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Contaminants in bald eagles of the upper Midwestern U.S.: A framework for prioritizing future research based on in-vitro bioassays☆

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ABSTRACT

Several organic contaminants (OCs) have been detected in bald eagle (*Haliaeetus leucocephalus)* nestling (eaglet) plasma in the upper Midwestern United States. Despite frequent and relatively high concentrations of OCs in eaglets, little is understood about potential biological effects associated with exposure. We screened an existing database of OC concentrations in eaglet plasma collected from the Midwestern United States against bioactivity information from the ToxCast database. ToxCast bioactivity information consists of concentrations expected to elicit responses across a range of biological space (e.g. cellular, developmental, etc.) obtained from a series of high throughput assays. We calculated exposure—activity ratios (EAR) by calculating the ratio of plasma concentrations to concentrations available in ToxCast. Bioactivity data were not available for all detected OCs. Therefore, our analysis provides estimates of potential bioactivity for 19 of the detected OCs in eaglet plasma. Perfluorooctanesulfonic acid (PFOS) EAR values were consistently the highest among all study areas. Maximum EAR values were $\geq$1 for PFOS, perfluorononanoic acid, and bisphenol A in 99.7, 0.53 and 0.26% of samples, indicating that some plasma concentrations were greater than what may be expected to elicit biological responses. About 125 gene targets, indicative of specific biological pathways, were identified as potentially being affected. Inhibition of several CYP genes, involved in xenobiotic metabolism, were most consistently identified. Other identified biological responses have potential implications for motor coordination, cardiac functions, behavior, and blood circulation. However, it is unclear what these results mean for bald eagles, given that ToxCast data are generated using mammalian-based endpoints. Despite uncertainties and limitations, this method of screening environmental data can be useful for informing future monitoring or research focused on understanding the occurrence and effects of OCs in bald eagles and other similarly-positioned trophic species.

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1. Introduction

The presence of organic contaminants (OCs) in aquatic environments has been widely documented (Ahrens, 2011; Dykstra et al., 2010; Elliott and VanderMeulen, 2017; Hites, 2006; Loos et al., 2009). Furthermore, many OCs, including legacy and current use, have the potential to elicit biological effects in exposed organisms. There is evidence that some OCs accumulate in organisms such as mussels (Booij et al., 2002; Dodder et al., 2014; Kimbrough et al., 2009) and fish (Flanagan Pritz et al., 2014; Stahl et al., 2014; Zhang et al., 2013) leading to potential transfer up the food chain to higher trophic organisms such as bald eagles (*Haliaeetus leucocephalus*). In fact, several current-use OCs, such as perfluorinated

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chemicals (PFCs) and flame retardants have been detected in bald eagle nestling (eaglet) plasma (Dykstra et al., 2010; Route et al., 2014a; Route et al., 2014b; Venier et al., 2010) throughout the upper Midwestern United States. Bald eagle nestlings are particularly useful indicators of local contamination because they are fed from a relatively small territory (1–2 km^2; Stalmaster, 1987).

Effects of exposure to legacy OCs, such as DDT, DDE, and PCBs, are widely known and include egg-shell thinning and developmental deformities (e.g. Bowerman et al., 2003; Gilbertson and Morris, 1976; Kozie and Anderson, 1991). Productivity (number of young produced) of bald eagles was inversely correlated with DDE and PCB concentrations in eggs (Wiemeyer et al., 1984) and eaglet blood plasma (Bowerman et al., 2003). With the ban of DDT, many bird populations began to recover during the 1980's and 1990's. Furthermore, with declining concentrations of PCBs and other organochlorine chemicals, the relationships between concentrations and reproductive success largely disappeared (Donaldson et al., 1999). Although many bird populations recovered, recovery was not uniform throughout the Great Lakes region (Bowerman et al., 2003; Dykstra et al., 1998; Grim and Kallemeyn, 1995; Kozie and Anderson, 1991), for reasons that are not fully understood. Productivity and reproductive success appear to be increasing or stable, but OCs are still present in the environment with the potential to pose a hazard to the health of bald eagles.

While there is some information on the biological effects of OCs on organisms using traditional laboratory exposure methods, data currently include a very limited number of current-use chemicals. Furthermore, testing methods and endpoints are not consistent among chemicals making it difficult to compare the relative hazard from different OCs. The U.S. Environmental Protection Agency's (USEPA) ToxCast database contains bioactivity data (chemical concentrations expected to affect specific gene targets or biological pathways) from a consistent set of assays for thousands of OCs. Data are generated using high-throughput in-vitro assays and provide pathway-specific responses of screened chemicals. Although the assays are primarily targeted toward mammalian processes, the ToxCast database provides an efficient way to screen for potential biological responses in other organisms and provides context for the relative hazards of chemicals found in the environment (Kavlock et al., 2012). Using chemical-endpoint interaction data from ToxCast, initial screening can be used to prioritize future research or management activities to better understand or help mitigate the potential hazards associated with exposure to harmful chemicals. For example, Blackwell et al. (2017) used ToxCast bioactivity data to screen environmental concentrations of various OCs in water samples, providing an indication of which chemicals presented the greatest relative hazard to aquatic biota health, which biological pathways were more likely affected, and which of the sampled sites warrant more study. Use of the ToxCast database in this manner provides an efficient method for screening environmental data for a large number of chemicals in a consistent manner.

