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Mass balance study of brominated flame retardants in female captive peregrine falcons†

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Little is known about brominated flame retardant (BFR) dynamics in birds, especially large molecules such as decabromodiphenyl ether (BDE-209). In particular, bioaccumulation from food and transfer dynamics to eggs are poorly understood. Therefore, an input–output mass balance study of tri–decaBDEs, DBDPE and HBCDD was performed in three female peregrine falcons from a captive breeding program by analyzing their naturally contaminated food (quail, chicken (cockerels)), plasma, feces and eggs. Predominant BFRs in cockerels and quail were BDE-209 and DBDPE, as well as HBCDD in quail. The predominant BFRs found in falcon plasma were BDE-209, -153 and -183, in eggs, HBCDD, BDE-209 and -153 and in feces, BDE-209. Mean absorption efficiencies (AE) for the tetra-octabrominated BDEs ranged from 84–100% and 70% for HBCDD. The AEs for BDE-206, -207, -208 and -209 varied due to the large variability seen for feces fluxes. All egg/plasma ratios for BDEs were similar and greater than one (range 1.1–2.7), including for BDE-209, indicating efficient transfer from females to the eggs. Excretion *via* egg-laying was approximately 6.0–29% of the initial, pre-breeding body burden of individual penta–decaBDE congeners, (15–45% for BDE-206). HBCDD was not detected in plasma but was found in eggs, also indicating efficient transfer and excretion *via* eggs. Input fluxes from food exceeded the output fluxes (feces, eggs) indicating considerable metabolism for tetra–octaBDEs, possibly also for the nona–decaBDEs and HBCDD. Bioaccumulation factors calculated from lipid weight concentrations in plasma and food (BAF$_p$) were highest for BDE-208 (31), -153 (23), -209 (19) and -207 (16) and from eggs and food (BAF$_e$), were highest for HBCDD (140), BDE-153 (41), -208 (42), -207 (24) and -209 (21). BAF$_e$ and BAF$_p$ values were below 10 for BDE-47, -99 and -100. For one falcon, egg results were available from three different years and estimated half-lives were 65 d (BDE-99), 624 d (BDE-153), 31 d (BDE-154), 349 d (BDE-183), 77 d (BDE-196) and 89 d (BDE-197).

Environmental significance

Results from mass balance studies help in understanding the dynamics of organic contaminants in living organisms. Very few mass balance studies of brominated flame retardants have been carried out, particularly in birds. High concentrations of penta- and hexaBDEs, as well as decabromodiphenyl ether (BDE-209) and hexabromocyclododecane (HBCDD) have been found in wild peregrine falcon eggs. It is not clear if concentrations of higher brominated BFRs in eggs reflect body burden and it is therefore important to understand the extent that BFRs bioaccumulate from food, metabolize, are excreted in feces and transfered to eggs, particularly in high trophic level birds of prey. Such understanding of how dietary exposure translates to body burdens and egg concentrations helps in interpreting monitoring data from wild birds, which are often based on data from eggs. Using captive peregrine falcons as a surrogate for wild peregrine falcons also provides such data for a species that has high exposure to BFRs, where correlations have been seen between BDE concentrations and reproductive effects and that has previously been endangered due to biomagnification of other organic contaminants.

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Introduction

The polybrominated diphenyl ether (PBDE) technical products pentaBDE, octaBDE, decaBDE, hexabromocyclododecane (HBCDD) and decabromodiphenyl ethane (DBDPE) are additive brominated flame retardants (BFRs) used in textiles and polymers. PBDEs and HBCDD cause effects on neurobehavioral development, reproduction and the thyroid system in laboratory animals and associations with PBDEs have been seen for similar effects in humans.[1–20] Due to the increasing concerns about the effects of PBDEs on the environment and human health, the penta- and octaBDE technical products, containing tri–octaBDEs, were banned within the EU in 2004 [21] and globally within the Stockholm Convention on Persistent Organic Pollutants (POPs) in 2009.[22] In 2008, the decaBDE mixture, containing nona–decaBDEs was banned for use in electronic and electrical equipment in the EU,[23] major producers in the US discontinued decaBDE mixture production and use at the end of 2013 [24] and it was listed on the Stockholm Convention in 2017 (http://chm.pops.int/). HBCDD was added to the Stockholm Convention on POPs in 2014. DBDPE is currently not regulated and is marketed as a replacement for the decaBDE mixture.

In Sweden, tri–decaBDE congeners and HBCDD have been found in wild peregrine falcon (*Falco peregrinus*) eggs as well as in a captive breeding population, although in lower concentrations.[25,26] Environmentally relevant concentrations of PBDE and HBCDD are associated with immunotoxic, thyroid, reproductive effects and eggshell thinning in laboratory studies of American kestrels (*Falco spaverius*)[27–35] and zebra finches (*Taeniopygia guttata*).[36–38] A significant negative correlation between BFR concentrations in eggs and reproduction has been seen in the wild Swedish peregrine falcons.[26] A recent review of numerous studies of toxicological endpoints in many different bird species found raptors to be the most sensitive to flame retardant exposure.[39]

The presence of tri–decaBDE congeners and HBCDD in wild and captive Swedish falcon eggs implies exposure of adults *via* their diet, uptake from the gastrointestinal tract, accumulation and transfer to the eggs. DBDPE has not been studied in Swedish falcon eggs previously. Due to the differences in their hydrophobicity and molecular size, different BDE congeners have different uptake, excretion and accumulation rates, as seen in mass balance studies of rats and lactating cows.[40–42] In the closely-related American kestrel, the half-lives of BDE-47, -99, -100 and -153 have been determined to be 72, 175, 178 and 572 days, respectively,[43] which were comparable to half-lives of 100 days for BDE-99, -100 and 153 estimated for herring gulls (*Larus argentatus*).[44] The long half-lives of these BDE congeners in birds indicate that they could be taken up and accumulated in peregrine falcons as well. However, in starlings and kestrels, the half-life of BDE-209 was determined to be only 13–14 days,[45,46] possibly due to a higher rate of metabolism for this congener. A similar short half-life for BDE-209 has been seen in humans as well.[47] In a recent study on kestrels, the half-life for HBCDD was found to be 14 days.[48] For BDE-209 in

particular, but for other BFRs as well, there are very few studies in birds of bioaccumulation from food or of transfer dynamics to eggs and none have studied the entire process of intake, accumulation, and excretion. To address this, an input–output mass balance study of tri–decaBDEs, HBCDD and DBDPE in captive female peregrine falcons was carried out to determine uptake and accumulation *via* the gastrointestinal tract, distribution to blood and excretion *via* eggs and feces.

Experimental section

Sampling

A captive breeding population of peregrine falcons was maintained at Nordens Ark (Hunnebostrand, Sweden) within the auspices of a Swedish Society for Nature Conservation falcon reintroduction project, with permission of the Swedish Environmental Protection Agency. The three females sampled in this study were hatched in captivity and information about them and the eggs collected are given in Table S1 in the ESI.† All experimental work including sampling of the peregrine falcons was performed in compliance with relevant Swedish Regulations and Guidelines on Laboratory Animals (Djurskyddsmyndighetens föreskrifter och allmänna råd (DFS 2004:4) om djurförsök m.m.) and was approved by the Swedish Animal Ethics Agency (Djurskyddsmyndigheten Permit Dnr 13-2005). Paired males and females were housed in separate outdoor cages with perches. The pairs were fed a diet predominated by commercially obtained one-day-old cockerels (*Gallus gallus*) (75% of diet) and quail (*Coturnix coturnix*) (16%), with a small proportion of white mice (9%) given sporadically. Blood samples were collected from the female peregrine falcons by a veterinarian after light carbon dioxide anesthesia during an ordinary health check on 16 February 2006, before the start of the breeding season. Blood was collected from the wing vein using a 2 mL syringe coated with liquid heparin. Immediately after drawing the blood it was transferred to a 5 mL Vacutainer tube to prevent clotting. A field blank of 5 mL NaCl (0.9%) was also prepared and treated identically with the plasma samples. The blood samples were centrifuged, the plasma removed and stored frozen at −20 °C until analysis. Six dead cockerels (mean weight 13.9 g each) and five dead quail (mean weight 91.8 g each) were collected at the same time as the blood samples were taken and stored frozen at −20 °C until homogenization. Feces samples were scraped off the metal plating on the cage walls and placed in brown tinted glass jars previously washed and burned at 450 °C and originated from both the male and the female sharing a cage. A small sample of the metal plating was taken as well. The females laid eggs in mid-May 2006 and unfertilised eggs were collected on June 6, 2006. Egg contents were removed immediately after collection and stored in tinted glass jars at −20 °C.

