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BROMINATED FLAME RETARDANTS (BFR) IN EGGS FROM BIRDS OF PREY FROM SOUTHERN GERMANY, 2014

W. Vetter[1], A. Schlienz[1], C. Gallistl[1], J. Müller[1], K.T. Von Der Trenck[2]

[1]*University of Hohenheim, Institute of Food Chemistry, Stuttgart, Germany*

[2]*Institute for the Environment, Measurements, and Nature Protection of the German State of Baden-Württemberg (LUBW) Griesbachstr. 1, D-76185 Karlsruhe, Germany*

Introduction

Polybrominated flame retardants are regularly detected in environmental samples throughout the world. Within the terrestrial animal kingdom, birds of prey belong to the top predators of diverse foodwebs. These animals are accumulating persistent organic pollutants (POPs) and BFRs in the body burden, and mothers transfer lipophilic contaminants to the yolk, depending on the amount of lipids invested in a clutch of eggs, which differs between species.[1,2] Birds of prey are responding sensitively to POPs, but sensitivity varies considerably with respect to compound and species.[3]

In this study we analyzed ~15 samples of birds of prey (peregrine falcon, eagle owl, osprey, and sea eagle) for the contamination level with polybrominated compounds. Main emphasis was put on the quantitation of different brominated flame retardants including classic compounds (PBDEs, HBCD, HBB) and less frequent and novel ones (DPTE, PBEB, PBT). In addition, one sample was also comprehensively analyzed by means of the novel non-targeted GC/ECNI-MS-SIM method of Hauler & Vetter[4]. Non-target analysis (NT) aims to analyze samples unbiased for all contaminants with maximal possible sensitivity. This was accomplished by using the GC/ECNI-MS-SIM method presented in 2015[4]. With this method the mass range of polybrominated compounds is distributed in three segments of 15 u each (1 overlapping mass), which are screened in eight subsequent runs.[5] This NT-GC/ECNI-MS-SIM method offers excellent sensitivity for polyhalogenated compounds.

Materials and methods

Samples and Chemicals. Fifteen bird eggs that failed to hatch were collected between March and July 2014 at different locations in Baden-Wuerttemberg, Southern Germany. The samples included eggs from the peregrine falcon (n=11), sea eagle (n=1), eagle owl (n=3) and osprey (n=2).

Sample Cleanup. About 100 mg of extracted egg fat was dissolved in 25 mL ethyl acetate/cyclohexane (54/46, w/w). A portion of 5 mL was separated and 20 mL extract (corresponding to ~80 mg egg fat) was supplemented with 20 µL of the internal standard α-PDHCH (10.7 ng/µL, IS). Then the solvent was changed to n-hexane. The sample was cleaned by adsorption chromatography (3 g silica gel, deactivated with 30% water, placed in a 1 cm i.d. glass column, elution of the target compounds with 60 mL n-hexane).[6] The solvent was evaporated to a final volume of 1 mL before further separation of target compound groups by a second adsorption chromatography step (8 g activated silica gel, placed in a 1 cm i.d. glass column).[6] Target compounds were eluted as followed: Fraction 1 (target compounds: PCBs) was eluted with 48 mL n-hexane, Fraction 2 (target compounds: polybrominated compounds and chloropesticides) was eluted with 50 mL n-hexane/ethyl acetate and Fraction 3 (target compounds, phenolic compounds) was eluted with 50 mL ethyl acetate. With focus on polybrominated compounds, we only analyzed Fraction 2 in this study.

GC/MS parameters. GC/ECNI-MS analyses were performed with a 7890/5975C system (Agilent) using the parameters described by Bendig et al. [6] A 30 m, 0.25 mm i.d. and 0.25 df DB-5 ultra inert column (Agilent) was used and heated from 50 °C (1 min hold time) at 10 °C/min to 300 °C (hold time 4 min).6 In the GC/ECNI-MS full scan mode, m/z 50-800 was recorded. Identification and quantification of different polyhalogenated compounds by GC/ECNI-MS-SIM was reported elsewhere.6

The NT-GC/ECNI-MS-SIM method was performed in eight GC runs according to Hauler & Vetter[4] except that a fourth time window was set up, taking into account the higher upper mass range of up to m/z 652. In brief, run 1 featured in SIM-mode from 8-18.8 min m/z 300-314, from 18.8-23.5 min m/z 350-364, from 25.3-26 min m/z 450-464, and from 26-40 min m/z 540-554. The second run started with the final mass of the previous run in all time windows and covered also 15 subsequent SIM ions (e.g. from 18.8-23.5 min m/z 364-378) a.s.o.

