Comparative Body Compartment Composition and In Ovo Transfer of Organophosphate Flame Retardants in North American Great Lakes Herring Gulls

ARTICLE *in* ENVIRONMENTAL SCIENCE AND TECHNOLOGY · JUNE 2014

Impact Factor: 5.33 · DOI: 10.1021/es501334w · Source: PubMed

2 AUTHORS:

Alana K Greaves
Carleton University
6 PUBLICATIONS **72** CITATIONS
SEE PROFILE

Robert J Letcher
Environment Canada
331 PUBLICATIONS **12,386** CITATIONS
SEE PROFILE

Environmental Science & Technology

Article

pubs.acs.org/est

Comparative Body Compartment Composition and *In Ovo* Transfer of Organophosphate Flame Retardants in North American Great Lakes Herring Gulls

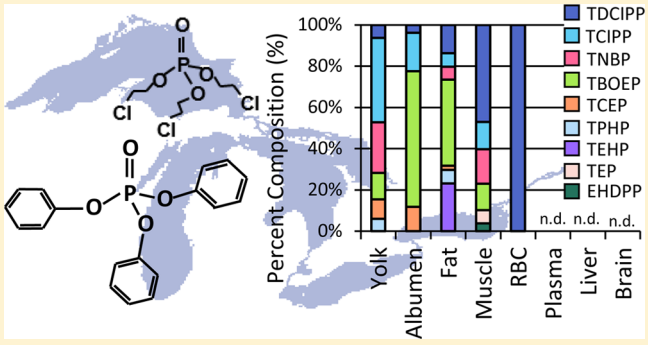
Alana K. Greaves[†,‡] and Robert J. Letcher*[,†,‡]

[†]Department of Chemistry, Carleton University, Ottawa, Ontario K1S 5B6, Canada

[‡]Ecotoxicology and Wildlife Health Division, Science and Technology Branch, Environment Canada, National Wildlife Research Centre, Carleton University, Ottawa, Ontario K1A 0H3, Canada

Supporting Information

ABSTRACT: Although recent usage of organophosphate (OP) flame retardants has increased substantially, very few studies have reported on OPs in biota including wildlife, and essentially there is no information on OP body compartment composition and *in ovo* or *in utero* transfer for any given wildlife species. Concentrations and patterns of 16 OP triesters were presently screened for and/or determined in six body compartments from female herring gulls (*Larus argentatus*; n = 8) and the separate egg yolk and albumen of their entire clutches of eggs (n = 16) (collected in 2010 from a Lake Huron colony site, Laurentian Great Lakes of North America). Fat (32.3 ± 9.8 ng/g wet weight; ww) contained the highest ΣOP concentration, followed by egg yolk (14.8 ± 2.4 ng/g ww) ≈ egg albumen (14.8 ± 5.9 ng/g ww) > muscle (10.9 ± 5.1 ng/g ww) ≫ red blood cells (1.00 ± 0.62 ng/g ww), whereas in liver, blood plasma, and brain all OPs were not detectable. Nine OPs accumulated in herring gulls, but the concentrations and proportions of OPs were dependent on the body and egg compartment. For example, tris(2-butoxyethyl) phosphate (TBOEP) accounted for 66% of the ΣOP concentration in albumen, but only for 13% in yolk. Tri-*n*-butyl phosphate (TNBP) accounted for 25% of the ΣOP concentration in yolk, but was not detected in albumen. Estimates showed that overall OP burdens in the body (3.5 μg) were greater than in the whole egg (1.2 μg), although depuration via *in ovo* transfer was substantial.



INTRODUCTION

With the production phase-out and regulation of brominated flame retardants (FRs) such as polybrominated diphenyl ethers (PBDEs), the use of alternative and replacement flame retardants such as organophosphate (OP) flame retardants has increased significantly. Total worldwide consumption of all flame retardant substances was 1.5 million T in 2004, with 14% attributable to OPs.[1] Also, OPs accounted for 20% of all FR consumption in Europe in 2006. In addition to their use as FRs (in, e.g., polyurethane foams and textiles), OPs are also often used as plasticizers for applications in floor polishes, lacquers, engineered thermoplastics, and epoxy resins, among others.[2] Multiple abiotic studies have shown that OPs such as tris(2-chloroethyl) phosphate (TCEP), tris(2-butoxyethyl) phosphate (TBOEP), tris(2-chloroisopropyl) phosphate (TCIPP), and tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) are present in air, water, and sediment samples collected throughout the northern hemisphere, and recently from the East Antarctic ice sheet.[3−15]

A growing number of toxicological studies have shown that OPs such as TBOEP, TCIPP, TDCIPP, triethyl phosphate (TEP), and tris(methylphenyl)phosphate (TMPP) elicit effects on embryonic development, mRNA expression, thyroid hormones, circulating bile acid concentrations, and the neurological system in fish, birds, rodents, and/or humans.[16−25]

Relatively few studies have investigated OPs in wildlife, particularly in birds. Eulaers et al.[26] very recently screened for six OPs in feathers and blood plasma of white-tailed eagle nestlings from Norway. TCEP and TCIPP were dominant in feathers (110 and 91 ng/g dry weight, respectively), while TCIPP and tris(1,3-dicholoro-2-propyl) phosphate (TDCIPP) were the only OPs detected in plasma (0.22 ng/g ww, each). Ma et al.[27] recently reported OPFR concentrations in muscle tissue of domestic chickens (*Gallus gallus domesticus*) and ducks (*Anas platyrhynchos domesticus*). TNBP, TCEP, TBOEP, and triphenyl phosphate (TPHP) were present at highest concentrations (up to 281 ng/g lipid weight; ~14 ng/g wet weight, ww). Chen et al.[28] also showed that of 12 OPs analyzed for, five were frequently quantifiable (including TPHP and

Received: March 18, 2014
Revised: June 2, 2014
Accepted: June 6, 2014
Published: June 6, 2014

Table 1. Mean (± Standard Error) Organophosphate (OP) Flame Retardant Concentrations Across All Herring Gull Tissues[a]

