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ABSTRACT

We recently reported that air cell administration of penta-brominated diphenyl ether (penta-BDE; DE-71) evokes biochemical and immunologic effects in chicken (*Gallus gallus*) embryos at very low doses, and impairs pipping (i.e., stage immediately prior to hatching) and hatching success at 1.8 μg g^{-1} egg (actual dose absorbed) in American kestrels (*Falco sparverius*). In the present study, absorption of polybrominated diphenyl ether (PBDE) congeners was measured following air cell administration of a penta-BDE mixture (11.1 μg DE-71 g^{-1} egg) or an octa-brominated diphenyl ether mixture (octa-BDE; DE-79; 15.4 μg DE-79 g^{-1} egg). Uptake of PBDE congeners was measured at 24 h post-injection, midway through incubation, and at pipping in chicken, mallard (*Anas platyrhynchos*), and American kestrel egg contents, and at the end of incubation in black-crowned night-heron (*Nycticorax nycticorax*) egg contents. Absorption of penta-BDE and octa-BDE from the air cell into egg contents occurred throughout incubation; at pipping, up to 29.6% of penta-BDE was absorbed, but only 1.40–6.48% of octa-BDE was absorbed. Higher brominated congeners appeared to be absorbed more slowly than lower brominated congeners, and uptake rate was inversely proportional to the log K_{ow} of predominant BDE congeners. Six congeners or co-eluting pairs of congeners were detected in penta-BDE-treated eggs that were not found in the dosing solution suggesting debromination in the developing embryo, extraembryonic membranes, and possibly even in the air cell membrane. This study demonstrates the importance of determining the fraction of xenobiotic absorbed into the egg following air cell administration for estimation of the lowest-observed-effect level.

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1. Introduction

There has been considerable interest in the increasing concentrations of polybrominated diphenyl ethers (PBDEs) in the environment over the last 25 years. These compounds have been commonly used as fire retardants in textiles, electronics, polymers and other materials since the 1970s. Congeners detected in penta- and octa-BDE formulations bioaccumulate and biomagnify in food chains (de Wit, 2002), and appear to cause some adverse effects on free-ranging wildlife (Henny et al., 2009).

Detailed investigations using American kestrels (*Falco sparverius)* have documented developmental, endocrine, immunologic and reproductive effects of environmentally relevant concentrations of PBDEs (Fernie et al., 2005a,b, 2006, 2008). We recently reported that air cell administration of penta-brominated diphenyl ether (penta-BDE; DE-71) evokes biochemical and immunologic effects in chicken (*Gallus gallus*) embryos, and impairs pipping and hatching success in American kestrels (McKernan et al., 2009). By measuring the fraction of the air cell administered dose that was absorbed into the contents of the egg, the lowest-observed-effect level (LOEL) on reproduction observed in kestrels may occur at 1.8 μg penta-BDE g^{-1} egg (McKernan et al., 2009). Levels of PBDEs detected in eggs of free-ranging birds approach and even exceed this LOEL (Norstrom et al., 2002; Rattner et al., 2004; Toschik et al., 2005; Chen et al., 2008), and one field study has suggested that PBDE concentrations exceeding 1 μg g^{-1} egg wet weight (w.w.) impairs reproductive success of ospreys (*Pandion haliaetus*) (Henny et al., 2009).

Several controlled exposure studies have described accumulation, distribution, excretion, and metabolism of PBDEs in birds. When laying chickens were fed the commercial penta-BDE formulation DE-71, the quantity of PBDE on a lipid weight basis was found to be generally similar in liver and abdominal fat (Pirard and De

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Pauw, 2007). Bioconcentration factors between feed and abdominal fat for higher brominated congeners (BDE-100, -153, and -154) ranged from 1.8 to 2.2, while the ratios of BDE-47, -99, -100, -153 and -154 were similar between feed and eggs. Lower brominated congeners (BDE-47, -99 and -100) were more readily excreted, and their source was attributed in part to reductive debromination of BDE-153, -154 and -183. Using a combination of egg injection and dietary exposure in American kestrels, carcass retention factors for juvenile birds were greater for BDE-99, -100 and -153 when compared to BDE-47 (Drouillard et al., 2007). Decabromodiphenyl ether (BDE-209) release from silastic implants resulted in its accumulation in muscle and liver of European starlings (*Sturnus vulgaris*), and notably the appearance of lower brominated congeners in these tissues which was suggestive of debromination (Van den Steen et al., 2007). Studies in laboratory mammals and fish have demonstrated Phase I and Phase II metabolism and debromination of PBDEs (reviewed by Hakk and Letcher, 2003; Stapleton et al., 2004, 2006; Chen et al., 2006; Sanders et al., 2006).

The avian egg has been used extensively for toxicity testing (e.g., pesticides, industrial compounds, petroleum crude oil, metals) and has provided critical data for ecological risk assessments. However, there has been little information on the absorption of air cell injected lipophilic compounds into avian egg contents. In order to establish toxicity thresholds, it is important to quantify exposure to the administered dose. To address this issue, chicken, mallard (*Anas platyrhynchos*), American kestrel and black-crowned night-heron (BCNH; *Nycticorax nycticorax)* eggs were injected with DE-71 or DE-79, and chemically analyzed at selected days of incubation. The goal of this study was to provide data on the rate of uptake and potential metabolism of air cell administered PBDEs by embryos from several different species of birds.

2. Materials and methods

2.1. Egg management and PBDE injection

All procedures involving animals were approved by the Institutional Animal Care and Use Committees of the Patuxent Wildlife Research Center and the University of Maryland. Fertile mallard eggs were obtained from Whistling Wings (Hanover, IL, USA) and white leghorn chicken eggs were obtained from CBT Farms (Chestertown, MD, USA). American kestrel eggs were collected fresh from a captive colony at the Patuxent Wildlife Research Center. Black-crowned night-heron eggs (1/nest) were collected from recently completed clutches at Chincoteague Bay, VA, USA (Middle Mouth Marsh; N 37°56′44″ W 75°24′13″), a site that has been reported in several studies to be relatively free of persistent organic pollutants (e.g., Ohlendorf et al., 1978; Rattner et al., 1997). All eggs were washed with a 1% Betadine® solution (Purdue, Wilson, NC, USA) at 40 °C, weighed, and labeled with a number two graphite pencil. Eggs were then stored in a cooler at 13 °C for up to 3 d. Eggs were allowed to equilibrate to room temperature before placement into incubators.

Eggs were artificially incubated (Kuhl Incubator Company, Flemington, NJ, USA) in trays that were adapted to turn horizontally oriented eggs 180° hourly, as previously described (McKernan et al., 2007, 2009). Chicken eggs were incubated at 37.6 °C and all other species were incubated at 37.5 °C. Moisture loss was determined by weighing eggs at 3–4 d intervals. Relative humidity within the incubator was adjusted so mean egg weight loss by the end of incubation was 14–16%. Eggs were candled at the time of weighing to confirm viability, and unfertilized or dead eggs were removed.

Corn oil (Sigma–Aldrich, St. Louis, MO, USA) was used as the vehicle for this study because it causes relatively little toxicity and mortality in developing avian embryos (Heinz et al., 2006; McKernan et al., 2007). A penta-PBDE mixture (Great Lakes DE-71; LGC Promochem, Teddington, UK) and an octa-PBDE mixture (Great Lakes DE-79; LGC Promochem) that both contain congeners commonly reported in avian eggs and tissues were tested. Injection solutions were prepared by dissolving neat PBDE in acetone, and following the addition of corn oil vehicle, solutions were mixed for 3 h. The final dosing solutions contained 1% acetone by volume, and their PBDE concentrations were analytically verified (see below).

Chicken eggs were incubated for 4 d, and mallard, kestrel and BCNH eggs were incubated until they were developmentally equivalent to a 4-d-old chicken embryo, at which point they were candled to confirm fertility. Any infertile, non-viable, or cracked eggs were discarded. Eggs were then randomly assigned to groups of vehicle-injected, DE-71 or DE-79 treatments. Vehicle or PBDE was injected at a constant volume (0.5 μl g^{-1} egg) in the air cell (injection site at blunt end of egg), as previously described (McKernan et al., 2007, 2009). Briefly, a hole was drilled into the cap end of the egg and the vehicle or PBDE mixture (DE-71 at 11.1 μg g^{-1} egg or DE-79 at 15.4 μg g^{-1} egg) was injected. Immediately following injection, the hole was sealed with ethylene vinyl acetate adhesive using a hot glue gun and the eggs were kept in a vertical position for 30 min to allow the oil to spread over the air cell membrane. Eggs were then placed horizontally in trays and returned to the incubator. Survival throughout incubation was monitored by candling or with a viability-detecting instrument (“Buddy”; Avitronics, Cornwall, England) until the embryos were sampled, had pipped or died.

