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INDIVIDUAL VARIATION IN BODY BURDEN, LIPID STATUS, AND REPRODUCTIVE INVESTMENT IS RELATED TO MATERNAL TRANSFER OF A BROMINATED DIPHENYL ETHER (BDE-99) TO EGGS IN THE ZEBRA FINCH

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Abstract—Avian eggs are exposed to hydrophobic contaminants through maternal transfer. How maternal transfer of contaminants within a species is influenced by individual variation in characteristics such as body burden, yolk precursor levels, or reproductive investment is not understood. The authors investigated sources of variation in the maternal transfer of 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99) in zebra finches (*Taeniopygia guttata*). The authors dosed adult female zebra finches with levels of BDE-99 relevant to exposure in wild birds (0, 33.7 or 173.8 ng/g body wt/d) for three weeks prior to pairing. Maternal BDE-99 and very-low-density lipoprotein (VLDL) in plasma were measured during egg formation and at clutch completion, and BDE-99 was measured in the corresponding egg. The lipid-normalized egg-to-maternal tissue BDE-99 relationship decreased with increasing maternal burden. Individual variation in maternal VLDL was related to BDE-99 transfer to the eggs when BDE-99 was at background levels in control birds, but not when BDE-99 was elevated in dosed birds. The decrease in maternal plasma BDE-99 over the laying period was only significant ($p < 0.05$) in the high-dose birds. Finally, the decrease in BDE-99 in maternal plasma during egg-laying was significantly positively correlated with clutch mass in the high-dose group. These results suggest that the relationship between maternal and egg contaminant levels can be highly variable. This has significant implications for using eggs as indicators of adult or environmental concentrations. Environ. Toxicol. Chem. 2013;32:345–352. © 2012 SETAC

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INTRODUCTION

Hydrophobic contaminants can accumulate in the lipid-rich yolk of the avian egg, and consequently avian eggs are often used as a biomonitor for concentrations, distribution, and long-term trends of hydrophobic contaminants in the environment [1-3]. It is clear that contaminants found in eggs are of maternal origin, with females off-loading some of their own contaminant burden to their eggs during egg production [4,5]. However, the specific mechanisms of this maternal transfer of contaminants and the factors that might affect the rate or amount of contaminant transfer from the mother to the egg are not well understood. This information is important when using contaminant concentrations in eggs to infer contaminant levels in the environment, at the sampling site, or as a proxy of maternal body burdens because variation in egg contaminant levels could reflect differences in the physiological state or reproductive effort of individual females that laid the sampled eggs rather than variation in site or maternal contaminant residues per se.

If transfer of hydrophobic contaminants is solely regulated by a passive partitioning process among lipid-rich tissue compartments, the lipid-normalized egg-to-maternal tissue contaminant ratio is expected to be 1 [6]. However, in birds there is significant variability in the lipid-normalized egg-to-maternal tissue contaminant ratios among species, and ratios often deviate from 1 [4,6]. It has been suggested that this variability in maternal transfer among species might be related to the differences in reproductive strategies and level of egg investment [4]. Although there have been some within-species studies on the effects of laying order and chemical structure of the contaminant on maternal transfer [5,7,8], the influence of individual variation in characteristics such as body burden and yolk precursor levels on maternal transfer has received less attention. During egg production the lipid status of laying females changes dramatically due to the hepatic synthesis and secretion into plasma of large amounts of the lipid-rich yolk precursors (vitellogenin and very-low-density lipoproteins [VLDLs]) that serve as the main source of yolk lipids [9]. For example, plasma lipid concentrations increase from approximately 3 mg/ml in non-laying turkeys (*Meleagris gallopavo*) to 21 mg/ml in laying turkeys [10], and similar changes have been documented in free-living birds [11–13]. Importantly, there is marked interindividual variation (8–10-fold) in plasma yolk precursor levels for any given egg or follicle size [14]. In addition, in some species at least, there can be two- to fourfold variation in the size and number of eggs laid among females within populations and among years [15]. Thus, in addition to differences in maternal body burden of contaminants, both the lipid status and level of reproductive investment among females could affect the dynamics of maternal transfer of contaminants to eggs.

In the present study, we investigated sources of variation in the maternal transfer of the polybrominated diphenyl ether (PBDE) congener, 2,2′,4,4′,5-pentabromodiphenyl ether (BDE-99), to eggs using the zebra finch (*Taeniopygia guttata*) as a model songbird species. Polybrominated diphenyl ethers are a group of hydrophobic and bioaccumulative chemicals that find use as flame retardants, and have become ubiquitous in