Compound	Full Name	Yolk (n = 16)	Albumen (n = 16)	Fat (n = 8)	Muscle (n = 8)	Red Blood Cells (n = 8)	Blood Plasma (n = 8)	Liver (n = 8)	Brain (n = 8)
TBOEP	tris(2-butoxyethyl) phosphate	1.89 ± 0.69	8.09 ± 2.49	13.4 ± 5.6	1.37 ± 0.78	nd−4.69 (38)	nd−2.25 (13)	nd	nd
TCEP	tris(2-chloroethyl) phosphate	1.38 ± 0.31	1.46 ± 0.77	0.65 ± 0.25	nd−1.51 (38)	nd	nd	nd	nd
TCIPP	tris(2-chloroisopropyl) phosphate	6.05 ± 1.36	2.31 ± 1.64	2.14 ± 0.73	1.42 ± 0.61	nd	nd	nd	nd
TDCIPP	tris(1,3-dichloro-2-propyl) phosphate	0.93 ± 0.34	0.47 ± 0.29	4.43 ± 1.87	5.04 ± 3.69	1.00 ± 0.62	nd−0.10 (13)	nd−0.37 (25)	nd−1.28 (25)
TNBP	tributyl phosphate	3.63 ± 0.58	nd−28 (25)	2.02 ± 0.91	1.79 ± 0.58	nd	nd	nd	nd
TPHP	triphenyl phosphate	0.89 ± 0.25	nd−4.18 (38)	2.11 ± 1.11	nd−1.03 (38)	nd−1.00 (13)	nd	nd	nd
TEHP	tris(2-ethylhexyl) phosphate	nd	nd−1.94 (31)	7.49 ± 6.48	nd−0.22 (25)	nd	nd	nd	nd
TEP	triethyl phosphate	nd−0.81 (38)	nd−23.0 (38)	nd−5.89 (25)	0.68 ± 0.36	nd	nd	nd−0.93 (13)	nd
EHDPP	2-ethylhexyl-diphenyl phosphate	nd−2.49 (19)	nd−10.9 (25)	nd−2.11 (25)	0.41 ± 0.09	nd−0.31 (13)	nd−0.18 (25)	nd	nd
TPP	tripropyl phosphate	nd	nd	nd	nd	nd	nd	nd	nd
TMPP	trimethylphenyl phosphate	nd	nd	nd	nd	nd	nd	nd	nd
TDBPP	tris(1,3-dibromo-2-propyl) phosphate	nd	nd	nd	nd	nd	nd	nd	nd
T2B4MP	tris(2-bromo-3-methylphenyl) phosphate	nd	nd	nd	nd	nd	nd	nd	nd
T4B3MP	tris(4-bromo-3-methylphenyl) phosphate	nd	nd	nd	nd	nd	nd	nd	nd
T3B4MP	tris(3-bromo-4-methylphenyl) phosphate	nd	nd	nd	nd	nd	nd	nd	nd
V6	2,2-bis(chloromethyl)propane-1,3-diyl tetrakis(2-chloroethyl) bis(phosphate)	nd	nd	nd	nd	nd	nd	nd	nd
Σ-OPs		14.8 ± 2.4	14.8 ± 5.9	32.3 ± 9.8	10.9 ± 5.1	1.00 ± 0.62	nd	nd	nd

[a]For compounds detected in less than half of samples for a given tissue, concentration ranges are shown (min−max), with detection frequency in parentheses. All concentrations are given in ng/g wet weight.

dx.doi.org/10.1021/es501334w | Environ. Sci. Technol. 2014, 48, 7942−7950

TDCIPP) in n = 13 individual herring gull eggs (*Larus argentatus*) collected in 2010 from Channel-Shelter Island (Lake Huron). TCIPP (0.21 to 4.1 ng/g ww), TCEP (0.02 to 0.55 ng/g ww), and TBOEP (0.16 to 2.2 ng/g ww) were most concentrated of all OPs detected. Among five colony sites in the Laurentian Great Lakes, Letcher et al.[29,30] analyzed 2010-collected herring gull egg pools for the same 12 OPs and reported the detection of TBOEP, TPHP, TCIPP, and TCEP. ΣOP concentrations ranged among the five sites from 2.02 (Chantry Island, Lake Huron) to 6.69 ng/g ww (Agawa Rock, Lake Superior). Salamova et al.[3] very recently reported on the levels of 12 OPs in air particle phase samples collected at five sites in the Great Lakes basin from March to December 2012. ΣOPFR concentrations ranged from 120 ± 18 to 2100 ± 400 pg/m^3, and were dominated by chlorinated OPs at urban sites, and by nonchlorinated OPs at remote sites. These ΣOP concentrations were around 2−3 orders of magnitude higher than for other brominated flame retardants in similar particle phase samples.

There is generally a dearth of reports on OPs in wildlife and fish,[2] and to our knowledge there are no published reports on OP body compartment composition and *in ovo* or *in utero* transfer for any given wildlife species. One laboratory study with male Wistar rats showed that tris(methylphenyl) phosphate (TMPP; formally known as tricresyl phosphate (TCrP)), is distributed to the adipose, liver, and kidney shortly after acute exposure.[31] The present study is a body compartment composition investigation on the deposition and burden of 16 OPs that were analyzed for in multiple tissues and blood (plasma and red blood cells) of female herring gulls (Lake Huron, Great Lakes of North America), as well as an examination of the *in ovo* transfer of these OPs into yolk and albumen of their entire clutches of eggs.

MATERIALS AND METHODS

Chemicals and Standards. A full list of the 16 target compounds, along with their full chemical names can be found in Table 1. TEP, TPP, TNBP, TCEP, TBOEP, TPHP, TEHP, TDBPP, and EHDPP were all purchased from Sigma-Aldrich (St. Louis, MO). TCIPP was purchased from AK Scientific (Union City, CA); TDCIPP and TMPP were purchased from TCI America (Portland, OR); and T2B4MP, T4B3MP, and T3B4MP were synthesized by GL Chemtech (Oakville, Canada). The OP 2,2-bis(chloromethyl-propane-1,3-diyl tetrakis(2-chloroethyl)bis(phosphate) (V6) was generously donated by Dr. Heather Stapleton (Duke University, NC). All possible sources of isotopically enriched OP standards were investigated, and five such internal standards were found. The d_{27}-TNBP and d_{15}-TEP internal standards were purchased from Cambridge Isotope Laboratories (Tewksbury, MA), and the d_{15}-TPHP internal standard was purchased from Wellington Laboratories (Guelph, Canada). d_{12}-TCEP and d_{15}-TDCIPP were prepared by and purchased from Dr. Vladimir Belov (Max Planck Institute for Biophysical Chemistry, Germany). All solvents, including dichloromethane, hexane, and HPLC-grade methanol were obtained from Caledon Laboratories (Georgetown, Canada), and ISOLUTE NH2 aminopropyl silica gel was obtained from Biotage (Charlotte, NC).

Sample Collection. Female herring gulls (*Larus argentatus*; n = 8), and their eggs (n = 16; 2 eggs total in each clutch per female), were collected from Chantry Island, Lake Huron (44° 19′ N, 81° 2′ W) in April 2010. Four tissues (liver, brain, fat, and muscle), red blood cells and plasma, and egg yolk and albumen were collected. All samples were collected within 1 h *post mortem* using chemically cleaned scalpels and scissors. Collected blood was separated into plasma and red blood cells on-site via a portable centrifuge. All tissues, with the exception of the eggs, were stored in chemically cleaned 2-mL cryovials, and placed in liquid nitrogen dry-shippers until their arrival at the National Wildlife Research Centre (NWRC, Ottawa, ON, Canada) for long-term storage at −40 °C until the time of analysis. Eggs were carefully removed from nests and placed in padded cases designed for herring gull eggs. Eggs were kept at ambient temperature (between 2 and 10 °C) for 24−48 h until their arrival at the NWRC. After separation of yolk and albumen, samples were stored at −40 °C until the time of analysis.