Our study evaluated an existing database of OC concentrations in bald eaglet plasma using bioactivity data from USEPA's ToxCast database to assess potential biological responses from OC exposure in the upper Midwestern United States. Our objective was to provide context for previously reported OC data by screening those data against concentrations suspected to affect specific biological processes. Although some studies have used ToxCast data to screen environmental samples such as water or sediment, few have screened concentrations of contaminants in plasma using this approach. Plasma contaminant concentrations allow for a direct comparison of contaminant burdens with the ToxCast data, removing uncertainties associated with potential trophic transport or biomagnification of OCs from sediment or water. This screening can be used to prioritize future monitoring efforts by identifying the relative importance of: (1) OCs posing a greater hazard to bald eagle health, (2) specific biological responses that may be elicited from OC exposure, and (3) sites where bald eagle health may be more threatened by exposure to OCs. This information can also be used to guide management activities focused on monitoring and minimizing the presence of OCs near areas with active bald eagle nests. For example, identification of a particular OC that may pose a hazard to eagle health could lead to actions that focus on reducing the loading of that OC to the environment.

2. Methods

Details regarding initial study design and results for most chemical data can be found in Dykstra et al. (2010) and Route et al. (2014a, 2014b). Results not previously reported in the aforementioned papers are provided in Supplementary Information. A brief description of methods follows to provide context for the current analysis.

2.1. Study areas

Eaglet plasma was collected from one eaglet at 159 eagle nests (sites) located within six study areas in the Upper Midwestern United States (Fig. 1, Table 1 and S1) from 2006 to 2015. Several sites (95 of 159, or 60%) were sampled in multiple years (Table S1). From 2006 to 2015, eaglets were sampled at four core study areas: Apostle Islands National Lakeshore (APIS), the upper St. Croix National Scenic Riverway (U-SACN), lower St. Croix National Scenic Riverway (L-SACN), and Mississippi National River and Recreation Area (MISS). Additional resources allowed for sampling along Wisconsin's Lake Superior South Shore (LSSS) in 2007 and 2008 and downstream from MISS in Pools 3 and 4 of the Mississippi River (Pools 3&4) in 2008 and 2009. Study areas were located within or adjacent to U.S. national parks and were chosen to represent a gradient of land-use characteristics. Because of the proximity of L-SACN, MISS, and Pools 3&4 to the Minneapolis-St. Paul metropolitan area, these study areas are strongly influenced by activities that accompany developed land use such as wastewater treatment plants, urban stormwater runoff, and other direct point sources (e.g. stormwater outfalls, industrial discharges, etc.) (Table 1 and S1). Land use in the other study areas is mostly forested with some agriculture. However, APIS and LSSS may also be under the influence from the port cities of Duluth, Minnesota and Superior, Wisconsin. Point sources may also contribute to atmospheric loading of some OCs providing a mechanism for transport and dispersion across a broader geographic area.

2.2. Sample collection and analysis

Plasma samples (n = 381) were collected from one five-to nine-week old eaglet at each site and analyzed for various contaminants. Sampling and analytical effort varied throughout the study dependent on available funding (Table 1, Table S1). Although analytical effort varied, the primary targeted OCs were 84 polychorinated biphenyl (PCB) congeners, 16 perfluorinated compounds (PFCs), 17 polybrominated diphenyl ether (PBDE) congeners, and DDT and its metabolites, DDE and DDD. Beginning in 2010, a suite of current-use OCs, including bisphenol A (BPA), octylphenol (OP), 4 phthalates, triclosan/triclocarban, and several more pesticides (e.g. chlordane, dieldrin, nonachlor) were added. All samples were analyzed at the Wisconsin State Laboratory of Hygiene. Primary targeted OCs were determined using gas chromatography/mass spectrometry (PBDEs, DDT, DDE, DDD, PCBs, nonachlor, and chlordane) or liquid chromatography/tandem mass

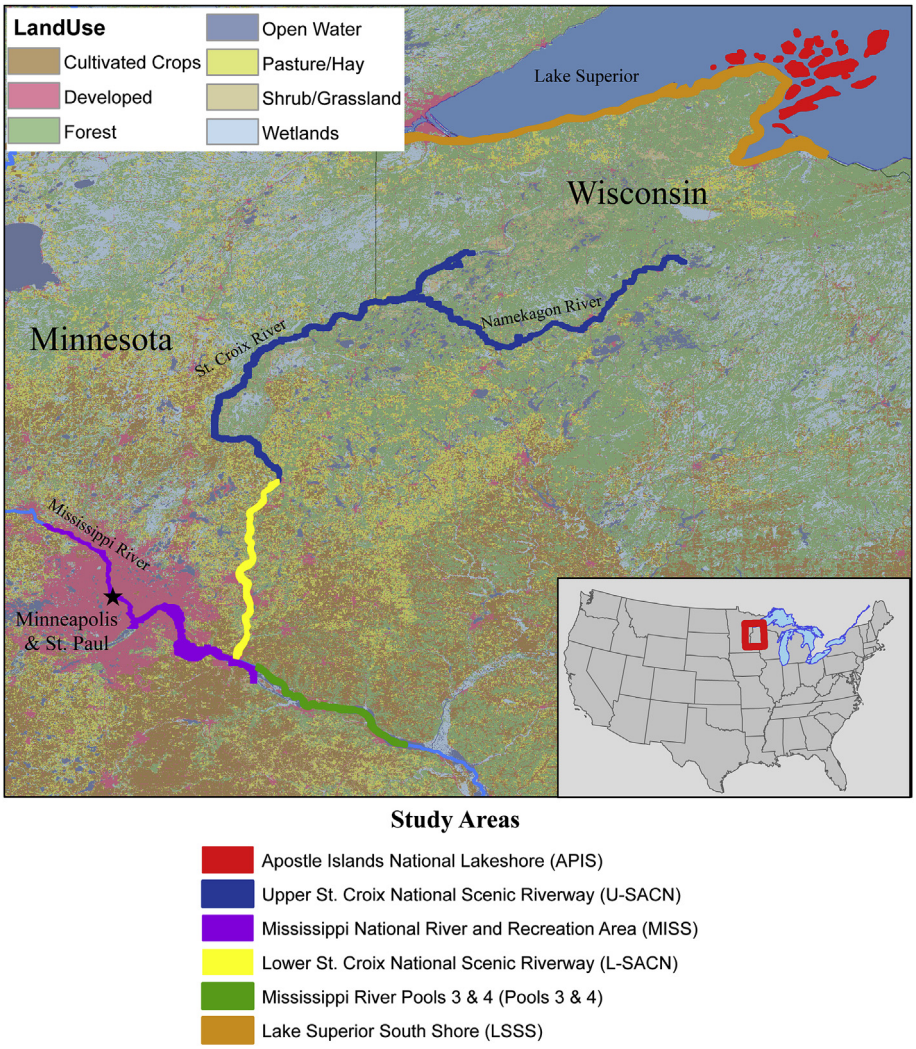

Fig. 1. Study areas where bald eaglet plasma was collected during 2006–2015.