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environmental, human, and wildlife samples [16]. One of the most pervasive congeners is BDE-99, which has been detected in avian tissue and egg samples throughout the world [17]. Female zebra finches were exposed to BDE-99 at three different dose levels (control, low, and high) prior to breeding. We then measured (1) BDE-99 and VLDL levels in maternal plasma during egg formation, (2) the BDE-99 concentration in the corresponding egg formed at the time the plasma sample was obtained, and (3) the BDE-99 and VLDL levels in maternal plasma at clutch completion to assess the extent to which maternal plasma burden was depleted during egg laying. The objectives of the present study were therefore (1) to examine how individual variation in body burden and yolk precursor levels influences the transfer of BDE-99 from the mother to the egg, (2) to determine the lipid-normalized egg-to-maternal plasma contaminant relationship, and (3) to determine how dosing level (i.e., body burden) interacts with individual variation in reproductive investment (clutch mass) to influence the reduction in maternal plasma BDE-99 over the laying period. Establishing the relationship between chemical concentrations in the mother and egg for a species can be useful for predicting the exposure of wild birds based on egg contaminant data. The relationship can also be useful in embryo toxicity studies: embryos could be dosed through maternal transfer, and the embryo exposure could be predicted from data on maternal body burden.

MATERIALS AND METHODS

Animals and husbandry

The present study was conducted on a captive colony of zebra finches maintained at the Simon Fraser University Animal Care Facility located in Burnaby, British Columbia, Canada. Zebra finches were housed in a controlled environment (temperature 19–23°C; humidity 35–55%; photoperiod 14 h light:10 h dark; lights on at 07:00). Nonbreeding birds were held in single sex cages $102 \times 39 \times 43$ cm. Breeding birds were housed in individual breeding cages ($51 \times 39 \times 43$ cm) equipped with an external nest box ($14 \times 14.5 \times 20$ cm). All birds were provided with mixed seed (panicum and white millet 1:2; 11.7% protein, 0.6% lipid, and 84.3% carbohydrate by dry mass), water, grit, and cuttlefish bone (calcium) ad libitum plus a multivitamin supplement in the drinking water once a week. Breeding pairs were also provided with an egg-food supplement (20.3% protein:6.6% lipid) daily from pairing to clutch completion (2 d after the last egg was laid). Experiments and animal husbandry were carried out under a Simon Fraser University Animal Care Committee permit (657B-96) in accordance with guidelines from the Canadian Committee on Animal Care.

Experimental protocol

All dosing was done with technical grade BDE-99 (> 98% purity, Cambridge Isotope Labs). The BDE-99 was analyzed via gas chromatography–mass spectrometry (GC–MS; electron capture negative ionization mode [ECNI]; see *Chemical analysis* section below), and the only bromide ion that was quantifiable was BDE-99. The BDE-99 was dissolved in safflower oil (Spectrum Organics), and the microliter amounts of BDE-99 dissolving solvent (nonane) were evaporated off using a steady stream of purified nitrogen gas. Females were treated with BDE-99 prior to breeding at two dose levels that we predicted would result in environmentally relevant body burdens based on a previous study in zebra finches [18]: 33.7 ng/g body weight/d ($n = 14$) and 173.8 ng/g body weight/d ($n = 14$). A safflower oil–only control group comprised $n = 15$ females. The BDE-99 levels in the safflower oil group were below the detection limits of our quantification method, and no PBDE congeners other than BDE-99 were detected in any of the dosing solutions. Adult females were randomly assigned to a dose group. Dose groups were kept in separate cages to prevent cross-contamination. Birds were orally dosed with BDE-99 or safflower oil daily for 21 d using a micropipette. Doses were adjusted daily according to female mass, with the dose volume being 10 μl/g body weight.

Following completion of the 21-d dosing period, females were paired with randomly selected males. All birds used in the breeding trials were experienced individuals that had previously produced at least one clutch. Nest boxes were checked daily between 09:00 and 11:00 for egg laying, and new eggs were numbered in consecutive order and weighed (0.001 g).

All plasma and egg samples were collected between 09:00 and 11:00. A blood sample was collected from each female on the day she laid her first egg (1E). All birds were blood-sampled from the brachial vein following puncture with a 26-G needle, and blood was collected into heparinized capillary tubes. Blood samples were centrifuged at 3,000 *g* for 10 min to separate plasma from the red blood cells, and plasma was then stored frozen (–80°C). In zebra finches, the day and night after a female lays her first egg corresponds to the deposition of yolk for the third-laid egg [19], and so the third egg was collected. Eggs were collected the morning that they were laid, that is, before significant incubation of each egg, so embryo development should not have affected egg composition. The collected egg was replaced by a dummy egg in the nest. Whole eggs were removed from the shell and stored frozen (–80°C) in chemically cleaned glass vials. A second blood sample was taken from each female at clutch completion (CC), defined as 2 d after the last egg was laid.

