germany, 21079, [3] Fraunhofer Institute for molecular biology and applied ecology (Fraunhofer IME), Schmallenberg, Germany, 57392, [4] Trier University, Biogeography, Trier, Germany, 54296, [5] German Environment Agency, Berlin, Germany, 14193

Introduction

In the past, conventional brominated flame retardants (BFRs) such as polybrominated diphenyl ethers (PBDEs) were identified as persistent organic pollutants (POPs) and subsequently regulated. Fire safety regulations became more stringent over the same period and regulated flame retardants were replaced by other compounds, often called novel, alternative or emerging flame retardants (eFRs). Many of these eFRs are highly chlorinated or brominated as well, and their fate and effects in the environment may be similar to those of their regulated counterparts.

The German Environmental Specimen Bank (ESB) is an instrument for environmental monitoring of the German Federal Ministry for the Environment, Nature Conservation, Building and Nuclear Safety (BMU) and is technically and administratively managed by the Federal German Environment Agency (UBA). The German ESB systematically collects and archives environmental samples inter alia to support chemical management, e.g. to a) monitor the effectiveness of environmental regulations, b) identify chemicals of emerging concern and to assess the needs for risk management options and comprehensive environmental monitoring and c) apply novel analytical techniques to historical samples for a better understanding of trends in pollution. Long-term sample storage at cryogenic conditions preserves the chemical and biological integrity of the samples over decades. Therefore, the archive allows for retrospective investigations of substances which were not known to be toxic at the time of sampling or which were not detectable due to technical limitations.

To investigate and monitor the pollution of the marine environment, eelpout, blue mussel and herring gull eggs from selected sites located at the German North- and Baltic Sea coast are regularly sampled. The objective of the current study was the investigation of eFRs as well as PBDEs in such samples over time and space.

Materials and methods

For the present study, ESB samples from 2015 were investigated for 43 flame retardants (Figure 1), i.e. eelpout, blue mussel and herring gull egg samples from the Wadden Sea National Park, North Sea at the Jade Bay region (NS1) and the Meldorf Bay region (NS2) as well as from the Western Pomerania Lagoon Area National Park, Baltic Sea (BS1). Furthermore, retrospective trend analysis for over 25 years was performed with blue mussel and herring gull egg samples from NS1 and BS1.

Blue mussels (*Mytilus edulis*) are collected bimonthly at both North Sea sites NS1 and BS, and twice a year at BS1 in the Baltic Sea (June and November). Directly after sampling and biometric characterization the samples are deep-frozen above liquid nitrogen (Wagner et al. 2016). Eelpout (*Zoarces viviparus*) is sampled once per year in early summer (May – June) before the mating season. Immediately after sampling the fish are biometrically characterized and dissected under clean air conditions. Biometric data of the samples are given on the German ESB webpage www.umweltprobenbank.de/en. The liver and both skinless fillets are deep-frozen at < -130°C (Klein et al. 2016). Herring gull eggs are collected in spring at the bird sanctuary islands Mellum (NS1), Trischen (NS2) and Heuwiese (BS1). Each second egg of a clutch is sampled for the ESB programme, transported to the lab facilities at 4°C where the egg content of approximately 30 eggs is pooled and cooled to <