2.2. Sample collection

In both the DE-71 and DE-79 injection trials, eggs were randomly sampled at three stages of development: 24 h post-injection ($n = 3$), midway through incubation ($n = 3$), and at pipping ($n = 3$–4) in chicken, mallard and kestrel eggs. Black-crowned night-heron eggs ($n = 6$) were only sampled at pipping. Eggs were removed from the incubator and weighed. The narrow end of the egg was gently removed so that the contents of the egg (yolk, albumen, embryo and the chorioallantoic membrane) could be poured out into a chemically-clean jar and weighed. Samples were frozen at −20 °C until chemical analysis.

The fraction of the administered dose of PBDE retained at the injection site was also assessed by collecting the air cell membrane in chicken eggs. Air cell membranes were dissected from the eggs, blotted to remove blood and albumen, weighed, and stored at −20 °C. Air cell membranes were pooled by day of incubation (5, 10 and 21 d), and these three pools were chemically analyzed.

2.3. Analytical methods

Concentrations of DE-71 and DE-79-associated congeners in dosing solutions and eggs were determined analytically by gas chromatography/mass spectrometry (GC/MS) using a Varian 3400 GC/Varian 4D ion trap MS (Sugar Land, TX, USA), operated in electron ionization (EI) mode, or an Agilent 6890 N GC (Santa Clara, CA)/JEOL GC Mate double-focusing magnetic sector MS (Peabody, MA), operated in electron-capture negative ionization (ECNI) mode, respectively. Congener peak areas were compared to that of an internal standard; *p*-terphenyl for EI- and decachlorodiphenyl ether for ECNI-GC/MS. Congener response factors were determined using authentic PBDE standards (AccuStandard, Inc., New Haven, CT, USA). DE-71 congener separations were done on a DB-5 column (60 m length × 0.32 mm inner diameter and a 0.25 μm film thickness; J&W Scientific, Folsom, CA, USA). Carrier gas was He for all GC work. Injections on the Varian GC were made in the splitless mode. The GC column was initially held at 75 °C for 1 min, and then increased at a rate of 4 °C min^{-1} to a final temperature of 350 °C, and held for 15 min. Initial congener identifications were confirmed

by MS in the full-scan, electron–ionization mode. Quantification was performed in selected-ion storage (ion trap) or selected ion monitoring (magnetic sector) MS using the sum of the areas of the three major ions of each PBDE congener (85 congeners for DE-71 and 49 congeners for DE-79) versus that of the internal standard. The following GC column temperature program was used for the ECNI-based DE-79 determinations (49 congeners): initial column temperature 90 °C for 4 min, increased at a rate of 30 °C min^{-1} to 150 °C, increased at 10 °C min^{-1} to 300 °C, then at a rate of 30 °C min^{-1} to 350 °C, and held for 5 min. In this case a 15 m (0.25 mm i.d., 0.1 μm film thickness) DB-5-HT GC column with a pressure pulse mode of injection was used (Chen et al., 2008). The quantification limits for congeners in the dosing solutions were estimated to be 10–50 ng mL^{-1}, as a function of the degree of bromination.

Corn oil dosing solutions were diluted in hexane and directly analyzed by GC/MS. Egg contents were analyzed using previously described methods (Chen et al., 2008; McKernan et al., 2009). The eggs were lyophilized, and subsamples were spiked with surrogate standard PCB 204 (Ultra Scientific, North Kingstown, RI, USA). Blanks were run coincidentally to evaluate possible laboratory contamination. Egg samples were subjected to enhanced solvent extraction (Dionex ASE 200, Sunnyvale, CA, USA) with methylene chloride. Large molecular weight compounds were separated from the PBDEs in the extracts on an Envirosep size-exclusion chromatography column (350 mm length × 21.2 mm diameter with 60 × 21.1 mm guard column; Phenomenex, Torrance, CA, USA). The PBDE-containing fraction was then purified on a 2000 mg, silica gel, solid-phase extraction glass column (Enviroprep, Burdick and Jackson, Muskegon, MI, USA). The PBDEs in the purified extracts were separated by GC/MS as previously described. Data were corrected based on the recovery of surrogate standard PCB 204 in each sample. Mean recovery of surrogate PCB 204 from the eggs was 72.9%. The limit of quantification in egg was 100 pg g^{-1} w.w.

2.4. Statistical analysis

Data were analyzed using SAS® (SAS Institute Inc., NC, USA). Polybrominated diphenyl ether concentrations were tested for normality with the Shapiro–Wilk (W) statistic and for homogeneity of variance. Prior to analysis, concentrations of total PBDE and individual congeners were log10 transformed (when necessary, 1 was added to each value to deal with 0 values), percent uptake was arcsine transformed, and several parametric analyses were then conducted. Concentrations of PBDE, percent uptake of administered dose absorbed into egg contents, and the ratios of congener concentrations absorbed into egg contents to the quantity present in dosing solutions were compared using factorial analysis of variance (ANOVA; 2 PBDE formulations × 3 incubation stages × 3 species and interactions) and Tukey's HSD method of multiple comparison. Analysis of covariance was performed to test for species differences and to estimate the uptake rate of log10 total PBDE and individual congeners versus number of days since administration. A quadratic model (versus linear model) was the best fit for all of the variables. The linear slope estimates of log10 BDE congener concentrations per day for the DE-71 and DE-79 treatments were compared to the log K_{ow} by simple linear regression.

3. Results

3.1. Background concentrations of PBDE and administered dose

Vehicle-treated chicken, mallard and kestrel eggs contained exceedingly low concentrations of PBDE (BDE congeners 28/33, 47, 99, 100, 153, 154, or 183 were detected in 11 of 27 samples; total PBDE in 1 sample was 83.4 ng g^{-1} w.w., 1 sample contained 22.0 ng g^{-1} and the remaining nine samples contained <3 ng g^{-1}). Of the four vehicle-treated BCNH eggs, total PBDE (mean ± SE) was 1.37 ± 0.496 ng g^{-1} w.w. (BDE-47 was detected in all samples; BDE-99 and -153 were detected in a single egg). Concentrations of total PBDE in these vehicle control samples were less than 1% of the administered dose of DE-71 or DE-79.

The DE-71 dose was analytically verified to be 11.1 μg g^{-1} egg. The dose was composed of 24 individual congeners, with BDE-47, -99 and -100 dominating the profile at 22%, 37% and 18%, respectively (Fig. 1a). The DE-79 dose was analytically verified to be 15.4 μg g^{-1} egg, and was composed of 13 congeners with BDE-183, -197, -196, -153 and -207 dominating the profile at 27%, 16%, 12%, 11 and 10%, respectively (Fig. 2a).

3.2. DE-71 in egg contents

Absorption of air cell injected DE-71 continued throughout incubation in a similar manner for all three species (Table 1, Tukey's HSD comparison, $p < 0.05$ for early versus mid-incubation).