Extraction and Cleanup. In the Letcher Lab−Organic Contaminants Research Laboratory (OCRL; NWRC, Ottawa, ON, Canada), the extraction and cleanup method followed that of Chen et al.[28] with minor modifications, including the use of additional deuterated OP internal standards. Briefly, approximately 0.6 g of a given sample to be analyzed was ground with 3 g of diatomaceous earth, and loaded into a 15 mL stainless steel extraction cell. All five deuterated OP internal standards (d_{12}-TCEP, d_{15}-TDCIPP, d_{15}-TPHP, d_{27}-TNBP, and d_{15}-TEP) were spiked at 5 ng each to all samples. The spiked samples were extracted for OPs using accelerated solvent extraction (Dionex ASE 200) operating at 100 °C and 1500 psi, using a 1:1 mixture of dichloromethane (DCM) and hexane. Anhydrous sodium sulfate (1 g) was added to the resulting samples to remove any water, and the solution was concentrated to 10 mL. Ten percent was removed for gravimetric lipid determination, while the remaining 90% was concentrated to 0.5 mL and loaded onto a preconditioned solid-phase extraction (SPE) cartridge containing 1 g of ISOLUTE NH2 aminopropyl silica gel. After rinsing the cartridge with 3 mL of 20:80 DCM/hexane, OPs were eluted using 3 mL of 20:80 DCM/hexane, followed by 8 mL of DCM, and finally 4 mL of 90:10 DCM/methanol. The eluate was evaporated to dryness, reconstituted in methanol, and analyzed via high-performance liquid chromatography−electrospray (positive mode)−tandem quadrupole mass spectrometry (LC−ESI(+)−MS/MS).

Quantification. All extracted sample fractions were analyzed for the suite of 16 OPs (Table 1) using LC−ESI(+)−MS/MS. Analysis was performed using a Waters 2695 HPLC coupled to a Waters Quatro Ultima triple quadrupole. All target compounds, with the exception of V6, were analyzed using a Waters Symmetry C_{18} column (100 mm L × 2.1 mm i.d., 3.5 μm particle size). V6 was analyzed using a Phenomenex LUNA C_{18} column (50 mm L × 2 mm i.d., 3 μm particle size). All compounds were identified based on their retention times compared to authenticated standards, and their characteristic mass transitions observed by multiple reaction monitoring. An eight-point calibration curve was performed daily to ensure linearity of response and to allow for quantification. Quantification was done using MassLynx 4.0 (Waters, 2002). Additional details regarding HPLC analysis, including all monitored transitions, can be found in the Supporting Information (SI) Table S1.

The five deuterated internal standards spiked at the start of the sample extraction accounted for matrix effects and sample-to-sample variations. Mean percent recoveries (±standard error; SE) of higher molecular weight ISs (d_{12}-TCEP, d_{15}-TDCIPP, d_{15}-TPHP, d_{27}-TNBP) were highly quantitative and

very similar (89% ± 1, 88% ± 2, 93% ± 1, and 91% ± 1, respectively), whereas the more volatile d_{15}-TEP had an overall lower mean recovery but with very good replicate precision (40% ± 2). All target OP concentrations were inherently corrected for any recovery inefficiencies as the internal standard quantification approach was used. Target compounds for which there was not a matched isotopically enriched internal standard available were assigned one of the five ISs as a surrogate IS. Surrogate ISs were chosen based on similarity of chemical structure to the target compound: d_{15}-TDCIPP was chosen as a surrogate IS for TCIPP and TDBPP; d_{15}-TPHP was chosen as a surrogate for TMPP, EHDPP, and all three TBMP isomers; and finally d_{27}-TNBP was chosen as a surrogate for TPP, TBOEP, and TEHP.

Quality Control and Assurance. Three replicate procedural blanks were run with every batch of eight samples to determine any background OP contamination. Procedural blanks were treated identically to all samples, with the exception that blanks did not contain sample tissue, but rather 0.25 g of chicken egg homogenate, previously determined to contain nondetectable levels of all target OPs. Chicken egg homogenate was added to the blanks since percent recoveries were quite low without it (28% ± 12, compared to 100% ± 8 with the chicken egg homogenate acting as a keeper). All solvents used during this experiment, including dichloromethane, hexane, and especially methanol, contained quantifiable levels of multiple OPs. Over 90% of procedural blanks (n = 30) contained TBOEP (15.0 ± 1.1 ng/g wet weight; ww), TCIPP (12.4 ± 7.6 ng/g ww), TNBP (5.6 ± 0.5 ng/g ww), TCEP (2.2 ± 0.2 ng/g ww), TEP (2.0 ± 0.4 ng/g ww), TPHP (1.5 ± 0.1 ng/g ww), EHDPP (1.2 ± 0.1 ng/g ww), TDCIPP (1.0 ± 0.09 ng/g ww), and TEHP (0.4 ± 0.04 ng/g ww). All sample concentrations were therefore blank-corrected on a batch-by-batch basis using the average of the three procedural blanks in each batch. A control was also run with every batch of eight samples to ensure reproducibility across all batches. To our knowledge, a biological standard reference material is not yet commercially available for OP analysis. Therefore, an in-house standard reference material was created for the purposes of this project: chicken egg homogenate, predetermined to be free of OP contamination, was spiked with a cocktail of all 16 OP target compounds (Table 1) assessed for in this study, such that the concentration of each OP in the tissue was 10 ng/g wet weight. Controls were reproducible, with a mean relative standard deviation of 20% across all target compounds. The Letcher Lab—OCRL participated in 2012 in the first international NORMAN PFR (OP) Interlaboratory Study (ILS). For this QA/QC exercise, the OP results from the OCRL complied within 10% of the NORMAN PFR certified data.

Statistical Analysis. All statistical analysis was performed using Statistica 8.0 (StatSoft, 2008). Normality of the data was tested using a Shapiro-Wilk test, with α set to 0.05. Initial testing found that the majority of OPs were not normally distributed; however after log-transformation, all compounds were normally distributed ($p \geq 0.05$). Statistical analysis was performed only for compounds where concentrations were above the method limit of quantification (MLOQ) in at least half of the samples. A full list of MLOQs can be found in SI Table S1. For compounds where at least half of the samples had quantifiable concentrations, any individual sample below the MLOQ was randomly assigned a concentration between zero and the MLOQ for statistical purposes. All correlations, including correlations between OP concentrations and tissue lipid content, were evaluated using a Pearson product-moment correlation, with $p \leq 0.05$ indicating statistical significance, and a Pearson product-moment correlation coefficient, $r \geq 0.5$ indicating a strong correlation. The influence of laying order on egg OP concentrations was examined using one-way ANOVA, followed by a Tukey honest significant difference test, with $p \leq 0.05$ indicating statistical significance. Finally, principle component (PC) analysis was used to determine potential patterns and trends in the data set, such as covariation of compounds among tissues. To ensure a normalized data set, all PC analyses were done using percent composition data.

