Pharmaceuticals in water, fish, and osprey nestlings in Delaware River and Bay

Thomas G. Bean[1], Barnett A. Rattner[2]ǂ, Rebecca S. Lazarus[2], Daniel D. Day[2], S. Rebekah Burket[3], Bryan W. Brooks[3], Samuel P. Haddad[3] and William W. Bowerman [1]

1. Department of Environmental Science and Technology, University of Maryland, College Park, MD, USA
2. U.S. Geological Survey Patuxent Wildlife Research Center, Laurel MD, USA
3. Department of Environmental Science, Baylor University, Waco, TX, USA

ǂ Contact Information for Corresponding Author:
U.S. Geological Survey, Patuxent Wildlife Research Center
BARC-East, Building 308, RM 213
Center Road
Beltsville, MD 20705
Telephone: 301-497-5671
FAX: 301-497-5624
brattner@usgs.gov

Abstract

Exposure of wildlife to Active Pharmaceutical Ingredients (APIs) is likely to occur but evidence of hazard and risk is limited. One exposure pathway that has received attention is trophic transfer of APIs in a water-fish-osprey food chain. Samples of water, fish plasma and osprey plasma were collected from Delaware River and Bay, and analyzed for 21 APIs. Only 2 of 21 analytes exceeded method detection limits in osprey plasma (acetaminophen and diclofenac) with plasma levels typically 2-3 orders of magnitude below human therapeutic concentrations (HTC). We built upon a screening level model used to predict osprey exposure to APIs in Chesapeake Bay and evaluated whether exposure levels could have been predicted in Delaware Bay had we just measured concentrations in water or fish. Use of surface water and BCFs did not predict API concentrations in fish well, likely due to fish movement patterns, and partitioning and bioaccumulation uncertainties associated with these ionizable chemicals. Input of highest measured API concentration in fish plasma combined with pharmacokinetic data accurately predicted that diclofenac and acetaminophen would be the APIs most likely detected in osprey plasma. For the majority of APIs modeled, levels were not predicted to exceed 1 ng/mL or method detection limits in osprey plasma. Based on the target analytes examined, there is little evidence that APIs represent a risk to ospreys nesting in Delaware Bay. If an API is present in fish orders of magnitude below HTC, sampling of fish-eating birds is unlikely necessary. However, several human pharmaceuticals accumulated in fish plasma within a recommended safety factor for HTC. It is now important to expand the scope of diet-based API exposure modeling to include alternative exposure pathways (e.g., uptake from landfills, dumps and wastewater treatment plants) and geographic locations (developing countries) where API contamination of the environment may represent greater risk.

Keywords: active pharmaceutical ingredient predictive model read-across water-fish-osprey food chain wildlife

Capsule: Low-level exposure of ospreys to pharmaceuticals via diet was detected in Delaware Bay, concentrations in plasma were predicted using a pharmacokinetic model.

Introduction

The Delaware River and Bay (DRB) is the longest undammed watercourse in the eastern United States (DRBC 2016a). The main stem of the channel runs from Hancock, NY, some 531 km south to the mouth of the bay where it meets the Atlantic Ocean at Lewes, DE. The DRB watershed includes New York, New Jersey, Pennsylvania, Delaware and Maryland (DRBC 2016a, Philadelphia Water Department 2007). South of the Chesapeake & Delaware Canal (C&D Canal) and Reedy Island, the 'River' becomes the 'Bay'. South of Reedy Island the channel becomes wider, deeper and more brackish, human population density is lower, and industry is largely replaced by agriculture and tourism. Over 15 million people rely on DRB for water (DRBC 2016). The section from Trenton, NJ (designated river mile 133 (DRBC 2011)) to New Castle County, DE (river mile 63) includes centers of high population density and industry (petroleum refineries, chemical manufacturing and processing, including pharmaceuticals (Toschik et al. 2005, DRBC 2016a and b). As of 2017, there were 128 National Pollutant Discharge Elimination System permits along DRB (92 are south of Trenton), discharging a total of 34.3 million m^3/d (with 33.0 million m^3/d south of Trenton) (Kent Barr (DRBC) Personal Communication April 6th 2017). Pollution of the watershed has been documented for over 200 years (DRBC 2016a and b). In the second half of the 20th century, the contribution of chemical contaminants (petrochemicals, PCBs, PBDEs, organochlorine and organophosphorus pesticides, lead and mercury) to poor water quality in DRB was recognized.