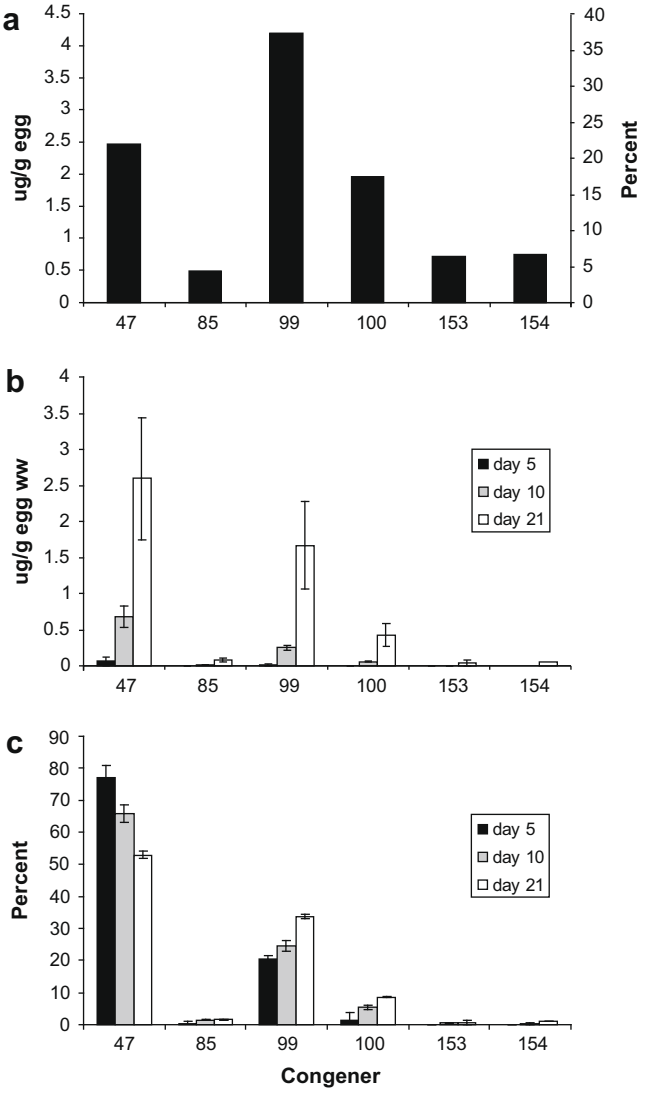

Fig. 1. (a) Concentration and relative contribution of congeners in administered dose of DE-71; (b) concentration and (c) relative contribution of congeners in embryonated chicken eggs on a fresh egg weight basis.

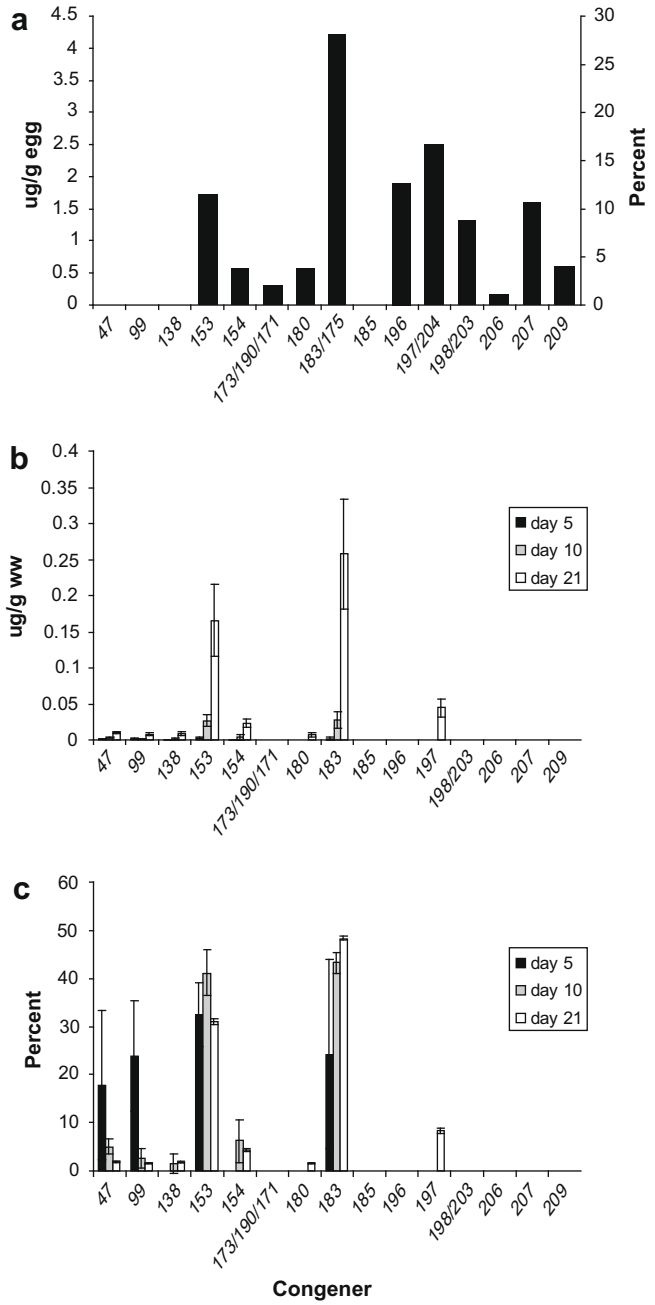

Fig. 2. (a) Concentration and relative contribution of congeners in administered dose of DE-79; (b) concentration and (c) relative contribution of congeners in embryonated chicken eggs on a fresh egg weight basis.

By pipping, 18.8–29.6% of DE-71 was absorbed into the egg contents in chickens, mallards, kestrels, and BCNHs. The dominant congeners from DE-71 absorbed into egg contents at all three time points were BDE-47, -99 and -100 (Fig. 1b and 1c for chicken, Fig. 3 for mallard and kestrel, Supplemental material Fig. SM-1 for BCNH), and by pipping substantial quantities BDE-47, -85, -99, -100, -153 and -154 present in the dosing solution were absorbed into egg contents (see egg to dosing solution ratios in Table 1). Although the absolute concentration of BDE-47 in egg contents increased over time, its relative contribution to total PBDE in egg contents was slightly lower at mid-incubation and pipping as the absorption of BDE-99 and -100 had increased. There were no significant differences in uptake rate of total PBDE and BDE congeners 47, 85, 99, 100, 153 and 154 among chickens, mallards and kestrels (quadratic ANCOVA, $p > 0.08$, for species term and species × linear and quadratic terms), and there were highly significant ($p < 0.0001$) linear and quadratic effects in uptake rate.

A total of six congeners or co-eluting pairs of congeners, and two metabolites (two to five per species) were detected at mid-incubation or at pipping that were not detected in the dosing solution (Table 2). Congeners 88 and 97/118 were detected in most species at pipping, while the other congeners were detected in only some of the species. Each of these congeners accounted for less than 0.05% of total PBDE measured in egg contents. Based upon their structure, as many as six of these congeners could have been formed by debromination of congeners present in the dosing solution (i.e., BDE-37 from congener -85; BDE-71 from -119; BDE-48 from -102 or -153; BDE-88 from -139 or -183; BDE-118 from -153; BDE-104 from -184).

3.3. Attempt to account for administered DE-71 dose

In preliminary range finding studies, corn oil injected into the air cell of mallard eggs was observed to spread well beyond the perimeter of the air cell, between the inner shell (contacts the albumen) and outer shell membranes (located between inner shell membrane and egg shell). The translucent nature of the eggshell for mallards (but not the chicken and kestrel) probably facilitated this observation. The spreading of the injection solution took several days and seemed to be complete by mid-incubation. Using the surface area equation for eggs from Paganelli et al. (1974), the inner shell membrane of our chicken eggs was estimated to be about 73.0 cm^2. At pipping, the air cell membrane of the chicken eggs averaged 8.65 cm^2, while the remaining inner membrane attached to the shell for eggs would account for approximately 64.35 cm^2.

As previously described, DE-71 was administered at a dose of 11.1 $\mu g\ g^{-1}$ egg (i.e., 669 μg/egg, average for three eggs). At pipping, 29.6% (196 μg) of administered dose of DE-71 was detected in chicken egg contents (Table 1). Total PBDE remaining associated with the air cell membrane of the chick egg accounted for 4.3% (28.8 μg; 3.34 $\mu g\ cm^{-2}$ of air cell membrane) of the administered dose. Assuming that the DE-71 spread evenly around the inner shell membrane remaining attached to the shell (i.e., 64.35 cm^2 of the total 73.0 cm^2), and was at the same concentration as found on the air cell membrane (i.e., 3.34 $\mu g\ cm^{-2}$), then 214 μg or 32.0% of the administered dose might be associated with the attached inner shell membrane of the chicken egg. If this assumption is correct, then 65.6% of the administered dose can be accounted for in the DE-71 treated chicken eggs.