Falcons at Nordens Ark swallow day-old cockerels whole but are fed quail where the legs have been removed. The falcons often remove the quail gastrointestinal tract before feeding. For chemical analysis, cockerels were thawed, plucked and the legs removed. The quail were thawed, plucked, and the legs, wings and gastrointestinal tract removed. The six cockerels were

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ground together in a stainless steel meat grinder and then homogenized with an ultra turrax homogenizer. The five quail were likewise combined, ground and homogenized. Three subsamples of each homogenate were removed and stored frozen at −20 °C until analysis.

Chemicals

Dichloromethane (DCM), *n*-hexane (both LiChrosolv), iso-propanol (LiChrosolv), acetone (Suprasolv), hydrochloric acid (HCl, 37% fuming, pro analysis), phosphoric acid (pro analysis) and silica gel 60 (0.063–0.200 mm) were obtained from Merck (Darmstadt, Germany). Diethyl ether (DEE) and iso-octane (both HPLC grade) were from LabScan (Gliwice, Poland). Potassium chloride (KCl, reagent grade) was from Scharlau Chemie S.A. (Barcelona, Spain), sodium chloride (NaCl, Anala R Normapur) from VWR Prolabo (Haasrode, Belgium), and sulfuric acid (H$_2$SO$_4$, 95–97%, pro analysis) from Fluka Chemie, Buchs, Switzerland.

Single congeners of BDEs -28, -47, -99, -100, -153, -154, -183, and -209 (99% purity, Cambridge Isotope Laboratories (CIL), Andover, MA), BDE-203, -205 (97–100% purity, AccuStandard, New Haven, CT, USA), BDE-184, -191, -196, -197, -206, -207 and decabromodiphenyl ethane (DBDPE) (>98% purity, Wellington Laboratories, Guelph, ON, Canada) and technical HBCDD (95.5 ± 0.5% purity, Dr Ehrenstorfer, Augsburg, Germany) were used as reference standards. As surrogate standards for the PBDEs, BDE-138 (tetra- to heptaBDEs), and ^{13}C$_{12}$-BDE-209 (octa- to decaBDEs and DBDPE, both 99%, from CIL) were used. For HBCDD, Dechlorane 603 (Occidental Chemicals, previously Hooker Chemical, Dallas, TX, USA) was used as surrogate standard. ^{13}C$_{12}$-heptachlorobiphenyl (CB)-180 (>98% from CIL) was used as recovery standard. Reference standards were prepared in iso-octane at 5–11 levels covering the concentration ranges of interest.

Extraction and cleanup

Surrogate standards (100 μL in iso-octane) were added to the samples which were allowed to equilibrate overnight. Peregrine falcon egg homogenates, peregrine falcon feces, cockerel and quail homogenates (approximately 5 g wet weight) were liquid–liquid extracted according to Jensen *et al.*[49] The extraction was done in 50 mL centrifuge tubes, using centrifugation instead of filtering for separation. Plasma samples were extracted according to the method described by Hovander *et al.*[50] Solvent amounts were reduced to half of the volumes described in that method due to the smaller amount of sample in this study. Lipid determination was done gravimetrically and extracted lipids were then re-dissolved in iso-octane.

All the samples were treated first with approximately 10 mL of concentrated sulfuric acid and then centrifuged for 10 min, at 200 rpm. The sample volume was reduced to 0.5 mL and then cleaned-up on an acidified silica column (0.5 g, SiO$_2$: H$_2$SO$_4$, 2 : 1). The column was washed with 10 mL *n*-hexane and the sample then eluted with 10 mL of *n*-hexane. This clean-up was sufficient for the egg samples but not for the cockerel, quail, feces and plasma samples so these were subjected to further clean-up on a silica column (1 g, deactivated with 2% water). After a 4 mL pre-fraction (*n*-hexane, discarded), the analytes were eluted with 13 mL of DCM (collected). A recovery standard was added prior to the analysis and the sample volumes adjusted to 100 μL.

GC-MS analysis

Analysis was done by gas chromatography – mass spectrometry (GC-MS). The system used was a Trace GC Ultra coupled to a DSQ II MS (both Thermo Scientific, Waltham, USA). Initially a split–splitless (SSL) injector (held at 280 °C, constant flow, closed for 1.5 min after injection) and later a programmable temperature vaporiser (PTV) injector was used. For PTV conditions, see Sahlström *et al.*[51]

The GC columns were DB-5MS fused-silica (J&W Scientific, Folsom, CA, USA) with helium (purity 4.6, Aga, Lidingö, Sweden) as the carrier gas. For octa- to decaBDEs and DBDPE a shorter column (15 m, 0.25 mm i.d., 0.1 μm film thickness) was used in order to minimize thermal degradation of the higher brominated compounds. The oven temperature program started at 80 °C (held for 2 min), then increased by 20 °C min^{-1} to 200 °C followed by 6 °C min^{-1} to 315 °C, which was held for 5 min. For tetra–heptaBDEs and HBCDD, a longer column (30 m, 0.25 mm i.d., 0.25 μm film thickness) was used to achieve better chromatographic separation. The oven temperature program started at 80 °C (held for 2 min), then increased with 25 °C min^{-1} to 200 °C, followed by 4 °C min^{-1} to 315 °C, which was held for 40 min.

With this method the HBCDDs elute in one peak and only the total HBCDD could be determined. Previous analyses with LC-MS showed that only the α-HBCDD congener was present in Swedish peregrine falcon eggs.[52]

The MS was run in electron capture ionisation mode measuring the negative ions formed (ECNI). Ammonia (purity 5.0, Aga, Sweden) was used as moderating gas. The electron energy was 70 eV and ion source temperature 180 °C. Detailed information on instrumental settings can be found in Johansson *et al.*[26] The MS was operated in selected ion monitoring (SIM) mode recording the bromide ions (*m/z* −78.9 and −80.9) for BDE-28, -35, -47, -49, -66, -77, -85, -99, -100, -138, -153, -154,-173, -183, -184, -191, -196, -197, -203, -205, HBCDD and DBDPE. Phenoxide fragment ions with 4 and 5 bromine atoms were recorded for octa- to decaBDEs: *m/z* −409 for native BDE-196 and -197, *m/z* −484.6 and 486.6 for native BDE-206, -207, -208 and -209. For ^{13}C-labelled octa- to decaBDEs, the *m/z* −415, −494.6 and −496.6 ions were recorded, and Dechlorane 603 was measured using the *m/z* −236.7 and −238.7 ions. The quantification was performed with XCalibur 2.0.7 (Thermo Finnigan, San Jose, CA, USA).

Quality assurance

UV-light protection was mounted on all light fixtures and windows in the laboratory to minimize degradation of BDE-209. Brown glassware was used when possible; otherwise glassware was covered with aluminum foil. Glassware was heated to 450 °C overnight before use. On every extraction occasion, one

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laboratory blank and one in-house lab reference material (LRM) sample were extracted in parallel. The LRM samples were aliquots of a large salmon muscle homogenate used as a QA/QC sample for analyses within the national environmental monitoring program performed at Stockholm University. The average lipid percent in the LRM samples ($n = 3$) was 13.5% with a relative standard deviation of 1%. Only BDE-28, -47, -49, -66, -99, -100, -153, -154 and -183, as well as DBDPE and HBCDD were detected in the LRM samples in concentrations above the limit of detection (LOD)/limit of quantitation (LOQ) (relative standard deviations 4–15% for all except BDE-183 (32%)).

A chromatographic peak was considered quantifiable when the signal-to-noise ratio was ≥5. For compounds present in the blanks, the LOD and LOQ were set as the average blank value +3 and +5 times the standard deviation, respectively.

GC-MS analysis was performed, mixing samples and calibration standards randomly. Samples were quantified using ≥5 point calibration curves. Compounds were positively identified if the mass isotope ratio was correct and the relative retention time (versus the surrogate standard) differed no more than 0.005 compared to the calibration standards.

Small amounts of BDE-209 are degraded to octa- and nonaBDEs during sample processing/analysis. These amounts are often so small that they do not notably influence the quantification of BDE-209. The quantification of octa- and nonaBDEs can however be affected since these most often/always are present in the samples at much lower concentrations than BDE-209. The extent of the degradation was determined by measuring the amounts of ^{13}C-octa- and nonaBDEs that were formed from the ^{13}C-BDE-209 that was added to all samples as a surrogate standard, assuming that the native and the ^{13}C-labelled BDE-209 are degraded equally.

In this study, corrections for degradation of BDE-209 were necessary for BDE-197, -206, -207 and -208. The corrections were performed for each sample individually with the actual degradation percentage of BDE-209 in that sample using relative peak areas. The degradation of BDE-209 into octa- and nonaBDEs was on average 0.71% (BDE-197), 0.37% (BDE-206), 1.6% (BDE-207) and 0.88% (BDE-208). When the peregrine falcon egg samples were analysed, the phenoxide ions for BDE-197 were not monitored and because of this, the average degradation of BDE-197 in the other samples (0.71%) was used for correction.