The DRB provides internationally important habitats for migratory and resident waterbirds (DRBC 2016b, Toschik et al. 2005). Some species of birds (osprey (*Pandion haliaetus*), bald eagle, (*Haliaeetus leucocephalus*) peregrine falcon *Falco peregrinus,* great blue heron *Ardea herodias*) suffered declines in productivity during the second half of the 20th century. Reproductive effects including eggshell thinning and diminished productivity

were attributed to *p,p'*-DDE and to a lesser extent PCBs (Wiemeyer et al. 1988, Steidl et al. 1991a,b,c Parsons and McColpin 1995). The last large scale wildlife toxicology study in DRB was conducted over a decade ago (Toschik et al. 2005) using the piscivorous osprey as a sentinel of environmental health (Grove et al. 2009). The greatest concentrations of organochlorine pesticides, the most toxic PCB congeners and PBDEs occurred between Trenton and the C&D Canal. Furthermore, osprey productivity north of the C&D Canal was only marginal for sustaining the population (i.e., 0.8 to 1.15 fledgling per active nest; Spitzer 1980, Poole 1989, Toschik et al. 2005). Plans to improve DRB water quality began as early as 1961, and by 1967 the most stringent water quality standards of any U.S. inter-state watershed were developed (DRBC 2016a). However, it is only since the turn of the millennium that decline in legacy contaminants and recovery of breeding populations of osprey and bald eagle have been observed in DRB (Toschik et al. 2005, Nye 2010, Clark and Wurst 2015, Rattner et al. 2016, Smith and Clark 2016, Gross and Brauning 2017, DRBC 2017).

In the last 2 decades, active pharmaceutical ingredients (APIs) in the environment have emerged as contaminants of concern (Daughton and Ternes 1999). Understanding risks of APIs to wildlife has more recently been identified as a priority research need (Boxall et al. 2012, Rudd et al. 2014). Unfortunately, studies of pharmaceutical occurrence and bioaccumulation in estuarine and marine systems are relatively limited compared to freshwater systems (Gaw et al. 2014). Pharmaceuticals, including acetaminophen, carbamazepine, diltiazem, diphenhydramine and sulfamethoxazole, were detected in a tributary of the Delaware (Assunpink Creek, in the early 2000s) (Alvarez et al. 2005). The DRBC has compiled a list of priority emerging contaminants that includes a diverse range of APIs (MacGillivray 2007, 2014).

100 The main source of environmental APIs is widely accepted as excretion of parent
101 compound and active metabolites by humans and livestock (Halling-Sorensen et al. 1998).
102 Wastewater treatment plants (WWTPs) represent an important sink, source and API exposure
103 pathway for wildlife. Exposure to APIs from WWTPs could result from birds and bats
104 foraging on (i) invertebrates in or emerging from filter beds, (Markman et al. 2007, 2011;
105 Bean et al. 2014, Park et al. 2009); (ii) foraging on plants, fruits, seeds or invertebrates on
106 land amended with biosolids or irrigated with effluent (McClellan and Halden 2010,
107 Washburn and Begier 2011, Jordan, et al. 1997, Dalkmann et al. 2012, Carter et al. 2014a and
108 b; 2015); and (iii) trophic transfer of APIs from effluent influenced surface waters (Kasprzyk-
109 Hordern et al. 2009, Almeida et al. 2014) into piscivorous species via diet (Lazarus et al.
110 2015, Richards et al. 2011). In DRB other important sources of APIs might include direct
111 discharge from pharmaceutical manufacturers, run-off from agriculture (e.g. poultry farms),
112 and septic systems.