3.4. DE-79 in egg contents

For all species, significantly less DE-79 was absorbed compared to DE-71 ($p = 0.0174$, 3-way ANOVA, PBDE formulation × incubation stage interaction). Absorption of DE-79 into egg contents (concentration, percent uptake and the ratio of BDE congeners absorbed into egg to that present in the dosing solution) varied considerably among species (Table 3). There were no significant differences in uptake rate of total PBDE and BDE congeners 153, 154 and 183 among chickens, mallards and kestrels (quadratic ANCOVA, $p > 0.15$, for species term and species × linear and quadratic terms), and there were highly significant ($p < 0.0001$) linear and quadratic effects in uptake rate. For BDE-47 and -99, there were significant species differences in uptake rate (BDE-47, quadratic ANCOVA, $p < 0.05$, for species × quadratic term; BDE-99, quadratic ANCOVA, $p < 0.0005$, for species term and species × linear and quadratic terms).

By mid-incubation and at pipping, the percentage of DE-79 absorbed in a given species was markedly less than for DE-71 (Tables

Table 1
Uptake of air cell administered DE-71 into egg contents during incubation in chicken, mallard, kestrel and black-crowned night-heron eggs.[a]

	Stage of incubation		
	24 h post-injection	Midway	Pip
Chicken			
DE-71 uptake into egg contents (μg total PBDE g^{-1} egg)[b]	0.088 ± 0.0459[A]	1.03 ± 0.111[B]	4.93 ± 0.994[B]
DE-71 uptake into egg contents (% of dose)	0.64 ± 0.356[A]	7.71 ± 0.857[B]	29.6 ± 4.56[C]
Ratio of congener concentration in egg to dose			
BDE-47	0.026 ± 0.0135[A]	0.280 ± 0.0351[B]	1.065 ± 0.2001[B]
BDE-85	0.002 ± 0.0015	0.030 ± 0.0031	0.164 ± 0.0303
BDE-99	0.004 ± 0.0021[A]	0.061 ± 0.0045[B]	0.403 ± 0.0849[B]
BDE-100	0.001 ± 0.0012[A]	0.029 ± 0.0024[A]	0.219 ± 0.0472[B]
BDE-153	ND	0.008 ± 0.0002	0.059 ± 0.0362
BDE-154	ND	0.005 ± 0.0026	0.072 ± 0.0187
Mallard			
DE-71 uptake into egg contents (μg total PBDE g^{-1} egg)	0.345 ± 0.0777[A]	2.42 ± 0.272[B]	4.58 ± 0.174[B]
DE-71 uptake into egg contents (% of dose)	2.62 ± 0.628[A]	16.94 ± 2.077[B]	27.7 ± 1.23[B]
Ratio of congener concentration in egg to dose			
BDE-47	0.088 ± 0.0196	0.553 ± 0.0365	0.709 ± 0.0621
BDE-85	0.013 ± 0.0025	0.082 ± 0.0148	0.193 ± 0.0074
BDE-99	0.023 ± 0.0053[A]	0.213 ± 0.0378[B]	0.468 ± 0.0294[B]
BDE-100	0.011 ± 0.0026[A]	0.089 ± 0.0150[A,B]	0.252 ± 0.0216[B]
BDE-153	0.001 ± 0.0015	0.032 ± 0.0049	0.181 ± 0.0336
BDE-154	ND	0.030 ± 0.0055	0.141 ± 0.0238
American kestrel			
DE-71 uptake into egg contents (μg total PBDE g^{-1} egg)	0.208 ± 0.1129[A]	2.43 ± 0.452[B]	2.80 ± 0.498[B]
DE-71 uptake into egg contents (% of dose)	1.64 ± 0.871[A]	18.42 ± 3.318[B]	18.8 ± 3.04[B]
Ratio of congener concentration in egg to dose			
BDE-47	0.058 ± 0.0327[A]	0.591 ± 0.0877[B]	0.381 ± 0.0362[B]
BDE-85	0.007 ± 0.0034[A]	0.087 ± 0.0209[A,B]	0.277 ± 0.1018[B]
BDE-99	0.010 ± 0.0054[A]	0.166 ± 0.0407[B]	0.205 ± 0.0246[B]
BDE-100	0.005 ± 0.0027[A]	0.087 ± 0.0211[A,B]	0.245 ± 0.0683[B]
BDE-153	0.003 ± 0.0008	0.026 ± 0.0066	0.188 ± 0.0722
BDE-154	0.002 ± 0.0005[A]	0.023 ± 0.0058[A]	0.205 ± 0.0825[B]
Black-crowned night-heron			
DE-71 uptake into egg contents (μg total PBDE g^{-1} egg)	–	–	3.16 ± 0.761
DE-71 uptake into egg contents (% of dose)	–	–	21.8 ± 5.24
Ratio of congener concentration in egg to dose			
BDE-47	–	–	0.648 ± 0.1483
BDE-85	–	–	0.137 ± 0.0349
BDE-99	–	–	0.256 ± 0.0638
BDE-100	–	–	0.144 ± 0.0387
BDE-153	–	–	0.070 ± 0.0200
BDE-154	–	–	0.057 ± 0.0160

[a] DE-71 was administered at an analytically verified dose of 11.1 μg g^{-1} egg. Values presented are arithmetic mean ± SE, n = 3/stage of incubation for chicken, mallard, kestrels, n = 6 for black-crowned night-herons; ND = not detected; – = not measured.
[b] Groups with the same capital letter superscripts in a row are not significantly different ($p > 0.05$; 3-way ANOVA, Tukey's comparison on 3-way interaction).

1 and 3; $p = 0.0088$, 3-way ANOVA, PBDE formulation × species × incubation stage). This is apparent at all three stages of incubation, with concentrations of DE-71 exceeding those of DE-79 by five- to ten-fold. Absorption of the DE-79 into egg contents ranged from 1.40% to 6.48% of the administered dose in chicken, mallard, kestrel, and BCNH eggs at pipping.

Congeners BDE-153 and -183 dominated the profiles in all species (Fig. 2 for chicken, Supplemental material, Fig. SM-2 for mallard and kestrel, Fig. SM-3 for BCNH) and congener 197 was detected only at pipping. Although the absolute concentration of lower brominated congeners increased during incubation, their relative contribution was decreased due to slower absorption of higher brominated congeners (BDE-183 and -197) that were present in the dosing solution at greater quantities.

There was limited evidence of in ovo biotransformation of DE-79; congeners detected in egg contents were also present in the dosing solution or as background in uninjected eggs. Total PBDE detected in air cell membranes was similar at various stages of incubation, and accounted for a larger percentage of the administered dose of DE-79 (24.9–36.4%) compared to that of DE-71 treated eggs.

Analytical measurements accounted for 38.7% (2.3% in egg contents and 36.4% in air cell membrane) of the total administered dose of DE-79 in chicken eggs by pipping. Using the aforementioned methodology for estimating PBDE associated with inner and outer shell membranes (previous section), recovery estimates exceeded 250% of administered dose. This overestimate may be attributable to low mobility of higher brominated congeners through, and between, the inner and outer shell membranes, as evidenced by their slower absorption into egg contents, and perhaps their limited migration from the air cell.

3.5. Relation between uptake rate and log K_{ow} of BDE congeners

The rate of uptake of most of the congeners was similar among species. However, for the DE-79 formulation, congeners 47 and 99 exhibited different uptake rates among species, and therefore were excluded from this analysis. For the remaining congeners, an inverse relationship was found between their uptake rate and log K_{ow} (Fig. 4, $r^2 = 0.45$, $p < 0.05$).

