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Exposure and Food Web Transfer of Pharmaceuticals in Ospreys (*Pandion haliaetus*): Predictive Model and Empirical Data

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ABSTRACT

The osprey (*Pandion haliaetus*) is a well-known sentinel of environmental contamination, yet no studies have traced pharmaceuticals through the water–fish–osprey food web. A screening-level exposure assessment was used to evaluate the bioaccumulation potential of 113 pharmaceuticals and metabolites, and an artificial sweetener in this food web. Hypothetical concentrations in water reflecting "wastewater effluent dominated" or "dilution dominated" scenarios were combined with pH-specific bioconcentration factors (BCFs) to predict uptake in fish. Residues in fish and osprey food intake rate were used to calculate the daily intake (DI) of compounds by an adult female osprey. Fourteen pharmaceuticals and a drug metabolite with a BCF greater than 100 and a DI greater than 20 μg/kg were identified as being most likely to exceed the adult human therapeutic dose (HTD). These 15 compounds were also evaluated in a 40 day cumulative dose exposure scenario using first-order kinetics to account for uptake and elimination. Assuming comparable absorption to humans, the half-lives ($t_{1/2}$) for an adult osprey to reach the HTD within 40 days were calculated. For 3 of these pharmaceuticals, the estimated $t_{1/2}$ in ospreys was less than that for humans, and thus an osprey might theoretically reach or exceed the HTD in 3 to 7 days. To complement the exposure model, 24 compounds were quantified in water, fish plasma, and osprey nestling plasma from 7 potentially impaired locations in Chesapeake Bay. Of the 18 analytes detected in water, 8 were found in fish plasma, but only 1 in osprey plasma (the antihypertensive diltiazem). Compared to diltiazem detection rate and concentrations in water (10/12 detects, <method detection limits [MDL]–173 ng/L), there was a lower detection frequency in fish (31/233 detects, <MDL–2400 ng/L); however when present in fish, all values exceeded the maximum diltiazem concentration found in water. Diltiazem was found in all 69 osprey plasma samples (540–8630 ng/L), with 41% of these samples exceeding maximum concentrations found in fish. Diltiazem levels in fish and osprey plasma were below the human therapeutic plasma concentration (30 000 ng/L). Effect thresholds for diltiazem are unknown in ospreys at this time, and there is no evidence to suggest adverse effects. This screening-level exposure model can help identify those compounds that warrant further investigation in high-trophic level species. *Integr Environ Assess Manag* 2015;11:118–129. © 2014 SETAC

Keywords: Bioaccumulation Diltiazem Ospreys Pharmaceuticals Screening-level exposure assessment

INTRODUCTION

In parallel with human population growth and a myriad of veterinary and human health uses, pharmaceuticals and their metabolites primarily enter the environment through wastewater from bulk drug production, sewage plants and septic systems, and in biosolids applied to agricultural lands (Kolpin et al. 2002; Ramirez et al. 2009). The development of advanced analytical techniques and widespread monitoring has revealed the presence of pharmaceuticals in a variety of environmental matrices (sediments, sewage sludge, water, and fish). Pharmaceuticals may not be completely removed by traditional wastewater treatment systems, and with constant wastewater inputs, even labile compounds may exhibit pseudo-persistence in surface waters (Daughton and Ternes 1999; Celiz et al. 2009). Their detection in the environment has raised concerns about bioaccumulation, transfer through the food web, and potential effects that pharmaceutical "cocktails" may elicit on ecosystems.

Understanding ecological risks of pharmaceuticals to free-ranging wildlife (amphibians, reptiles, birds, and mammals) remains a major research need (Boxall et al. 2012), the one exception being the nonsteroidal anti-inflammatory drug (NSAID) diclofenac used to treat livestock. Diclofenac use resulted in nontarget poisoning and endangerment of several species of Asian vultures feeding on carcasses of cattle that had

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been treated with this drug (Oaks and Watson 2011). The catastrophic effects of diclofenac on old world vultures resulted in detailed investigations of several NSAIDs in birds. A recent workshop evaluated the risk of pharmaceuticals to wildlife and identified major information gaps including the need to conduct food web exposure modeling and environmentally realistic risk assessments (Arnold et al. 2013). Hernout et al. (2011) also suggested that prioritizing chemicals (e.g., metals, pesticides, pharmaceuticals) in a food web framework before intensive and costly investigation would be beneficial to natural resource managers and policymakers.

Ospreys (*Pandion haliaetus*) are a high trophic level species that have served as sentinels of ecosystem health and environmental change (Grove et al. 2009). Their eggs and blood are excellent matrices to document spatial and temporal trends and to elucidate exposure, bioaccumulation, and biomagnification of contaminants. Ospreys are strictly piscivorous and this aspect makes their diet easy to monitor and link to sources of localized contaminant exposure. Their diet can vary with salinity (Glass and Watts 2009), prey availability, and trophic position and can range from anadromous fish in polyhaline regions to nonmigratory fish in oligohaline waters. Ospreys are adaptable to human landscapes and can be found nesting in highly industrialized and urbanized areas and even in proximity to wastewater treatment plants (WWTPs).

To date, no studies have examined the bioaccumulation of pharmaceuticals and their fate in the water-fish-osprey food web. This study describes a framework and the findings of a screening-level exposure assessment to estimate the daily and cumulative 40 day intake of pharmaceuticals that are being analyzed by some environmental research laboratories (Du et al. 2012; Furlong et al. 2014). This was complemented by empirical analyses of 23 compounds and an artificial sweetener analyzed in water and blood plasma of fish and osprey nestlings from sites located along potentially impaired waterways in Chesapeake Bay.

METHODS

Screening-level exposure model: Daily intake

The daily intake (DI) of 113 pharmaceuticals, metabolites, and an artificial sweetener (Table S1) by an adult female osprey was calculated to determine which compounds reached or exceeded the human therapeutic dose (HTD) (assumes comparable intestinal absorption for both ospreys and humans) (Figure 1). These compounds are quantified at the US Geological Survey National Water Quality Laboratory (Furlong et al. 2014) and some of these compounds are analyzed in fish plasma (Du et al. 2012). Three hypothetical exposure regimes (10, 100, and 1000 ng/L) were chosen (ranging from "dilution dominated" high flow to "wastewater effluent dominated" low flow) (Brooks et al. 2006) and modeled across 3 pH values (pH 6, pH 7, pH 8) that are representative of surface water gradients at field study sites. The pH consideration is important, because the drugs examined are all potentially ionizable and their bioconcentration factors (BCFs) are pH-specific and dependent on log D (a measure taking into account ionized and un-ionized forms of a molecule) (Meylan et al. 1999; Fu et al. 2009). These factors can influence bioaccumulation and toxicity in fish (Valenti et al. 2009, 2011, 2012), and ultimately, their absorption (bioaccessibility) in the gastrointestinal tract of birds. Predicted BCFs for each substance (ACS 2014) were used to calculate the quantity of a pharmaceutical accumulated in a generic fish in 24 hours (Berninger et al. 2011).