[13]C$_{12}$-CB-180 was added to the samples before the analysis in order to evaluate the absolute recoveries of the surrogate standards. The average ± SD recoveries were 69 ± 7% (BDE-138), 77 ± 7% ([13]C$_{12}$-BDE-209), and 65 ± 5% (dechlorane) (Table S2, ESI†). The higher absolute recoveries in the blanks were due to the absence of a sample matrix and do not affect the relative recoveries. The relative recoveries of the analytes versus the surrogate standards were determined as described in the ESI† and were 88–109% for the PBDEs and 121% for HBCDD (Table S3, ESI†). No corrections for recoveries were made in the final results.

Calculations for the mass balance

In the following calculations, if analyte values were below the LOD in a matrix, the value was replaced by zero. Values that were above the LOD but below the LOQ, were replaced by the LOQ divided by the square root of 2.[53]

The falcons were assumed to be in steady state based on their constant, long-term diet with low PBDE contamination. Concentrations of analytes in plasma lipids were also assumed to be in equilibrium with other body lipids. Total body lipid content was not available for peregrine falcons, but has been quantified in the closely-related American kestrel to be 10.8% so this value was used in the calculations.[27] The total body burden in each falcon before breeding was calculated using the lipid weight concentrations of each analyte found in plasma multiplied by the estimated total body lipid content per kg body weight. The body burdens should thus be considered best estimates.

Input fluxes were calculated as the amount (g) of food ingested per day multiplied by the mean analyte concentrations found in the triplicate cockerel and quail homogenates weighted for the average number and body weight of each food type eaten daily over the three month period prior to blood sampling (see ESI† for details of calculations). As no analytical data were available for mice, a weighted average concentration based on the proportions of cockerels and quail ingested was used. These data were obtained from feeding diaries kept on the individual falcon pairs at Nordens Ark. As food was given to the breeding pair simultaneously, the female was assumed to eat half of each food type given.

Fecal excretion fluxes were calculated using the fecal output per day multiplied by the concentration of each analyte found in feces. The BFR concentrations in feces were assumed to be representative for the female, as the males were also hatched in captivity and fed the same diet. Excretion of BFRs via pellet egestion (consisting of feathers, bones) was considered negligible. As fecal output was not possible to measure in this study, it was estimated using data from the literature (6.5 g dw per kg body weight and day) as described in the ESI.† Egg-laying is also an excretion route for contaminants, and for the falcons, egg-laying occurred three months after the initial sampling was performed. Previous studies in peregrine falcons showed no statistically significant differences in organochlorine concentrations[54,55] or in PBDE concentrations[56,57] between eggs in the same clutch. Therefore, excretion via egg-laying was calculated from the weight of the egg contents multiplied by the concentrations found in the egg and the number of eggs produced by each female in 2006 (three each for two females, two clutches of four for one female). The amounts excreted were converted to fluxes (ng day^{-1}) by dividing the total amount excreted in all eggs for each female by 365 days. When a specific analyte was quantifiable for all matrices, an input–output mass balance for the pre-breeding time point was calculated for each female peregrine falcon using the input fluxes from diet (cockerels, quail, mice), calculated body burdens and the output fluxes from feces.

The absorption efficiency (AE) from the gut for each falcon was calculated as the fraction of each analyte ingested that was not excreted in feces:

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$$AE = 1 - \left(\frac{\text{feces flux}}{\text{input flux}}\right) \quad (1)$$

Bioaccumulation factors (BAFs) for the different analytes were calculated as the ratios between lipid weight concentrations in plasma and food (BAF_p) or eggs and food (BAF_e). The lipid weight concentrations of each analyte in food were weighted averages calculated based on the weighted average dietary intake of cockerels, quail and mice. Transfer to eggs was calculated as the ratio between egg concentrations and plasma concentrations (E/P) on a lipid-weight basis. Metabolic rate constants (MRCs) for individual analytes were estimated as the metabolism flux (total input flux minus feces flux) divided by the estimated body burden for each falcon.

Results

Concentrations of BFRs

Lipid weight (lw) concentrations of individual BDE congeners, HBCDD and DBDPE in the different matrices from each of the three peregrine falcons and in the triplicate homogenates of cockerels and quail are given in Table 1. Fresh weight concentrations are given in Table S4 (ESI†). The predominant BFRs in cockerels and quail were BDE-209 (means of 0.28 and 0.32 ng g^{-1} lw, respectively) and DBDPE (means of 2.8 and 0.72 ng g^{-1} lw, respectively) as well as HBCDD in quail (0.2 ng g^{-1} lw) (Table 1 and Fig. S1–S3 (ESI†)). The quail also contained low concentrations of BDE-47, -49, -99, -100, -183, -197, -206, -207 and -208. The cockerels contained low concentrations of BDE-47, -99, -100, -153, -183, -196, -197, -206, -207 and -208, but HBCDD was not detected, and the concentrations for each BDE congener were 5–10 times higher than in quail.

Concentrations were below the detection limit (LOD) in all falcon samples for BDE-28, -66, -77, -85, -173, -184, -191 and -205. BDE-35 was detected in one falcon egg sample (0.019 ng g^{-1} lw). BDE-154 (which co-elutes with BB-153) was found in falcon plasma and eggs and BDE-203 in two falcon eggs. BDE-99, -153, -183, -197, -207, -208, and -209 were detected in most sample types (Table 1). Other BFRs that were detected in some of the sample types were BDE-47, -100, -196, -206, and HBCDD. DBDPE was below the detection limits in all falcon samples.

The predominant BFRs in falcon plasma were BDE-209 (4.1–6.2 ng g^{-1} lw), followed by BDE-153 (1.3–3.0 ng g^{-1} lw) and −183 (0.71–1.1 ng g^{-1} lw) (Table 1 and Fig. S1–S3 (ESI†)). In eggs, the predominant BFRs were HBCDD (5.9–15 ng g^{-1} lw) followed by BDE-209 (5.2–6.3 ng g^{-1} lw) and −153 (2.3–6.2 ng g^{-1} lw). BDE-209 was the predominant BFR in feces (4.7–21 ng g^{-1} lw). Indications of DBDPE were found in one falcon feces sample but due to high background concentrations from the metal plating itself, it was not possible to quantify DBDPE in the other feces samples. When present, the concentration of each individual BDE congener, as well as HBCDD, was found to be similar in each of the three peregrine falcon's plasma as well as in eggs, usually within a factor of 2–3 (Table 1). However, for

feces this was only true for tetra–octaBDEs. For BDE-206, -207, -208 and -209, the feces concentrations varied 20- to 40-fold (dry weight basis) or 4- to 7-fold (lipid weight basis) between the individual falcons, or in the case of falcon 466, between two separate feces samples (Tables 1 and S4†). HBCDD was only detected in one of the four feces samples.

The congener profiles for major components of tetra-hexa, octa- and nonaBDE congeners found in falcon food, plasma, eggs and feces are compared to profiles of these congeners in the penta- (Bromkal 70-5DE), octa- (Bromkal 79-8DE) and dec-aBDE (Bromkal 82-0DE, Saytex 102E) technical mixtures[59] in Fig. 1. For pentaBDE congeners, proportions of BDE-153 increase and BDE-47 and -99 decrease going from food to plasma and eggs (Fig. 1a), and when compared to the technical product. For octaBDE congeners, the proportion of BDE-196 increases and BDE-197 decreases going from technical product to food and plasma (Fig. 1b). Feces have the highest proportion of BDE-196. For the nonaBDEs, the proportion of BDE-206 decreases and BDE-208 increases from food to plasma and eggs, and this is quite pronounced compared to the technical decaBDE products (Fig. 1c). In general, food and feces patterns were more similar to each other, and plasma and egg patterns were more similar to each other.

Mass balance results

Calculated input–output fluxes and body burdens for individual analytes in each peregrine falcon as well as the means are presented in Table 2. Due to the large differences in fecal concentrations of BDE-206, -207, -208 and -209, the fecal excretion fluxes were more variable, and in some cases (falcon 398, falcon 466, feces 1) were larger than the input fluxes. Excretion *via* egg-laying was approximately 6.0–29% of the initial, pre-breeding body burden of individual penta–decaBDE congeners, (15–45% for BDE-206). As the concentrations of HBCDD in plasma samples were below the LOD of 1 ng g^{-1} lw, it was not possible to estimate excretion *via* egg-laying. When compared to mean excretion *via* feces in ng d^{-1} (Table 2), mean excretion in eggs was more important than fecal excretion for BDE-197, -154, -153 and HBCDD (feces flux/egg flux ratios below 1). Both fluxes were similar for BDE-99. Fecal excretion was more important for BDE-47, -100, -183, and -196 (1.8–7.6 times higher feces flux/egg flux ratios). Fecal excretion may play a role for nona–decaBDEs (15–100 times higher feces flux/egg flux ratios, Table 2), but due to the high variability in concentrations found for these BDEs, this should be viewed with some caution.