4. Discussion

Findings from this study demonstrate that up to 29.6% of air cell injected PBDE is absorbed through the air cell membrane into the

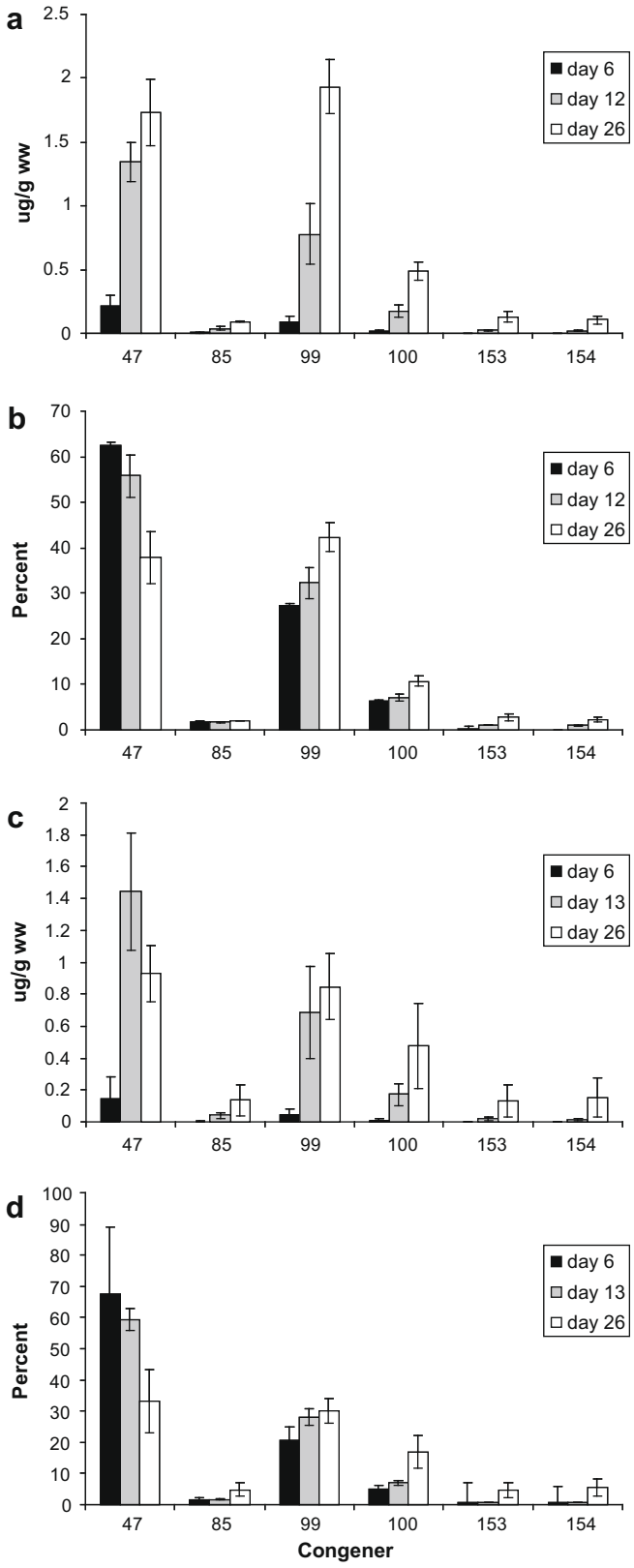

Fig. 3. (a) Concentration and (b) relative contribution of congeners in embryonated mallard eggs administered DE-71; (c) concentration and (d) relative contribution of congeners in embryonated kestrel eggs administered DE-71.

contents of chicken, mallard, kestrel, and BCNH eggs. The DE-71 formulation was more readily absorbed than DE-79. There was some evidence of biotransformation of PBDEs in egg contents (embryo, extraembryonic membranes, and possibly even the air cell membrane), as some congeners detected in eggs were not present in either the dosing solution or in vehicle-injected eggs.

4.1. Uptake of air cell administered PBDE

To the best of our knowledge, the present work and a related study (McKernan et al., 2009) are the first to describe the quantities of air cell administered PBDE or other halogenated compounds absorbed into the contents of avian eggs. There was evidence of gradual (but not exponential) absorption of DE-71 and DE-79 in chicken and mallard eggs over the entire exposure period. Absorption of lower brominated DE-71 was greater than higher brominated DE-79 in chickens, mallards, and kestrels. By the time of pipping, 18.8–29.6% of DE-71 and only 1.4–6.5% of DE-79 was absorbed into egg contents. The greater molecular volume and lower water solubility of the higher brominated congeners may impede their absorption through the air cell membrane. Furthermore, it has been demonstrated that there is preferential protein binding of higher brominated congeners (Morck et al., 2003). Such characteristics were perhaps evidenced by more DE-79 being retained by the air cell membrane compared to DE-71.

The DE-71 and DE-79 congener profiles of egg contents at 24 h post-injection are markedly different than those of the dosing solution. As metabolic capabilities are limited in early development (DE-71 metabolites only appeared in egg contents toward the end of incubation), it seems likely that this different profile reflects preferential uptake of lower brominated congeners. At mid-incubation and pipping, congener profiles of DE-71 and DE-79 in egg contents continued to differ from the dosing solutions, and this could be due to both preferential uptake and biotransformation, as some unique congeners were detected. The mechanisms (preferential uptake and/or biotransformation) accounting for the differential PBDE congener profiles of the dosing solutions and egg contents might be best resolved using radio-labeled single component exposures.

Comparison of findings from our test system (air cell injected bird eggs) to feeding studies involving more advanced life stages and different vertebrate classes is tenuous, but still worthy of discussion. In the present study, dominant congeners absorbed into egg contents following DE-71 air cell administration included BDE-47, -99 and -100, which differs considerably from findings in chickens fed DE-71 for which the ratios of BDE-47, -99, -100, -153 and -154 were similar between feed and eggs (Pirard and De Pauw, 2007). Using an exposure regime of egg injection and daily gavage during the nestling period of American kestrels, Drouillard and coworkers (2007) found greater carcass retention of higher brominated congeners (BDE-99, -100 and -153), possibly due to preferential metabolism and clearance of lower brominated congeners. In contrast to differential uptake of air cell administered DE-71 and -79 in the present study, carcass and liver retention of these formulations was nearly equal in male rats following a 21-d dietary exposure (Huwe et al., 2002, 2007). Burreau et al. (1997) fed BDE-47, -99, and -153 to juvenile pike (*Esox lucius*) and found lower uptake efficiency with increasing congener bromination, which is similar to the present findings.

Few studies report the relationship between administered dose and actual exposure following treatment of avian or reptilian eggs. De Roode and van den Brink (2002) injected PCBs into the yolk of chicken eggs, and found an exponential increase in uptake into the embryo, with 18% of the administered dose absorbed by day 19 of incubation. Similarly, Maervoet and coworkers (2005) noted an exponential uptake after yolk injection of PCB congeners 77, 153 and 180 into the chicken embryo during the last week of incubation. The exponential uptake of PCBs following yolk injection is

Table 2
PBDE congeners detected in egg contents that were not detected in DE-71 dosing solution or control egg.[a]

Congener	Stage of incubation				
	24 h post-injection	Midway (ng g^{-1} w.w. ± SE)	% of BDE congeners in sample	Pip (ng g^{-1} w.w. ± SE)	% of BDE congeners in sample
Chicken					
BDE-120	3ND	3ND		1.7 ± 0.3	0.03%
BDE-88	3ND	3ND		2ND, 1.9	⩽0.03%
BDE-97/118	3ND	3ND		2ND, 0.7	⩽0.02%
Mallard					
BDE-104/121	3ND	3ND		2ND, 1.6	⩽0.04%
BDE-120	3ND	3ND		2ND, 1.8	⩽0.04%
BDE-88	3ND	2ND, 1.5	⩽0.05%	1ND, 1.7, 2.3	⩽0.05%
BDE-97/118	3ND	3ND		2ND, 1.1	⩽0.02%
5′ MeO BDE-100[b]	3ND	3ND		2ND, 1.0	⩽0.02%
American kestrel					
BDE-88	3ND	1ND, 0.7, 1.2	⩽0.04%	3ND, 0.8	⩽0.04%
BDE-97/118	3ND	1ND, 0.4, 1.1	⩽0.03%	3ND, 0.6	⩽0.04%
Black-crowned night-heron					
BDE-35	–	–		4ND, 0.7, 0.7	⩽0.02%
BDE-37	–	–		5ND, 0.2	⩽0.01%
BDE-97/118	–	–		1.2 ± 0.4	0.04%
2′ MeO BDE-68	–	–		5ND, 0.2	⩽0.01%

[a] Detection limit (DL) = 100 pg g–1 w.w. ND = not detected; n = 3–6 per stage of incubation.
[b] MeO = methoxy.