Lipid and yolk precursor analysis

Total lipid content of plasma was determined colorimetrically using olive oil as the calibration standard [20]. The average intra-assay coefficient of variation (CV) was 3.7% ($n = 2$ replicates), and the inter-assay CV was 2.6% ($n = 2$ assay plates). Plasma triglyceride was measured as an index of total plasma VLDL using an analytical assay for free glycerol and total glycerol (Sigma-Aldrich). This index of VLDL was developed in domestic fowl [21] and has been validated in zebra finches [22]. Plasma triglyceride was calculated as the difference between total glycerol and free glycerol. Average intra-assay CV was 3.5% ($n = 2$ replicates), and inter-assay CV was 4.5% ($n = 3$ assay plates) for plasma triglyceride. Total egg lipid content was determined gravimetrically from a 1-ml aliquot of the dichloromethane (DCM)/hexane sample extract (see *Chemical analysis* section below). The extract was placed on a preweighed aluminum dish and the solvent was evaporated; the dish was then reweighed to determine the total mass of the lipid.

Chemical analysis

All PBDE standards (BDE-17, -28/33, -47, -49, -66, -85, and -99) were purchased from Wellington Laboratories. Plasma, eggs, and dosing solutions were analyzed for BDE-17, -28, -47, -49, -54, -66, -71, and -99.

Plasma samples (0.038–0.120 g), egg samples (0.48–1.03 g), and dosing solutions (200 μl) were accurately weighed, and neutral fractions were extracted and cleaned up using established methodologies [23,24]. In brief, plasma samples were

spiked with 50 ng of each of the internal standards (BDE-30 and -156), acidified, denatured, and liquid–liquid extracted with 50% (v/v) methyl tert-butyl ether/hexane. The organic phase layer containing the PBDEs was separated and collected. Egg samples were ground with approximately 25 g of anhydrous sodium sulfate and extracted with 50% DCM/hexane using an accelerated solvent extraction system (Dionex ASE 200). The extraction columns were spiked with 20 ng of each internal standard. The column extraction eluant was concentrated to 10 ml, and a 10% portion was removed for gravimetric lipid determination. The remaining extracts were cleaned by gel permeation chromatography (GPC) and eluted from the GPC column with 50% DCM/hexane. The first fraction (140 ml) containing lipids and biogenic material was discarded, and the second fraction (200 ml) containing PBDEs was concentrated to a volume of approximately 4 ml. Dosing solution samples were initially processed with GPC and did not go through ASE extraction. The dosing solutions were spiked with 20 ng of each internal standard.

All samples were cleaned up using a silica solid phase extraction column (J.T. Baker). The column was conditioned with successive washes of 10% (v/v) methanol (6 ml) in DCM and then 8 ml of 5% DCM in hexane. The sample was then loaded onto the cartridge and eluted with 8 ml of 5% DCM/hexane. The eluant was then concentrated, and the solvent was exchanged with isooctane to a final volume of approximately 175 μl. The exact mass of each sample was recorded, and the final volume was determined by dividing by the density of 2,2,4-trimethylpentane (0.69 g/ml).

The PBDEs in the isolated chemical fractions were analyzed using GC-MS working in electron capture negative ionization mode (GC/ECNI–MS). Analytes were separated and quantified on an Agilent 6890 series GC equipped with a 5973 quadrupole MS detector (Agilent Technologies). The analytical column was a 15 m × 0.25 mm × 0.10 μm DB-5HT fused-silica column (J & W Scientific). Helium and methane were used as the carrier and reagent gases, respectively. A sample volume of 1 μl was introduced to the injector operating in pulsed-splitless mode (injection pulse at 25 psi until 0.50 min; purge flow to split vent of 96.4 ml/min to 2 min; gas save flow of 20 ml/min at 2 min), with the injector held at 240°C. The GC oven ramping temperature program was as follows: initial 100°C for 4 min, 25°C/min. until 260°C, 2.5°C/min until 280°C for 10 min, 25°C/min until 325°C and hold for a final 7 min. The GC-to-MS transfer line was held at 280°C, the ion source temperature was 200°C, and the quadruple temperature was 150°C.

The PBDE congeners were monitored using the bromine anions of m/z 79 and 81. Analytes were identified by comparison of retention times and ECNI mass spectra to those of the authentic standards.

Mean internal standard recoveries for the BDE-30 were 95 ± 2% SE for plasma analysis and 89 ± 2% SE for egg analysis. Mean internal standard recovery for BDE-156 was 110 ± 4% SE for plasma analysis, and 95 ± 4% SE for egg analysis.

Analytes were quantified using an internal standard approach; thus all reported values were inherently recovery-corrected. The method limits of quantification for BDE-99, based on a signal-to-noise ratio of 10, was 0.07 ng/g wet weight for plasma analysis, and 0.02 ng/g wet weight for egg analysis. Method blanks ($n = 19$) were included for each sample batch to assess background interference and possible contamination, and a blank subtraction was done for BDE -28, -47, -49, and -99. Duplicate analysis of the samples was not possible as all plasma and egg tissues were consumed to ensure quantifiable analyte levels. In-house standard reference material (polar bear [*Ursus maritimus*] plasma for plasma analysis, double-crested cormorant [*Phalacrocorax auritus*] egg for egg analysis) was also included in each sample batch to ensure consistency of data acquisition (within two standard deviations of in-house mean).