Table 1
Summary of select landscape characteristics and spatial distribution of eaglet plasma samples collected throughout the upper Midwestern United States, 2006–15. NPDES, National Pollutant Discharge Elimination System.

Study Area	Average distance to nearest city (population ≥10,000), kilometers	Average distance to nearest NPDES discharge, kilometers	Number of sites sampled	Number of samples collected
Apostle Islands National Lakeshore (APIS)	107.6	13.6	27	58
Lake Superior South Shore (LSSS)	73.8	4.7	15	16
Upper St. Croix Scenic Riverway (U-SACN)	72.7	11.2	31	65
Lower St. Croix Scenic Riverway (L-SACN)	14.2	4.4	22	64
Mississippi National River and Recreation Area (MISS)	5.3	1.8	43	145
Mississippi River Pools 3 & 4 (Pools 3&4)	13.7	2.9	21	33
Totals			159	381

spectrometry (PFCs) methods. Analytical methods for determination of primary targeted OCs are detailed in Dykstra et al. (2010) and Route et al. (2014a, 2014b). Current-use OCs were determined using a high-performance liquid chromatography-triple quadrupole mass spectrometric method based on methods detailed in Silva et al. (2003), USEPA (1995, variously dated), and Ye et al. (2008). A volume of 0.5 mL serum was buffered to low pH followed by liquid/liquid extraction with methyl tert-butyl ether. Current-use OCs were extracted on a Phenomenex Strata-X column and eluted using methanol:acetonitrile (1:1) containing 1% acetic acid, evaporated to dryness and reconstituted in 100 μL methanol. Contaminants were then separated using binary gradient elution reversed phase chromatography and analyzed using a triple quadruple mass spectrometer in the atmospheric pressure chemical ionization negative ionization mode.

2.3. Quality assurance/quality control

Laboratory matrix-spike samples were analyzed with environmental samples to monitor method performance (Table S2). With

few exceptions, percent recovery of most OCs fell within 70–130%. PCB3 had consistently low percent recoveries (31–68%), perfluorododecanoic acid, perfluorotridecanoic acid, and perfluorotetradecanoic acid had at least one high (>200%) recovery, and percent recovery of isononylphenol was 150% in 2014. Concentrations were not corrected for recovery for this analysis.

2.4. ToxCast database

The ToxCast database is a publicly accessible database containing high-throughput screening data for over 9000 unique chemicals. Chemicals tested in ToxCast come from a single source and are prepared and screened in a consistent, standardized manner (Richard et al., 2016), greatly increasing the comparability of data between assays. All chemicals are analyzed in dose-response, allowing for point-of-departure estimates to be determined for each chemical-assay pair, and chemicals can be ranked in terms of relative potency within a given assay. The current version of the database (v2, October 2015) (USEPA, 2015) includes 12 assay batteries encompassing cell-free, biochemical-based in vitro assays, cell-based in vitro assays, and high-throughput whole organism assays (USEPA, 2015). ToxCast assays cover a range of biological space, including nonspecific endpoints (cytotoxicity, oxidative stress, cell morphology), and endpoints associated with over 200 unique signaling pathways (e.g. aryl hydrocarbon receptor, androgen receptor, pregnane X receptor). The bioactivity data can be prioritized by specific intended gene targets or broader intended target families (groups of gene targets related to similar biological pathways) to assess potential biological responses. Data in ToxCast are obtained by testing mammalian cells but can be used to provide context for environmental data and an indication of the types of biological responses that may be expected in other biota.

2.5. Data analysis

Comparisons of OC concentrations to bioactivity data available from ToxCast were analyzed in R (v.3.4.0; R Core Team, 2015) using the toxEval package (DeCicco et al., 2018). Plasma concentrations were compared against activity cutoff concentration (ACC) values obtained from the ToxCast database (USEPA, 2015) to calculate exposure—activity ratios (EAR). The ACC represents the concentration at which a threshold of response is achieved from in vitro tests and was used because it is uniform for all chemicals tested within an assay (Blackwell et al., 2017). Data originating from Apredica and Bioseek assays were excluded from this analysis because they target mostly nonspecific endpoints that we concluded would not be beneficial for evaluating specific biological responses to OCs in bald eagles. Assays classified as "background measurement" were also excluded from analysis for a similar reason. Individual endpoints are annotated by 'intended target family' (ITF) in ToxCast, which can be used to group endpoints according to function. We included all ITFs available in ToxCast in our analysis, except for 'zebrafish' and 'undefined' because we determined them to be unrepresentative for this analysis. Additionally, PCB congener pairs and triplets are not represented in the current version of ToxCast, so were removed from the dataset prior to comparisons with bioactivity data.

Data quality flags indicating false positive OC-endpoint matches in ToxCast were used to further filter the dataset for our analysis. EAR values calculated with an ACC containing any of the following flags were excluded from analysis: only highest concentration above baseline (baseline here refers to the noise of an assay, Filer et al., 2016), active; only one concentration above baseline, active; noisy data; borderline active; gain AC50 < lowest concentration & loss AC50 < mean concentration; and hit-call potentially confounded by overfitting. Several OC-endpoint matches resulted in relatively high (>100) EAR values. The dose-response curves associated with these OC-endpoint matches were examined to assess if the curve followed a logical dose-response relationship. We determined the dose-response curves associated with the high EAR values to be of sufficient quality to include in analysis.