-130°C. Herring gull eggs, eelpout fillets – respectively livers – and mussel soft bodies are pooled to annual samples (Rüdel et al. 2009) and cryo-archived.
The analytical procedure for the determination of flame retardants as well as target analytes are described in detail by Neugebauer et al. (2018). Samples were freeze-dried and homogenized and spiked with nineteen mass-labelled standards. Samples were extracted by accelerated solvent extraction with hexane:dichloromethane 1:1 (v:v) followed by a multicolumn clean-up: 1st 2 g Na2SO4 upon 2 g silica, eluted by toluene:hexane 1:1 (v:v); 2nd, 33g BioBeads SX-3, eluted by ethylacetate:cyclohexane 1:1 (v:v); 3rd 2 g Florisil (5% water deactivated), eluted by n-hexane and n-hexane-toluene 1:1 (v:v). Instrumental detection of alternative HFRs occurred by gas chromatography coupled to tandem mass spectrometry operated with atmospheric pressure ionization (GC-API-MS/MS). 19 eFRs of different substance classes were determined in two separate runs. Among them were 24 PBDEs, Dechlorane 60x, Dechlorane Plus incl. selected degradation products, as well as emerging BFRs of different size or degree of bromination such as decabomodiphenylethane (DBDPE), dibromopropyltribromophenylether (DPTE), tribromoanisol (TBA) or pentabromotoluene (PBT). Separation and detection of 24 PBDEs was conducted by gas chromatography coupled to mass spectrometry (GC-MS).

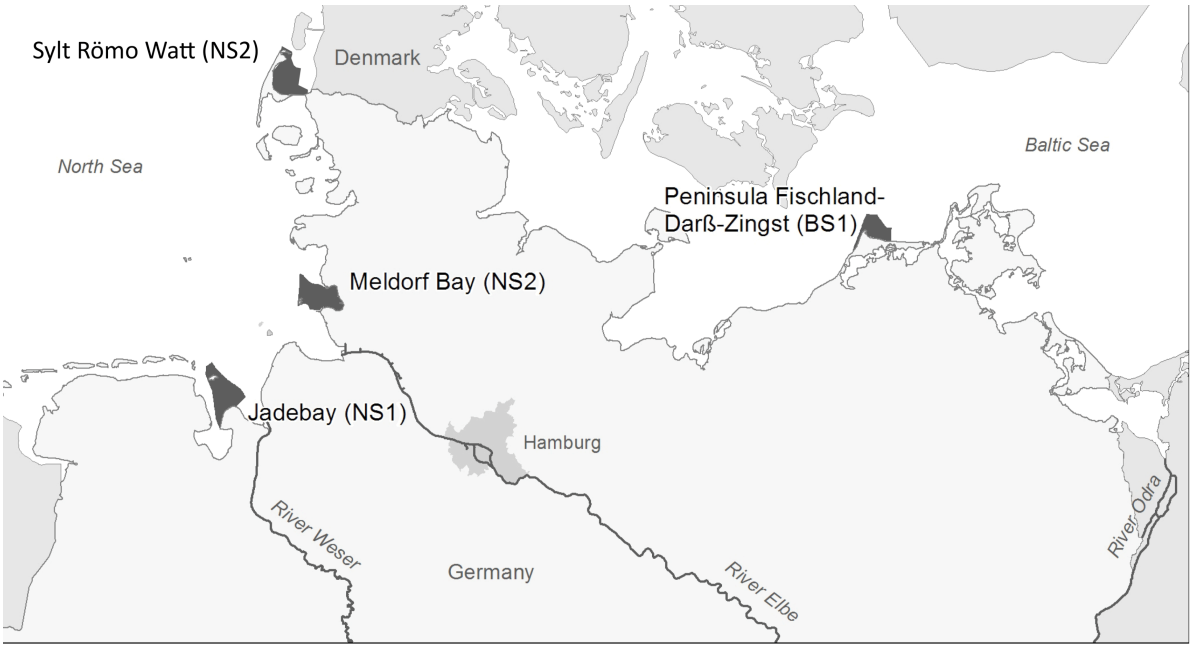

Figure1: Sampling locations

Results and Discussion
Selected results are presented in Figures 2 and 3. PBDE concentrations of most recent samples taken in 2015 ranged from about 4000 pg g^{-1} lipid weight (lw) in eelpout fillet from the Baltic Sea site (BS1) to about 250000 pg g^{-1} lw in herring gull egg from one North Sea site (NS1). Except for herring gull egg, PBDE concentrations were higher in samples from the Meldorf Bay (NS2) than from the Jade Bay (NS1). Except for mussel samples from NS1 and BS1 (high levels of BDE209), PBDE profiles were dominated by the 7 main PBDEs. Total concentrations of investigated eFRs were lower than total PBDE concentrations and ranged from 750 pg g^{-1} lw in eelpout fillet from one North Sea site (NS2) to about 52000 pg g^{-1} lw in blue mussel from the same region. High values observed in the latter mussel samples originated from elevated levels for DPTE and BEHTBP. Herring