Statistical analysis

All statistical analyses were carried out using SAS 9.1.3 (SAS Institute, 2003). Data were found to meet assumptions of normality and homogeneity of variance following Shapiro–Wilk's and Levene's tests, and by inspecting q-q plots and residual plots. The effect of dose was assessed using generalized linear models, and post hoc tests for differences between means were adjusted for multiple comparisons following the Tukey–Kramer method. Correlations were measured by Pearson's correlation coefficients. The lipid-normalized egg-to-maternal plasma BDE-99 relationship was analyzed using linear and nonlinear regressions. We assessed the relationship between egg BDE-99 and maternal 1E VLDL or plasma lipid using linear regression, and we controlled for the effect of maternal BDE-99 on egg BDE-99 by using the residuals from the linear regression of egg BDE-99 on maternal BDE-99. We analyzed difference in maternal plasma from 1E to the CC stage using the repeated statement in the mixed procedure, controlling for clutch mass as a covariate. Unless otherwise stated, all analysis was done on lipid-normalized BDE-99 concentrations.

RESULTS

Influence of individual variation in body burden and yolk precursor levels on maternal transfer

Maternal treatment with BDE-99 had a significant dose-dependent effect on maternal plasma concentrations, which were significantly higher in the high-dose group compared to the control and low-dose group at both the 1E and CC stages (Table 1). High-dose birds had approximately eight times more BDE-99 than the low-dose birds at the 1E stage, and approximately 10 times more at the CC stage. Egg concentrations were also significantly affected by dose, but not to the same extent as

Table 1. Lipid-normalized BDE-99 concentrations (ng/g lipid wt; [mean and standard error]) in maternal plasma at the first egg (1E) and clutch completion (CC) stage, and in the third egg laid[a]

		Dose (ng BDE-99/g body wt/d)				
Sample	% Lipid	0	33.7	173.8	$F_{2,43}$	p
1E plasma	2.31	35.53 (9.91)A	1,083.70 (143.62)A	8,818.21 (1061.14)B	64.58	< 0.0001
CC plasma	2.00	29.50 (3.66)A	571.75 (61.01)A	5,688.45 (646.60)B	74.48	< 0.0001
Third egg	7.35	68.20 (11.88)A	2,148.37 (206.49)B	5,034.44 (408.78)C	94.00	< 0.0001

[a] Significant differences between groups are indicated by different upper case letters ($p < 0.05$).

maternal concentrations, because a smaller proportion of the maternal plasma burden was transferred to eggs in the high-dose group compared to the low-dose group. The high-dose group eggs had approximately 2.3 times more BDE-99 than low-dose group eggs (Table 1).

The lipid normalized egg-to-maternal plasma BDE-99 ratio was assessed by looking at the relationship between the BDE-99 concentration in the third egg (ng/g lipid wt) and the BDE-99 concentration in 1E maternal plasma (ng/g lipid wt) for each dose group. The control and high-dose group had a significant positive egg-to-maternal plasma BDE-99 relationship (control: $r^2 = 0.604$, $p = 0.0007$, slope = 0.93; high dose: $r^2 = 0.693$, $F_{1,12} = 27.12$, $p = 0.0002$, slope = 0.321), and the low-dose group had a marginally nonsignificant positive relationship, which may have improved if sample size were increased ($r^2 = 0.262$, $p = 0.061$, slope = 0.709). The slope of the egg-to-maternal plasma relationship in the control and low-dose groups was not significantly different from 1 ($t = 0.330$, $p = 0.748$ and $t = 0.847$, $p = 0.414$, respectively), and in the high-dose group the slope was significantly lower than 1 ($t = 11.029$, $p < 0.0001$). Because the slope of the egg-to-maternal plasma BDE-99 relationship decreased from control to low dose and from low to high dose, we tested the overall fit of an equation based on a saturation binding curve (*egg concentration = [A × maternal plasma concentration]/[B + maternal plasma concentration]*, where A is the maximum egg concentration, and B is the maternal plasma concentration needed to achieve half of the maximum egg concentration) to the full data set (Fig. 1). The parameter estimates for the nonlinear regression were A = 7,506 ± 674.2 SE and B = 3,488 ± 844.0 SE. The point of the curve at which the slope is 1 is when the maternal plasma BDE-99 concentration is 1629 ng/g lipid weight. Both the nonlinear and linear regressions significantly fit the full data set (adjusted $r^2 = 0.879$, $F_{2,41} = 351.95$, $p < 0.0001$ and adjusted $r^2 = 0.823$, $F_{1,41} = 196.19$, $p < 0.0001$, respectively); however, the fit of the nonlinear relationship was significantly better than the linear relationship ($F_{1,41} = 20.26$, $p < 0.0001$).