Calculated pharmaceutical residues in fish from each scenario were used to estimate the DI for a 1568 g adult female osprey (USEPA 1993). Due to the complexities in modeling cumulative exposure of a growing osprey nestling (e.g., logistic growth plateauing at 40 days, changing food intake, and metabolic demands, etc.), a 40 day exposure assessment for an adult osprey was conducted. Food intake rate (FIR) was estimated 2 ways. The first estimate used osprey bite size and body weight (BWt) to calculate a FIR of 329 g of fish wet weight (ww) per day (Poole 1985; USEPA 1993). The second estimate used dry weight (dw) consumption rates based on the relationship between BWt and metabolic energy for birds (FIR g dw/day= $0.648BWt^{0.651}$) (Nagy 1987; USEPA 1993). The FIR for an adult female osprey (77.94 g fish dw/day) converted to ww (assuming 75% water content for a generic fish) was 312 g/day. These 2 estimates yielded similar results and the metabolic-based estimate was selected for use. The DI (μg pharmaceutical/kg Bwt) was calculated using Equation 1

$$\mathrm{DI} = (\text{residue in fish})(\mathrm{FIR})/(\text{kg BWt}). \quad (1)$$

The DIs for varying degrees of absorption were compared to the oral HTD for an adult. Human therapeutic doses were obtained as the minimum daily dose to exert a therapeutic effect (RxList 2008; FDA 2012; Drugsite Trust 2014).

Screening-level exposure model: 40 day cumulative intake

To estimate cumulative body burden of ospreys, assumptions included that diet was the principal exposure route, BWt and FIR were constant, and intestinal absorption was comparable between ospreys and humans. Clearance was incorporated assuming a first-order kinetic elimination equation to calculate total exposure (i.e., μg/kg BWt) because the majority of ionizable pharmaceuticals follow this type of elimination (Bardal et al. 2011). Using DI (t = 1 d), exposure (E) oscillated following a saw-tooth pattern between peak (E_{peak}) and trough (E_{trough})

$$E_{peak} = (DI_{remaining})e^{-kt} + \mathrm{DI}\,(\text{just after meal}), \quad (2)$$

$$E_{trough} = (DI_{remaining})e^{-kt} + (\mathrm{DI})e^{-kt}\,(\text{just before a meal}), \quad (3)$$

$$t_{1/2} = \ln(2)/k\,(\text{half-life elimination constant}). \quad (4)$$

There are limited data on the half-lives ($t_{1/2}$) of pharmaceuticals in birds to apply in these equations. To place the 40 day exposure into perspective, the drug $t_{1/2}$ in ospreys needed to reach or exceed the HTD within 40 days at most extreme scenario (1000 ng/L concentration, pH 8, complete absorption) was back calculated. Equation 3 (daily exposure at nadir) was used to conservatively estimate cumulative daily body burden. The back calculated $t_{1/2}$ for ospreys was compared to the $t_{1/2}$ in humans (Ebadi 2008; Wishart et al. 2008; FDA 2012).

Empirical pharmaceutical exposure data

Study sites were selected in urbanized areas in proximity to WWTPs, combined sewer outflows, and effluent dominated low flow sites. These sites include the Susquehanna River (MD, PA), Back River (MD), James River (VA), and the US

