Environmental Toxicology

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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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.nt0n9in.rnmfd2 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 maternalaffeof6-286.5(the)-682.polybrotamigated6-487.diphenylts

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

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

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 signi“cant positive egg-to-maternal plasma BDE-99 relationship (control: $r^2 ¼ 0.604$,

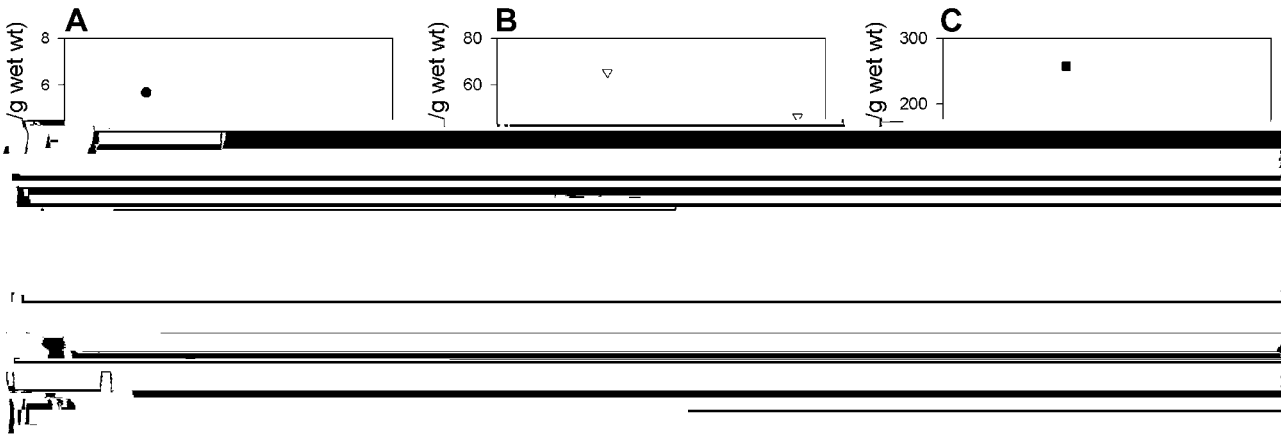

Fig. 2. Relationship between BDE-99 in the third egg (ng/g wet wt) and maternal plasma very-low-density lipoprotein (VLDL; mg triglyceride/ml) for each dose group, controlling for the effect of maternal 1E plasma BDE-99 on egg BDE-99. The slope (unstandardized coefficient) and associated probability 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

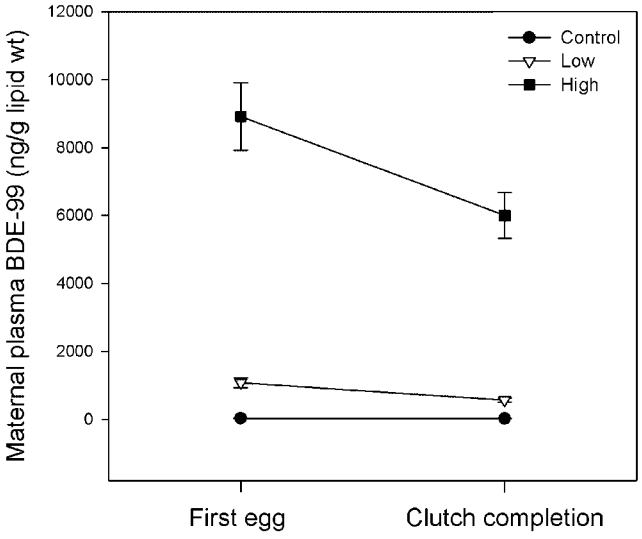

Fig. 3. Decrease in maternal plasma burden over laying period. Maternal plasma BDE-99 (ng/g lipid wt) concentration the day the first egg (1E) was laid and at clutch completion (CC). A significant decrease in plasma concentration occurs over the laying period, controlling for clutch size ($p = 0.001$). 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.

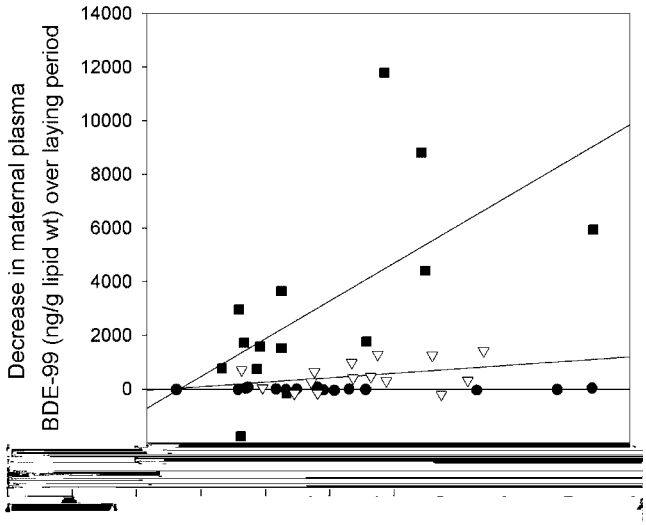

Fig. 4. Effect of clutch mass (total mass of all eggs laid) on the decrease in maternal plasma BDE-99 (ng/g lipid wt) over the laying period (from the day the first egg is laid to clutch completion). The high-dose group (solid squares) had a significant positive relationship between clutch mass and maternal 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 =

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

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