The mass burdens of all detected OPs were estimated based on empirical data collected during collection and sample processing, as well as previously documented data. Mean masses (± SE) for whole body (946 g ± 15) and egg (82 g ± 2) were recorded for all samples. The total mass of fat was not recorded, although Norstrom et al.[32] found that body fat accounts for approximately 8.2% of the total body weight of a herring gull. Therefore, the average mass of body fat in our bird samples was 78 g ± 1. The total mass of muscle was also not recorded. To our knowledge, there has not been a study to determine the total muscle mass in herring gulls. However, the largest muscle in herring gulls, and the muscle from which our samples were taken, is the pectoralis muscle. In the California Gull (*Larus californicus*), the pectoralis muscle accounts for approximately 10.8% of total body weight.[33] Using this figure as an estimate, the mean pectoralis muscle mass was found to be 102 g ± 2, which acts as a lower bound muscle mass estimate in herring gulls.

RESULTS AND DISCUSSION

Tissue-Specific Accumulation of OP Flame Retardants in Herring Gulls. OP concentrations varied greatly among body and egg compartments. ΣOP concentrations (mean ± SE) were highest in fat (32.3 ± 9.8 ng/g ww), followed by yolk (14.8 ± 2.4 ng/g ww), albumen (14.8 ± 5.9 ng/g ww), and muscle (10.9 ± 5.1 ng/g ww). Red blood cells contained a low but quantifiable ΣOP concentration (1.00 ± 0.62 ng/g ww) (Table 1), and blood plasma, liver, and brain all had OP concentrations below the detection limits (SI Table S1). The lack of OPs in the brain strongly suggests that the blood—brain barrier, a highly selective barrier that filters incoming and outgoing blood to the brain, is effective at preventing OPs from entering the brain. Furthermore, the presence of OPs in fat and muscle (long-term storage tissues), but not in liver or blood plasma (short-term mobilization tissues) suggests rapid metabolism of OPs.

OP triesters, like the ones analyzed in this study, are thought to be easily dealkylated to their respective diesters via hydrolysis in the body.[1,34,35] These OP triesters are assumed to dealkylate either spontaneously or enzymatically via α-esterases and phosphorylphosphatases.[1] Many factors may affect their biodegradability, including bulky alcohol or halogenated moieties causing steric effects, and high lipophilicity.[8] Chu et al.[35] recently demonstrated that rat liver microsomes metabolized TDCIPP to its diester, bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), very rapidly and effectively (over 95% conversion within 100 min). Sasaki et al.[34] and Chapman et al.[36] also assessed OP triester metabolism in rat liver microsomes, and found that TPHP was readily metabolized to diphenyl phosphate (DPHP), and TCEP was partially metabolized to bis(2-chloroethyl) phosphate (BCEP). TNBP was also partially metabolized to dibutyl-*n*-phosphate

(DNBP), however its major metabolites were hydroxylated forms of TNBP (i.e., TNBP−OH, and TNBP−OH_2).[34] Further evidence of metabolism has been seen in avian hepatocytes, zebrafish embryos, and chicken embryos, which when exposed to TDCIPP and/or TCIPP showed upregulation of phase 1 and/or phase 2 metabolism enzymes.[19,20,37] Although the metabolism of the OPs under review in this study has yet to be tested in herring gulls, it is likely that these triesters are readily metabolized in these birds. Therefore, it is important to note that all OP concentrations seen in this study are likely residual levels, the result of factors such as, e.g., selective deposition to a given tissue, enzyme-mediated metabolism, and/or chemical degradation in the body.

It has been shown that the first-laid egg in a clutch is always the largest and heaviest, and eggs sequentially laid are of decreasing weight, with up to a maximum of three eggs per clutch.[38] From the recorded egg lengths and masses of all collected eggs, it is possible to distinguish which of the two eggs from each clutch was laid first. The effect of laying order on OP concentrations was examined across all egg clutches in the present study. No significant ($p > 0.33$) differences between first- and second-laid eggs were observed for the ΣOP concentration or for any individual OP concentration. For Norwegian glaucous gulls (*Larus hyperboreus*), Verreault et al.[38] reported that concentrations of Σ-polybrominated diphenyl ethers (PBDEs), Σ-polychlorinated biphenyls (PCBs), and Σ-organochlorine (OC) contaminants did not statistically vary between first-, second-, or third-laid eggs in clutches. As Verreault et al.[38] hypothesized, the lack of intraclutch variability could indicate constant movement and shuttling of these compounds in the body, as well as a constant exposure to these compounds via the environment and/or diet.

Of the 16 OPs analyzed for in the present study, to our knowledge, V6, TEP, T3B4MP, and T4B3MP have not been screened for or reported in any free-ranging wildlife or fish species. Regardless, these OPs were not detectable in the present herring gull body or egg compartment samples. The OPs detected and quantifiable in more than one body or egg compartment were TBOEP, TCEP, TCIPP, TDCIPP, TNBP, and TPHP. These six OPs are also the major ones that have been found in multiple abiotic studies worldwide, including surface water, sewage treatment plants, and sediment.[2,5,6,8] Eulaers et al.[26] very recently examined for six OPs (TDCIPP, TCEP, TCIPP, TBOEP, TPHP, and EHDPP) in feathers and blood plasma of white-tailed eagle nestlings from Norway. TCEP and TCIPP were dominant in feathers (110 and 91 ng/g dry weight, respectively), while TCIPP and TDCIPP were the only OPs detected in plasma (0.22 ng/g ww, each). Salamova et al.[3] found that these same six OPs (TBOEP, TCEP, TCIPP, TDCIPP, TNBP, and TPHP) were detected in the particle phase of air from five collection sites (in 2012) around the Great Lakes. Urbanized areas (i.e., Cleveland, and Chicago) contained overall higher ΣOP concentrations (maximum of 2100 ± 400 pg/m^3) than more remote areas (i.e., Sturgeon Point, Eagle Harbor, and Sleeping Bear; minimum of 120 ± 18 pg/m^3). Furthermore, recent investigations by Letcher et al.[29,30] showed that these same six OPs, in particular TBOEP, TCEP, and TCIPP, were found in multiple aquatic food web species (including mysis, plankton, and multiple fish species) from western Lake Erie and Lake Ontario.