Results and discussion

Quantitative data.

In a targeted approach, the samples were screened mainly for the polybrominated compounds and compound classes shown in Table 1. In all samples, the sum PBDE concentration (40 congeners screened) was much higher than the sum of all other BFRs. Typical sum PBDE concentrations were in the range >100 to ~500 ng/g fat (Table 1). The highest PBDE levels in individual peregrine falcons was >1,000 ng/g fat. PBDE levels were dominated by BDE 47. Typically for bird eggs, hexaBDEs (BDE 153, BDE 154) also played an important role, and also the heptaBDE BDE 183 was detected in five peregrine falcons but in none of the other birds. Although the exact origins of the bird eggs were known to us, no detailed data analysis was possible (see however below) because of the unknown age and pollution level of the mother animal, which has also an impact on the pollutant transferred to the egg. Aside from PBDEs, DPTE was the most prominent BFR in peregrine falcons based on the mean concentration. DPTE was detected in nine of the eleven peregrine falcons with up to 60 ng/g fat. The median (29 ng/g fat) was only slightly lower abundant than the mean (Table 1). In addition, we also detected the DPTE metabolites BATE in six and ATE in four peregrine falcons. In all samples (with positive detections) the concentration was highest for DPTE > BATE > ATE. BATE and ATE never exceeded a concentration of 10 ng/g fat. It appears from our data, that DPTE is more persistent in the birds (or bird eggs) than BATE and ATE. Contrary to previous reports,[7] DPTE is still being used in Germany, and we also found DPTE frequently as pollutant in German kitchens.[6,8]

HBCD was only detected in three peregrine falcons (26-66 ng/g fat) and one eagle owl (Table 1). This pointed to an uneven distribution of this BFR (compared to PBDEs). Interestingly, one of the peregrine falcon eggs with high HBCD level was collected close to the factory of a German car producer and a second one on a church tower. This verifies the urban/industrial impact on the HBCD level.

Three peregrine falcons and one eagle owl showed hexabromobenzene (HBB) levels of 12-26 ng/g fat. HBB is a classic BFR frequently used in Japan and possible other parts in Asia.[9] Thus, these residues likely resulted from the import of Japanese/Asian electronic equipment and/or cars.

In addition we detected 2,4,6-TBP in a few and its metabolite 2,4,6-tribromoanisole (TBA) in most of the samples. TBP and TBA are both anthropogenic pollutants and halogenated natural products.[10] In marine environments the bulk is usually assigned to natural sources while terrestrial levels are characterized by a predominant anthropogenic impact. The South German birds in this study have no access to the marine food chain and (other) halogenated natural products (HNPs) were not detected in any of the samples. In this respect our eggs differ from eggs of the same species from Norway which partly showed the presence of HNPs.[2]

Finally, we were able to detect the modern BFRs pentabromoethylbenzene (PBEB, n=4) and pentabromotoluene (PBT, n=2) in birds of prey (Table 1). Although the concentrations were low (maximum PBEB 3.9 ng/g fat, PBT 14 ng/g fat, these positive detections indicate that novel BFRs are more and more entering the environment. Figure 1 illustrates that several of the novel BRFs could already be detected as abundant peaks in the chromatograms. These examples also indicate differences in the pattern of polyhalogenated compounds (e.g. higher relative abundance of BDE 47 in peregrine falcons and generally more polybrominated compounds in sea eagle (Figure 1). For this reason we chose the sea eagle sample shown in Figure 1b for the comprehensive nontarget analysis on polybrominated compounds.

Nontarget analysis.