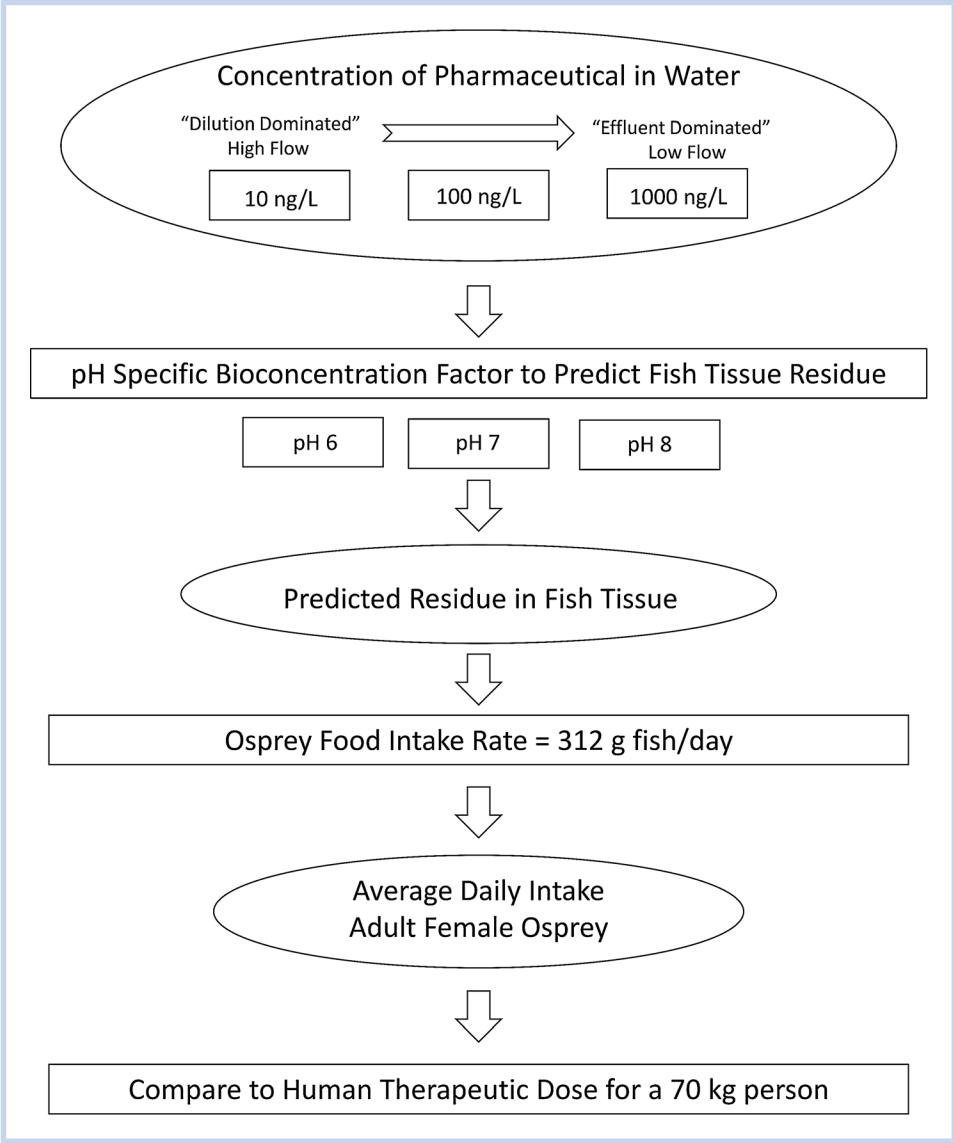

Figure 1. Theoretical screening-level exposure assessment framework used to model the daily intake (DI) for an osprey.

Environmental Protection Agency (USEPA) designated Regions of Concern (Baltimore Harbor [MD], Anacostia River/middle Potomac [DC, MD, VA], and the Elizabeth River [VA]), all of which appear on the 303d list for impaired waterways (Figure 2) (USEPA 2013). Sampling was undertaken during osprey nesting seasons of 2011, 2012, and 2013. The Paul S. Sarbanes Ecosystem Restoration Project at Poplar Island (MD), a remote mid-Bay location, was used as a reference site.

Duplicate water samples were collected from 12 select sampling sites (2–3 locations along a stretch of the Susquehanna River, Back River, Anacostia/middle Potomac Rivers, James River, and at Poplar Island). Surface water samples were collected in clean 4 L amber glass jugs. Field blanks were taken by opening an empty jar to account for other sources of contamination. Water quality parameters (pH, dissolved O_2, temperature, conductivity) were measured concurrently (YSI Multimeter Yellow Springs OH). Water samples were stored on wet ice and shipped overnight to Baylor University.

All procedures involving fish and ospreys were conducted under approval of the Institutional Animal Care and Use Committees of the US Geological Survey (USGS) and the University of Maryland, and appropriate scientific collection permits. Game camera (Bushnell 8MP Trophy Cam, Overland Park, KS) images of prey items delivered to osprey nests, direct observations, and identification of scraps were used to reconstruct osprey diet and identify target species for sampling. Based on osprey diet reconstruction, a combination of gizzard shad (*Dorosoma cepedianum*), catfish (blue catfish *Ictalurus furcatus*, brown bullhead *Ameiurus nebulosus*, and channel catfish *Ictalurus punctatus*), and carp (*Cyprinus carpio*) were sampled on the Susquehanna, Anacostia/middle Potomac, and James Rivers. Atlantic menhaden (*Brevoortia tyrannus*), striped bass (*Morone saxatilis*), and white perch (*Morone americana*) were sampled at the more saline Poplar Island site, and a combination of carp, catfish, gizzard shad, and white perch were sampled on Back River. These fish species reflect different trophic levels ranging from primary consumers (herbivorous) such as Atlantic menhaden and gizzard shad, to secondary consumers (carnivorous) including white perch and striped bass, to catfish and carp (omnivorous) representing a combination of both primary and secondary consumers. Fish were captured by electroshocking in upriver sites. At Poplar Island,

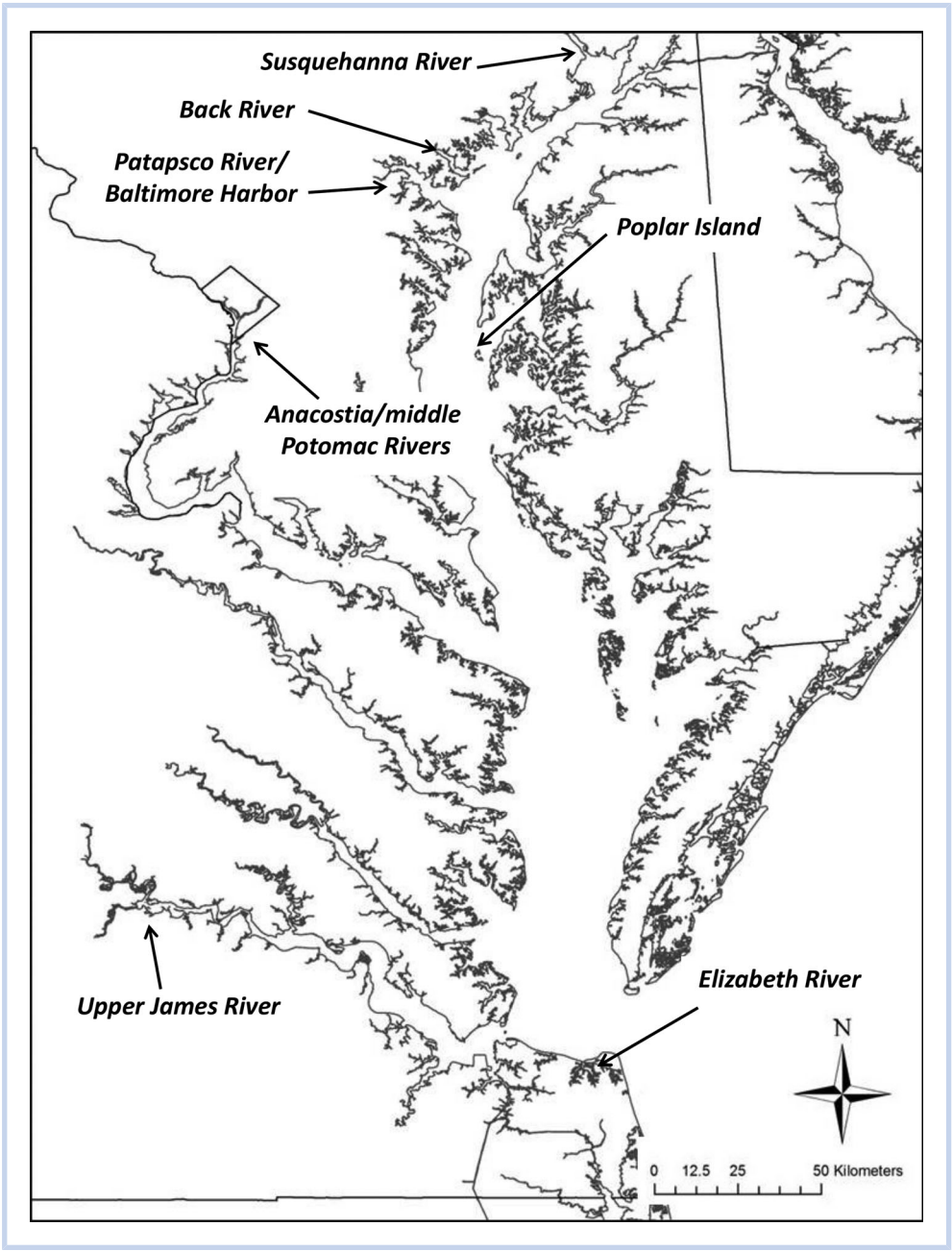

Figure 2. Map detailing the locations of Chesapeake Bay study sites.