Summary statistics of EAR values were calculated: number of OC-endpoint matches, minimum EAR (EAR_{min}), average EAR (EAR_{mean}), and maximum EAR (EAR_{max}). EAR values associated with the same assay within a given sample were summed to provide an indication of total biological response to chemical mixtures (EAR_{mix}). This method assumes simple mixture additivity of detected chemicals (i.e. individual chemicals may cause the same biological response). Additionally, a total EAR (EAR_{Tot}) was calculated for each eaglet sample to identify sites with the greatest overall potential to elicit biological responses. This was calculated by summing EAR values for every chemical-endpoint match identified in each eaglet.

3. Results and discussion

3.1. Organic contaminant presence

A summary of chemical concentrations for all OCs detected in at least one sample is included in Table S3. Of the detected OCs, 19 (including congeners) had associated bioactivity data in ToxCast and will be the focus of the following discussion (Table 2, S3). Detected concentrations of the 19 OCs screened against ToxCast ranged from 0.83 (PCB187) to 4200 (perfluorooctanesulfonic acid or PFOS) μg/L. The greatest concentrations were generally detected for PFCs; PFOS and perfluorononanoic acid (PFNA) were detected at a maximum of 4200 and 160 μg/L, respectively. Maximum concentrations of PBDEs, phthalates, PCBs, and other PFCs were 20, 22, 5.3, and 110 μg/L, respectively. For comparison, Venier et al. (2010) reported a maximum PBDE concentration of 6.96 μg/L in bald eagle plasma collected across several Great Lakes, substantially lower than observed in our study. Concentrations of total PCBs in plasma detected in our study were often within ranges reported in bald eagles across several Great Lakes (Bowerman et al., 2003; Venier et al., 2010). In general, OCs were detected more often and in greater concentrations in eaglet plasma collected near urban centers. Specifically, high PFC concentrations reflected known contamination plumes in the Minneapolis-St. Paul metropolitan area where high concentrations have been detected in groundwater, surface water, birds and fish (Custer et al., 2010; Monson, 2013; Oliaei et al., 2013; Route et al., 2014b; Yingling, 2015).

3.2. Organic contaminant prioritization

EAR_{max} values for individual OC-endpoint matches ranged from 0.0004 (perfluorohexanoic acid or PFHxA) to 907 (PFOS; Table S4) across all samples. Individual EAR_{max} values were orders of magnitude <1 for 16 of the 19 (84%) OCs. EAR_{max} values for PFHxA and perfluoroheptanoic acid (PFHpA) were consistently low, compared to the other OCs (Fig. 2). However, EAR_{max} was >1 for PFOS, BPA, and PFNA, indicating that plasma concentrations were greater than ACC concentrations in at least one sample at some sites. PFOS consistently had the highest EAR values among the OCs for which chemical-endpoint interaction data exist. Although analyzed in relatively few samples (n = 24 among APIS, U-SACN, L-SACN, and MISS), BPA concentrations resulted in EAR_{max} values similar to those observed for many of the PFCs. Several other OCs had EAR_{max} values that were ≥10% of at least one ACC [e.g. perfluorodecanoic acid = 0.37, perfluoroundecanoic acid (PFUnA) = 0.30, OP = 0.14], indicating that focused work on these

Table 2
Select summary statistics for the 19 organic contaminants detected in bald eaglet plasma samples from the upper Midwestern United States that were screened against available bioactivity information in ToxCast. Concentrations are micrograms per liter.

Chemical	Minimum concentration	Median concentration	Maximum concentration
Flame retardant			
PBDE99	0.12	1.1	15
PBDE47	0.19	3.5	20
Alkylphenol			
4-*tert*-octylphenol	2.95	2.95	2.96
Phthalate			
Bis(2-ethylhexyl) tetrabromophthalate	0.4	0.74	1.6
Mono(2-ethylhexyl) phthalate	0.27	1.2	22
Antioxidant			
Bisphenol A	0.53	1.05	5.8
Paraben			
Butylparaben	0.27	0.27	0.28
Propylparaben	0.51	0.73	3.57
Pesticide			
Dieldrin	0.5	0.945	5.7
Polychlorinated biphenyl			
PCB187	0.83	1.55	5.3
Perfluorinated compound			
Perfluorodecanoic acid	1.1	15	85
Perfluoroheptanoic acid	0.12	0.18	6.6
Perfluorohexanoic acid	0.13	4.9	5.7
Perfluorononanoic acid	0.82	3.7	160
Perfluorooctanoic acid	0.12	0.5	14
Perfluorooctanesulfonic acid	7.5	335	4200
Perfluoroundecanoic acid	1.1	7.9	110
Antimicrobial			
Triclocarban	0.27	0.31	0.58
Triclosan	0.82	0.95	1.8

specific OCs may be warranted to more fully understand their distributions and potential hazards to eagle health. Taking into account all OC-endpoint matches for a given chemical within a sample, three OCs stand out because the total EAR_{max} is > 1: PFOS, PFUNA, and BPA (Fig. 2).