The full mass balance could be calculated for BDE-99, -153 (only cockerel intake), -183, -196, -197, -206, -207, -208 and -209. Partial mass balances could be calculated for BDE-47, -100, -154/BB-153 and HBCDD. For BDE-47, -99, -100, -153, -183, -196 and -197, the input fluxes exceeded the output fluxes, but for BDE-206, -207, -208 and -209, this varied. For falcon 223 and 466 (feces sample 2), input exceeded output, but for falcon 398 and 466 (feces sample 1), output exceeded input for these congeners.

The mean results for AEs for the three falcons are given in Table 3. The tetra–octabrominated BDEs have high mean AEs

Table 1 Concentrations of individual BDE congeners, HBCDD and DBDPE (ng g^{-1} lipid weight) in plasma, feces and eggs of three female peregrine falcons and means for triplicate homogenates of their food (chicken cockerels, quail). Concentrations below the LOQ but above the LOD are given as a *range (in italics)*. Concentrations below the LOD are given as *< values in italics*

BDE congener group	Sample type	Lipid %	Tetra BDE-47	Tetra BDE-49	Penta BDE-99	Penta BDE-100	Hexa BDE-153	Hexa BDE-154[a]	Hepta BDE-183	Octa BDE-196	Octa BDE-197	Octa BDE-203	Nona BDE-206	Nona BDE-207	Nona BDE-208[b]	Deca BDE-209	DBDPE	HBCDD
Chicken (mean, n = 3)	Food	8.3	0.071	0.0034	0.16	0.045	0.12	*<0.005*	0.17	0.026	0.062	*<0.04*	0.014	0.029	0.011	0.28	2.6	*<0.08*
Range		7.45–9.79	*0.055–0.082*	*<0.002–0.0034*	*0.13–0.18*	*0.037–0.049*	*0.10–0.13*	*<0.004–0.006*	*0.14–0.18*	*0.024–0.028*	*0.052–0.072*	*<0.03–0.04*	*<0.003–0.015*	*0.019–0.036*	*0.0064–0.012*	*0.18–0.31*	*2.1–3.7*	*<0.07–0.08*
Quail (mean, n = 3)	Food	10.4	0.014	0.0041	0.026	0.005	*<0.01*	*<0.004*	0.0087	*<0.006*	0.002	*<0.03*	0.004	0.015	0.0042	0.32	0.57	0.20
Range		10.3–10.5	*0.013–0.016*	*0.0035–0.0046*	*0.025–0.027*	*0.0039–0.0056*	*<0.01–0.01*	*<0.004–0.005*	*<0.006–0.013*	*<0.004–0.007*	*0.0015–0.0037*	*<0.03–0.03*	*<0.003–0.0066*	*0.013–0.016*	*<0.003–0.0051*	*0.14–0.66*	*<0.3–1.2*	*0.20–0.20*
Falcon identification code																		
223	Plasma	1.01	*<0.7*	*<0.1*	*0.2–0.7*	*<2*	3.0	0.80	1.1	*0.2–0.5*	0.30	*<2*	*0.02–0.05*	0.55	0.38	6.2	*<2*	*<1*
398	Plasma	1.17	*<0.5*	*<0.09*	*<0.4*	*<2*	2.4	0.53	1.1	*0.1–0.4*	0.28	*<1*	*0.007–0.02*	0.32	0.24	4.1	*<1*	*<1*
466	Plasma	1.41	*<0.5*	*<0.08*	0.70	*<2*	1.3	0.41	0.71	*0.1–0.4*	0.23	*<1*	*0.01–0.03*	0.41	0.29	5.7	*<1*	*<1*
223	Egg	5.64	0.091	*<0.006*	1.0	0.40	6.2	1.4	1.7	0.47	0.56	0.22	0.073	0.70	0.46	6.2	*<0.8*	15
398	Egg	4.95	*0.03–0.09*	*<0.007*	0.43	0.16	3.6	0.71	1.5	0.43	0.48	0.17	0.046	0.54	0.40	5.2	*0.3–1*	5.9
466	Egg	5.59	0.14	*<0.006*	1.0	0.35	2.3	0.65	0.84	0.32	0.36	*0.07–0.2*	0.055	0.67	0.38	6.3	*<0.8*	12
223	Feces	0.983	*0.1–0.4*	*<0.04*	*0.1–0.3*	0.071	0.44	*0.03–0.1*	0.21	0.14	0.047	*<0.5*	0.18	0.25	0.13	5.4	nd[c]	*<0.9*
398	Feces	5.64	*0.07–0.2*	*<0.02*	0.22	0.079	0.36	*0.02–0.07*	0.21	0.32	*<0.02*	*<0.3*	0.88	1.2	0.92	21	nd[c]	*<0.6*
466	Feces	4.27	*<0.4*	0.11	*<0.4*	0.070	0.32	*0.07–0.2*	0.34	*0.07–0.2*	*<0.04*	*<0.8*	0.71	0.93	0.57	19	2–7	3.2
466	Feces	2.19	*<0.3*	*<0.04*	*0.1–0.3*	0.072	0.32	*0.03–0.1*	0.15	0.16	0.043	*<0.5*	0.14	0.27	0.18	4.7	nd[c]	*<0.9*

[a] Quantified on peak height. Not baseline separated from other compound. [b] Estimated with BDE-206 and relative response factors. [c] Not detected due to high background concentrations from sampling trays.

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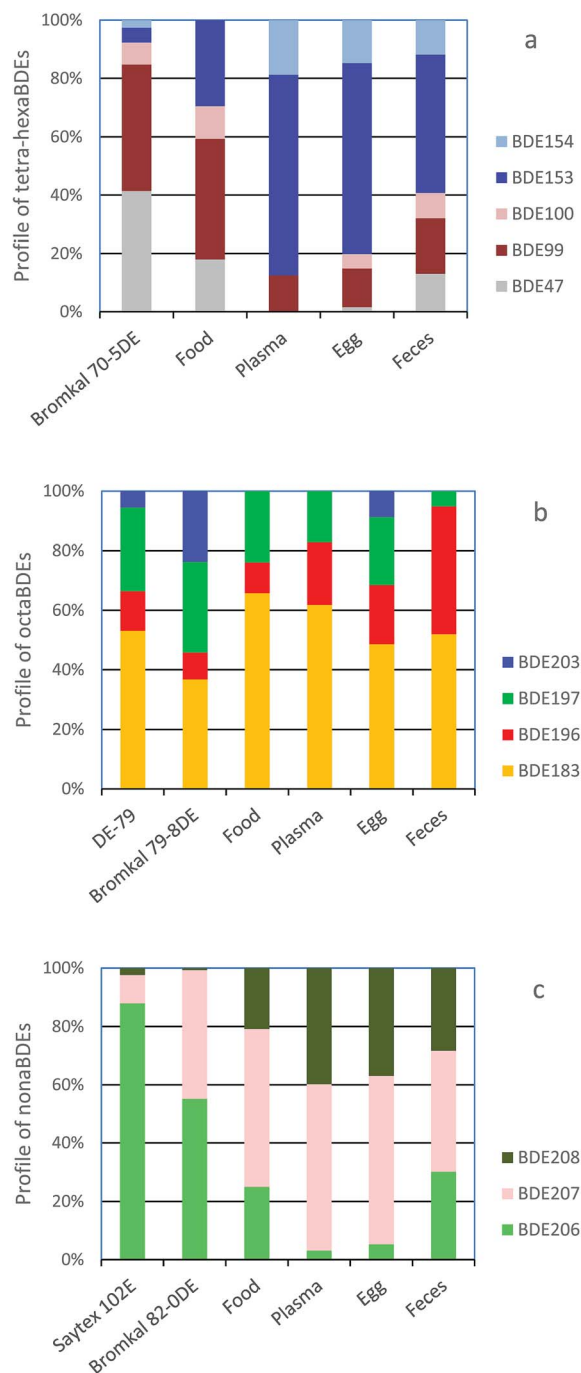This journal is © The Royal Society of Chemistry 2019

Fig. 1 Comparison of (a) tetra–hexaBDE, (b) octaBDE and (c) non-aBDEcongener profiles in peregrine falcon eggs with those of commercial penta-, octa- and decaBDE mixtures.[59]

ranging from 84–100%. HBCDD has a somewhat lower mean AE of 70%. The AEs for BDE-206, -207, -208 and -209 varied due to the large variability seen for feces fluxes. When using the two lower feces fluxes, the AEs were 84 and 94% for BDE-206, 87 and 96% for BDE-207, 76 and 94% for BDE-208 and 79 and 92% for BDE-209. However, when the two higher feces fluxes were used, these were −52 and −170%, 7 and −69%, −50 and −240% and −74 and −170% for BDE-206, BDE-207, BDE-208 and BDE-209, respectively.

