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Analysis of TBBP-A and HBCD in peregrine falcon eggs

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Table of Contents

1. Introduction 3

2. Materials and Methods 3

2.1 Soxhlet extraction, GPC and LC-MS analysis 3

3. Results and Discussion 3

5. References 5

Annex: 1

1. Introduction

The brominated flame retardants (BFRs) tetrabromobisphenyl-A (TBBP-A) and hexabromocyclododecane (HBCD) are widely used in Europe and may be transported to northern regions by long range transport, as has been modelled for other BFRs [Wania and Dugania, 2003] or via bird migration from southern source areas [Lindberg *et al.* 2003]. Due to their physical-chemical properties, these chemicals have the potential to bioaccumulate and cause toxicity in exposed organisms. Biomagnification of TBBP-A and HBCD in the food chain puts predators high in the chain, such as the peregrine falcon *(Falco peregrinus)*, at increased risk of exposure and accumulation. Recent studies have demonstrated substantial levels of different flame retardants, including HBCD, in eggs of Swedish populations of *F. peregrinus* [Lindberg *et al.,* 'Research ASAP'] and guillemot *(Uria algae)* eggs collected over the last three decades [Sellström *et al.,* 2003].

The objective of the present study was to analyse concentrations of hexabromocyclododecane (HBCD) and tetrabromobisphenol-A (TBBP-A) in 34 peregrine falcon eggs collected in Greenland from 1986 to 2003.

2. Materials and Methods

Frozen peregrine falcon egg samples (34) were received from NERI for the analysis of HBCD and TBBP-A. The method of extraction, clean up and chemical analysis is described in the literature [De Boer *et al.* 2001].

2.1 Soxhlet extraction, GPC and LC-MS analysis

Frozen eggs were thawed, dried by mixing with sodium sulphate and then extracted by Soxhlet extraction. Following Soxhlet, internal recovery standards were added to the egg extracts. For TBBP-A the internal standard was ^{13}C-TBBP-A. For HBCD, the three diastereomers of ^{13}C-HBCD were used. Extracts were purified by gel permeation chromatography (GPC) and silica column. NERI provided lipid content data for the samples.

The purified egg extracts were measured in three series by liquid chromatography/mass spectrometry (LC/MS) and LC/MS/MS as well as gas chromatography/mass spectrometry (GC/MS). Each series included the egg samples (one per series in duplicate), two blanks, internal reference material samples (eel and sediment), and a recovery standard. Quantification was performed using external standards of TBBP-A and HBCD diastereomers. Detection limits for the LC/MS method are under 10 ng/g wet weight. However, for the convenience of comparison to GC/MS and literature values based on lipid weight, the detection limits for LC/MS data are reported here in units of ng/g lipid weight.

Quality Control
The method performance was assessed through internal quality control, which included new calibration curves, procedural blanks, internal standards and internal reference materials (eel and sediment) for each sample batch. The method has been tested by participation in an interlaboratory test in collaboration with QUASIMEME, with good results [De Boer *et al*. 2002].

3. Results and Discussion

TBBP-A

In all of the peregrine falcon egg samples from Greenland, TBBP-A was not detected with the LC/MS or LC/MS/MS analytical methods. The limits of detection are given in Table 1. A flame retardant with the largest production volume of all BFRs, TBBP-A is used extensively in Europe, America, and Asia [BSEF website]. Nevertheless, the undetectable levels of TBBP-A in eggs may result from a lack of exposure in the Greenlandic populations or low biomagnification. Two other possibilities for the non-detectable levels of TBBP-A are i) a low rate of maternal transfer of the compound to the eggs or ii) an efficient metabolisation of TBBP-A. The former was observed in quail after an oral dosage of TBBP-A to laying birds [Halldin *et al.* 2001]. The radiolabelled TBBP-A administered was also readily excreted resulting in low body residues after nine days [Halldin *et al.* 2001].

In several Greenlandic egg samples, a possible metabolite of TBBP-A, dimethyltetrabromo-bisphenyl-A (Me-TBBP-A), was detected (see Appendix). Me-TBBP-A is more hydrophobic than the parent compound and thus can be expected to bioaccumulate more effectively [Hakk & Letcher 2003]. Published data on Me-TBBP-A body residues are rare, although a Japanese study in the 1980's reported that while no TBBP-A was found in the tissues of mussels, the metabolite Me-TBBP-A was present [Wantanabe, *et al*. 1983]. The authors suggested that the metabolism was microbial, although the metabolism by macroinvertebrates or other animal species cannot be ruled out. Other flame retardant peaks, such as BDEs previously measured by NERI, were also present in the chromatograms.

HBCD

Individual HBCD diastereomers were not detected in the peregrine falcon egg samples with LC/MS or LC/MS/MS, but total HBCD was detected in over half of the egg samples with GC/MS (see Appendix). The highest HBCD concentration was detected in an egg sample collected in 1990 at Skyggesø (230 ng/g lipid). The range of HBCD concentrations in this study is somewhat lower compared to the findings of a recent egg study in Sweden, where HBCD concentrations ranged from <4 to 2400 ng/g lipid in Swedish peregrine falcon eggs [Lindberg *et al.* 2003]. Sellström *et al.* [2003] detected HBCD concentrations in Swedish guillemot eggs between 34 and 300 ng/g lipid in a study of eggs collected over the past two decades. They found large variation between birds, and that concentrations were generally higher during the 1990's than in earlier samples.