Based on EAR magnitudes among the different OCs, PFCs, and in particular PFOS, stand out as potentially the most hazardous to bald eagles. Furthermore, the actual hazard from total PFCs may be greater than what our analysis shows because numerous PFCs that were detected in our study have not been tested in the ToxCast program. Relatively high (>1) EAR values associated with PFOS were observed in eaglet plasma across the entire study area. However, the highest values mostly occurred at sites near the Minneapolis-St. Paul urban area (Table S5) where PFCs are produced. High PFOS concentrations in water, sediment, fish, birds, and other biota have been documented in this area, as well as the dominance of PFOS in relation to detected PFCs (Custer et al., 2010; Custer et al., 2012a; Delinsky et al., 2010; Oliaei et al., 2013; Route et al., 2014b). Specifically, Pool 2 in the Mississippi River (within our MISS study area) is known to be a hot spot for PFOS, though concentrations appear to be decreasing over time (Monson, 2013; Route et al., 2014b). Additionally, similar mean concentrations of ∑PFCs were detected in tree swallow nestling plasma across the Great Lakes basin (Custer et al., 2017), but no association between PFC concentrations and egg failure was observed (Custer et al., 2012b). PFOS concentrations in 7 samples (5 from MISS, 2 from L-SACN) exceeded an avian toxicity reference value of 1700 μg/L in plasma, a value deemed to be protective of a tertiary avian predator based on gross pathological effects (Newsted et al., 2005). Although relatively few plasma concentrations exceeded this toxicity reference value, exposure to PFCs can cause sub-lethal effects. For example, hepatic PFOS concentrations (mean range 54–81 ng/g wet weight) were negatively correlated with expression of genes associated with molecular chaperones, ribonucleic acid (RNA) processes, and carbohydrate transport and metabolism in common cormorants (Nakayama et al., 2008). Similarly, a concentration of 1500 μg/L caused 0.5–2-fold changes in some thyroid gene expression in exposed chicken cells (Vongphachan et al., 2011). Other PFCs such as perfluorobutanesulfonic acid (PFBS), PFHxA, and perfluorohexanesulphonic acid (PFHxS) caused more than two-fold changes in thyroid gene expression at the same concentration. Comparable results were observed for herring gull cells exposed to similar concentrations of perfluorobutyrate, PFBS, PFHxA, PFHxS, PFHpA, and perfluoroheptanesulfonate (Vongphachan et al., 2011), all of which were detected in eaglet plasma in our study.

Similar to PFCs, PCBs and PBDEs are known to frequently occur in the environment and have frequently been detected in biota (Chen and Hale, 2010; Wenning et al., 2011; Van Ael et al., 2012). However, bioactivity information for many PCB and PBDE congeners included in this study is limited in the ToxCast database. For example, only 2 of 54 PCBs and 2 of 10 detected PBDEs had available information. A total of 53 PCBs (including congener pairs and triplicates) were detected in at least half of all samples. Considering the frequent detection of PCBs and limited bioactivity information in ToxCast, this analysis potentially greatly underestimates the hazard of PCB exposure to eagles. Because PCBs are legacy chemicals, their occurrence in the environment and potential effects have been fairly well studied (Bowerman et al., 2003; Gilbertson and Morris, 1976). Reduced nest site attentiveness was observed in glaucous gulls (*Larus hyperboreus)* with ∑PCB concentrations in blood as low as 50 μg/kg (Harris and Elliott, 2011). Geometric means of ∑PCBs in bald eaglet plasma assessed as part of our study were >50 μg/kg at LSSS, MISS, and L-SACN (Dykstra et al., 2010). Given evidence of frequent exposure, more information related to potential sub-lethal effects from exposure would be valuable for assessing eagle health.

Although there have been mixed results, PBDEs, and their hydroxylated forms, have been implicated in the interference of

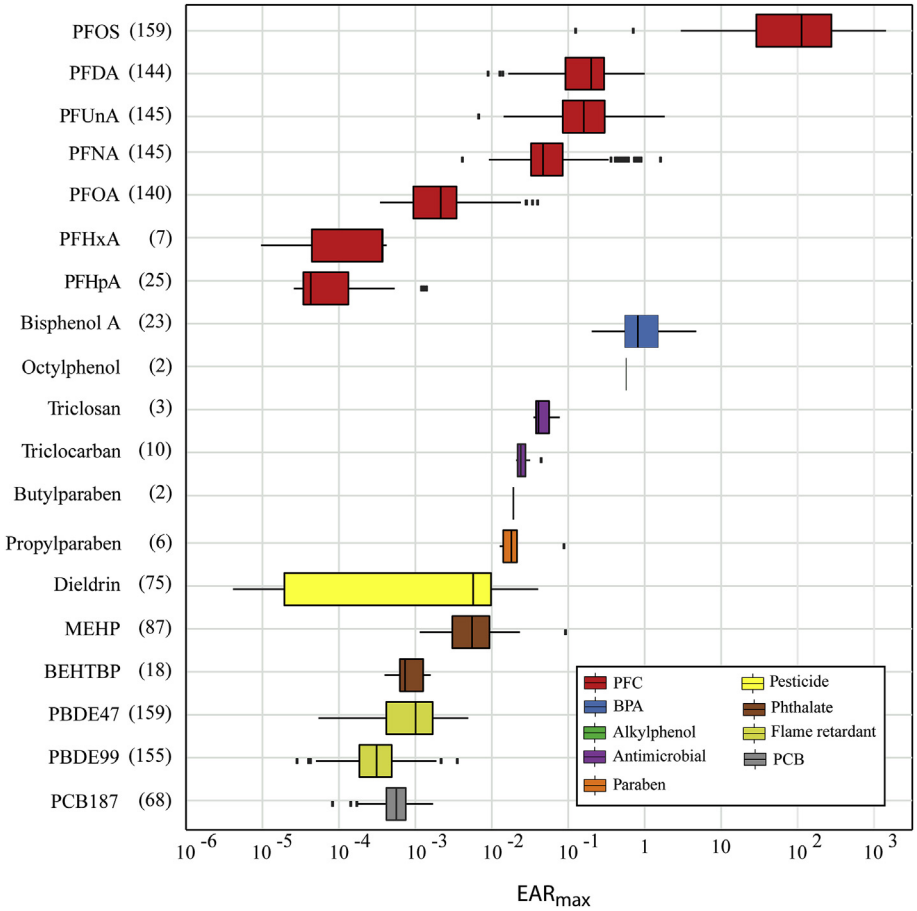

Fig. 2. Chemical summaries of maximum exposure—activity ratios (EAR_{max}) for organic contaminants detected in eaglet plasma collected from six study areas in the upper Midwestern United States, 2006–2015. Values represent the maximum of the sum of EAR for all organic contaminant-endpoint matches for a given chemical within a sample. Numbers in parentheses after chemical name represent the number of sites (eagle nests) at which the chemical was detected. Boxplot whiskers extend to the smaller of the maximum value and 1.5 times the interquartile range, and the larger of the minimum value and 1.5 times the interquartile range. Individual points represent values beyond the ends of the whiskers. PFC, perfluorinated chemical; BPA, bisphenol A; PCB, polychlorinated biphenyl.