113 Pharmaceutical exposure is potentially a cause for concern in wildlife as APIs are
114 biologically active molecules, designed to affect macromolecules, cells or even to kill
115 microorganisms in order to positively affect health, physiology or behavior. Pharmaceuticals
116 have had positive benefits in humans, livestock and companion animals, through improving
117 quality of life, growth and life expectancy (MEA 2005). Thus, API contamination of the
118 environment is unlikely to disappear in the near future (Boxall et al. 2012). Evolutionary
119 conservation of protein/DNA targets across species (Gunnarson et al. 2008) gives potential
120 for APIs to evoke therapeutic-like or other effects in free-ranging fish and wildlife. For
121 example, Valenti et al. (2012) and Margiotta-Casaluci et al. (2014) identified internal doses
122 of the antidepressants sertraline and fluoxetine for fish that exceed HTCs.

123 There are few examples of exposure, hazard and risk of APIs in wildlife. The best
124 characterized areas relate to hazard and risk posed by diclofenac (reviewed in Oaks and

125 Watson 2011) and other non-steroidal anti-inflammatory drugs (NSAIDs) (Naidoo et al.,
126 2010a,b, 2011, Fourie et al. 2015, Zorilla et al. 2015) in avian scavengers in response to
127 population declines of *Gyps* vultures in Asia. Another example of APIs causing mortality in
128 birds occurred in North America, where bald and golden eagles (*Aquila chrysaetos*)
129 consumed residues of barbiturates contained in carcasses of companion animals disposed in
130 landfills (Friend and Franson 1999, Russell and Franson 2014). Compared with wildlife
131 feeding at lower trophic levels, ospreys are expected to be exposed to greater levels of APIs.

132 Trophic transfer of APIs in a simple water-fish-osprey food-chain has been
133 investigated in Chesapeake Bay, USA (Lazarus et al. 2015). In that study, method detection
134 limits (MDL) were exceeded for 18 of 23 APIs in water, 7 of 23 in fish plasma, but only 1 of
135 23 in osprey plasma. The API detected in osprey was the calcium channel blocker diltiazem
136 (used to treat hypertension) (detected in all 69 samples). Diltiazem concentrations in osprey
137 plasma (0.54-8.63 ng/mL) were 1 to 2 orders of magnitude below HTC or maximum plasma
138 concentration (cMax). A screening level modeling exercise predicted diltiazem to be among
139 the top 15 APIs most likely to be found in Chesapeake Bay osprey nestlings. This modeling
140 exercise used three hypothetical surface water concentrations (10, 100 and 1000 ng/L),
141 uptake by fish at pH 8, daily intake of fish by ospreys, and assumed 100% API absorption
142 into blood. Risk was assessed using a theoretical elimination half-life required for ospreys to
143 accumulate HTC. However, the selection of the 15 APIs for the detailed modeling was based
144 on absolute concentration and not scaled relative to HTC or another estimate of hazard. This
145 meant that the model only included 3 of the 23 analytes actually quantified by mass
146 spectrometry (Lazarus et al. 2015). In the present study, the scope of the model was restricted
147 to 21 analytes in water, fish plasma and osprey plasma. The original model was built upon
148 using a read-across approach to fill in data gaps for pharmacokinetic parameters in birds that
149 are currently unavailable for the vast majority of drugs. The updated model incorporated

150 pharmacokinetic parameters to assess exposure through estimates of plasma concentration
151 rather than theoretical elimination half-lives as previously described in Lazarus et al (2015).
152 This would enable evaluation of whether avian sampling (for these analytes) was necessary to
153 predict exposure level and risk (relative to HTC as toxicity thresholds for APIs are largely
154 unknown for wild birds).

155 The aim of the present study was to further investigate API exposure via trophic
156 transfer for ospreys nesting in DRB. Water, fish plasma and osprey nestling plasma were
157 analyzed for 21 pharmaceuticals. We report API concentration and frequency of detection in
158 3 study regions (South, and Central and North DRB). We expected a spatial gradient of APIs
159 (decreasing from North to South) due to proximity to major sources of APIs (WWTPs, drug
160 manufacturers and human population centers) and previous patterns in DRB with other
161 contaminants (Toschik et al. 2005).