By means of the NT-GC/ECNI-MS-SIM method we detected >200 polyhalogenated compounds (substance classes such as PCBs and PBDEs counted as 1) in the samples (due to the presence of characteristic isotope patterns of the potential molecular ion). In the case of small peaks, the isotope patterns showed some difference from the theoretical value and structure information could not be unequivocally obtained. In this context it is worth noting that in GC/MS-SIM quantifications differences between quantifier and qualifier ion are requested to be within 10% for positive identification of a given compound. Such variations are however too high for getting an exact match of the isotope pattern. Therefore, the evaluation was limited to compounds providing a minimum peak area ("Initial Threshold" in the GC/MS software set to 9.8). With this restriction, the NT-runs featured 134 peaks from ~90 compounds for which we recorded retention times, the monoisotopic peak, the base peak and structure information.

The data set included peaks from chlordane and toxaphene but also many unknown compounds. With emphasis put on polybrominated compounds, we could identify two tetrabromotoluene isomers which are transformation products of PBT (which amounted to 11 ng/g fat in this egg, Table 1). However, the toluene backbone was verified in the laboratory, but the position of the hydrogen remained unknown. It is also unclear whether PBT was transformed biologically and/or in an abiotic way (e.g. by UV light). In the early elution range we found strong evidence for the presence of tribromoaniline, which had been recently described by us.[4]
It is known from bird eggs that in these are also bioaccumulating compounds with masses >550 Da (see also above), which are partly not bioavailable for (marine) mammals. For this reason the original NT method of Hauler & Vetter[4] was modified by adding a fourth time window in the upper mass range. Different unknown polybrominated compounds could be detected by this measure (NT01 No. 23-25; NT02 No. 23-25; NT03 No. 21&23; NT04 No.17-19; NT05 No.15-17; NT06 No.4; NT07 No.7; NT08 No.10-12). However, the structures of these compounds could not be determined until today. These compounds are most likely unknown (to us) BFRs or their metabolites (similarly to tetrabromotoluene). Two mass spectra of unknown polybrominated compounds are shown in Figure 2.
In conclusion, the bird egg sample contained a variety of unknown polybrominated compounds. Although we were unable to identify most of the polybrominated compounds, our results verify that there are a large number of potential POPs, which are not detected by classic targeted GC/MS-SIM analysis. Even without knowing the structure of the compounds, the GC/MS-SIM data can be used to set up a method for screening a further range of samples for them. In this way, the environmental relevance of the compounds can be documented and future measures for their avoidance envisioned where appropriate.

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References

1. Drouillard KG, Norstrom RJ (2001) New Zeal. J. Agric. Res. 44, 297-300.
2. Herzke D, Berger U, Kallenborn R, Nygård T, Vetter W (2005) Chemosphere 61: 441-449.
3. Hoffman DJ, Melancon MJ, Klein PN, Eisemann JD, Spann JW (1998) Environ. Toxicol. Chem. 17: 747-757.
4. Hauler C, Vetter W (2015) Rapid Comm. Mass Spectrom. 29: 619-628
5. Rosenfelder N, Van Zee NJ, Mueller JF, Gaus C, Vetter W (2010) Anal. Chem. 82: 9835-9842.
6. Bendig P, Hägele F, Vetter W (2013) Anal. Bioanal. Chem. 405: 7485-7496.
7. von der Recke R, Vetter W (2007) Environ. Sci. Technol. 41: 1590-1595.
8. Vetter W, Gallistl C, Lok B, Schlienz A (2016) Kitchen stories: polyhalogenated compounds in dishcloths
after regular use in households. Submitted to Dioxin 2016, Florence, Italy
9. Vetter W, Rosenfelder N (2008) Anal. Bioanal. Chem. 392: 489-504.
10. Vetter W (2006) Rev. Environ. Contam. Toxicol. 188: 1-57.

TABLE 1: Concentrations of polybrominated flame retardants (ng/g fat) in eggs of peregrine falcons (n=11) and other birds of prey from Southern Germany, 2014

	Peregrine falcon (n=11)*	Sea eagle #6	Eagle owl #7	Eagle owl #8	Eagle owl #9	Osprey #10	Osprey #11
PBDEs	480	320	340	390	130	140	150
DPTE	33	8.7	<	10	<	9.2	<
BATE	3.4	2.4	1.7	2.3	<	<	1.4
ATE	1.4	2.4	<	<	<	<	<
HBCD	12.4	<	<	81	<	<	<
HBB	6.7	<	<	<	12.1	<	<
2,4,6-TBA	2.2	2.1	0.6	<	2.3	3.8	4.0
2,4,6-TBP	3.9	<	<	<	<	<	<
PBEB	1.1	<	2.3	2.6	<	<	<
PBT	2.2	11.3	<	<	3.6	2.2	<

* mean values calculated by using LOD/2 for non-detectables.