thyroid circulation in bald eagles (Cesh et al., 2010). Other biological effects such as steroid hormone and retinol production show varied responses among species exposed to PBDEs and other similar flame retardants (Guigueno and Fernie, 2017). Additionally, patterns in P450 EROD activity among groups of terns generally followed patterns of contaminant concentrations such as ∑PCBs and ∑PBDEs (i.e. groups with higher contaminant concentrations exhibited greater P450 activity) (Herring et al., 2010). Although results from laboratory exposures of birds to PCBs and PBDEs show inconsistent biological responses, reduced antibody-mediated responses in American kestrels (*Falco sparverius*) exposed to PBDEs (Fernie et al., 2005) and adverse effects on cardiac development in domestic chickens (Carro et al., 2013) have been observed. The frequent occurrence of PCBs and PBDEs in bald eaglets combined with the limited availability of data related to biological responses in bald eagles and other birds suggests these chemicals should be a priority for research to better understand the potential biological effects resulting from exposure.

Several known or suspected endocrine disruptors (e.g. BPA, OP, phthalates) were detected. Of these, our analysis shows that the relative order in terms of highest potential for eliciting biological responses is BPA > OP > mono(2-ethylhexyl) phthalate > bis(2-ethylhexyl) tetrabromophthalate. The EAR_{max} for BPA was >1 for at least one assay endpoint. Biological effects (e.g. impaired growth and development, reduced thyroid hormone production, impaired reproduction, etc.) of exposure to these OCs have been documented in other vertebrates such as fish, humans, and other birds (e.g. Boas et al., 2010; Flint et al., 2012; Mankidy et al., 2016).

3.3. Biological response prioritization

Values of EAR_{mix} were used to assess potential biological responses. These values represent exposure to OC mixtures by accounting for every detected chemical associated with a specific endpoint (EAR>0). A total of 125 gene targets were identified as potentially being affected by detected OCs in this study with EAR_{mix} values ranging from <0.0001 to 908 (Fig. 3). The following discussion will focus on the 29 gene targets with associated $EAR_{mix} \geq 1$ for brevity (Table S5).

Processes related to several of the cytochrome P450 (CYP) genes were identified as potentially being inhibited; the highest observed EAR_{mix} values were associated with CYP gene targets (Fig. 3, Table S5). CYP genes can be related to metabolism of xenobiotic substances (Watanabe et al., 2013) and catalyzation of endogenous steroids in birds (Tsutsui et al., 2013). Although the CYP genes identified in this analysis are associated with the former, other CYP genes are not specifically covered by the available assays. Our results indicate potential for inhibited metabolism of foreign substances in eagles within the study area because of exposure to the measured OCs. Although some evidence suggests no clear

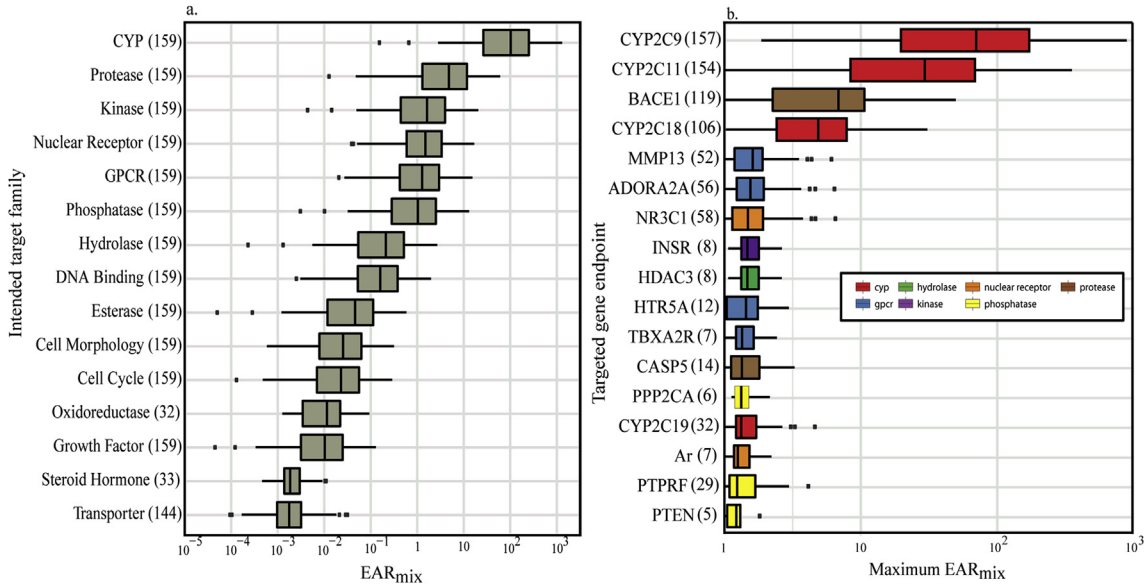

Fig. 3. Summaries of mixture exposure—activity ratios (EAR_{mix}) for organic contaminants detected in eaglet plasma collected from six study areas in the upper Midwestern United States, 2006–2015. Mixture EAR was calculated by summing the calculated EAR for every chemical identified as potentially affecting specific (a) intended target families and (b) targeted gene endpoints. For brevity, Fig. 3b only shows identified gene targets with an associated $EAR_{mix} \geq 1$ and sample size ≥ 5. Numbers in parentheses after target family or endpoint name represent the number of sites (eagle nests) affected. Boxplot whiskers extend to the smaller of the maximum value and 1.5 times the interquartile range, and the larger of the minimum value and 1.5 times the interquartile range. Individual points represent values beyond the ends of the whiskers.