In the present herring gulls, all body and egg compartments contained different proportions of OPs (Figure 1). For example, in the egg, TBOEP accounted for approximately

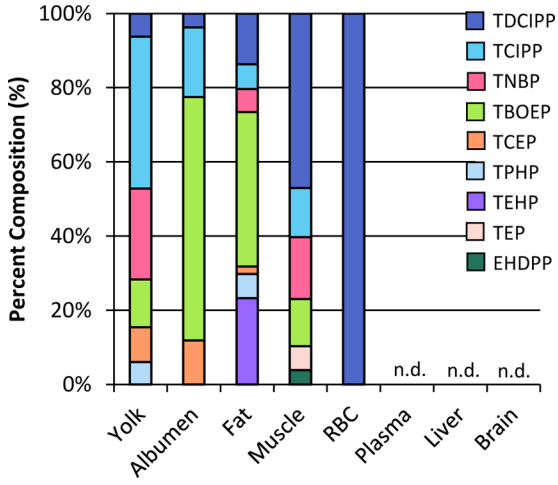


Figure 1. Percent proportions of the arithmetic mean ΣOP flame retardant concentrations for each tissue (n = 16 for yolk and albumen, n = 8 for all other tissues).

66% of the ΣOP concentration in albumen, but only for 13% in yolk; TNBP accounted for 25% of ΣOPs in yolk, but was not detected at all in albumen. In the maternal gull, TDCIPP accounted for 100% of the ΣOP concentration in red blood cells, but only approximately 46% in muscle, and only 14% in fat. The difference in OP patterns between tissues is further illustrated in the PCA loading and score plots (Figure 2). PC 1 and PC 2 (38.6% and 21.8%) account for >60% of the overall variability in the data. PCA showed that individual OPs were

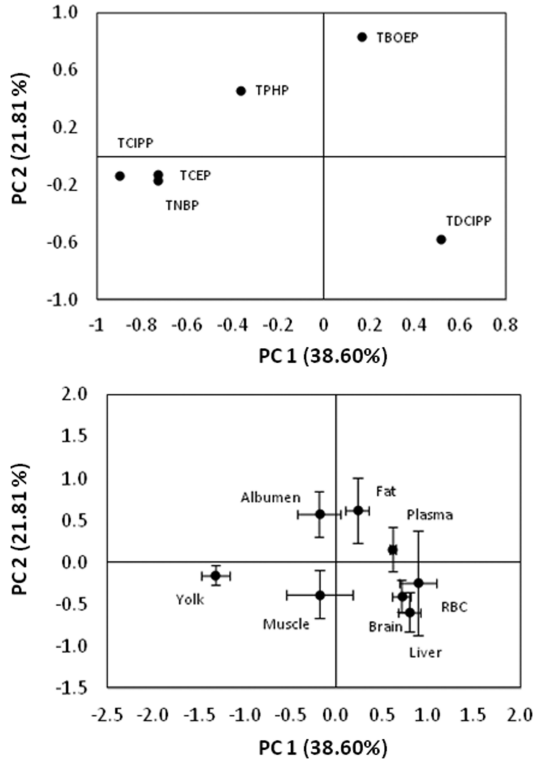


Figure 2. Principle components analysis (PCA) of the ratios of individual OP to ΣOP concentrations and showing the first and second principle components: PC 1 and PC 2 (top). Mean (± standard error) factor scores for all tissues are shown in the bottom plot. Variabilities associated with PC 1 and PC 2 are given.

dx.doi.org/10.1021/es501334w | Environ. Sci. Technol. 2014, 48, 7942−7950

generally well distributed across both factors, although TCEP, TCIPP, and TNBP were clustered together. The score plot (Figure 2) indicates that this clustering is driven by the yolk. Regardless, these tissue-specific accumulation patterns demonstrated that the depositional OP patterns are to some extent body- and egg compartment-specific.

In an attempt to understand these OP pattern differences between tissues, correlative relationships were examined among compound concentrations and various physical parameters (i.e., bioconcentration factors of OPs, extractable lipid content in samples, and partition coefficients of OPs). The bioconcentration factor (BCF) reflects the extent to which a compound will concentrate from the surrounding water into an aquatic organism. Authors van der Veen and de Boer[2] compiled a list of the known BCFs for OPs, including the major OPs detected in this study: TCEP, TCIPP, TDCIPP, TPHP, TNBP, and TBOEP. These six OPs have a large range of BCFs, from 1.37 for TCEP to 1080 for TBOEP. From examination of the correlations between OP concentrations and their BCFs, a weak but significant correlation was observed in yolk (p = 0.012, r = 0.28; SI Figure S1). A similar correlation was observed in albumen (p = 0.006, r = 0.39), although no significant correlative relationships were observed in the maternal gull. Overall, and considering that herring gulls have a diet comprising mainly fish, it appears that the BCFs do not play a significant role in the body compartment deposition and distribution of OPs in herring gulls.

Correlations between all OP concentrations and the lipid content of individual samples were examined to determine if OPs accumulated in a tissue as a function of extractable lipid content. Overall, only TDCIPP showed a significant but weak correlation with extractable lipid content (p = 0.01, r = 0.43). The final correlative parameter to be examined was the octanol–water partition coefficient (K_{OW}) of the OPs. The K_{OW} of a compound dictates the preference for compound accumulation in a polar versus a nonpolar environment. Correlations between OP concentrations and their log-K_{OW} values showed no significant relationships ($p \geq 0.41$). For example, even though the lipid content in yolk was more than 100 times higher than in albumen (31% vs 0.2% w/w, respectively), ΣOP concentrations in the yolk and albumen were statistically identical (14.8 ± 2.4 ng/g ww vs 14.8 ± 5.9 ng/g ww, respectively; p = 0.49).

Although hydrophobic in nature, OP triesters do not behave like typical hydrophobic organic contaminants. Their equivalent concentrations in yolk and albumen (Table 1), regardless of the enormous difference in extractable lipid contents in these egg compartments, suggests that their hydrophobicities do not play a role in their *in ovo* transfer and residue concentrations, but rather that other processes are at work such as protein-associated pathways.

To our knowledge, there has yet to be a study which investigates the binding affinity of OPs with various transport proteins in the body. Such information would be useful in understanding how OPs are distributed in the body, and why certain OPs accumulate preferentially in one tissue over another as our present results demonstrate. Fang et al.[39] showed that TPHP has a moderate binding affinity for the androgen receptor, yet has no binding affinity for the estrogen receptor. It has also been shown that TCEP has a weak binding affinity for acetylcholinesterase.[24] Multiple studies have examined the phenotypic effects of OPs in rodents, birds, and fish.[18−20,40−42] In most of these studies, even though valuable information has been gained regarding phenotypic changes upon exposure to OPs (such as endocrine disruption, developmental toxicity, and mRNA expression changes), very little is known regarding direct interactions between OPs and specific biomolecules.