162 **Methods**

163 ***Study area***

164 The study area was divided into three regions (Figure 1): (i) North (Delaware River
165 from Bristol, PA to just south of Reedy Island, DE; River mile 119 to 52.8); (ii) Central
166 (Delaware Bay south of Reedy Island, DE to Lewes, DE; River mile 52.80 to 0); (iii) South (a
167 coastal 'reference area' which includes the Inland Bays of Delaware). The North river
168 segment has a narrow channel, relatively low salinity, and is influenced by effluents from
169 major WWTPs in Philadelphia and Wilmington (ca. 1.78 million m^3/d from Philadelphia and
170 0.5 million m^3/d from Wilmington) (Stephens ND, Veolia 2017). The Central region is
171 characterized by a wide and deeper channel, brackish water and smaller WWTPs (river mile
172 23.1, discharge at Murderkill River 45,400 m^3/d) (DNREC 2010)). The Inland Bays are
173 shallow and separate from the main channel in the South region, with effluents of 2,650 m^3/d
174 and 4,160 m^3/d from Cities of Lewes and Rehoboth, respectively, and potentially inputs from

175 septic systems too (DNREC 2010). All procedures involving fish and ospreys were approved
176 by the Institutional Animal Care and Use Committees of the US Geological Survey (USGS)
177 and with appropriate Federal and State scientific collection permits.

178 ***Collection of surface water***

179 Sites were selected based on location of WWTP discharges. Duplicate surface water
180 samples were collected at 2 locations in the North (Neshaminy, PA and Delaware City, DE);
181 the two locations were located upstream and downstream of Philadelphia and Wilmington to
182 account for the effluent inputs of these major urban areas. In the Central region duplicate
183 samples were collected at the mouth of the Murderkill River, DE, and in the South, from the
184 center of Rehoboth Bay. Chemically-clean 4 L amber glass bottles were filled under water
185 facing the current, placed on wet ice and shipped the same day to Baylor University, Waco,
186 TX for analysis.

187 ***Collection of fish plasma***

188 Game cameras (Bushnell 8MP Trophy Cam, Overland Park, KS) were placed at 9
189 osprey nests (2 in North; 4 in Central and 3 in South) between May and August 2015 to
190 observe fish species being brought to nestlings. A total of 194 images where fish could be
191 identified (approximately 1 identifiable image per 1000) by staff of the US Fish and Wildlife
192 Service, Annapolis MD, and were combined with 20 images of fish scraps recorded when
193 visiting osprey nests. The 214 fish were categorized by region. We tailored our fish collection
194 efforts to 2-3 dominant prey species typically 25-35 cm long as preferred by ospreys (Poole
195 1989, see Supplementary Material 1 Figure S1). These were white perch (*Morone*
196 *americana*), Atlantic menhaden (*Brevoortia tyrannus*) and channel catfish (*Ictalurus*
197 *punctatus*) in the North (Gizzard shad *Dorosoma cepedianum* were more abundant than
198 catfish but had moved to more saline waters at time of fish collection); Atlantic menhaden,
199 white perch and gizzard shad in the Central region, and Atlantic menhaden and summer

flounder (*Paralichthys dentatus*) in the South. Fish were caught in July and August 2015 by various methods (hook and line, trawl and gill net). The length of each fish was measured and weight determined using a spring balance (Salter Brecknell, Smethwick, UK). Individual fish were anesthetized by placing each in a bucket containing 10 g/L MS222 (tricane methanesulfonate, Argent, Redmond WA), before collecting 1-3 mL of blood using a 1.5 inch 22 gage needle into a 3 mL heparinized syringe from the caudal vein or dorsal aorta. Blood was transferred into a lithium heparin-coated 3 mL vacutainer (BD), which was placed on wet ice. Blood samples were spun at 1060 × *g* for 10 min within 2 h of collection. Plasma was harvested, transferred to a cryotube (Corning, NY) and placed on dry ice before storage at -80°C. Frozen samples were shipped to Baylor University, stored at -80ºC and analyzed by LCMS/MS within 6 months.