orthologous (evolved from a common ancestor, separated by a speciation event, but retains the same function) relationship between bird and human CYP2C genes, these genes are not well-characterized in most birds (Watanabe et al., 2013). Inhibition of other CYP genes (e.g. CYP7B) can lead to reduced reproductive success in birds as a result of decreased sexual behavior (Tsutsui et al., 2013). Though it is unclear if this is the case for the eaglets sampled in this study, this information could be used to prioritize future monitoring efforts focused on identifying biological responses to validate these results.

The BACE1 endpoint was also often identified as potentially being inhibited, with both a high number of OC-endpoint matches and relatively high EAR_{mix} values. BACE1 is typically associated with the formation of Alzheimer's in humans (Dominguez et al., 2005). Although it is unclear what this might mean for other vertebrates, such as eagles, hyperactivity (Dominguez et al., 2005) or more adventurous activity (Harrison et al., 2003) was observed in mice expressing BACE1 compared to those not. Other evidence suggests that BACE1 may be required for formation and maturation of muscle spindles, so inhibition may result in alterations to motor coordination (Cheret et al., 2013).

Four G-protein-coupled receptors (GPCRs; ADORA2A, HTR5A, HTR7, and TBXA2R) were the second most commonly identified target family based on EAR magnitudes. These endpoints are associated with functions such as cardiac rhythm and circulation, blood flow, platelet aggregation, and behavioral functions. There is some evidence of ADORA and HTR7 being conserved in chickens, however an ortholog for TBXA2R is lacking (Lagerström et al., 2006).

Other gene targets with $EAR_{mix}>1$ represent biological pathways associated with steroidal [androgen receptor (AR), glucocorticoid receptor (GR)] and non-steroidal [pregnane-X receptor (PXR) and peroxisome proliferator activated receptor gamma (PPARG)] nuclear receptors, proteases [matrix metallopeptidase 13 (MMP13)], and phosphatases [protein tyrosine phosphatase receptor type F (PTPRF)]. Inhibition of some of these gene targets may result in behavioral, skeletal, or reproductive responses. For example, there is some evidence that AR plays a regulatory role in aggressive behavior in male song sparrows during the pre-breeding season (Sperry et al., 2010), in which an AR antagonist slightly decreased the number of flights associated with aggressive displays. These results indicate that birds exposed to chemicals that affect the AR may be outcompeted for nesting territory or mates because they are less likely to confront other males. MMP13 plays a role in recovery from tibial dyschondroplasia (a skeletal abnormality in birds) through its role in vascularization and ossification processes (Asawakarn and Asawakarn, 2012). Therefore, inhibition could result in longer recovery times or other skeletal abnormalities which may affect the ability of eagles to participate in certain behaviors. Although PTPRF has not been widely studied in birds, inhibition in human cells has been linked to proliferation of tumor growth (Bera et al., 2014).

3.4. Site prioritization

To prioritize sites where eagles may be more affected by exposure to the OCs included in our analysis, we used EAR_{Tot}, which sums EAR values from all OC-endpoint matches identified in each sample. Average values of EAR_{Tot} ranged from 0.8 to 1026. Sites with relatively high EAR_{Tot} values were consistently high across most samples collected at that site in different years (e.g. WH-012, WH-TX05), indicating that the eagles are being continuously exposed to OCs at these sites over extended periods of time. All but two sites (AS-106 and AS-TX-09, both in APIS) had average $EAR_{Tot} \geq 1$ (Table S6). Furthermore, 77, 88, and 91% of sites located within L-SACN, MISS, and Pools 3 + 4 had average $EAR_{Tot} \geq 100$.

Because PFOS was so prevalent throughout the study area, we also calculated EAR_{Tot} excluding PFOS ($EAR_{Tot\text{-}NoPFOS}$; Table S6) to explore the underlying variability in the rest of the chemical data. Values for $EAR_{Tot\text{-}NoPFOS}$ exhibited greater heterogeneity within study areas, compared to EAR_{Tot}, indicating that, for most OCs, contamination may be more site specific and related to local sources. Excluding PFOS substantially reduced average EAR values

(EAR ranged from 8.9E-5 to 5.2) however, average EAR values for 21 (13%) of the sites was still ≥1. Almost half (44%) of the sites with $EAR_{Tot\text{-}NoPFOS}$≥1 were located within APIS (Table S6, Fig. 4b). Additionally, two sites within U-SACN had average $EAR_{Tot\text{-}NoPFOS}$≥1. In consideration of EAR_{Tot}, sites within U-SACN were ranked low compared to the rest of the study areas. Using $EAR_{Tot\text{-}NoPFOS}$ values, the overall pattern of higher EAR values within L-SACN, MISS, and Pools 3 + 4 compared to the other study areas essentially reverses (Fig. 4). Although several sites within the more affected study areas may be given high priority, now sites within APIS and a few within U-SACN would be ranked higher on a priority list for further monitoring. When excluding PFOS, 18 (66%) APIS sites had $EAR_{Tot\text{-}NoPFOS}$>1, the highest percentage of the six study areas. This was largely driven by BPA, PFNA, and PFUnA EAR values.