Burden Estimate of Organophosphate Flame Retardants. The mass burden of each OP in all body and egg compartments (SI Table S2) was estimated based on OP concentrations (Table 1), and a combination of sample collection data and estimated tissue masses (additional detail in the Materials and Methods section). Overall, the OP tissue burdens were highest in fat (2.5 ± 0.7 μg), followed by muscle (1.1 ± 0.5 μg), yolk (0.8 ± 0.1 μg), albumen (0.35 ± 0.05 μg), and red blood cells (0.02 ± 0.01 μg), and were not detected in blood plasma, liver, and brain. These burdens were used to determine overall accumulation differences between mother (body) and offspring (egg) (Figure 3). It is important to note

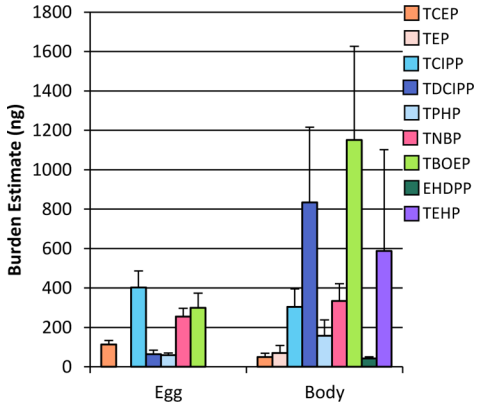


Figure 3. Combined arithmetic mean (±standard error) organophosphate (OP) flame retardant burdens for the egg (yolk and albumen) and maternal body (fat, muscle, red blood cells, plasma, liver, and brain).

that the burden estimates for the mother are a lower bound estimate, since the tissues that were sampled in this study (i.e., fat, muscle, liver, blood plasma, red blood cells, and brain) only accounted for approximately 30% of the maternal gull's total body mass. Even so, body burden estimates were comparable to, or higher than, egg burden estimates for most OPs.

It is highly probable that OPs undergo rapid metabolism in the maternal gull. Accumulated OP burdens in the maternal gull are likely postmetabolic residues, found largely in storage tissues (e.g., fat), as opposed to high metabolic activity tissues (e.g., liver). These residues are subsequently transferred *in ovo* to their eggs. Therefore, all OPs in the egg likely reflect to some extent residual OP burdens in the maternal gull. It should be noted that *in ovo* transfer of OPs seems to be to some degree compound-specific. Although the ΣOP burden was higher in the maternal gull, burdens in the gull were not proportional to burdens in the egg. For example, there was overall about one-third as much TCIPP as TDCIPP in the maternal gull, although there was about six times more TCIPP as TDCIPP in the egg, indicating a preferential transfer of TCIPP *in ovo*.

The same eight individual herring gulls analyzed in this study were also analyzed for perfluoroalkyl substances (PFASs).[43] PFASs are extremely stable to degradation, and have been shown to bioaccumulate and biomagnify in food webs, particularly perfluorooctanesulfonate (PFOS).[43] Overall body burden of ΣPFASs and ΣOPs was very similar (4.0 vs 3.5 μg,

respectively), but the egg burden of ΣPFASs was much higher than that of ΣOPs (8.6 vs 1.2 μg, respectively). Female herring gulls typically lay three eggs per clutch. Based on these estimated burdens, female herring gulls clear approximately 50% of their ΣOP burden per clutch, whereas they clear approximately 87% of their ΣPFAS burden per clutch. These burdens indicate that although *in ovo* transfer of OPs occurs to a much lesser extent than PFASs, *in ovo* transfer of OPs is still a significant depuration pathway for female herring gulls.

Implications. Multiple studies have examined the effects of OPs in rodents, birds, and fish.[18−20,40−42] Several OPs, including TDCIPP, TCEP, TCIPP, TBOEP, TPHP, and TMPP, have been shown to be endocrine disruptors in either zebrafish embryos, zebrafish H295R and MVLN cell lines, or chicken embryos.[18−20] For example, chicken embryos injected with 7640 ng TDCIPP/g egg showed a statistically significant decrease in plasma free thyroxine (T_4) concentrations compared to controls.[20] Furthermore, TDCIPP, TPHP, and TMPP were all shown to inhibit the binding of 17β-estradiol (E2) to its estrogen receptor in zebrafish MVLN cell lines.[19] Zebrafish embryos exposed to TDCIPP (exposure range from 10 to 600 μg TDCIPP/L) resulted in dose-dependent developmental toxicity, with end points including decreased body weight, decreased hatching rate (for the 600 μg TDCIPP/L group only), and increased rate of spinal malformation (for both the 300 and 600 μg TDCIPP/L groups).[18] Lastly, multiple mRNA transcripts have been found to be upregulated due to OP exposure. In zebrafish, exposure to TDCIPP and TPHP has been shown to cause an upregulation of cytochrome P450 (CYP) 17 and CYP 19A transcripts in both males and females.[19] In chicken embryos, upregulation of CYP 3A37 and CYP 2H1 was observed after TDCIPP exposure, and upregulation of liver fatty acid-binding protein and CYP 3A37 was observed after TCIPP exposure.[20]

The OP concentrations found in the air and water from the Great Lakes ecosystem are much lower than the dosing concentrations used in the above toxicity studies. Salamova et al.[3] very recently found that atmospheric concentrations of OPs from multiple sites across the Great Lakes were quite high (2−3 orders of magnitude higher than other brominated flame retardants), with ΣOP concentrations up to 2100 pg/m^3. Similarly, Jantunen et al.[44] found that ΣOP concentrations from rivers, streams, and creeks draining into the Great Lakes averaged over 600 ng/L. These concentrations are approximately 3 orders of magnitude lower than the dosing concentrations in studies that have elicited toxicity effects. However, it should be noted that a multitude of other contaminants are found in the Great Lakes basin (e.g., PBDEs, PCBs, OCs) which could have the possibility of producing synergistic effects with OPs. These types of cocktail effects may be possible, although they have not been tested in herring gulls.

In comparison to OP triesters, their metabolites are far less hydrophobic and are phosphoric acids.[8] It is thus very likely that the metabolites are quickly cleared from the body, and thus are not bioaccumulated in biota. Even so, concentrations of OP triesters in the Great Lakes ecosystem are high. With high exposure to OPs, and likely rapid metabolism, further research should consider analyzing biological tissue for OP diesters. Since they are thought to be the major metabolites of many OP triesters, their distribution and concentrations throughout the body would be highly valuable information, which could help researchers understand not only OP metabolism, but may also give insight into the pharmacokinetics of OP diesters and their chemical interactions in the body.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional information concerning all multiple reaction monitoring transitions used in this study and method limits of quantification for all target compounds; an example of the correlation between OP concentrations and bioconcentration factors. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +1 613-998-6696; fax: +1 613-998-0458; e-mail: Robert.Letcher@ec.gc.ca.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Major funding for this project was provided by Environment Canada's Chemicals Management Plan (CMP). Supplemental funding was provided by National Science and Engineering Research Council (NSERC) of Canada (to R.J.L.).We thank Craig Hebert, Doug Crump, and Kim Williams for the collection of all samples used in this study. We also thank Heather Stapleton for providing purified V6, and Lewis Gauthier for his time and effort spent helping to optimize the OP triester method.