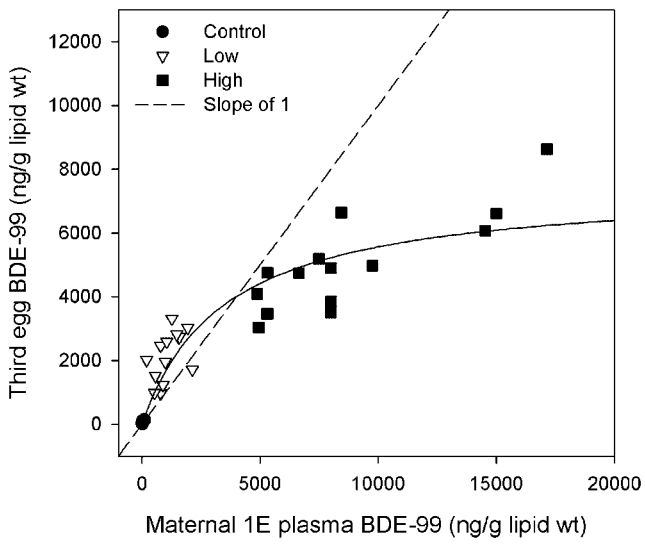

Fig. 1. Relationship between lipid-normalized BDE-99 concentration in the third egg, and the maternal plasma on the day that the first egg was laid (1E). The timing of yolk deposition for the third egg corresponds with the timing of the 1E blood sample. The lipid-normalized egg-to-maternal tissue BDE-99 relationship is described by the equation: *egg concentration = (7,506 × maternal concentration)/(3,488 + maternal concentration).*

Table 2. The relationship between egg BDE-99 (ng/g wet wt) and maternal plasma very-low-density lipoprotein (mg triglyceride/ml) controlling for the effect of maternal 1E plasma BDE-99 on egg BDE-99

	Unstandardized coefficients		Standardized coefficients			
Dose group	b	SE	β	*t*	*p*	R^2
Control	–0.289	0.131	–0.522	–2.2	0.046	0.272
Low	0.556	1.413	0.113	0.39	0.701	0.013
High	4.828	8.026	0.171	0.6	0.559	0.029

Maternal 1E plasma VLDL was negatively related to egg BDE-99 (ng/g wt wt) in the control group, but had no relationship with egg BDE-99 in either dose group (Table 2 and Fig. 2). Egg lipid was not correlated with BDE-99 concentration of the third egg (ng/g wet wt) in any dose group ($r < 0.125$, $p > 0.0671$), and was also not correlated with maternal plasma lipid or plasma VLDL at the 1E stage ($r = –0.175$, $p = 0.256$ and $r = –0.222$, $p = 0.148$, respectively). Mean maternal plasma lipid or plasma VLDL at the 1E stage was not significantly different between dose groups ($p > 0.070$).

Effect of dosing level and reproductive investment on maternal reduction in BDE-99

A significant decrease was found in maternal plasma BDE-99 over the laying period based on a repeated measures analysis, comparing 1E and clutch completion, including all doses and controlling for clutch size ($p = 0.001$). However, the interaction between dose group and plasma sample stage was highly significant ($p = 0.001$). Though there was a decrease in the maternal plasma concentrations of BDE-99 from the 1E to CC stage in all dose groups (Table 1), the decrease was only significant for the high-dose group ($p < 0.0001$; Fig. 3).

Total clutch mass (g) did not vary significantly with dose ($p = 0.709$). The decrease in maternal plasma BDE during egg-laying (i.e., the difference between 1E maternal plasma and CC maternal plasma BDE-99 concentrations) was significantly positively correlated with clutch mass in the high-dose group ($r^2 = 0.395$, $p = 0.016$) but not in the low ($r^2 = 0.275$, $p = 0.054$) or control dose groups ($r^2 = 0.007$, $p = 0.644$; Fig. 4). It is possible that the decrease in maternal plasma BDE-99 over the laying period is due to metabolism of BDE-99 rather than transfer to eggs. However, there were no significant differences in the BDE-47:BDE-99 ratio between the 1E and CC stages in any of the doses ($p > 0.131$ for all doses), something that would not be expected if metabolism of BDE-99 was responsible for the decrease.

DISCUSSION

In the present study, we found that individual variation in maternal plasma contaminant burden, yolk precursor levels, and reproductive effort (clutch mass) was related to maternal transfer of contaminants to eggs. Our dosing protocol resulted in environmentally relevant plasma burdens, with concentrations of up to 8,818.21 ng/g lipid weight in plasma, and up to 5,034.44 ng/g lipid weight in eggs. Concentrations of BDE-99 in free-living birds have been reported of up to 9,200 ng/g lipid weight in peregrine falcon (*Falco peregrinus*) eggs [25] and up to 26,600 ng/g lipid weight in the liver of sparrowhawks (*Accipiter nisus*) [26].