fish were captured using a midwater trawl and a commercial pound net. Plasma was sampled from the 2 to 3 dominant prey fish species found at each site that fell within the osprey foraging size range (25–35 cm) (Poole 1989). All fish ($n = 233$) were anesthetized (MS222, tricane methanesulfonate), weighed, measured, and 1 to 2 mLs of blood were sampled using a heparinized syringe. Fish blood was stored on wet ice and transported to the USGS-Leetown Science Center in WV. The blood was centrifuged at 2000 *g* at 4°C for 10 min on the same day of collection. Plasma was harvested for pharmaceutical analyses and stored at −80°C. Fish tissue was saved for analysis of organic contaminants as part of a concurrent study.

Osprey nests were identified in mid-March along a 25 to 35 km stretch of river. A sample egg was collected for analysis of legacy contaminants and nests were visited weekly to determine reproductive success as part of a concurrent study. Once nestlings reached 40 to 45 days of age, a single chick was briefly removed from the nest (<10 min). Body weight and culmen length were measured, and a 5 to 7 mL brachial blood sample was drawn into a heparinized syringe. Samples were stored on wet ice and centrifuged at 1500 *g* at 4°C for 10 min on the same day of collection. Plasma was harvested and samples ($n = 69$) were stored at the USGS-Patuxent Wildlife Research Center at −80°C. All plasma samples were shipped frozen to Baylor University for the quantification of pharmaceuticals.

Analysis of pharmaceuticals

A suite of 23 pharmaceuticals and metabolites and an artificial sweetener (Tables S1 and S2) were quantified in water and plasma samples from fish and osprey nestlings via isotopic dilution liquid chromatography-tandem mass spectrometry (LC-MS/MS). These compounds included analgesics (acetaminophen, codeine), antibiotics (erythromycin, sulfamethoxazole, trimethoprim), an anticoagulant (warfarin), antidepressants (paroxetine,

fluoxetine, sertraline, and primary metabolites norfluoxetine and desmethylsertraline), an antihistamine (diphenhydramine), antihypertensives (atenolol, diltiazem, propranolol), anti-inflammatories (celecoxib, diclofenac), an antilipemic (gemfibrozil), an antiseizure (carbamazepine), a parasiticide (ivermectin), psychostimulants (diazepam, methylphenidate), a stimulant (caffeine), and an artificial sweetener (sucralose; conservative tracer of effluent discharges) (Soh et al. 2011).

For water, sample filtration and extraction generally followed previously described protocols (Du et al. 2014). A mixture of 24 internal standards (deuterated analogues of target compounds, except for ivermectin for which abamectin was the internal standard) and 5 mL of methanol was added to 500 mL of each water sample before extraction and acidification (pH adjusted with 100 μL of 85% [v/v] phosphoric acid) (Lajeunesse et al. 2008). Resulting concentrations of internal standards were approximately 100 ng/g. Samples were subsequently loaded onto strong cation-exchange cartridges (Strata-SCX, 500 mg; Phenomenex, Torrance, CA) preconditioned with 4 mL of methanol and 8 mL of nano-pure water. Each cartridge was washed with 4 mL of HCl (0.1 *N*) and 4 mL of methanol, followed by elution of the 5 antidepressant serotonin reuptake inhibitors with 6 mL of 5% (v/v) NH_4OH in methanol. Extraction of 19 other analytes generally followed a previously reported protocol (Vanderford and Snyder 2006). Each sample (500 mL subsample) was spiked with a mixture of internal standards and loaded onto a preconditioned HLB cartridge (200 mg, Waters, Milford, MA). These loaded cartridges were air-dried and subsequently eluted with 5 mL methanol followed by 5 mL 10:90 (v/v) methanol-methyl tertiary butyl ether. The eluate from 2 separate extractions was evaporated to dryness under a stream of N and reconstituted in 1 mL of chromatographic mobile phase (i.e., methanol-0.1% [v/v] aqueous formic acid). Before LC-MS/MS analysis, samples were sonicated for 1 min and filtered using Pall Acrodisc hydrophobic Teflon Supor membrane syringe filters (13 mm diameter; 0.2 μm pore size; VWR Scientific, Suwanee, GA).

For plasma samples, a slightly modified extraction method was used (Fick, Lindberg, Parkkonen et al. 2010). An aliquot of fish and osprey plasma (typically 1 mL), combined with the same mixture of internal standards that was used for water, was diluted to 5 mL using 0.1% (v/v) aqueous formic acid and mixed thoroughly by sonication. The mixture was loaded on preconditioned (5 mL of methanol and 5 mL of nano-pure water) HLB SPE cartridges (200 mg, Waters). Each cartridge was air-dried and subsequently eluted with 5 mL of methanol. The eluate was reconstituted, and analytes were quantified by LC-MS/MS as previously described (Du et al. 2012).

For water and fish plasma, method detection limits (MDLs) were less than 11 ng/L with the exception of ivermectin and sucralose (Table S2). Osprey plasma from the reference site, spiked with the mixture of internal standards, was used to determine MDLs, which were similar to that of water and fish (Table S2). For quality control purposes, 1 pair of matrix spike samples and 1 method blank sample was added for each batch analysis. Spike recoveries ranged from 81% to 111% in water, 81% to 113% in fish, and 81% to 89% in ospreys.