■ REFERENCES

(1) Schindler, B. K.; Forster, K.; Angerer, J. Determination of human urinary organophosphate flame retardant metabolites by solid-phase extraction and gas-chromatography-tandem mass spectrometry. *J. Chromatogr. B* **2009**, *877* (4), 375−381.

(2) van der Veen, I.; de Boer, J. Phosphorus flame retardants: Properties, production, environmental occurrence, toxicity and analysis. *Chemosphere* **2012**, *88*, 1119−1153.

(3) Salamova, A.; Ma, Y.; Vernier, M.; Hites, R. A. High levels of organophosphate flame retardants in the Great Lakes atmosphere. *Environ. Sci. Technol. Lett.* **2014**, *1*, 8−14.

(4) Cheng, W.; Sun, L.; Huang, W.; Ruan, T.; Xie, Z.; Zhang, P.; Ding, R.; Li, M. Detection and distribution of Tris(2-chloroethyl) phosphate on the East Antarctic ice sheet. *Chemosphere* **2013**, *92*, 1017−1021.

(5) Cristale, J.; García Vázquez, A.; Barata, C.; Lacorte, S. Priority and emerging flame retardants in rivers: Occurrence in water, sediment, *Daphnia magna* toxicity and risk assessment. *Environ. Int.* **2013**, *59*, 232−243.

(6) Leonards, P.; Steindal, E. H.; van der Veen, I.; Berg, V.; Ove Bustnes, J.; van Leeuwen, S. *Screening of Organophosphor Flame Retardants 2010*; SPFO-Report 1091/2011, TA-2786/2011; Norwegian Climate and Pollution Agency (KLIF): Oslo, 2011.

(7) Evenset, A.; Leknes, H.; Christensen, G. N.; Warner, N.; Remberger, M.; Gabrielsen, G. W. *Screening of New Contaminants in Samples from the Norwegian Arctic*; NIVA Report 4351-1, SPFO-Report 1049/2009, TA-2510/2009; Norwegian Climate and Pollution Agency (KLIF): Oslo, 2009. ISBN: 978-82-449-0065-2.

(8) Reemtsma, T.; Quintana, J. B.; Rodil, R.; Garcia-Lopez, M.; Rodriguez, I. Organophosphorus flame retardants and plasticizers in water and air. I. Occurrence and fate. *Trends Anal. Chem.* **2008**, *27* (9), 727−737.

(9) Green, N.; Schlaback, M.; Bakke, T.; Brevik, E. M.; Dye, C.; Herzke, D.; Huber, S.; Plosz, B.; Remberger, M.; Schøyen, M.; Thelle Uggerud, H.; Vogelsang, C. *Screening of Selected Metals and New*