The lipid-normalized relationship between maternal plasma BDE-99 at the timing of yolk deposition and the BDE-99 in the

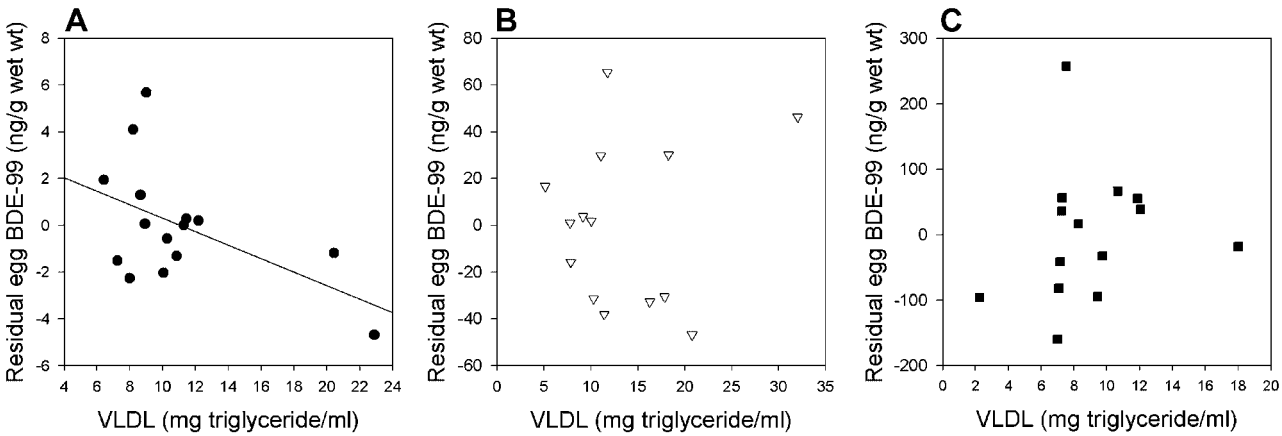

Fig. 2. Relationship between BDE-99 in the third egg (ng/g wet wt) and maternal plasma very-low-density lipoprotein (VLDL; mg triglyceride/ml) for each dose group, controlling for the effect of maternal 1E plasma BDE-99 on egg BDE-99. The slope (unstandardized coefficient) and associated probability for each relationship is given in Table 2. Egg BDE-99 was negatively related to maternal plasma VLDL in the control group (**A**), and had no relationship with egg BDE-99 in either the low (**B**) or high (**C**) dose group.

corresponding egg across all individuals followed a saturation curve, with the maximum predicted egg concentration being approximately 7,506 ng/g lipid weight. This relationship could have consequences for using eggs as indicators of adult contaminant burdens, as egg contaminant concentrations from highly exposed birds could underestimate adult concentrations. The mechanisms controlling egg:mother contaminant ratios are not well understood. Russell et al. [6] proposed that in oviparous organisms, transport of hydrophobic chemicals from the maternal tissues to the eggs is a passive process, and that the lipid-normalized egg-to-maternal tissue concentration ratio would equal 1. In birds, yolk precursors are transported by the plasma to the highly vascularized walls of the developing follicle, where they are then deposited into the developing oocyte via receptor-mediated endocytosis [9]. Being small, neutral, and hydrophobic molecules, PBDEs theoretically should be able to freely diffuse across biological membranes, such as the oocyte plasma membrane [27]. Because of the close association of the yolk with the blood stream, and because of the physicochemical properties of BDE-99, we would expect that the lipid-normalized egg contaminant and maternal plasma levels would be in equilibrium and have a 1:1 relationship. However, at high maternal BDE-99 concentrations the slope of the egg-to-mother relationship was significantly less than 1. One possible explanation for the relationship being less than 1 is that the transfer of BDE-99 to the eggs is not an entirely passive process. Contaminant molecules could bind to other lipophilic particles, such as the yolk precursors, and then be actively transported into the

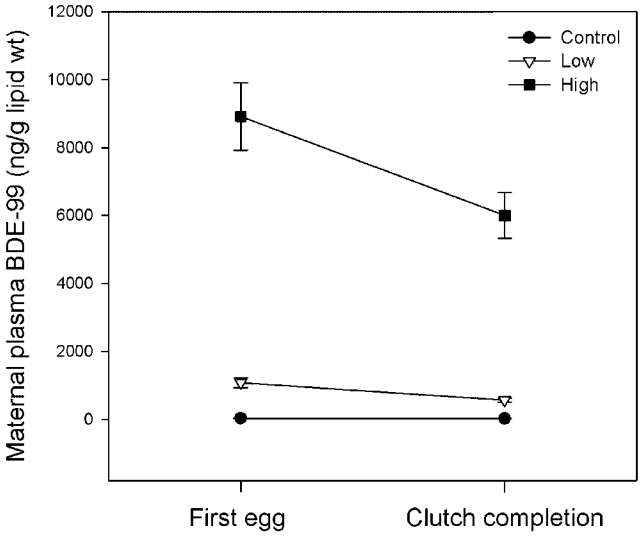

Fig. 3. Decrease in maternal plasma burden over laying period. Maternal plasma BDE-99 (ng/g lipid wt) concentration the day the first egg (1E) was laid and at clutch completion (CC). A significant decrease in plasma concentration occurs over the laying period, controlling for clutch size ($p = 0.001$). The interaction between dose and plasma sample is significant ($p = 0.001$). Maternal plasma BDE-99 did not differ significantly from the 1E to CC stage in the control or low-dose groups ($p = 0.991$ and $p = 0.352$, respectively), but there were significant differences between 1E and CC plasma BDE-99 concentrations in the high-dose group ($p < 0.0001$). Error bars represent standard error.