gull eggs of all sites were dominated by chlorinated FRs. Mussels and eelpout did not show a distinct profile. DBDPE as highly brominated compound was only quantified in mussels and herring gull egg from NS2 at concentrations close to the method quantification limit.
Concentrations of most PBDEs in mussels were declining over the past 25 years which was expected due to various regulation efforts on the different technical mixtures in the past (Venkatesaan & Halden 2015). Temporal trends were less clear for BDE209 or in herring gull egg samples. Declining trends were also observed for eBFR in mussel samples which was less expected as these compounds were thought to replace classical FRs such as PBDEs. In herring gull eggs from the Baltic Sea site, eBFR concentrations increased. Concentrations of dechloranes decreased over the last years at all sites.

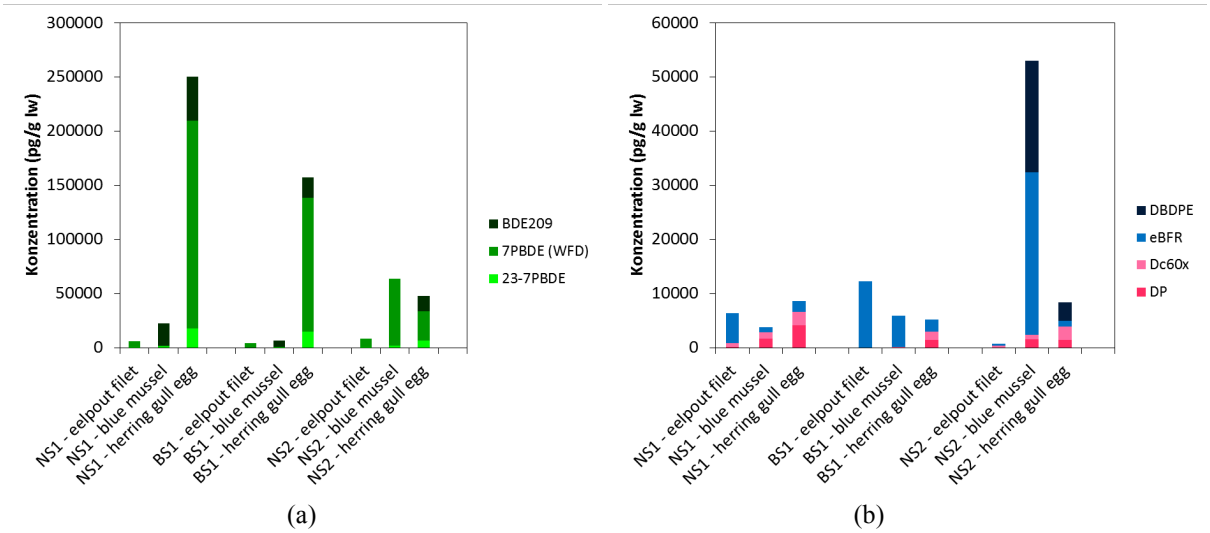

Figure 2: (a) PBDE concentrations (pg g-1 lw) in biota samples taken in 2015. 24 PBDEs analyzed were grouped into BDE209, sum of 7 PBDE of the water framework directive (7PBDE (WFD)) and the remaining PBDE (23-7PBDE). (b) Concentrations (pg g-1 lw) of eFRs in biota samples taken in 2015. The 19 eFRs were grouped into DBDPE, sum of 10 emerging BFR (eBFR), Dechlorane Plus incl. selected degradation products (DP) and sum of Dechlorane 602, 603, 604 (Dc60x). NS: North Sea. BS: Baltic Sea.