Organic Contaminants 2007; NIVA Report 5569-2008, SPFO-Report 1014/2008, TA-2367/2008; Norwegian Climate and Pollution Agency (KLIF): Oslo, 2008. ISBN: 978-82-577-5304-7.
(10) Quintana, J. B.; Rodil, R.; Reemtsma, T.; García-López, M.; Rodríguez, I. Organophosphorus flame retardants and plasticizers in water and air. II. Analytical methodology. *Trends Anal. Chem.* **2008**, *27* (10), 904−915.
(11) Saito, I.; Onuki, A.; Seto, H. Indoor organophosphate and polybrominated flame retardants in Tokyo. *Indoor Air* **2007**, *17*, 28−36.
(12) Martínez-Carballo, E.; González-Barreiro, C.; Sitka, A.; Scharf, S.; Gans, O. Determination of selected organophosphate esters in the aquatic environment of Austria. *Sci. Total Environ.* **2007**, *388*, 290−299.
(13) Andresen, J. A.; Grundmann, A.; Bester, K. Organophosphorus flame retardants and plasticisers in surface waters. *Sci. Total Environ.* **2004**, *332*, 155−166.
(14) Hartmann, P. C.; Bürgi, D.; Giger, W. Organophosphate flame retardants and plasticisers in indoor air. *Chemosphere* **2004**, *57*, 781−787.
(15) Fries, E.; Püttmann, W. Monitoring of the three organophosphate esters TBP, TCEP and TBEP in river water and ground water (Oder, Germany). *J. Environ. Monit.* **2003**, *5*, 346−352.
(16) Crump, D.; Porter, E.; Egloff, C.; Williams, K. L.; Letcher, R. J.; Gauthier, L. T.; Kennedy, S. W. 1,2-Dibromo-4-(1,2-dibromoethyl)-cyclohexane and tris(methylphenyl) phosphate flame retardants cause significant, yet variable, effects on embryonic development, mRNA expression, and circulating bile acid concentrations in domestic chicken embryos. *Toxicol. Sci.* **2014**, Accepted March 2014.
(17) Egloff, C.; Crump, D.; Porter, E.; Williams, K. L.; Letcher, R. J.; Gauthier, L. T.; Kennedy, S. W. Tris(2-butoxyethyl)phosphate and triethyl phosphate alter embryonic development, hepatic mRNA expression, thyroid hormone levels, and circulating bile acid concentrations in chicken embryos. *Toxicol. Appl. Pharmacol.* **2014**, Submitted March 2014.
(18) Wang, Q.; Liang, K.; Liu, J.; Yang, L.; Guo, Y.; Liu, C.; Zhou, B. Exposure of zebrafish embryos/larvae to TDCPP alters concentrations of thyroid hormones and transcriptions of genes involved in the hypothalamic-pituitary-thyroid axis. *Aquat. Toxicol.* **2013**, *126*, 207−213.
(19) Liu, X.; Ji, K.; Choi, K. Endocrine disruption potentials of organophosphate flame retardants and related mechanisms in H295R and MVLN cell lines in zebrafish. *Aquat. Toxicol.* **2012**, *114*−*115*, 173−181.
(20) Farhat, A.; Crump, D.; Chiu, S.; Williams, K. L.; Letcher, R. J.; Gauthier, L. T.; Kennedy, S. W. In ovo effects of two organophosphate flame retardants − TCPP and TDCPP − on pipping success, development, mRNA expression, and thyroid hormone levels in chicken embryos. *Toxicol. Sci.* **2013**, *134* (1), 92−102.
(21) Meeker, J. D.; Stapleton, H. M. House dust concentrations of organophosphate flame retardants in relation to hormone levels and semen quality parameters. *Environ. Health Perspect.* **2010**, *118* (3), 318−323.
(22) Flaskos, J.; McLean, W. G.; Fowler, M. J.; Hargreaves, A. J. Tricresyl phosphate inhibits the formation of axon-like processes and disrupts neurofilaments in cultured mouse N2a and rat PC12 cells. *Neurosci. Lett.* **1998**, *242*, 101−104.
(23) Matthews, H. B.; Eustis, S. L.; Haseman, J. Toxicity and carcinogenicity of chronic exposure to tris(2-chloroethy)phosphate. *Fundam. Appl. Toxicol.* **1993**, *20*, 477−485.
(24) Matthews, H. B.; Dixon, D.; Herr, D. W.; Tilson, H. Subchronic toxicity studies indicate that tris(2-chloroethyl)phosphate administration results in lesions in the rat hippocampus. *Toxicol. Ind. Health* **1990**, *6* (1), 1−15.
(25) Tilson, H. A.; Veronesi, B.; McLamb, R. L.; Matthews, H. B. Acute exposure to tris(2-chloroethyl)phosphate produces hippocampal neuronal loss and impairs learning in rats. *Toxicol. Appl. Pharmacol.* **1990**, *106*, 254−269.
(26) Eulaers, I.; Jaspers, V. L. B.; Halley, D. J.; Lepoint, G.; Nygård, T.; Pinxten, R.; Covaci, A.; Eens, M. Brominated and phosphorus flame retardants in white-tailed eagle *Haliaeetus albicilla* nestlings: Bioaccumulation and associations with dietary proxies (δ^{13}C, δ^{15}N, and δ^{34}S). *Sci. Total Environ.* **2014**, *478*, 48−57.
(27) Ma, Y.; Cui, K.; Zeng, F.; Wen, J.; Liu, H.; Zhu, F.; Ouyang, G.; Luan, T.; Zeng, Z. Microwave-assisted extraction combined with gel permeation chromatography and silica gel cleanup followed by gas chromatography-mass spectrometry for the determination of organophosphorus flame retardants and plasticizers in biological samples. *Anal. Chim. Acta* **2013**, *786*, 47−53.
(28) Chen, D.; Letcher, R. J.; Chu, S. Determination of non-halogenated, chlorinated and brominated organophosphate flame retardants in herring gull eggs based on liquid chromatography-tandem quadrupole mass spectrometry. *J. Chromatogr. A* **2012**, *1220*, 169−174.
(29) Letcher, R. J.; Chen, D.; Chu, S.; Gauthier, L. T. Organophosphate triester flame retardant and phosphoric acid diester metabolite analysis and spatial and temporal trends in herring gulls from the North American Great Lakes. *Organohalogen Comp.* **2011**, *73*, 1257−1260.
(30) Letcher, R. J.; Chen, D.; Backus, S.; McGoldrick, D.; Gauthier, L. T. Spatio-temporal trends of organophosphate flame retardants in Great Lakes herring gulls, as well as Lake trout and their convergent aquatic food webs. *Abstracts from the SETAC North American 33rd Annual Meeting*; Long Beach, CA, 2012.
(31) Kurebayashi, H.; Tanaka, A.; Yamaha, T. Metabolism and disposition of the flame retardant plasticizer tri-*p*-cresyl phosphate, in the rat. *Toxicol. Appl. Pharmacol.* **1985**, *77*, 395−404.
(32) Norstrom, R. J.; Clark, T. P.; Kearney, J. P.; Gilman, A. P. Herring gull energy requirements and body constituents in the Great Lakes. *Ardea, Off. J. Neth. Ornithol. Union* **1986**, *72*, 1−23.
(33) Carrier, D.; Leon, L. R. Skeletal growth and function in the California gull (*Larus californicus*). *J. Zoo.* **1990**, *222*, 375−389.
(34) Sasaki, K.; Suzuki, T.; Takeda, M.; Uchiyama, M. Metabolism of phosphoric-acid triesters by rat-liver homogenate. *Bull. Environ. Contam. Toxicol.* **1984**, *33*, 281−288.
(35) Chu, S. G.; Chen, D.; Letcher, R. J. Dicationic ion-pairing of phosphoric acid diesters post-liquid chromatography and subsequent determination by electrospray positive ionization-tandem mass spectrometry. *J. Chromatogr. A* **2011**, *1218*, 8083−8088.
(36) Chapman, D. E.; Michener, S. R.; Powis, G. Metabolism of the flame-retardant plasticizer tris(2-chloroethyl)phosphate by human and rat-liver preparations. *Fundam. Appl. Toxicol.* **1991**, *17* (2), 215−224.
(37) Crump, D.; Chiu, S.; Kennedy, S. W. Effects of tris(1,3-dichloro-2-propyl) phosphate and tris(1-chloropropyl) phosphate on cytotoxicity and mRNA expression in primary cultures of avian hepatocytes and neuronal cells. *Toxicol. Sci.* **2012**, *126*, 140−148.
(38) Verreault, J.; Villa, R. A.; Gabrielsen, G. W.; Skarre, J. U.; Letcher, R. J. Maternal transfer of organohalogen contaminants and metabolites to eggs of Arctic-breeding glaucous gulls. *Environ. Pollut.* **2006**, *144*, 1053−1060.
(39) Fang, M.; Webster, T. F.; Gooden, D.; Cooper, E. M.; McClean, M. D.; Makey, C.; Stapleton, H. M. Investigating a novel flame retardant known as V6: Measurements in baby products, house dust, and car dust. *Environ. Sci. Technol.* **2013**, *47*, 4449−4454.
(40) Liu, C.; Wang, Q.; Liang, K.; Liu, J.; Zhou, B.; Zhang, X.; Liu, H.; Giesy, J. P.; Yu, H. Effects of tris(1,3-dichloro-2-propyl) phosphate and triphenyl phosphate on receptor-associated mRNA expression in zebrafish embryos/larvae. *Aquat. Toxicol.* **2013**, *128*−*129*, 147−157.
(41) Umezu, T.; Yonemoto, J.; Soma, Y.; Suzuki, T. Tris(2-chloroethyl)phosphate increases ambulatory activity in mice: Pharmacological analyses of its neurochemial mechanism. *Toxicol. Appl. Pharmacol.* **1998**, *148* (1), 109−116.
(42) Morales, N. M.; Matthews, H. B. In vivo binding of the flame retardants tris(2,3-dibromopropyl) phosphate and tris(1,3-dichloro-2-propyl) phosphate to macromolecules of mouse liver, kidney and muscle. *Bull. Environ. Contam. Toxicol.* **1980**, *25*, 34−38.

(43) Gebbink, W. A.; Letcher, R. J. Comparative tissue and body compartment accumulation and maternal transfer to eggs of perfluoroalkyl sulfonates and carboxylates in Great Lakes herring gulls. *Environ. Pollut.* **2012**, *162*, 40−47.
(44) Jantunen, L.; Struger, J.; Backus, S. M.; Kraft, J.; Hung, H. Organophosphate flame retardants in Southern Ontario tributaries and precipitation. In *Abstracts of the 56th Annual Conference on Great Lakes Research*; International Association for Great Lakes Research: West Lafayette, IN, 2013.