The highest BAF_p values were found for BDE-208 (31), BDE-153 (23), BDE-209 (19) and BDE-207 (16) and highest BAF_e values were found for HBCDD (140), BDE-153 (41), BDE-208 (42), BDE-207 (24) and BDE-209 (21) (Table 4). Both BAF_e and BAF_p values were below 10 for BDE-47, -99 and -100. Where both BAF_e and BAF_p values were available, the BAF_e values were somewhat higher (up to 2 times) but generally the two values were similar (Table 4).

All egg/plasma ratios were similar and all were greater than one (range 1.1–2.7), including for BDE-209, indicating efficient transfer from females to the eggs (Table 3).

The mean MRC values for those analytes that could be calculated are presented in Table 3. As for AEs, the MRCs for BDE-206, -207, -208 and -209 showed large variability depending on the feces fluxes. When using the two lower feces fluxes, both MRC values were 0.05 d^{-1} for BDE-206, 0.006 d^{-1} for BDE-207, 0.003 d^{-1} for BDE-208, and 0.006 and 0.005 d^{-1} for BDE-209. However, when the two higher feces fluxes were used, these were −0.1 and −0.03 d^{-1}, −0.004 and −0.005 d^{-1}, −0.002 and −0.009 d^{-1} and −0.004 and −0.01 d^{-1} for BDE-206, -207, -208 and -209, respectively.

Discussion

When present, the concentrations of individual BDE congeners, as well as HBCDD, were similar in each of the three peregrine falcon's plasma and in the eggs (Table 1) reflecting similar exposure due to being fed the same diet. The most likely explanation for finding low concentrations of PBDEs and HBCDD in the cockerel and quail diet fed to the falcons is probably due to low levels present in the feed given to the hens and subsequent transfer from hens to eggs. The source of these contaminants into feed may possibly be from air deposition to plants[41,42] and the use of fish meal.[58]

Lower brominated PBDEs

The peregrine falcon's main exposure to tetra–hexaBDEs was from ingestion of cockerels (Table 2). The mean AEs for BDE-47, -99, -100 and -153 (93–97%) in the peregrine falcons in this study (Table 3) were similar to AEs (termed bioavailabilities) found in a study in rats that were dosed with a commercial pentaBDE mixture in food (88–95% for BDE-47 to BDE-154)[60] or fed tri–decaBDEs via diet contaminated with a high dose oil (90–94% for BDE-47 to -154).[40] These were also similar to net absorption rates of 95–99% for tri–hexaBDEs found in grey seals fed a diet of herring for three months.[61] In a mass balance study of tri–hexaBDEs in milk cows, somewhat lower AEs were seen, from 72% for BDE-47 to 35% for BDE-154.[42] Thus, the AEs determined in falcons agreed reasonably well with what has been seen in mammalian studies.

Table 4 presents biomagnification factors (BMFs) of BDE congeners from published bird studies calculated as the ratios between lipid weight concentrations in predator tissues (muscle, liver, eggs) and lipid weight concentrations in prey tissues (fat, liver, muscle), depending on the study. As for the peregrine falcon BAFs, Mo et al.[62] found biomagnification

Table 2 Input and output fluxes of individual BDEs and HBCDD (ng day^{-1}) in three female peregrine falcons and means for all three falcons. Values below the LOD are designated nd

	Units	BDE-47	BDE-99	BDE-100	BDE-153	BDE-154	BDE-183	BDE-196	BDE-197	BDE-206	BDE-207	BDE-208	BDE-209	HBCDD
Falcon 223														
Chicken	ng day^{-1}	0.57	1.3	0.36	0.95	nd	1.4	0.21	0.50	0.11	0.22	0.086	2.1	0.59
Quail	ng day^{-1}	0.031	0.055	0.010	nd	nd	0.018	0.10	0.0051	0.0092	0.031	0.0090	0.68	0.43
Mice	ng day^{-1}	0.054	0.12	0.033	0.085	nd	0.13	0.028	0.045	0.011	0.023	0.0085	0.25	0.092
Sum intake	ng day^{-1}	0.65	1.4	0.41	1.0	nd	1.5	0.34	0.55	0.13	0.28	0.10	3.1	1.1
Body burden	ng	nd	38	nd	230	60	83	26	23	2.6	42	29	470	nd
Eggs	ng per egg	0.17	2.0	0.77	12	2.7	3.2	0.87	1.1	0.14	1.3	0.87	12	28
Eggs ($n = 3$)	Total ng excreted	0.51	5.9	2.3	35	8.1	10	2.6	3.2	0.41	4.0	2.6	35	84
Eggs	ng day^{-1}	0.0014	0.016	0.0063	0.096	0.022	0.027	0.0071	0.0088	0.0011	0.011	0.0071	0.096	0.23
Feces	ng day^{-1}	0.013	0.0091	0.0032	0.020	0.0032	0.010	0.0059	0.0021	0.0082	0.011	0.0059	0.24	nd
Ratio feces flux/egg flux		9.3	0.6	0.5	0.2	0.1	0.4	0.8	0.2	7.3	1.0	0.8	2.5	—
Falcon 398														
Chicken	ng day^{-1}	0.57	1.3	0.37	0.96	nd	1.4	0.21	0.50	0.11	0.23	0.087	2.2	0.59
Quail	ng day^{-1}	0.030	0.054	0.010	nd	nd	0.018	0.10	0.005	0.009	0.030	0.0088	0.66	0.42
Mice	ng day^{-1}	0.052	0.11	0.032	0.082	nd	0.12	0.026	0.043	0.010	0.022	0.0082	0.24	0.087
Sum intake	ng day^{-1}	0.66	1.5	0.41	1.0	nd	1.5	0.33	0.55	0.13	0.28	0.10	3.1	1.1
Body burden	ng	nd	32	nd	270	60	120	32	32	1.6	37	27	470	nd
Eggs	ng per egg	0.10	0.71	0.27	6.1	1.2	2.5	0.71	0.82	0.078	0.92	0.68	8.8	9.9
Eggs ($n = 3$)	Total ng excreted	0.31	2.1	0.82	18	3.6	7.4	2.1	2.4	0.23	2.8	2.0	26	30
Eggs	ng day^{-1}	0.00085	0.0058	0.0022	0.049	0.0099	0.020	0.0058	0.0066	0.00063	0.0077	0.0055	0.071	0.082
Feces	ng day^{-1}	0.055	0.089	0.030	0.14	0.021	0.082	0.12	nd	0.34	0.47	0.36	8.2	nd
Ratio feces flux/egg flux		65	15	13	2.8	2.1	4.0	21	—	540	61	66	115	—
Falcon 466														
Chicken	ng day^{-1}	0.57	1.3	0.36	0.95	nd	1.4	0.21	0.50	0.11	0.23	0.086	2.2	0.59
Quail	ng day^{-1}	0.023	0.042	0.0080	nd	nd	0.014	0.080	0.0039	0.007	0.023	0.0068	0.51	0.33
Mice	ng day^{-1}	0.052	0.12	0.033	0.083	nd	0.12	0.025	0.044	0.010	0.022	0.0083	0.23	0.08
Sum intake	ng day^{-1}	0.64	1.4	0.40	1.0	nd	1.5	0.31	0.55	0.12	0.27	0.10	2.9	1.0
Body burden	ng	nd	74	nd	140	43	74	29	24	2.1	43	30	600	nd
Eggs	ng per egg	0.29	2.2	0.75	4.9	1.4	1.8	0.68	0.75	0.12	1.4	0.79	14	25
Eggs ($n = 8$)	Total ng excreted	2.4	17	6.0	39	11	14	5.4	6.0	0.94	11	6.3	110	200
Eggs	ng day^{-1}	0.0066	0.047	0.016	0.11	0.030	0.038	0.015	0.016	0.0026	0.030	0.017	0.30	0.55
Feces 1	ng day^{-1}	nd	nd	0.019	0.088	0.036	0.088	0.04	nd	0.19	0.25	0.15	5.0	0.88
Feces 2	ng day^{-1}	nd	0.0032	0.01	0.044	0.0088	0.020	0.022	0.0060	0.02	0.037	0.025	0.63	nd
Ratio feces 1 flux/egg flux		—	—	1.2	0.82	1.2	2.3	2.7	—	74	8.3	8.7	17	1.6
Ratio feces 2 flux/egg flux		—	0.069	0.61	0.41	0.29	0.52	1.5	0.365	7.8	1.2	1.4	2.1	—
Mean for falcons ($n = 3$)														
Chicken	ng day^{-1}	0.57	1.3	0.36	0.95	nd	1.4	0.21	0.50	0.11	0.23	0.086	2.2	0.59
Quail	ng day^{-1}	0.028	0.050	0.0093	nd	nd	0.017	0.093	0.0047	0.0084	0.028	0.0082	0.62	0.39
Mice	ng day^{-1}	0.052	0.12	0.033	0.084	nd	0.12	0.026	0.044	0.010	0.022	0.0083	0.24	0.086
Sum intake	ng day^{-1}	0.65	1.4	0.41	1.0	nd	1.5	0.33	0.55	0.13	0.28	0.10	3	1.1
Body burden	ng	nd	48	nd	210	54	92	29	26	2.1	40	29	510	nd
Eggs	ng per egg	0.19	1.6	0.60	7.7	1.8	2.5	0.75	0.89	0.11	1.2	0.78	11	21
Eggs	Total ng excreted	1.1	8.4	3.1	31	7.6	10	3.4	3.9	0.53	6.1	3.7	57	100
Eggs	ng day^{-1}	0.0030	0.023	0.0085	0.085	0.021	0.027	0.0093	0.011	0.0015	0.017	0.010	0.16	0.27
Feces	ng day^{-1}	0.023	0.025	0.016	0.072	0.017	0.05	0.048	0.0020	0.14	0.19	0.13	3.5	0.22
Ratio feces flux/egg flux		7.6	1.1	1.9	0.85	0.82	1.8	5.2	0.19	96	11	13	22	0.4