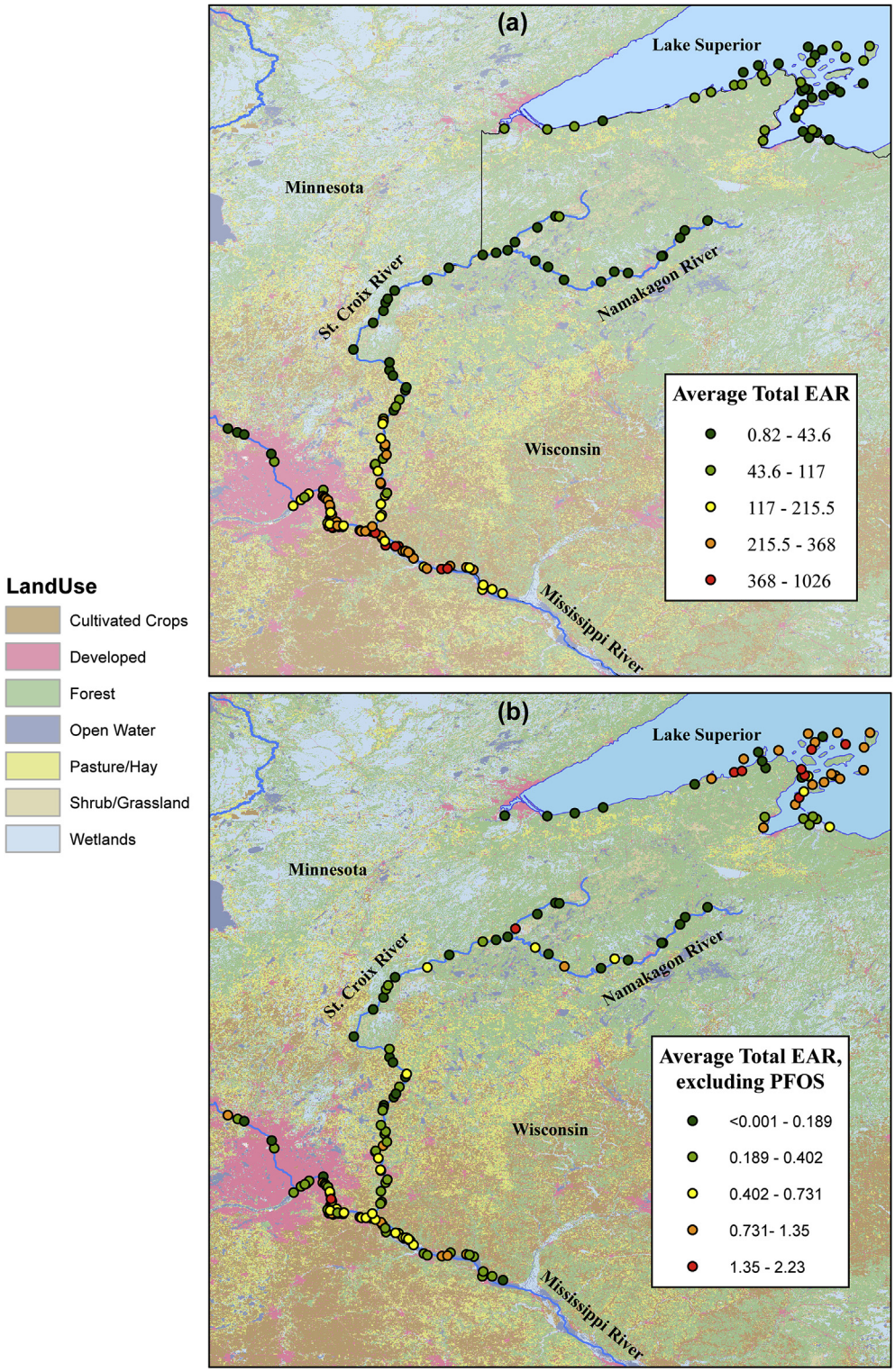

Fig. 4. Average total exposure—activity ratios (EAR) (a) including PFOS, and (b) excluding PFOS in bald eaglet plasma samples collected from six study areas in the upper Midwestern United States, 2006–2015. Total EAR values represent all chemicals detected within a sample for which activity concentrations exist.

There was little variation in EAR_{Tot} values among sites within a study area, indicating that eagles within a given study area are exposed to similar chemical profiles. Despite the low intra-area variability, we found evidence of high inter-area variability indicating that, because of different chemical profiles among sites, the hazards of OC exposure are not similar among different populations (Table S6, Fig. 4). Other researchers have documented the importance of contaminant profile heterogeneity among freshwater systems as factors influencing which OCs may bioaccumulate (Elliott et al., 2009).

4. Conclusions

We evaluated the potential sub-lethal biological responses of 19 OCs detected in bald eaglet plasma by using bioactivity information (chemical concentrations expected to elicit biological responses) from the USEPA ToxCast database. Concentrations of most OCs were substantially lower than concentrations expected to affect biological processes, but PFOS, PFNA, and BPA concentrations were above activity concentrations in at least one sample. Biological processes such as metabolism, behavior, development, and cardiac functions were identified as potentially being affected by OCs present in bald eaglet plasma. Some patterns emerged pointing to site-specific exposure differences, indicating the importance of local point sources, particularly with respect to PFOS. Although our analysis indicates that among the OCs tested PFOS should be given high priority for future monitoring, this screening was limited by the availability of contaminant occurrence data and bioactivity information in ToxCast. For example, bioactivity information for PCBs and PBDEs was sparse, potentially underestimating the overall hazard to eagle health. Additionally, it must be noted that identified biological responses do not necessarily mean there will be an observed effect. Further research needs to be conducted to assess how and if the identified alterations in biological activity result in gross health effects. Nonetheless, this screening provides information about expected biological responses from specific chemicals. The information can be used to prioritize chemicals and sites for future monitoring or research efforts focused on understanding the effects of OC exposure on bald eagles. This information could ultimately guide management efforts to mitigate OC influence on bald eagles.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2018.10.093.

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