Collection of osprey plasma

Osprey nests were surveyed at 7-10 day intervals between March and August 2015. A total of 29 plasma samples were collected across the study area according to availability of readily accessible nests (north: n=10, central n= 9, south n=10). Briefly, when young were approximately 40-45 d old, a single nestling from each study nest was removed (see methods in Lazarus et al. 2015), placed into a mesh bag and weighed with a spring balance. A 5-7 mL blood sample was drawn from the alar vein through a 23-gauge 1 inch needle into a heparinized monovette syringe (Sarstedt International, Newton, NC) and young were returned to their nests within 8 to 25 minutes. Tubes were rocked and placed on wet ice. Processing, storage and analysis were the same as for fish plasma.

Analytical methods

Chemicals

All chemicals and their corresponding isotopically-labelled analogs were obtained from various vendors. Acetaminophen, acetaminophen-*d*4, amitriptyline, amitriptyline-*d*3,

aripiprazole, aripiprazole-*d*8, benzoylecgonine, benzoylecgonine -*d*3, buprenorphine, buprenorphine-*d*4, caffeine, carbamazepine, carbamazepine-*d*10, diclofenac, diltiazem, diphenhydramine, diphenhydramine-*d*3, fluoxetine, fluoxetine-*d*6, methylphenidate, methylphenidate-*d*9, norfluoxetine, norfluoxetine-*d*6, promethazine, promethazine-*d*3, and sertraline were purchased as certified analytical standards from Cerilliant (Round Rock, TX, USA). Amlodipine, amlodipine-*d*4, caffeine-*d*9, desmethylsertraline, desmethylsertraline-*d*4, diclofenac-*d*4, diltiazem-*d*3, erythromycin-13C, d3, sertraline-*d*3, sulfamethoxazole-*d*4, trimethoprim and trimethoprim-*d*9 were purchased from Toronto Research Chemicals (Toronto, Ontario, Canada). Erythromycin, sucralose, and sulfamethoxazole were purchased from Sigma-Aldrich (St. Louis, MO, USA) and sucralose-*d*6 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). All chemicals were reagent grade and used as received. HPLC grade methanol (MeOH) and methyl tert-butyl ether (MTBE) were obtained from Fisher Scientific (Fair Lawn, NJ, USA), formic acid was purchased from VWR Scientific (Radnor, PA, USA), and a Thermo Barnstead™ Nanopure™ (Dubuque, IA, USA) Diamond UV water purification system was used throughout sample analysis to provide 18 MΩ water.

Water extractions

Sample filtration and extraction generally followed previously described protocols (Du et al. 2014a). Prior to solid phase extraction, each sample was filtered through 0.2 µm filter paper. A mixture of internal standards, including deuterated analogs of all target compounds, was added to 500 mL of water sample so that each sample contained 100 ng of every target analyte prior to extraction. For the analytes extracted using strong cation-exchange cartridges (Strata- SCX, 500 mg, Phenomenex, Torrance, CA), 5 mL of methanol was added to each water sample prior to extraction and acidification (pH adjusted with 100 µL of 85% (v/v) phosphoric acid, (Lajeunesse et al. 2008) Samples were then loaded onto