factors (BMFs) for tetra–pentaBDEs for the kingfisher-fish food chain to be below 10. These results for the tetra–pentaBDEs are in contrast to BMFs determined in several other bird species. For terrestrial bird species, Voorspoels et al.[63] found BMFs for BDE-47, -99 and -100 in a sparrowhawk-passerine food chain and a buzzard-rodent food chain to be greater than 10 (Table 4).

Table 3 Mean absorption efficiencies (AE), egg to plasma ratios (E/P) on a lipid weight basis and metabolic rate constants (MRC, d^{-1}) for three female peregrine falcons

Analyte (n)	AE (%)	E/P	MRC (d^{-1})
BDE-47 (2)	97	—	—
BDE-99 (3)	97	1.7	0.03
BDE-100 (3)	96	—	—
BDE-153 (3)	93	1.8	0.004
BDE-154 (3)	—	1.6	—
BDE-183 (3)	97	1.4	0.02
BDE-196 (3)	84	1.3	0.009
BDE-197 (3)	100	1.7	1.0
BDE-206 (3)	$90/-110^a$	2.7	$0.05/-0.07^a$
BDE-207 (3)	$92/-31^a$	1.6	$0.006/-0.005^a$
BDE-208 (3)	$85/-150^a$	1.4	$0.003/-0.005^a$
BDE-209 (3)	$86/-120^a$	1.1	$0.006/-0.007^a$
HBCDD (3)	70	—	

^{a} Based on low/high fecal output fluxes ($n = 2$ for each value).

These BMFs are 2–3 times higher than BAFs found for the falcons. In an osprey egg-fish food chain, BMFs were also higher for BDE-47, -99 and -100 compared to the falcons.[64,65] For a guillemot egg-herring food chain, BMFs for BDE-47 and -99 were higher than in the falcons, but BDE-100 had a BMF of 7.1, which is similar to that of the falcons.[66]

For BDE-153, the BAFs found in the peregrine falcons (Table 4) were similar to the BMFs found for sparrowhawks and buzzards,[63] and osprey,[64,65] but were higher than found in kingfishers[62] (Table 4). Voorspoels *et al.*[63] found that BMFs from the sparrowhawk-passerine food chain increased with increasing log K_{OW} for BDE-28 to BDE-183, whereas for the buzzard-rodent food chain, the BMFs increased from BDE-28 to BDE-153, and then dropped for BDE-183. For the peregrine falcons in the current study, the BAF_e also increased with increasing log K_{OW} from BDE-47 to BDE-153, with a decrease for BDE-183.

Metabolic rate constants of 0.03 d^{-1} for BDE-99 and 0.004 d^{-1} for BDE-153 found for the peregrine falcons are quite similar to MRCs of 0.01 and 0.03 d^{-1} for BDE-99 and 0.001 and

0.007 d^{-1} for BDE-153 from a mass balance study of tri–hexaBDEs in two lactating cows.[42] The lower MRC for BDE-153 indicates slower metabolism for this congener, which is supported by its higher BAF. This is also supported by the BDE congener profiles in Fig. 1a, which show a shift to higher proportions of BDE-153 and lower proportions of BDE-99 from food to plasma and egg.

The AEs, BAFs, and MRCs found for tetra–hexaBDEs in the peregrine falcons were within ranges reported for other studies and species discussed above.[40,42,60–66] These results thus support the assumptions that have been used in the input–output calculations of the tetra–hexaBDEs. These include the assumptions used for calculating the estimated food intakes, fecal excretion rates, and body burdens, as well as the assumptions that the falcons were in steady state and that concentrations were in equilibrium between plasma and body lipids. Additional support comes from a study of wild birds (buzzard, sparrow hawk, owls) where equilibrium between body fat and serum/plasma lipids was also seen for tri–heptaBDEs.[67]

In steady state, the input flux from dietary ingestion in pre-breeding birds should equal the output flux *via* fecal excretion as long as no metabolism occurs. Fig. 2 shows the mean results of the input/output mass balance in the peregrine falcons for tetra–hexaBDEs. The large discrepancies seen in the peregrine falcons indicate substantial metabolism of tetra–hexaBDEs (more than 90%). Even with the added excretion *via* eggs, total excretion of parent compounds was still low, but was somewhat higher for BDE-100, -153 and -196, reflecting the lower MRCs for BDE-153 and -196 (BDE-100 could not be calculated).

Several studies of PBDEs in rats also found discrepancies between the amounts administered and those recovered in tissues and feces, indicating that 40–60% of these congeners were metabolized.[40,60] In support of this, hydroxylated metabolites of several lower brominated PBDEs were also found in rat carcass and feces in one of these rat studies.[60] Likewise, in a mass balance study of tri–hexaBDEs in milk cows, metabolism was inferred for several tetraBDE congeners.[42] In an input–output study in grey seals, the input fluxes were also found to be much higher than output fluxes for tetra–hexaBDEs.[61]

Table 4 Mean bioaccumulation factors (BAF_p, ng g^{-1} lw in plasma: ng g^{-1} lw in food; BAF_e, ng g^{-1} lw in egg: ng g^{-1} lw in food) for the three captive peregrine falcons compared to biomagnification factors in other bird species from the literature

BDE	BAF_p	BAF_e	Sparrowhawk-passerine	Buzzard-rodent	Osprey-fish	Guillemot-herring	Kingfisher-fish
BDE-47	—	1.6	10	12	29	19	5.7
BDE-99	3.7	6.1	20	14	32	17	3.6
BDE-100	—	8.1	25	17	19	7.1	1.5
BDE-153	23	41	21	22	46		6.2
BDE-154	—	—	24		20		2.1
BDE-183	6.8	10	29	12			17
BDE-196	14	18					15
BDE-197	5.3	9.0					16
BDE-206	1.9	4.8					1.0
BDE-207	16	24					4.0
BDE-208	31	42					4.4
BDE-209	19	21					1.7
HBCDD	—	140					
Reference	This study	This study	Voorspoels *et al.*[63]	Voorspoels *et al.*[63]	Chen *et al.*[64,65]	de Wit *et al.*[66]	Mo *et al.*[62]

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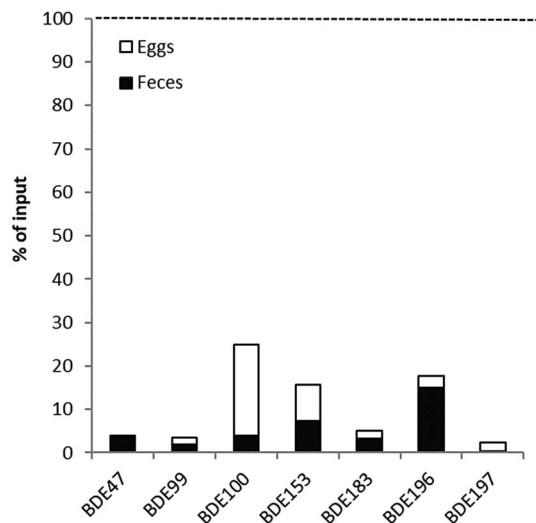*Environ. Sci.: Processes Impacts*, 2019, **21**, 1115–1131 | **1123**

Fig. 2 Mean results of the input–output mass balance of tetra–octaBDEs for the three peregrine falcons with outputs from eggs and feces plotted as the percentage of total input from food. The dotted horizontal line represents the total input from food (100%).