Statistical analyses

For the daily and 40 day screening-level exposure models, DI and half-life elimination constants were estimated using Microsoft Excel. For empirical exposure data, concentrations of pharmaceuticals and an artificial sweetener were first recovery corrected and only values above the MDL were reported. If the analyte was present in all samples, the arithmetic mean and standard deviation were obtained using SAS (SAS Institute, NC). If an analyte was detected in only 1 of the 2 duplicate water samples, one randomly selected value was included in the statistical analysis. If analytes were detected in over half (but not all) of the samples at a study location, the Kaplan-Meier method was used to estimate an interval that contains the theoretical mean (Helsel 2005, 2009).

Parametric statistics were conducted for those analytes detected in all samples. Continuously distributed analyte concentrations in water, and fish and osprey plasma were tested for homogeneity of variance (Levene's test) and normality (Shapiro-Wilk test). In 2 instances, variables were log or square root transformed to correct for normality or heterogeneous variances. A 1-way analysis of variance followed by Tukey's honestly significant difference method for multiple comparisons was performed ($\alpha = 0.05$). For those sites with nondetects, a generalized Wilcoxon nonparametric test was used (Helsel 2005).

For all detectable compounds, a hazard quotient (HQ) was calculated by dividing the maximum concentration found in fish or osprey plasma by the human therapeutic plasma concentration (C_{max}). The larger the HQ value, the greater the potential for a compound to exert a pharmacological effect in fish or ospreys.

RESULTS

Screening-level exposure model

Of 114 compounds, 31 had a BCF less than or equal to 1.00 (Table S1). These 31 compounds plus the bronchodilator tiotropium (no BCF available) were excluded from the model. Of the 83 remaining compounds, 15 had both a BCF greater than 100 and an estimated DI greater than 20 μg/kg-day and are predicted to have the greatest potential to bioaccumulate. The calculated DIs for these 15 compounds at concentrations of 10, 100, and 1000 ng/L in an adult female osprey are presented in Table 1. At concentrations of 1000 ng/L water at pH 8, the DI of orlistat, fenofibrate, tamoxifen, and loperamide would be 1.1 to 4.4 times greater than the oral HTD. Based on the information in Table 1, orlistat is the only compound that still exceeds the HTD even if the intestinal absorption in ospreys was only half that of humans (12 198 μg/kg BWt-day, 2.4 times greater than the HTD). Pharmaceutical concentrations in water and pH values selected in the model were environmentally realistic (analyte concentrations in Chesapeake Bay range from 0.029 to 10 249 ng/L and site pH ranged from 6.15 to 8.34) (Tables 3 and S3).

Cumulative 40 day exposure model

For the top 15 compounds (BCF > 100, DI > 20 μg/kg BWt-day), the theoretical half-life (calculated from the half-life elimination constant, k) for an osprey to reach the HTD within a 40 day period ranged from 1 to 231 days. For this subset of pharmaceuticals, the half-lives in humans ranged from 0.04 to 7 days. Notably, the half-lives in ospreys for fenofibrate, tamoxifen and ezetimibe were less than that in humans, with the HTD being exceeded in just 3 to 7 days (Table 1).

Empirical assessment of pharmaceuticals in water, fish, and osprey

Of the 24 analytes measured, 17 were detected in water and 8 of these were also detected in fish (Tables 2, S3, and S4).

Table 1. DI at 3 water concentrations and $t_{1/2}$ to accumulate an HTD within 40 days for 15 pharmaceuticals compared to human values

			DI osprey (100% absorption) (μg/kg BWt-day)[a]				$t_{1/2}$ ospreys to exceed HTD 100% absorption[a]	
			10 ng/L	100 ng/L	1000 ng/L		1000 ng/L	
Rank	Compound	BCF pH 8	pH 8	pH 8	pH 8	HTD (μg/kg BW)	pH 8	$t_{1/2}$ humans (d)
1	Orlistat	123 000	244	2440	24 396[a]	5143	1.79	0.04–0.08
2	Fenofibrate	15 100	29.9	299	2995[a]	1714	0.42[a] (HTD in 3 d)	0.83
3	Piperonyl butoxide	2400	4.76	47.6	476	Topical	NA[b]	NA
4	Tamoxifen	797	1.58	15.8	158[a]	143	1.00[a] (HTD in 4 d)	5–7
5	Ketoconazole	646	1.28	12.8	128	2857	22.8	0.14
6	Ezetimibe	589	1.16	11.6	117	143	1.19[a] (HTD in 7 d)	0.79–1.25
7	Iminostilbene[b]	556	1.10	11.0	110	5714	34.3	0.08–2.71
8	Loratadine	538	1.06	10.7	107	143	1.27	0.35
9	Loperamide	519	1.03	10.3	103[a]	57.1	0.67	0.45
10	Promethazine	358	0.71	7.10	71.1	357	4.07	0.67–0.79
11	Diltiazem	343	0.68	6.80	68.0	2571	231	0.12–0.18
12	Raloxifene	327	0.65	6.50	65.0	857	11.3	1.15
13	Dextromethorphan	228	0.45	4.52	45.2	571	11.3	0.05–0.16
14	Desmethylsertraline	213	0.42	4.20	42.3	NA	NA	2.58–4.33
15	Sertraline	142	0.28	2.82	28.2	357	11.5	1.04–1.08

DI = daily intake; HTD = human therapeutic dose; NA = not applicable; not ingested for therapeutic uses or a metabolite of a parent compound.
[a]Indicate the estimated DI or $t_{1/2}$ for ospreys exceeds HTD or human $t_{1/2}$.
[b]Iminostilbenes are a group of antiseizure drugs that includes carbamazepine and oxcarbamazepine. HTD and half-lives are given based on the lowest dose in this group that is needed for a therapeutic effect.

Diltiazem was the only compound detected in all 3 matrices (Table 3, all values presented on a ng/L basis). When compared to the human therapeutic plasma concentration (C_{max}), all detected compounds had a HQ less than or equal to 0.08.

In water, concentrations were averaged from the 2 to 3 sampling sites per tributary. There were 73 out of 77 instances where the analyte was detected in the duplicate water samples and the median relative percent difference between samples was 12.02%. Samples were collected from the reference site each year of the study, but diltiazem was only detected in water samples in 2011 and 2012. At Back River, 18 analytes were detected in water with concentrations being 2 to 154 times greater than other sites. Carbamazepine, diltiazem, sulfamethoxazole, diphenhydramine, and caffeine were detected in water at all intensively sampled sites. Statistical analysis revealed that the Anacostia/middle Potomac Rivers had the greatest carbamazepine (log transformed) and caffeine concentrations in water (Back River excluded from analysis as there was only a single sample), followed by the James, Poplar Island, and Susquehanna ($p < 0.04$). There were no differences in diltiazem concentrations in water among all 5 sites.