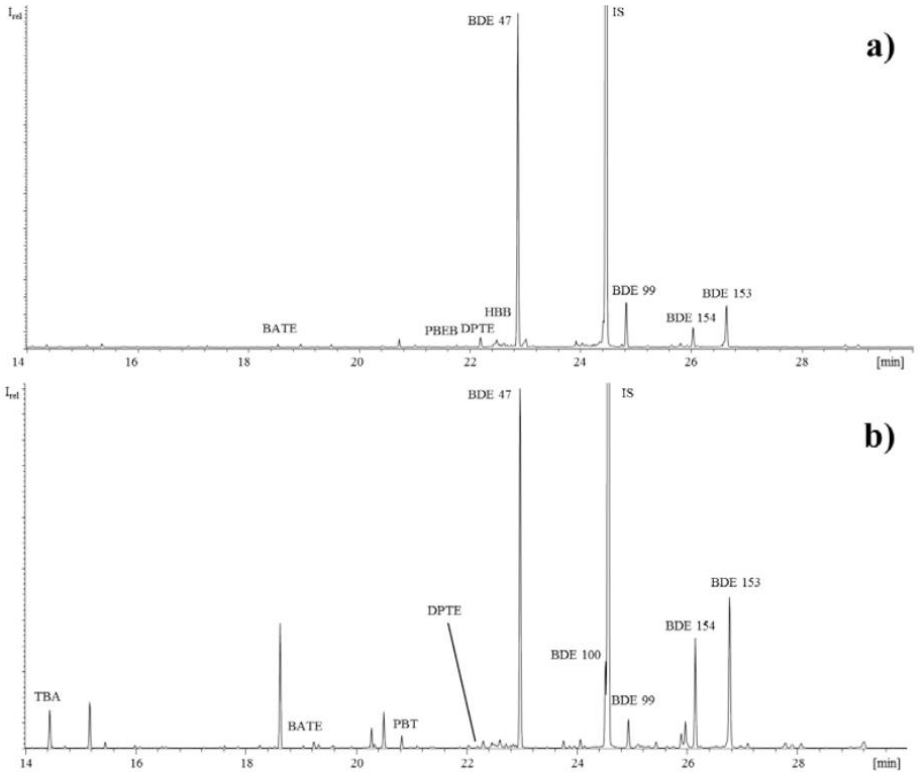

Figure 1: GC/ECNI-MS-SIM ion chromatograms (m/z 79) of (a) peregrine falcon and (b) sea eagle from Southern Germany (2014).

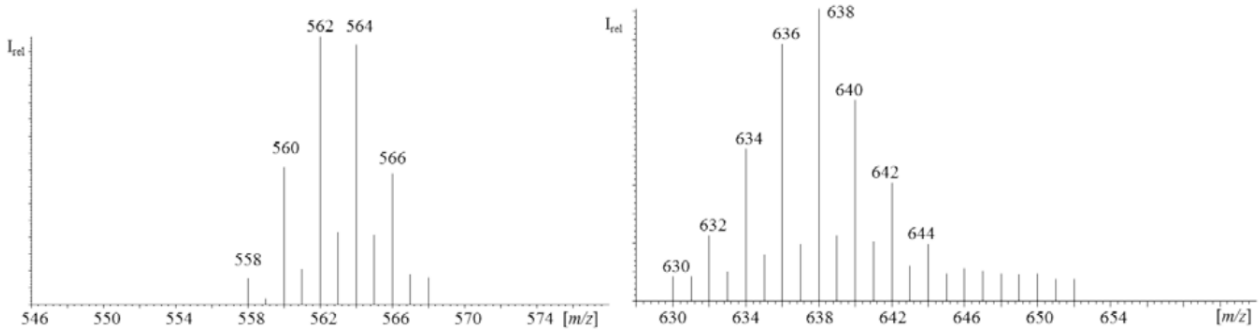

Figure 2. GC/ECNI-MS spectra of two polyhalogenated compounds detected in the sea eagle egg in non-target runs which were unknown to us (note that the mass spectrum on the right has been merged from two SIM runs).