BDE-47 and -99 were found to be metabolized to several OH–BDEs in chicken liver microsomes.[68] In a previous study, rapid clearance of BDE-47 was seen in dosed kestrels.[43] Several OH–BDEs originating from lower brominated BDEs were also found in plasma in wild peregrine falcon nestlings in Canada.[69] Thus, these results indicate that chickens, kestrels and peregrine falcons are able to metabolize lower brominated BDEs, including to hydroxylated metabolites in chickens and falcons. However, no OH–BDE metabolites were seen in starlings dosed with a pentaBDE mixture *via* silastic implants.[70] This may indicate differences in metabolic capacity and/or different metabolic pathways between different bird taxa. This has been shown in a few studies. For example, hepatic cytochrome P450 activity was found to be higher in omnivorous birds and lower in fish-eating and predatory birds such as kestrels and sparrowhawks.[71,72] For PCBs, northern fulmar had higher hepatic phase I and II metabolic activity, and higher concentrations of OH–PCB metabolites than kittiwakes, indicating differences in metabolic capacity between these two bird species.[73] Thus, the congener profile changes seen in Fig. 1a going from food to plasma, egg and feces were probably due to metabolism of some congeners.

Hepta–decaBDEs

Exposure to hepta–decaBDEs was primarily from ingestion of cockerels (Table 2). The variable concentrations found in feces seem to indicate that exposure to and excretion of BDE-206, -207, -208, -209 were more variable. This led to both positive and negative estimates of AEs and MRCs for these congeners. The positive values found for the low fecal output could possibly be considered reasonable estimates of longer term exposure, whereas the negative values obtained from the high fecal output may be more reflective of sporadic short term exposure (Table 3). These differences could be due to a source of technical

decaBDE contamination in the breeding pen that the birds ingest episodically or due to fluctuations in concentrations in the food. In support of the latter, Moser and McLachlan[74] found that short term ingestion of food contaminated with high concentrations of polychlorinated furans led to higher concentrations in feces and higher feces flux in humans, particularly for the highly chlorinated and hydrophobic octa-chlorinated dibenzofuran (OCDF).

The congener profile for the nonaBDEs seen in the four feces samples was quite similar and did not match any technical decaBDE products despite the large differences in concentrations (Fig. 1c). The congener profile for nonaBDEs was also quite similar in food as in feces (Fig. 1c). These results would seem to rule out direct ingestion of a technical decaBDE, in favor of the hypothesis that food with sporadically higher contamination levels was a more likely source of the high feces concentrations. A possible explanation for sporadic high concentrations in food could be that decaBDE-related congeners vary in concentration in cockerels and quail, but since individuals were pooled and homogenized, only a mean concentration for intake was available for each. Another possible explanation could be that the mice that the falcons were fed were more contaminated with decaBDE-related congeners than cockerels and quails. Mice were fed sporadically and only occasionally to the falcons and this could have led to short-term ingestion and excretion of a high decaBDE-related congener dose on these occasions. This could also explain why both low and high concentrations of nona–decaBDEs were found in feces samples from the same falcon. However, since no mice were analyzed, it was not possible to confirm this. As the most likely explanation for the high feces concentrations and fluxes was due to short-term sporadic ingestion of nona–decaBDEs from food, we assumed that the lower feces concentrations and fluxes were more representative of the long term exposure in the three captive peregrine falcons. We have therefore used the positive values found for AEs and MRCs in the following discussion.

The AEs in falcons for BDE-183, -196 and -197 ranged from 84–100%. For BDE-206, -207, -208 and -209, based on the lower feces fluxes, the AEs were 85–92% (Table 3). Rats fed with tri–decaBDEs *via* diet contaminated with a high dose oil, had AEs of 71–78% for BDE-183 and -197, which were lower than for tetra–hexaBDEs. The lowest AEs in the rats were for BDE-196 (46%), BDE-206 (32%), BDE-207 (45%) and BDE-209 (37%),[40] which were considerably lower than found for the falcons. However, using radioactively-labelled BDE-209 in rats, Mörck *et al.*[75] found 10% of the single oral dose to be absorbed, 90% of the dose to be excreted in feces, and 65% of the fecal excretion to be metabolites, indicating that uptake could actually be much higher. Similarly, Sandholm *et al.*[76] found BDE-209 uptake in rats to be 26%, but the presence of numerous hydroxylated metabolites indicated even higher uptake. In support of the high AEs found in falcons, in particular for BDE-209, grey seals fed a supplement of BDE-209 in cod liver oil for one month had a mean net absorption of 89%.[61]

For BDE-183, BAFs/BMFs were similar for peregrine falcons, sparrowhawk, buzzard and kingfisher (Table 4). For other

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higher brominated BDEs, the only BMF data to compare to come from the kingfisher study. The BAF/BMF for BDE-196 was similar in both bird species, the BAF for BDE-197 was somewhat lower in the falcons than in kingfisher, and the BAFs for BDE-206, -207, -208 and -209 were from 2–10 times higher in the falcons than the corresponding BMFs in kingfisher (Table 4). These differences for the nona–decaBDEs could be due to differences in ability to metabolically debrominate BDE-209 or differences in diet.

BDE-197 had a high MRC ($1.0~d^{-1}$), indicating more efficient metabolism of this congener. The MRC of BDE-183 was intermediate ($0.02~d^{-1}$) and BDE-196 had a relatively low MRC ($0.009~d^{-1}$), which was similar to that of the more recalcitrant BDE-153, indicating that this congener may be more slowly metabolized (Table 3). The congener profiles from food to plasma and egg (Fig. 1b) support this, as there was a shift to somewhat higher proportions of BDE-196 and lower proportions of BDE-197. The positive values for MRCs for BDE-206, -207, -208 and -209 were low and in the same ranges as most of the other BDE congeners, indicating that these congeners were also probably metabolized. This is supported by comparison of the BDE congener profiles from food to plasma and egg (Fig. 1c), where there is a shift to higher proportions of BDE-208 and lower proportions of BDE-206.

If the falcons were in steady state, the input flux from dietary ingestion in pre-breeding birds should equal the output flux via fecal excretion as long as no metabolism occurs. Fig. 2 shows the mean results of the input/output mass balance in the three peregrine falcons for hepta–octaBDEs. The large discrepancies seen in the peregrine falcons for BDE-183, -196 and -197 indicate substantial metabolism of these BDEs. The results for all analyzed BDEs including BDE-206, -207, -208 and -209 for each individual falcon and feces sample are shown in Fig. S4.† Substantial metabolism is indicated for the nona–decaBDEs when using the lower feces outputs, but when the higher feces outputs were used, these result in higher output than input.

Huwe et al.[40] also found discrepancies between the amounts of several octa–decaBDEs dosed via dust or oil in rats and those recovered in tissues and feces, indicating that some of these were also metabolized. As stated previously, Mörck et al.[75] and Sandholm et al.[76] also found evidence of BDE-209 metabolism to numerous hydroxylated metabolites containing five to nine bromines, as well as traces of nonaBDEs.

Reductive debromination of BDE-209 in mammals and birds has also been shown to occur. In rats dosed with commercial decaBDE containing predominantly BDE-209 over 21 days and then allowed to depurate, only 5% of the parent BDE-209 was found in the body and only 4% was found in feces, indicating substantial metabolism.[77] BDE-197 and -207 were also recovered in higher amounts than in the dose given, indicating reductive debromination of BDE-209. Similarly, in dairy cattle, metabolic debromination of BDE-209 to BDE-196, -197 and -207 after absorption from feed was also indicated.[41]

Previous laboratory studies in starlings and kestrels have shown debromination of BDE-209 to lower brominated BDEs. Van den Steen et al.[45] found BDE-196, -197, -206, -207, and -208 as debromination products of BDE-209 in serum from starlings

with BDE-209 silastic implants. Letcher et al.[46] estimated that 80% of BDE-183, -196, -197, -206, -207, and -208 amounts later found in kestrel tissues and plasma originated from the metabolic debromination of BDE-209 given via diet. The formation of OH–BDEs was not included in these studies. Debromination of BDE-209 to lower brominated BDEs was also indicated by Holden et al.[78] as wild peregrine falcon eggs had different octa–nonaBDE congener profiles than technical octa- and decaBDE products. The octa- and nonaBDE congener profiles seen in the captive peregrine falcon eggs (Fig. 1b and c) were very similar to those seen by Holden et al.,[78] and thus metabolic debromination is also indicated in the captive falcons.