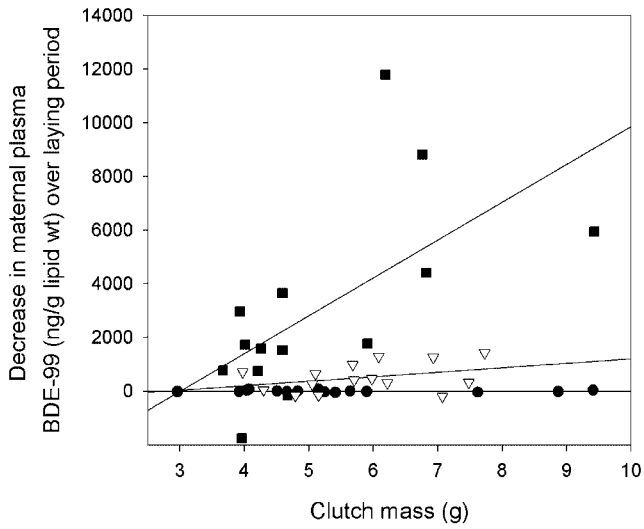

Fig. 4. Effect of clutch mass (total mass of all eggs laid) on the decrease in maternal plasma BDE-99 (ng/g lipid wt) over the laying period (from the day the first egg is laid to clutch completion). The high-dose group (solid squares) had a significant positive relationship between clutch mass and maternal plasma BDE-99 reduction ($r^2 = 0.395$, $p = 0.016$ slope = 1408.750), and the low-dose group (open triangles) had a weak positive relationship ($r^2 = 0.275$, $p = 0.054$, slope = 245.466). No relationship was found in the control group (solid circles, $r^2 = 0.007$, $p = 0.644$, slope = –1.638.

yolk in piggy-back fashion complexed with vitellogenin or VLDL molecules via receptor-mediated endocytosis [28]. If contaminant transfer to the egg is a receptor-mediated process, higher concentrations of BDE-99 could be saturating relative to lower concentrations, which corresponds with the relationship that we observed. Alternatively, if BDE-99 transfer to the eggs is entirely through passive diffusion, a possible explanation for egg-to-mother contaminant ratios less than 1 could be that during the rapid growth phase of the yolk there is insufficient time for BDE-99 to reach equilibrium across compartments. This is not likely the case, as the egg-to-maternal plasma BDE-99 relationship was able to reach a slope similar to 1 in the control and low-dose groups. It has also been proposed that lipid-normalized egg-to-maternal tissue contaminant ratios less than 1 are the result of dilution of egg lipid contaminants from maternal dietary lipids or newly synthesized lipids from the liver [29,30]. However, we measured circulating maternal BDE-99 rather than storage tissue BDE-99, which should account for any dilution from additional lipids. Overall, our data suggest that the maternal transfer of BDE-99 is at least partially through a saturable transport process, rather than exclusively passive diffusion.

It has also been suggested that the extent of egg contaminant dilution is related to reproductive investment [4], and birds that invest low quantities of maternal lipids in eggs will have ratios less than 1, whereas birds that invest large quantities of maternal lipids in eggs and use more endogenous sources of lipid for yolk formation will have ratios closer to 1 [4,29]. However in the present study, reproductive investment (clutch mass) was the same across dose groups, yet we still observed differences in the egg:mother contaminant ratios. Maternal plasma burden rather than reproductive investment influenced the egg-to-maternal tissue contaminant ratio in our zebra finches, and the lipid-normalized egg-to-maternal tissue contaminant ratio decreased with increasing maternal burden. In contrast to our findings, a study of maternal transfer of contaminants in white leghorn chickens (*Gallus domesticus*) found that the lipid-normalized egg:mother polychlorinated biphenyl ratio was not affected by body burden [5]. These contrasting results may be accounted for by differences in dosage, as our cumulative administered low and high doses were 707.7 and 3,649.8 ng/g body weight (33.7 and 173.8 ng/g body wt/d for 21 d), whereas in the leghorn chicken study total dosage ranged from 312.5 to 937.5 μg, which in a 1.5-kg hen would be equivalent to 208 to 625 ng/g body weight. It is possible that the dosage in the leghorn chicken study was not high enough for saturation to occur and the egg-to-maternal tissue ratio to start decreasing. Additional studies with different species examining the effect of individual variation in maternal burden and reproductive investment on the egg-to-maternal tissue contaminant ratios are needed to identify whether the saturating relationship we saw with BDE-99 is observed for other hydrophobic contaminants and in other species, which could help elucidate potential mechanisms of contaminant transfer.