Although 7 pharmaceuticals and sucralose were found in fish, detection frequency was low, rarely exceeding half of the samples per site. Thus, parametric statistical analyses could not be conducted among analytes, fish species, and sites (Table S4). By inspection of these data, diphenhydramine and diltiazem were present in fish from all study sites. Both the Anacostia/middle Potomac and Back Rivers had the largest suite of pharmaceuticals detected. Diltiazem was found in 13% of fish samples and present in all species, with the greatest plasma concentration (2.4 ng/mL) found in a catfish collected on the Susquehanna (Table 3).

Diltiazem was detected in all 69 osprey nestling plasma samples (1.6–24.6 times greater than the MDL). Osprey nestlings on the Anacostia/middle Potomac, Baltimore Harbor, and Back River had higher ($p < 0.04$) diltiazem plasma concentrations (square root transformed) compared to the James, Elizabeth, Susquehanna Rivers, and the Poplar Island reference site. Plasma diltiazem concentrations at Poplar were higher than those on the Elizabeth and James Rivers ($p < 0.007$). For the Potomac River, which had osprey nests evenly spaced downriver from Blue Plains WWTP, diltiazem in nestling plasma did not exhibit a spatial concentration gradient ($p > 0.35$).

DISCUSSION

Screening-level exposure assessment

Whereas several studies have examined uptake of pharmaceuticals from water by fish (Brown et al. 2007; Ramirez et al. 2009), their transfer to high trophic level wildlife has not been evaluated. The likelihood for a broad suite of potentially ionizable pharmaceuticals to bioaccumulate in a water–fish–osprey food web was modeled using their concentration in

Table 2. Summary of compounds detected in water and fish across study sites[a]

	Analgesic		Antibiotic			Anticoagulant	Antihistamine	Psychostimulant	
Site/class	Acetaminophen	Codeine	Sulfamethoxazole	Trimethoprim	Erythromycin	Warfarin	Diphenhydramine	Methylphenidate	Diazepam
Poplar Island									
Water			+				+		
Atlantic menhaden									
Striped bass							+		
White perch							+		
Anacosita/middle Potomac Rivers									
Water	+		+	+	+		+	+	
Catfish sp.							+		
Gizzard shad							+		
Carp							+		
Back River									
Water	+	+	+	+	+	+	+	+	+
Catfish sp.							+		
Gizzard shad		+					+		
Carp							+		
White perch							+		
James River									
Water			+	+			+		
Catfish sp.							+		
Gizzard shad							+		
Susquehanna River									
Water			+				+		
Catfish sp.									
Gizzard shad									

	Antihypertensive		Anti-inflammatories			Antilipemic	Antiseizure	Artificial Sweetener	Stimulant
	Atenolol	Diltiazem	Propanolol	Celecoxib	Diclofenac	Gemfibrozil	Carbamazepine	Sucralose	Caffeine
Poplar Island									
Water		+					+	+	+
Atlantic menhaden		+							
Striped bass		+		+					
White perch		+							
Anacosita/Middle Potomac Rivers									
Water	+	+			+	+	+	+	+
Catfish sp.							+		
Gizzard shad	+	+						+	
Carp		+							

Table 2. (*Continued*)

	Antihypertensive		Anti-inflammatories			Antilipemic	Antiseizure	Artificial Sweetener	Stimulant
	Atenolol	Diltiazem	Propanolol	Celecoxib	Diclofenac	Gemfibrozil	Carbamazepine	Sucralose	Caffeine
Back River									
Water	+	+	+	+	+	+	+	+	+
Catfish sp.		+							
Gizzard shad		+					+		
Carp							+		
White perch									
James River									
Water	+	+				+	+	+	+
Catfish sp.		+							+
Gizzard shad		+							
Susquehanna River									
Water							+	+	+
Catfish sp.		+							
Gizzard shad									

[a]+Compound detected.

water, pH-specific BCF and FIR of an adult female osprey. Those compounds with high pH-specific BCFs and long half-lives near low flow point sources (i.e., low dilution scenario) were predicted to exceed the HTD.

This screening-level assessment identified a subset of 15 of 114 compounds that warrant further investigation based on their potential to exceed the HTD. Over a narrow range of pH (6–8), there was little effect on the BCF of 6 of these compounds (orlistat, fenofibrate, piperonyl butoxide, ezetimibe, iminostilbene, and loratadine), with BCFs fluctuating by less than 20% (Table S1). Although ionizable at pH extremes (low or high pKa), these 6 compounds were in their neutral state from pH 6–8 and predicted to be the most bioaccumulative. It has been suggested that compounds with such characteristics could evoke pharmacological responses and possibly toxicity in invertebrates and fish at their isoelectric point (Ebadi 2008; Rendal et al. 2011). The remaining 10 compounds (mean pKa 8.37) are not ionizable until pH exceeds environmentally relevant conditions. The use of pH-specific BCFs appears to be a valuable tool to identify and prioritize pharmaceuticals and metabolites that have the greatest potential to bioaccumulate at environmentally relevant conditions.

An estimate of the half-life is required to model first-order kinetic elimination of drugs over a specific period of time and provides a measure of the persistence of a xenobiotic. Based on our screening-level exposure model, HTDs for fenofibrate, tamoxifen, and ezetimibe were exceeded in adult ospreys at theoretical half-lives (0.24–1.19 days) that were less than their half-lives in humans (0.83–1.25 days) (Ebadi 2008; Wishart et al. 2008). Such theoretical half-lives are not unreasonable. For the aforementioned compounds, it might be possible for an osprey to accumulate a HTD within 3 to 7 days of exposure in a low-flow scenario.

Uncertainty factors and model assumptions

There are a suite of model assumptions and sources of uncertainty that influence DI. This model only takes into account dietary exposure, which is generally acknowledged to be the principal exposure route in fish-eating birds (USEPA 1993), and that the ingested compounds are absorbed at a rate comparable to that of humans. Although this assessment assumed a constant body weight and FIR, this is unlikely because of variations in prey availability, tidal influences, and movement of fish in relation to point source, osprey foraging success, and sibling competition. Environmental factors, including weather and duration and intensity of sunlight (UV and visible), can affect the compound before it enters the prey and its disposition in both prey and predator. There are a series of factors that must be considered in extrapolating uptake (i.e., total tissue and plasma concentrations) and effect thresholds among species within a vertebrate class (body weight and surface area [Davidson et al. 1986], biochemical, genetic, physiological, and behavioral factors [Dorrestein and Van Miert 1988; Toutain et al. 2010]) and are even more tenuous among classes.