Table 3
Uptake of air cell administered DE-79 into egg contents during incubation in chicken, mallard, kestrel and black-crowned night-heron eggs.[a]

	Stage of incubation		
	24 h post-injection	Midway	Pip
Chicken			
DE-79 uptake into egg contents (μg total PBDE g^{-1} egg)[b]	0.008 ± 0.0010^{A}	0.065 ± 0.0141^{B}	0.532 ± 0.0930^{C}
DE-79 uptake into egg contents (% of dose)	0.041 ± 0.0100^{A}	0.345 ± 0.0748^{A}	2.31 ± 0.401^{A}
Ratio of congener concentration in egg to dose			
BDE-153	0.001 ± 0.0005^{A}	0.016 ± 0.0026^{B}	0.097 ± 0.0169^{C}
BDE-154	ND	0.007 ± 0.0036	0.040 ± 0.0063
BDE-183	0.0004 ± 0.00029	0.007 ± 0.0016	0.061 ± 0.0105
Mallard			
DE-79 uptake into egg contents (μg total PBDE g^{-1} egg)	0.016 ± 0.0038^{A}	0.162 ± 0.0260^{B}	1.49 ± 0.584^{C}
DE-79 uptake into egg contents (% of dose)	0.086 ± 0.2106^{A}	0.813 ± 0.1378^{A}	6.48 ± 2.524^{B}
Ratio of congener concentration in egg to dose			
BDE-153	0.004 ± 0.0011^{A}	0.036 ± 0.0067^{B}	0.127 ± 0.0149^{B}
BDE-154	ND	0.019 ± 0.0037	0.060 ± 0.0116
BDE-183	0.002 ± 0.0005^{A}	0.018 ± 0.0032^{A}	0.174 ± 0.0519^{B}
American kestrel			
DE-79 uptake into egg contents (μg total PBDE g^{-1} egg)	0.011 ± 0.0668^{A}	0.073 ± 0.0169^{B}	0.486 ± 0.0734^{B}
DE-79 uptake into egg contents (% of dose)	0.058 ± 0.0378^{A}	0.402 ± 0.0958^{A}	2.24 ± 0.346^{A}
Ratio of congener concentration in egg to dose			
BDE-153	0.002 ± 0.0005^{A}	0.018 ± 0.0042^{B}	0.071 ± 0.0117^{C}
BDE-154	0.001 ± 0.0015	0.010 ± 0.0013	0.035 ± 0.0065
BDE-183	0.0009 ± 0.00088	0.008 ± 0.0017	0.052 ± 0.0088
Black-crowned night-heron			
DE-79 uptake into egg contents (μg total PBDE g^{-1} egg)	–	–	0.286 ± 0.0561
DE-79 uptake into egg contents (% of dose)	–	–	1.40 ± 0.274
Ratio of congener concentration in egg to dose			
BDE-153	–	–	0.047 ± 0.0093
BDE-154	–	–	0.020 ± 0.0030
BDE-183	–	–	0.032 ± 0.0067

[a] DE-79 was administered at an analytically verified dose of 15.4 μg g^{-1} egg. Values presented are arithmetic mean ± SE, n = 3/stage of incubation for chicken, mallard, kestrels, n = 6 for black-crowned night-herons; ND = not detected; – = not measured.
[b] Groups with the same capital letter superscripts in a row are not significantly different ($p > 0.05$; 3-way ANOVA, Tukey's comparison on 3-way interaction).

in contrast to the gradual uptake of air cell administered PBDE observed in the present study. Portelli et al. (1999) reported dose-dependent uptake of topically administered DDT (dichlorodiphenyltrichloroethane) into snapping turtle (*Chelydra serpentina serpentine*) eggs within 3 d of application, however only a small fraction (1.6–20%) of the total dose was absorbed. Gale et al. (2002) found that 4–10% of topically administered 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 3,3′,4,4′,5-pentachlorobiphenyl was absorbed into egg contents of red-eared sliders (*Trachemys scripta elegans*) over a 16-d period. The fraction of air cell administered PBDE absorbed into avian egg contents is similar to fractional uptake following topical application of contaminants observed in turtle eggs.

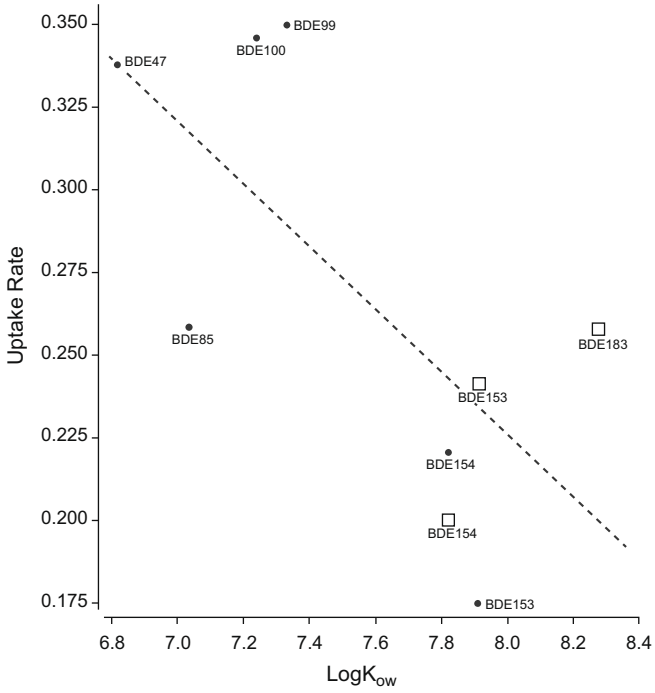

Fig. 4. Relation of congener uptake averaged among species (slope of log10 transformed uptake per day) and log K_{ow} of BDE congeners 47, 85, 99, 100, 153, 154 and 183 in DE-71(•) and DE-79 (□) injection trials.

4.2. Biotransformation

Six individual or co-eluting BDE congener pairs, and two metabolites, were detected in egg contents of chicken, mallard, kestrel, or BCNH that were not detected in the DE-71 dosing solution or control eggs. Tomy et al. (2004) fed juvenile lake trout (*Salvelinus namaycush*) a mixture of PBDEs and detected three congeners (an unknown penta, BDE-140, and an unknown hexa) in carcass that were not present in control fish or spiked food samples. The six congeners detected in the present study suggest PBDE debromination in mid-incubation and pipping bird embryos. Methoxylated BDE congeners were also detected, but in only two samples. It is possible that the 2′methoxy-BDE-68 congener could be from a naturally occurring source in the food chain of the BCNH hen, as it has been detected in marine sponges (Teuten et al., 2005). Notably, a recent report described the formation of highly toxic hydroxylated BDEs from methoxylated BDEs in an in vitro assay system using microsomes from chickens and other species (Wan et al., 2009). There was little to no evidence of biotransformation of the DE-79 dose, presumably due to its low absorption into egg contents.

Polybrominated diphenyl ethers undergo debromination, and while the mechanism is poorly understood, some have suggested that the pathway may be related to the deiodination of thyroxine and other thyronines (Stapleton et al., 2004, 2006; Benedict et al., 2007). Stapleton et al. (2004, 2006) reported debromination in common carp (*Cyprinus carpio*) fed BDE congeners, with congener 99 debrominated to BDE-47, and congener 183 debrominated to BDE-154 (Stapleton et al., 2006). Furthermore, when juvenile trout (*Oncorhynchus mykiss*) were fed BDE-209, several hepta-, octa-, and nona-BDE congeners were detected in tissues (Stapleton et al., 2006). Debromination of PBDEs has also been reported in European starlings (*Sturnus vulgaris*) exposed to BDE-209 using silastic implants, as octa-, nona- and hexa-BDEs were detected in muscle and liver tissues (van den Steen et al., 2007).

Mammalian and fish studies implicate Phase I cytochrome P450-associated enzymes in hydroxylation of PBDEs (Hakk and Letcher, 2003; Sanders et al., 2006). Hydroxylated and methoxylated PBDE congeners have been detected in free-ranging wildlife (Marsh et al., 2004; McKinney et al., 2006; Verreault et al., 2005, 2007; Gebbink et al., 2008). This class of flame retardants has also been reported to be metabolized through Phase II conjugation processes (e.g., uridine diphosphate glucoronosyl-transferases and glutathione S-transferases) (Hakk and Letcher, 2003).