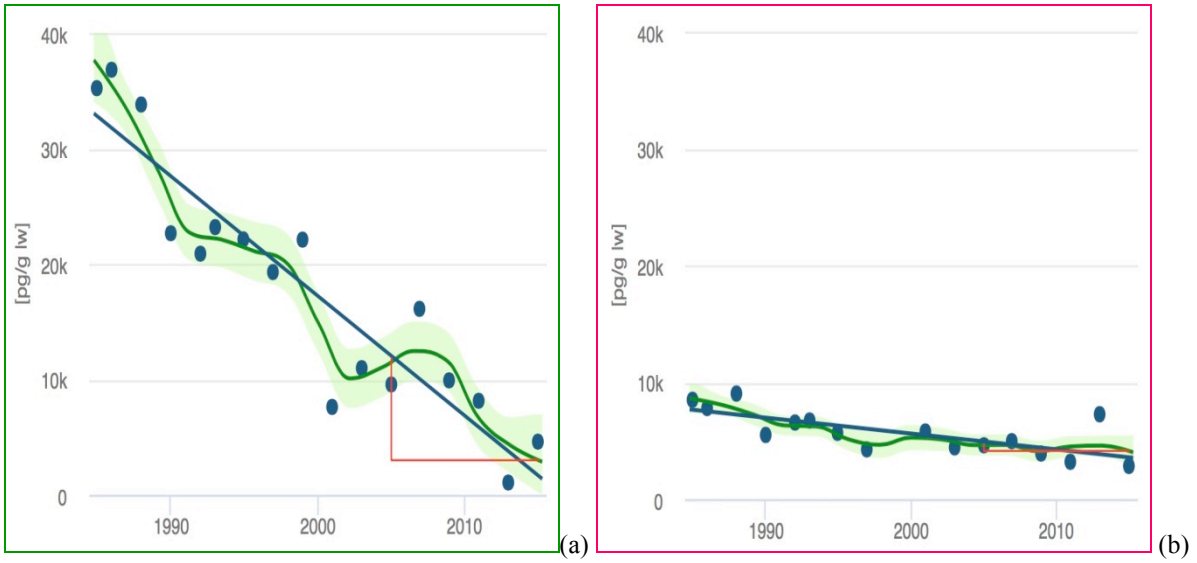

Figure3: Temporal trends of a: 7PBDE (WFD) in blue mussel and b: dechlorane60x+Dechlorane Plus in blue mussel.

Acknowledgements
We thank our laboratory staff for their dedicated work on the project, particularly Judith Söhler and Steffi Rolle. The German Environment Agency is acknowledged for funding (AZ 93 04/25).

References
1. Klein R, Bartel M, Paulus M, Tarricone K, Teubner D, Wagner G (2018); Richtlinie zur Probenahme und Probenbearbeitung, Aalmutter (Zoarces viviparus). *www.umweltprobenbank.de* (accessed 05/2018)
2. Neugebauer F, Dreyer A, Lohmann N, et al. (2018); *ABC*. 410: 1375–1387
3. Paulus M, Klein R, Tarricone K, Teubner D (2018); Richtlinie zur Probenahme und Probenbearbeitung Silbermöwe (Larus argentatus). *www.umweltprobenbank.de* (accessed 05/2018)
4. Rüdel H, Uhlig S, Weingärtner M (2009): Guidelines for Sampling and Sample Processing: Pulverisation and Homogenisation of Environmental Samples by Cryomilling. *www.umweltprobenbank.de* (accessed 05/2018)
5. Wagner G, Klein R, Teubner D, Paulus M (2016); Richtlinie zur Probenahme und Probenbearbeitung - Miesmuschel (Mytlilus edulis Komplex). *www.umweltprobenbank.de* (accessed 05/2018)
6. Venkatesan A, Halden R. (2015); *Int. J. Environ. Res. Public Health, 12:10549-10557*