Interspecific differences in drug absorption, distribution, metabolism, and elimination can be used to place species sensitivities into perspective. Once a compound is ingested, it is subject to pH changes in the gut and intestines that can influence a drug's ionizable state. Although our model accounted for environmentally relevant water pH and used pH-specific BCFs, it did not include the effects of digestive tract pH. Of the compounds with the greatest potential for

Table 3. Diltiazem concentrations in water, fish, and osprey on ng/L basis

Site	Water (ng/L) Detects/*n* Mean ± SD Extremes	Fish (ng/L plasma) Detects/*n* Mean ± SD Extremes HQ[b]						Osprey (ng/L plasma) Detects/*n* Mean ± SD[a] Extremes HQ[b]
		Catfish	Gizzard shad	Carp	Rockfish	Menhaden	Perch	
Poplar Island	2/3	NS	NS	NS	3/17	1/10	1/10	13/13
	1.06–1.14[c]				—	—	—	2199 ± 1524[C]
	<MDL–2.05				<MDL–1800	<MDL–410	<MDL–410	605–4458
					0.06	0.01	0.01	0.15
Anacosita/middle Potomac Rivers	2/3	0/30	3/33	1/18	NS	NS	NS	13/13
	1.08–1.62[c]	—	—	—				4517 ± 1384[A]
	<MDL–2.47	—	<MDL–410	<MDL–330	NS	NS	NS	3503–8630
			0.01	0.01				0.29
Back River	1/1	1/2	1/9	0/9	NS	NS	0/5	7/7
	—	—	—	—			—	2353 ± 1207[B,C]
	173	<MDL–350	<MDL–420	—			—	1049–4288
		0.01	0.01					0.29
James River	3/3	8/27	3/27	NS	NS	NS	NS	12/12
	5.85 ± 2.51	—	—					912 ± 225[D]
	2.96–7.49	<MDL–770	<MDL–570					537–1355
		0.03	0.02					0.05
Susquehanna River	2/2	9/18	0/18	NS	NS	NS	NS	10/10
	1.67 ± 0.45	—	—					1434 ± 372[C,D]
	1.35–1.99	<MDL–2400	–					4049–2099
		0.08						0.07
Additional sites where only osprey nestlings sampled								
Elizabeth River								6/6
								966 ± 352[D]
								564–1320
								0.04
Baltimore Harbor/ Patapsco River								8/8
								3786 ± 714[A,B]
								2885–5110
								0.17

— = indicates no mean calculated, contaminant was detected in fewer than half of the samples; HQ = hazard quotient; MDL = method detection limit; NS = not sampled because fish species was not a large component of osprey diet at a particular site; SD = standard deviation;

[a]Means with different captial letter superscripts are significantly different ($p < 0.05$).

[b]HQ is the upper extreme concentration found in fish or osprey plasma divided by the human therapeutic plasma concentration (C_{max}) for diltiazem (30 000 ng/L).

[c]If nondetects were present in <50% of the samples, the Kaplan-Meier method was used to estimate the extremes of the mean followed by a generalized Wilcoxin nonparametric test.

bioaccumulation (Table 1), 9 have a pKa greater than 7.5 and 2 have a pKa less than 6.9, thus remaining in their neutral state in the small intestine favoring increased bioaccessibility. A great deal is known about metal bioaccessibility in birds (Martinez-Haro 2009), but far less is known about the absorption efficiency and potential interspecific differences of pharmaceuticals and many other organic compounds in the avian gastrointestinal tract. Additional information is required before bioaccessibility can be included in the model.

Pharmaceuticals have much shorter environmental half-lives than persistent organic pollutants, although studies suggest some pharmaceuticals can exhibit pseudo-persistence under effluent dominated scenarios (Daughton 2002; Brooks et al. 2006). Furthermore, some pharmaceuticals may be poorly metabolized by fish, resulting in increased potential for bioaccumulation (Connors et al. 2013). Unlike mammals, pharmacokinetic parameters (including $t_{1/2}$) have been estimated for only a few classes of drugs used in disease prevention and treatment (e.g., antibiotics, antifungals, antivirals, analgesics, parasiticides, and sedatives) in a limited number of avian species (domestic poultry and waterfowl and companion animals including psittacines) (Goetting et al. 2011; Guzman 2014). However, for free-ranging avian wildlife, pharmacokinetic parameters are unknown, with the exception of NSAIDs. Notably, diclofenac half-lives vary by over an order of magnitude among old world vultures (*Accipitridae*), new world vultures (*Cathartidae*), and domestic poultry (*Galliformes)*, and other NSAIDs exhibit a wide range of half-lives among orders of birds (Baert and Backer 2003; Naidoo et al. 2008, 2009). Once a xenobiotic enters the body, cytochrome P450s are the primary system used to metabolize foreign compounds (phase I metabolism) with monooxygenase activity varying across species, sex, age, diet (feeding guild), season, and disease (Walker 1980). Notably, low monooxygenase activity is found in fish-eating birds (Walker 1980; Toutain et al. 2010).

Empirical findings in water and fish

Eighteen pharmaceuticals and an artificial sweetener were detected in water samples from Chesapeake Bay. Frequency of detection and concentrations were greatest in water samples collected on the Back River, which receives appreciable WWTP input (180 million gallons/day from 1.3 million residents from Baltimore) (Baltimore County Watershed Management Program 2012). Despite greater input from Blue Plains WWTP and population size (330 million gallons/day from 2.1 million residents of the Washington District of Columbia metropolitan area) (District of Columbia Water and Sewage Authority 2014), concentrations were seemingly lower on the Anacostia/middle Potomac Rivers.