Most of the Greenlandic peregrine falcon eggs were collected in this period, although the contamination patterns in Greenland may be different than in the Baltic Sea area, particularly if Greenland is more under the influence of North American sources where HBCD is used significantly less than in Europe [BSEF website].

4. Conclusion

The low or undetectable residues of both HBCD and TBBP-A in most of the egg samples suggest that there should be low risk of adverse effects on the growth and development of peregrine falcon chicks and falcon populations due to presence of HBCD or TBBP-A. However in several egg samples, a possible metabolite of TBBP-A was found to be contributing to the internal BFR concentrations. To our knowledge, such results have not yet been reported for avian species. If the Me-TBBP-A is a persistent metabolite of TBBP-A, there could be implications for the risk assessment of the parent compound.

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Appendix RIVO report C007/03

Concentrations (ng/g lipid) of flame retardants TBBP-A, HBCD and Me-TBBP-A measured in peregrine falcon eggs.
HBCD diastereomers and TBBP-A measured with LC/MS.
Total HBCD and Me-BBP-A measured with GC/MS.

No.	year	location	NERI code	RIVO code	% l.w.	d.w. (%)	HBCD total	α–HBCD	β-HBCD	γ-HBCD	TBBP-A	Me-TBBP-A
1	1986	Igaliko	01-1593	1621	6,96	19,40	<0.8	<45	<11	<13	<16	49
2	1987	Igaliko	01-1608	1622	9,41	22,02	34	<49	<13	<14	<17	120
3	1990	Igaliko	01-1623	1623	4,85	17,36	9	<101	<27	<29	<35	160
4	1990	Skyggesø	01-1619	1624	6,26	20,92	4,1	<48	<13	<14	<18	12
5	1991	Kirkeruin	01-1598	1625	7,52	18,40	<1.1	<52	<13	<15	<19	15
6	1992	Skyggesø	01-1621	1626	6,00	15,89	<1.1	<70	<18	<20	<25	750
7	1994	Havnen	01-1632	1627	6,78	18,35	2,1	<46	<12	<13	<16	270
8	1995	Igaliko	01-1603	1628	6,54	19,53	<0.8	<57	<15	<17	<19	440
9	1998	Upernaviarsuk	01-1613	1629	7,62	21,61	<0.8	<47	<12	<14	<14	28
10	1999	Upernaviarsuk	01-1614	1630	6,64	17,98	<0.9	<41	<10	<12	<18	520
11	2001	Egaluit	01-1645	1631	6,40	18,62	<0.8	<50	<14	<14	<17	360
12	1989	Skyggesø	01-1617	1632	10,69	22,42	7,8	<47	<13	<14	<58	<0.1
13	1991	Bagerfalken	01-1611	1633	6,84	18,97	10	>83	<21	<24	<29	<0.1
14	1991	Hosp.dal	01-1629	1634	5,15	17,45	22	<118	<31	<33	<41	940
15	1992	Eqaluit	01-1601	1635	9,31	18,25	1,2	<62	<16	<18	<21	<0.1
16	1994	Eqaluit	01-1602	1636	7,48	22,36	2,4	<76	<20	<21	<27	<0.1
17	1995	Lejren	02-1785	1637	6,30	18,96	3,2	<87	<22	<25	<30	2
18	1998	Sdr. Igaliko	01-1604	1638	5,80	34,20	2,6	<100	<26	<29	<34	24
19	1999	Lejren	01-1626	1639	6,02	17,26	<1.2	<93	<25	<27	<33	0,4
20	2000	Upernaviarsuk	01-1615	1640	3,94	13,65	2,7	<132	<36	<38	<46	<0.1
21	2002	Qanisartut	02-1787-1	1641	5,27	15,91	<1.0	<104	<27	<30	<36	1,7
22	n.a.	n.a.	02-1790*	1643	n.a.	n.a.	0,2	<5	<1.3	<1.4	<1.8	0,2
23	2002	Enoraq	02-1788	1642	6,87	20,05	1,6	<87	<23	<25	<31	0,9
24	1990	Skyggesø	01-1618	1644	7,27	21,14	230	<84	<22	<25	<30	400
25	1992	Skyggesø	01-1622	1645	5,63	15,58	32	<103	<27	<30	<36	480
26	1992	Skyggesø	01-1620	1646	6,84	17,74	26	<82	<22	<23	<29	430
27	1994	Havnen	01-1630	1647	6,74	17,97	77	<88	<24	<25	<31	230
28	1994	Havnen	01-1631	1648	7,92	20,85	67	<77	<20	<21	<27	240
29	1994	Havnen	01-1633	1649	5,73	15,54	<0.1	<105	<28	<30	<37	270
30	1999	Upernaviarsuk	02-1789-1	1650	3,98	16,04	<0.1	<150	<40	<43	<53	905
31	2000	Sdr. Igaliko	01-1607	1651	3,58	13,34	14	<162	<42	<47	<56	700
32	2000	Upernaviarsuk	01-1616	1652	4,63	17,54	<0.1	<125	<32	<37	<45	900
33	2003	Skyggesø	03-0542-1	1653	6,04	18,18	<0.1	<99	<26	<28	<35	290
34	2003	Enoraq	03-0543	1654	7,02	20,38	27	<85	<23	<24	<30	760

n.a. = not available

*This sample, concentrations expressed as ng/g wet weight