4.3. Utility of air cell injection studies

The avian embryo has been used extensively as a toxicity bioassay for environmental contaminants. As a time and cost saving measure, many investigators have administered toxicants by injection into the air cell or yolk, and there is some evidence that such techniques mimic toxicity observed when contaminants are maternally deposited into the egg. For example, embryotoxic responses elicited by egg injection of PCBs compare favorably to those observed following natural exposure (Hoffman et al., 1996). However, methylmercury injected into mallard eggs seems to be slightly more toxic than dietary methylmercury, as air cell administration of 1.6 μg g^{-1} egg resulted in comparable toxicity to that observed following maternal deposition at 5.5 μg g^{-1} w.w. (Heinz et al., 2009). Air cell injected compounds must cross the air cell membrane, blood vessels, albumen, and yolk to reach the embryo. It is unknown if air cell administered compounds mimic the distribution of naturally deposited contaminants in eggs, although they do evoke similar types of toxic responses (e.g., teratogenic effects, edema, histopathology, enzyme induction). However, the migration and metabolism of air cell injected compounds must be carefully characterized to ascertain true exposure.

Clearly, the PBDE dose to which the embryo was exposed was much less than the administered dose. This difference should be taken into account when designing egg injection studies and using findings for risk assessments. Additionally, degree of halogenation (bromination, chlorination) must be considered when using air cell injection as a route of embryonic exposure as larger congeners may not readily cross the air cell membrane. Previous reports of LOELs and LD50s of air cell injections of PCBs and other persistent organic pollutants may underestimate toxicity if test compounds are incompletely absorbed. In our previous study, we demonstrated that at an administered concentration of 10 μg DE-71 g^{-1} egg, kestrel pipping and hatching success was significantly reduced, yet much less PBDE was actually absorbed into egg contents, and thus the estimated LOEL was approximately 1.8 μg g^{-1} egg w.w. (McKernan et al., 2009). Although air cell administered compounds are not naturally incorporated into the yolk or albumen, determining their concentration in egg contents is critical in predicting toxic effects in eggs of free-ranging birds.

In the present study, we observed that 36.4% of the DE-79 dose was associated with the air cell membrane at pipping, and recognized that a significant fraction of the remaining dose may be sequestered in the inner shell membrane, outer shell membrane, and porous eggshell. We performed a rough estimate of the quantity of PBDE that could have spread between the inner and outer shell membranes in an attempt to account for the entire DE-71 and -79 doses. A total of 65.6% of the injected DE-71 dose was accounted for, however the same calculations resulted in over 250% of the DE-79 dose. This overestimate for DE-79 may be due to lower mobility of higher brominated congeners around the inner and outer shell membranes. In future studies, we recommend analyzing the remaining air cell membrane, eggshell, and membranes attached to the eggshell, as well as egg contents, to more accurately estimate mass balance. In addition, researchers may want to consider analyzing specific tissues of the avian embryo in order to generate information on distribution of air cell injected compounds.

Biotransformation (e.g., debromination, hydroxylation, methoxylation) of PBDE congeners confounds efforts to measure uptake of parent compounds following dietary administration, or in this case, air cell injections. Increased concentrations of lower brominated congeners (or even differences in ratios of congeners) by pipping may reflect differential absorption of the dosing solution, or possibly the result of debrominated parent compounds which would supplement lower molecular weight congeners in the dosing solution. Formation of other metabolites that are not analyzed may reduce percent recovery in mass balance studies. For example, in the PBDE mass balance study of Huwe et al. (2007), 20–40% of the total dose fed to rats for 21 d was not recovered, and was attributed to biotransformation. Similarly, in a related mass balance study, only 48–80% of DE-79 fed to rats for 21 d was recovered (Huwe et al., 2002).

5. Conclusions

This study demonstrates differential uptake of air cell administered PBDEs into the avian egg, and the importance of confirming the actual fraction absorbed. In addition, evidence is provided that PBDEs can be debrominated, and perhaps methoxylated, in the avian embryo. Production of penta- and octa-BDE formulations has been discontinued due to the potential toxicity of lower brominated congeners. However, deca-BDE is still in production. It is being released into the environment and has been detected in tissues of wildlife (Chen et al., 2008). Phasing out of penta- and octa-mixtures may not fully protect free-ranging wildlife, as fish and birds have been shown to be capable of debrominating deca-BDE (Stapleton et al., 2006; van den Steen et al., 2007).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemosphere.2009.12.023.

References

Benedict, R.T., Stapleton, H.M., Letcher, R.J., Mitchelmore, C.L., 2007. Debromination of polybrominated diphenyl ether-99 (BDE-99) in carp (*Cyprinus carpio*) microflora and microsomes. Chemosphere 69, 987–993.

Burreau, S., Axelman, J., Broman, D., Jakobsson, E., 1997. Dietary uptake in pike (*Esox lucius*) of some polychlorinated biphenyls, polychlorinated naphthalenes and polybrominated diphenyl ethers administered in natural diet. Environ. Toxicol. Chem. 16, 2508–2513.

Chen, L.-J., Lebetkin, E.H., Sanders, J.M., Burka, L.T., 2006. Metabolism and disposition of 2,2′,4,4′,5-pentabromodiphenyl ether (BDE99) following a single or repeated administration to rats and mice. Xenobiotica 36, 515–534.

Chen, D., La Guardia, M.J., Harvey, E., Amaral, M., Wohlfort, K., Hale, R.C., 2008. Polybrominated diphenyl ethers in peregrine falcon (*Falco peregrinus*) eggs from the northeastern US. Environ. Sci. Technol 42, 7594–7600.

de Roode, D.F., van den Brink, N.W., 2002. Uptake of injected PCBs from the yolk by the developing chicken embryo. Chemosphere 48, 195–199.

de Wit, C.A., 2002. An overview of brominated flame retardants in the environment. Chemosphere 46, 583–624.

Drouillard, K.G., Fernie, K.J., Letcher, R.J., Shutt, L.J., Whitehead, M., Gebink, W., Bird, D.M., 2007. Bioaccumulation and biotransformation of 61 polychlorinated biphenyl and four polybrominated diphenyl ether congeners in juvenile American kestrels (*Falco sparverius*). Environ. Toxicol. Chem. 26, 313–324.

Fernie, K.J., Mayne, G., Shutt, L.J., Pekarick, C., Grasman, K.A., Letcher, R.J., Drouillard, K.G., 2005a. Evidence of immunomodulation in nestling American kestrels (*Falco sparverius*) exposed to environmentally relevant PBDEs. Environ. Pollut. 138, 485–493.

Fernie, K.J., Shutt, L., Mayne, G., Hoffman, D.J., Letcher, R.J., Drouillard, K.G., Ritchie, I.J., 2005b. Exposure to polybrominated diphenyl ethers (PBDEs): changes in thyroid, vitamin A, glutathione homeostasis, and oxidative stress in American kestrels (*Falco sparverius*). Toxicol. Sci. 88, 375–383.

Fernie, K.J., Shutt, L.J., Ritchie, J.L., Letcher, R.J., Drouillard, K.G., Bird, D.M., 2006. Changes in the growth, but not the survival, of American kestrels (*Falco sparverius*) exposed to environmentally relevant polybrominated diphenyl ethers. J. Toxicol. Environ. Health Part A 69, 1541–1554.

Fernie, K.J., Shutt, J.L., Letcher, R.J., Ritchie, J.I., Sullivan, K., Bird, D.M., 2008. Changes in reproductive courtship behaviors of adult American kestrels (*Falco sparverius*) exposed to environmentally relevant levels of the polybrominated diphenyl ether mixture, DE-71. Toxicol. Sci. 102, 171–178.

Gale, R.W., Bergeron, J.M., Willingham, E.J., Crews, D., 2002. Turtle sex determination assay: mass balance and responses to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 3,3′,4,4′,5-pentachlorobiphenyl. Environ. Toxicol. Chem. 21, 2477–2482.

Gebbink, W.A., Sonne, E., Dietz, R., Kirkegaard, M., Riget, F.F., Born, E.W., Muir, D.C.G., Letcher, R.J., 2008. Tissue-specific congener composition of organohalogen and metabolite contaminants in east Greenland polar bears (*Ursus maritimus*). Environ. Pollut. 152, 621–629.

Hakk, H., Letcher, R.J., 2003. Metabolism in the toxicokinetics and fate of brominated flame retardants – a review. Environ. Int. 29, 801–828.