Sulfamethoxazole, diphenhydramine, diltiazem, carbamazepine, sucralose, and caffeine were frequently detected in water samples, and 3 of these (diphenhydramine, diltiazem, and carbamazepine) were often detected in fish plasma (Tables S3 and S4). In fish, detection frequency, concentrations, and HQs were low and far less than critical environmental concentrations hypothesized to cause pharmacological effects in fish (Schwab et al. 2005; Fick, Lindberg, Tysklind et al. 2010; Du et al. 2014). This is not unexpected as other reports indicate that both sucralose and caffeine do not bioaccumulate in fish. Sucralose was detected at lower concentrations in fish than in water samples from the Anacostia/middle Potomac Rivers and did not bioconcentrate (maximum detected concentration in fish/maximum detected concentration in water = 0.50; Tables S3 and S4). Notably laboratory studies have reported a BCF less than 1 for sucralose in zebrafish (*Danio rerio*) (Lillicrap et al. 2011) and is not that different from literature estimates (BCF = 1, Table S1) (ACS 2014). Of the compounds most frequently detected in fish, our estimated BCFs were within an order of magnitude compared to literature values presented in Table S1 (diphenhydramine: estimated 79.1 compared with literature value of 16.7; carbamazepine: 44.1 versus 16.2; diltiazem: 319 versus 343). Interestingly, antidepressants were not found in the present study despite being detected in many urban rivers in North America (Brooks et al. 2005; Ramirez et al. 2009; Lajeunesse et al. 2011; Schultz et al. 2010).

Diltiazem was the only analyte detected in water, fish, and biota. For diltiazem, concentrations in water were low (mean 2.44 ng/L), and with the exception of Back River (173 ng/L), was generally an order of magnitude below those found in urban inland waters of the United States and Sweden (36–1800 ng/L) (Kolpin et al. 2002, 2004; Fick, Lindberg, Parkkonen et al. 2010; Du et al. 2014). In Chesapeake Bay, diltiazem fish plasma concentrations were 2 times greater than those observed at 3 WWTPs in Sweden (MDL-1000 ng/L) (Fick, Lindberg, Parkkonen et al. 2010). Out of 10 commonly used pharmaceuticals tested in *Daphnia magna* and Japanese medaka (*Oryzias latipes*), diltiazem exhibited the greatest acute toxicity (96 h LC50 = 8.2 and 15.0 mg/L, respectively) (Kim et al. 2007). The predicted no-effect concentration for diltiazem based on the lowest acute EC50 values was estimated to be 8.2 μg/L (Kim et al. 2007), which is over an order of magnitude greater than the maximum value observed in the Back River. Evaluating these data in a more complete assessment should also include chronic responses linked to therapeutic hazard (Brausch et al. 2012; Valenti et al. 2012). The aquatic hazards and risk of diltiazem and many other pharmaceuticals remain poorly characterized (Brooks 2014).

Several interspecific differences in pharmaceutical bioaccumulation were found among fish species (Table S4). For example, diltiazem was detected in channel catfish, but not gizzard shad from the Susquehanna River, whereas carbamazepine was observed in blue and channel catfish, but again not gizzard shad from the Anacostia/middle Potomac Rivers. Spatial variations in fish migration patterns may explain such differences in pharmaceutical bioaccumulation. For example, anadromous gizzard shad migrate downstream to deeper waters in the winter, whereas catfish remain in upper estuarine sites where they may be continuously exposed to wastewater discharge. The influence of trophic position (e.g., herbivorous gizzard shad and omnivorous catfish) on pharmaceutical bioaccumulation in fish is not well understood.

Empirical findings in osprey nestlings

This screening-level exposure assessment suggests that only 3 of 24 analytes quantified in osprey plasma (diltiazem, sertraline, and desmethylsertraline) are likely to exceed the HTD. Of these 3, diltiazem has the highest pH-specific BCF (343 at pH 8) and was detected in all osprey nestling plasma at low concentrations (0.56–8.63 ng/mL plasma), with the maximum value being 28% of the HTD. Although present in all osprey samples, there were no overt signs (therapeutic or toxicological) observed in our companion study examining reproductive success. Ospreys are thriving in Chesapeake Bay, including the

most contaminated sites, and reproduction is generally adequate to sustain stable populations (>1.15 fledglings per active nest) (Lazarus et al. 2012).

Of the 15 compounds identified in the screening-level model as having the greatest potential to bioaccumulate, 3 were measured in osprey plasma (Table 1; diltiazem 11, desmethylsertraline 14, and sertraline 15), and only diltiazem was detected. The accumulation of diltiazem and other antidepressants is theoretically pH-dependent. The bioaccumulation characteristics (partition coefficients log p and log D at pH 8) of sertraline (log $p = 5.08$ and log D = 3.60) and its metabolite desmethylsertraline (log $p = 4.89$ and log D = 3.73), are not unlike diltiazem (i.e., log $p = 4.73$ and log D = 3.90). Thus, diltiazem may be bioaccumulating not only because of its high pH-specific BCF, but also because of other biological characteristics including specific binding mechanisms. It is clear that diltiazem concentrations were greatest in osprey nestlings followed by fish and water concentrations. Across sites, the maximum diltiazem concentrations in water, fish plasma, and osprey plasma were averaged for each matrix to approximate a biomagnification factor. Diltiazem concentrations in fish plasma were 21.6 times greater than those in water, and osprey plasma concentrations were 4.71 times greater than fish. It should be noted that the biomagnification factor from fish to osprey most certainly varies with osprey diet composition.

CONCLUSIONS

This screening-level exposure assessment identified 15 out of 113 pharmaceuticals and an artificial sweetener that warrant further investigation in fish-eating birds due to their high BCF and DI. Some of these compounds might even exceed the HTD. The antihypertensive drug diltiazem was detected in all osprey nestling plasma samples in several tributaries of Chesapeake Bay. Twelve additional analytes that were predicted to bioaccumulate, but not measured in environmental samples should receive priority for further investigation. Although diltiazem in ospreys did not exceed the HTD and was well below the C_{max}, our findings indicate that it can bioaccumulate to levels that are over 4 times greater than values in fish plasma. Even though empirical concentrations of drugs in the present study are well-below therapeutic levels for humans, the paucity of effect threshold data for birds and lower vertebrates makes interpretation of these observations challenging. Our knowledge of mammalian pharmacology can assist in extrapolation of effects to wildlife (Huggett et al. 2003), but in some (and hopefully rare) instances, birds and other perhaps other classes of vertebrates may be sensitive to low-level environmental exposures (Oaks and Watson 2011).

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SUPPLEMENTAL DATA

Table S1. List of 114 compounds for the screening-level exposure assessment, their CAS number, and bioconcentration factor (BCF)

Table S2. Analytes quantified in water and plasma from fish and ospreys and their method detection limits (MDL)

Table S3. Analytes detected in water samples

Table S4. Concentrations of pharmaceuticals in fish plasma at each study site

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