To further investigate the relationship between maternal and egg BDE-99, we considered the effects of maternal yolk precursor (VLDL) levels on egg BDE-99 at the time of yolk deposition within each dose group. Mothers with more plasma VLDL transferred less BDE-99 in the control group, but there was no relationship between maternal VLDL and egg BDE-99 when maternal BDE-99 levels were elevated above background levels. Avian VLDL is 87% lipid and 13% protein, and is the main source for yolk lipids [9]. Very low density lipoprotein is taken up by the plasma membrane of growing oocytes via receptor-mediated endocytosis [31]. When VLDL levels in the plasma have saturated the transport mechanisms, increased plasma VLDL does not result in increased egg lipids. In this situation, as maternal plasma VLDL levels increase, the relative proportion of lipids in the plasma compared to the eggs would increase, and fewer hydrophobic contaminants would be transferred to the egg if contaminant transfer were passive. We found no correlation between maternal VLDL, or plasma lipids, and egg lipids. This is consistent with previous studies that have failed to find strong relationships between individual variation in plasma yolk precursor levels and egg composition [13,32], and suggests that levels of VLDL are typically above those needed to saturate the transport mechanisms in zebra finches. The negative relationship between maternal VLDL and egg BDE-99, such as would be expected if BDE-99 transfer was passive, was only observed in the control group when BDE-99 was at background levels, and the relationship dissociated at elevated BDE-99 concentrations. This pattern supports the idea that at higher concentrations the egg-to-mother BDE-99 relationship is not solely driven by passive partitioning, as we have suggested above.

We found that maternal dose group affected the extent of the reduction in maternal plasma BDE-99 from the 1E to CC stage. Maternal plasma BDE-99 significantly decreased from the 1E to the CC stage in the high-dose group but not in the low-dose group. Thus, whereas the total reduction in plasma BDE-99 was greater in the high-dose group (3,129.76 ng/g in the high-dose group, 511.95 ng/g lipid wt in the low-dose group), the proportion of BDE-99 lost was greater in the low-dose group (35.5% in the high-dose group, 47.2% in the low-dose group), which corresponds with the pattern of high-dose birds transferring proportionally less BDE-99 to their eggs. Maternal plasma BDE-99 stayed at low levels throughout the laying period in control birds. We also found that maternal dose group affected whether or not the reduction in maternal plasma burden over the laying period was related to reproductive investment (measured by clutch mass), as only the high-dose group showed a significantly positive relationship. The reduction in maternal plasma BDE-99 over the 3- to 7-d laying period is most likely due to elimination through maternal transfer, as there is evidence in birds that BDE-99 is persistent. with an estimated half-life of 100 to 175 d [3,33]. In addition, we observed no increase in the BDE-47:BDE-99 ratio, which suggests that metabolism by debromination did not contribute significantly to the reduction in maternal plasma BDE-99 burden. Several studies that report lower concentrations of contaminants in females than in male birds suggest that this is due to the female having an additional contaminant elimination route through egg laying [34–36]. However, differences in male and female contaminant concentrations are not always observed [37,38]. Our results show that although females can eliminate large amounts of their burden to the eggs, the reduction may not be significant in birds with low initial body burdens or small clutch masses. This could explain why differences in male and female contaminant burdens are not always observed. The relationships of contaminant exposure level and clutch mass with the reduction in maternal burden over the laying period could also have consequences on laying order effects, if the decreasing burden results in declining egg concentrations. Reviews of studies on laying order effects on egg contaminant concentrations have found that there is no consistent pattern for egg contaminants over the laying sequence, and the majority of studies show no significant laying order effects [7,39]. This is not surprising, as individual variation in maternal contaminant

burden and reproductive investment could result in variation in laying order effects among individuals. In addition, if the saturating egg-to-mother contaminant relationship that we observed for BDE-99 occurs for other contaminants, proportionally fewer contaminants will be transferred to the egg at higher maternal burdens, which could also minimize laying order effects.

In summary, we found a significant effect of plasma burden on maternal transfer, with more highly exposed birds transferring proportionally less BDE-99 to their eggs. These data suggest that maternal transfer of BDE-99 involves a saturable transport process. This has significant implications for using eggs as indicators of adult or environmental concentrations, as even within a species the egg-to-maternal tissue relationship can vary significantly, and eggs from highly exposed birds may underestimate adult exposure. Maternal burden also affects whether individual variation in female lipid status, as measured by yolk precursor levels of the mother, can influence contaminant transfer to the egg. Mothers with higher plasma VLDL levels transfer less BDE-99 to their eggs only when BDE-99 is at background levels. Finally, the decreases in maternal plasma BDE-99 burden over the laying period, as contaminants are transferred to eggs, was affected by dose level (i.e., body burden) and reproductive investment, with only the high-dose group showing a significant reduction burden, and the reduction being greater in birds with larger clutches. These results suggest that the presence of sex differences in adult contaminants or laying order effects on egg contaminants can be highly variable, even within a species.

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