Heinz, G.H., Hoffman, D.J., Kondrad, S.L., Erwin, C.A., 2006. Factors affecting the toxicity of methylmercury injected into eggs. Arch. Environ. Contam. Toxicol. 50, 264–279.

Heinz, G.H., Hoffman, D.J., Klimstra, J.D., Stebbins, K.R., Kondrad, S.L., Erwin, C.A., 2009. Species differences in the sensitivity of avian embryos to methylmercury. Arch. Environ. Contam. Toxicol. 56, 129–138.

Henny, C.J., Kaiser, J.L., Grove, R.A., Johnson, B.L., Letcher, R.J., 2009. Polybrominated diphenyl ether flame retardants in eggs may reduce reproductive success of ospreys in Oregon and Washington, USA. Ecotoxicology 18, 802–813.

Hoffman, D.J., Rice, C.P., Kubiak, T.J., 1996. PCBs and dioxins in birds. In: Beyer, W.N., Heinz, G.H., Redmon-Norwood, A.W. (Eds.), Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations. CRC Press, Boca Raton, Florida, USA, pp. 165–208.

Huwe, J.K., Hakk, H., Lorentzsen, M., 2002. A mass balance feeding study of a commercial octabromodiphenyl ether mixture in rats. Organo. Comp. 58, 229–232.

Huwe, J., Hakk, H., Lorentzsen, M., 2007. Bioavailability and mass balance studies of a commercial pentabromodiphenyl ether mixture in male Sprague-Dawley rats. Chemosphere 66, 259–266.

Maervoet, J., Beck, V., Roelens, S.A., Covaci, A., Voorspoels, S., Geuns, J.M.C., Darras, V.M., Schepens, P., 2005. Uptake and tissue-specific distribution of selected polychlorinated biphenyls in developing chicken embryos. Environ. Toxicol. Chem. 24, 597–602.

Marsh, G., Athanasiadou, M., Bergman, A., Asplund, L., 2004. Identification of hydroxylated and methoxylated polybrominated diphenyl ethers in Baltic Sea salmon (*Salmo salar*) blood. Environ. Sci. Technol. 38, 10–18.

McKernan, M.A., Rattner, B.A., Hale, R.C., Ottinger, M.A., 2007. Egg orientation affects toxicity of air cell administered polychlorinated biphenyl 126 (3,3′,4,4′,5-pentachlorobiphenyl) in chicken (*Gallus gallus*) embryos. Environ. Toxicol. Chem. 26, 2724–2727.

McKernan, M.A., Rattner, B.A., Hale, R.C., Ottinger, M.A., 2009. Toxicity of polybrominated diphenyl ethers (DE-71) in developing chicken (*Gallus gallus*), mallard (*Anas platyrhynchos*), and American kestrel (*Falco sparverius*) embryos and hatchlings. Environ. Toxicol. Chem. 28, 1007–1017.

McKinney, M.A., Cesh, L.S., Elliott, J.E., Williams, T.D., Garcelon, D.K., Letcher, R.J., 2006. Brominated flame retardants and halogenated phenolic compounds in North American west coast bald eaglet (*Haliaeetus leucocephalus*) plasma. Environ. Sci. Technol. 40, 6275–6281.

Morck, A., Hakk, H., Orn, U., Klasson-Wehler, E., 2003. Decabromodiphenyl ether in the rat: absorption, distribution, metabolism and excretion. Drug Metab. Dis. 31, 900–907.

Norstrom, R.J., Simon, M., Moisey, J., Wakeford, B., Weseloh, D.V., 2002. Geographical distribution (2000) and temporal trends (1981–2000) of brominated diphenyl ethers in Great Lakes Herring gull eggs. Environ. Sci. Technol. 36, 4783–4789.

Ohlendorf, H.M., Klaas, E.E., Kaiser, T.E., 1978. Environmental pollutants and eggshell thinning in the black-crowned night heron. Wading Birds 7, 63–82.

Paganelli, C.V., Olszowka, A., Ar, A., 1974. The avian egg: surface area, volume, and density. Condor 76, 310–325.

Pirard, C., De Pauw, E., 2007. Absorption, disposition and excretion of polybrominated diphenyl ethers (PBDEs) in chicken. Chemosphere 66, 320–325.

Portelli, M.J., de Solla, S.R., Brooks, R.J., Bishop, C.A., 1999. Effect of dichlorodiphenyltrichloroethane on sex determination of the common snapping turtle (*Chelydra serpentina serpentine*). Ecotox. Environ. Safe. 43, 284–291.

Rattner, B.A., Melancon, M.J., Rice, C.P., Riley Jr., W., Eisemann, J., Hines, R.K., 1997. Cytochrome P450 and organochlorine contaminants in black-crowned night-herons from the Chesapeake Bay region, USA. Environ. Toxicol. Chem. 16, 2315–2322.

Rattner, B.A., McGowan, P.C., Golden, N.H., Hatfield, J.S., Toschik, P.C., Lukei Jr., R.F., Hale, R.C., Schmitz-Afonso, I., Rice, C.P., 2004. Contaminant exposure and reproductive success of ospreys (*Pandion haliaetus*) nesting in Chesapeake Bay regions of concern. Arch. Environ. Contamin. Toxicol. 47, 126–140.

Sanders, J.M., Chen, L.-J., Lebetkin, E.H., Burka, L.T., 2006. Metabolism and disposition of 2,2′,4,4′-tetrabromodiphenyl ether following administration of single or multiple doses to rats and mice. Xenobiotica 36, 103–117.

Stapleton, H.M., Letcher, R.J., Baker, J.E., 2004. Debromination of polybrominated diphenyl ether congeners BDE 99 and BDE 183 in the intestinal tract of the common carp (*Cyprinus carpio*). Environ. Sci. Technol. 38, 1054–1061.

Stapleton, H.M., Brazil, B., Holbrook, R.D., Mitchelmore, C.L., Benedict, R., Konstantinov, A., Potter, D., 2006. In vivo and in vitro debromination of decabromodiphenyl ether (BDE 209) by juvenile rainbow trout and common carp. Environ. Sci. Technol. 40, 4653–4658.

Teuten, E.L., Xu, L., Reddy, C.M., 2005. Two abundant bioaccumulated halogenated compounds are natural products. Science 307, 917–920.

Tomy, G.T., Palace, V.P., Halldorson, T., Braekevelt, E., Danell, R., Wautier, K., Evans, B., Brinkworth, L., Fisk, A.T., 2004. Bioaccumulation, biotransformation and biochemical effects of brominated diphenyl ethers in juvenile lake trout (*Salvelinus namaycush*). Environ. Sci. Technol. 38, 1496–1504.

Toschik, P.C., Rattner, B.A., McGowan, P.C., Christman, M.C., Carter, D.B., Hale, R.C., Matson, C.W., Ottinger, M.A., 2005. Effects of contaminant exposure on reproductive success of ospreys (*Pandion haliaetus*) nesting in Delaware River and Bay, USA. Environ. Toxicol. Chem. 24, 617–628.

Van den Steen, E., Covaci, A., Jaspers, V.L.B., Dauwe, T., Voorspoels, S., Eens, M., Pinxten, R., 2007. Accumulation, tissue-specific distribution and debromination of decabromodiphenyl ether (BDE 209) in European starlings (*Sturnus vulgaris*). Environ. Pollut. 148, 648–653.

Verreault, J., Gabrielsen, G.W., Chu, S., Muir, D.C.G., Andersen, M., Hamaed, A., Letcher, R.J., 2005. Flame retardants and methoxylated and hydroxylated polybrominated diphenyl ethers in two Norwegian arctic top predators: glaucous gulls and polar bears. Environ. Sci. Technol. 39, 6021–6028.

Verreault, J., Shahmiri, S., Gabrielsen, G.W., Letcher, R.J., 2007. Organohalogen and metabolically-derived contaminants and associations with whole body constituents in Norwegian arctic glaucous gulls. Environ. Int. 33, 823–830.

Wan, Y., Wiseman, S., Chang, H., Zhang, X., Jones, J.D., Hecker, M., Kannan, K., Tanabe, S., Hu, J., Lam, M.H.W., Geisy, J.P., 2009. Origin of hydroxylated brominated diphenyl ethers: natural compounds or man-made flame retardants? Environ. Sci. Technol. 43, 7536–7542.