Reductive debromination of BDE-209 would therefore lead to endogenous production of some of these octa–nonaBDE congeners in the falcons. This would mean that the plasma concentrations measured were a combination of accumulation from food intake and debromination of BDE-209, and this would also lead to higher concentrations in eggs. This might lead to overestimation of true BAFs based on plasma and egg concentrations, which might explain why these were fairly high for BDE-183, -196, and -197, and particularly for nonaBDEs (BDE-207 and -208) in the Swedish falcons but does not explain the high BAF for BDE-209 (Table 4).

HBCDD

Technical HBCDD contains mainly γ-HBCDD (75–89%) with small proportions of α- (10–13%) and β-HBCDD (1–12%),[79] but falcons have only α-HBCDD in eggs.[52,80] The main exposure to HBCDD was from ingestion of quail but as only total HBCDD was quantified, we do not know which stereoisomers were present in quail. HBCDD concentrations were below the detection limits in plasma (<1 ng g^{-1} lw, <0.01 ng g^{-1} ww) but it was found in all eggs and one of four feces samples, indicating that uptake from diet occurs and it is excreted in both feces and eggs.

The AE was 70% which is similar to absorption seen for α-, β- and γ-HBCDD stereoisomers in rats (73–83%).[81] The BAF$_e$ was high, 140 (Table 4). The BAF$_e$ was probably an overestimation of actual bioaccumulation by at least a factor of six and possibly more, as indicated by the high egg/plasma partitioning ratios (6.1–18) found in the toxicokinetic study of HBCDD stereoisomers in American kestrels by Letcher et al.[48] and from estimated minimum egg/plasma ratios calculated (5.9–15) using the falcon plasma LOD of 1 ng g^{-1} lw. If the LOD of 1 ng g^{-1} lw were used, the BAF$_p$ (plasma LOD/weighted food concentration on a lipid weight basis) for the peregrine falcons would be estimated to be a minimum of 12–13. It was not possible to calculate MRC values for HBCDD. For the one falcon with detectable amounts of HBCDD in feces, Fig. S4† indicates that some metabolism occurred. The lack of detection in plasma samples may also be a sign that HBCDD stereoisomers are metabolized, which has previously been seen for treated kestrels,[48] chicken liver microsomes in vitro[68] and in rats.[81]

Transfer to eggs

Synthesis of yolk precursor lipids occurs in the liver with subsequent secretion to plasma and deposition in egg follicles over

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a short period of time.[82,83] The similar concentrations and congener profiles of tetra–decaBDEs in both plasma and eggs of the falcons indicate that the plasma and egg lipids are in reasonable equilibrium with each other. These results confirm that tetra–decaBDE concentrations in eggs reflect the female's body concentration, and eggs are thus a good monitoring matrix for these contaminants in peregrine falcons. Although the egg/plasma ratios decrease somewhat with increasing bromination degree (Table 3) and log K_{ow}, no significant correlation was found. Van den Steen et al.[70] also found similar congener profiles between serum and eggs of starlings for the tetra–hexaBDEs present in the pentaBDE technical mixture, indicating similar transfer for the different BDE congeners from maternal stores to eggs. In a dosing study of BDE-99 in zebra finches, Eng et al.[83] determined egg/plasma ratios based on plasma samples collected at the time of laying the first egg. For the control and low-dose groups, the ratio was approximately 2, similar to the mean ratio of 1.7 (range 1.4–2.1) found for BDE-99 in the falcons.

The egg/plasma ratios for α-HBCDD seen in the kestrel study[48] (6.1 and 18) and estimated from the current study (5.9–15) seem to indicate that transfer of HBCDD from the female to eggs is quite efficient during egg development in these two *Falconid* species, and that HBCDD transfer may be higher than for PBDEs in the falcons. Letcher et al.[48] speculated that this may be due to protein-specific binding and transport to eggs. Another possible explanation could be that HBCDD is metabolized quickly in plasma so that no equilibrium is established between plasma and egg. In support of this, Letcher et al. recently found a half-life of 15 days for HBCDD in kestrels.[48] Also HBCDD was not detected in serum from bald eagles[84] and was found at very low concentrations (median 0.05 ng g^{-1} ww) in 7 of 15 bald eagles[85] and in wild peregrine falcon nestlings (GM of 0.39 ng g^{-1} ww).[69]

Half-lives

Eggs from female 223 laid in 1998 and 1999 have previously been analysed for PBDEs and HBCDD.[26] Comparison of these data with those from the egg laid in 2006 analysed with the same methods (Fig. S5†), indicates that concentrations of the lower brominated BDEs (BDE-99, -153, -154/BB-153, -196,

-197) have declined with time. However, the higher brominated BDEs such as BDE-209, as well as HBCDD, were detected in the 2006 egg, but not in the earlier eggs. The appearance of BDE-209 and HBCDD is likely due to the lack of regulation of these technical products in 2006 and their increasing concentrations in the environment,[86,87] whereas the declines in the lower brominated BDEs reflect the effect of previous restrictions and bans on environmental levels. Thus, these results reflect changes in use patterns of the different technical products and diffuse contamination into feed and food sources. These results are supported by temporal trend studies showing declines for BDEs present in technical penta- and octaBDE, but increases for BDEs present in technical decaBDE as well as HBCDD in wild peregrine falcons in Sweden.[87]

By plotting the data from these three time points it was possible to roughly estimate the half-lives of several BDEs in this peregrine falcon using linear regression (Fig. S6†). The results are presented in Table 5 together with those for kestrels and herring gulls taken from the literature. Generally, the half-lives found in the peregrine falcons were similar to those found for both kestrels and herring gulls. The half-lives in falcon 223 were probably a reflection of being fed cleaner food over time (due to reduced environmental contamination) and thus depuration, as the values differ considerably for related congeners. In a previous study, a female peregrine falcon, originally wild but also kept in the captive breeding program, had eggs from two consecutive years analysed and the half-life for BDE-153 was multiyear but could not be quantified.[25] No estimate of the half-life for BDE-209 in peregrine falcons could be made, but the half-life has been found to be 13 days in starlings,[45] 14 days in kestrels[46] and 8.5–13 d in grey seals.[61]

Weaknesses of the study

A number of assumptions were used in calculating the input and output variables for the peregrine falcons. The study was a snapshot of the contaminant status and assumed that the falcons were in steady state and thus there was equilibrium

Table 5 Estimated or measured half-lives ($t_{1/2}$) in days of BDE congeners and HBCDD in different bird species

BDE congener	$t_{1/2}$ d Peregrine falcon	$t_{1/2}$ d Kestrel	$t_{1/2}$ d Herring gull
BDE-47		72	
BDE-99	65	175	100
BDE-100		178	100
BDE-153	624	572	100
BDE-154	31		
BDE-183	349		
BDE-196	77		
BDE-197	89		
BDE-209		14[a]	
HBCDD		15[b]	
Reference	This study	Drouillard et al.,[43] [a,b]Letcher et al.[46,48]	Norstrom et al.[44]

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between body lipids and plasma. However, Huwe et al.[88] found more of the higher brominated BDEs in plasma than adipose in a 21 d dosing study of tri–decaBDEs in rats using dust or oil. Thus, the assumption of equilibrium may not be correct, which would lead to overestimations of the uptake and body burdens of higher brominated BDEs. Tri–heptaBDEs and HBCDD have been found in low concentrations in feathers of terrestrial raptors and concentrations generally correlate with internal concentrations.[89–93] Thus, annual moulting is an additional excretion pathway for tri–heptaBDEs and HBCDD. This occurs in the autumn in peregrine falcons, and was not considered in the mass balance. However, due to the low concentrations that are found in feathers, this is probably not a significant excretion route. Other assumptions included using an estimated total body lipid content based on that of kestrels, that food intake for females was assumed to be 50% of what was offered and that missing data for contaminants in mice were estimated from data for cockerels and quail.

Conclusions

The input–output mass balance study showed efficient dietary uptake, bioaccumulation and transfer to eggs of most BDE congeners, including BDE-209, as well as HBCDD. Egg-to-plasma ratios were between 1.1 and 2.7, confirming that egg concentrations reflect body burdens, even for higher brominated BDEs. The high concentrations of BDE-209 that have been found in wild peregrine falcon eggs are thus reflective of body burdens. The input fluxes from food exceeded the output fluxes from feces and eggs, indicating considerable metabolism of tetra–octaBDEs and possibly for nona–decaBDEs and HBCDD. Despite the weaknesses in the study, the input–output variables estimated for tetra–heptaBDEs were supported by results from other published studies. For the octa–decaBDEs, there is more uncertainty due to large fluctuations in feces concentrations. For HBCDD, results for some variables are also more tentative due to the lack of detection in plasma and most feces samples. There is a need for more studies of metabolism in birds, including the formation of OH–BDEs and reductive debromination products of BDE-209. In general, more studies of HBCDD in raptors are needed.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

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