250 cartridges pre-conditioned with 4 mL of methanol and 8 mL of nano-pure water. Next, each
251 SCX cartridge was washed with 4 mL of 0.1 N HCl and 4 mL of methanol, followed by
252 elution of antidepressant serotonin reuptake inhibitors with 6 mL of 5% (v/v) NH_4OH in
253 methanol. Extraction of other analytes generally followed the protocol of Vanderford and
254 Snyder (2006). After filtration and fortification with 100 ng internal standard, samples (500
255 mL sub-sample) were loaded on HLB cartridges preconditioned with 5 mL methyl tertiary
256 butyl ether, 5 mL methanol, and 5 mL nanopure water (6 mL/200 mg, Waters Corp., Milford,
257 MA). These loaded cartridges were air-dried and eluted with 5 mL methanol followed by 5
258 mL 1:9 (v/v) methanol-methyl tertiary butyl ether. For each extraction, the eluate was
259 evaporated to dryness under a stream of nitrogen and reconstituted in 1 mL of the mobile
260 phase (i.e., methanol-0.1 % (v/v) aqueous formic acid). Prior to LC-MS/MS analysis,
261 samples were sonicated for 1 min and filtered using Pall Acrodisc® hydrophobic Teflon
262 Supor membrane syringe filters (13-mm diameter; 0.2-µm pore size; VWR Scientific,
263 Suwanee, GA).

264 *Plasma extractions*

265 For fish and osprey plasma samples, a slightly modified extraction method was used
266 (Fick et al. 2010a, Scott et al. 2016). Typically a 1 mL aliquot of plasma was combined with
267 internal standards and diluted to 5 mL using 0.1% (v/v) aqueous formic acid. The mixture
268 was sonicated and loaded on pre-conditioned (5 mL methanol and 5 mL of nano-pure water)
269 HLB SPE cartridges (6 mL/200 mg, Waters Corp., Milford, MA). Each cartridge was dried
270 with N_2 gas and eluted with 5 mL of methanol. The eluate was reconstituted, and analytes
271 were quantified by LC-MS/MS as previously described (Du et al. 2012).

272 *Instrumental analysis*

273 Samples were analyzed using isotope-dilution liquid chromatography-tandem mass
274 spectrometry (LC-MS/MS) with an Agilent Infinity 1260 autosampler/quaternary pumping

system, Agilent jet stream thermal gradient electrospray ionization source, and model 6420 triple quadrupole mass analyzer. A binary gradient method consisting of aqueous 0.1 % formic acid as solvent A, and MeOH as solvent B, was used. Separation was performed using a 10 cm × 2.1 mm Poroshell 120 SB-AQ column (120Å, 2.7 µm, Agilent Technologies, Santa Clara, CA, USA) preceded by a 5 mm × 2.1 mm Poroshell 120 SB-C18 attachable guard column (120Å, 2.7 µm, Agilent Technologies, Santa Clara, CA, USA). The flow rate was held constant at 0.5 mL/min. The column temperature was maintained at 60 °C. The injection volume was 10 µL. Analytes were ionized in positive and negative mode using electrospray ionization. MRM transitions for the target analytes and associated instrument parameters were automatically determined using MassHunter Optimizer Software by flow injection analysis (Supplementary Material 1, Table S1).

In the present study, method detection limits (MDLs) represented the lowest concentrations of an analyte that were reported with 99% confidence that the concentration is different from zero in a given matrix. The EPA guideline (40 CFR Part 136, Appendix B, USEPA 2017) for generating method detection limits was followed to generate the current set of MDLs. The experimental design used 8 replicates and the spiking level for each analyte was 1 ng/L. After analysis, MDLs were calculated by multiplying the standard deviation resulting from 8 replicates by the one-sided Student's *t* value for the corresponding number of samples. Corresponding MDLs and instrument limit of detection (LOD) can be found in Supplementary Material 1, Table S2.

Quantitation was performed using an isotope dilution calibration method. Calibration standards, containing mixture of internal standards and variable concentrations of target compounds, were prepared in 95:5 0.1% (v/v) aqueous formic acid–methanol. The linear range for each analyte was confirmed from plots of sensitivity (i.e., response factor; RF) versus analyte concentration. Our criterion for linearity required that the relative

300 standard deviation of RFs for standards spanning the noted range was ≤ 15%. Internal
301 standard calibration curves were constructed for each analyte using eight standards that were
302 within the corresponding linear range. Calibration data were fit to a linear regression, and
303 correlation coefficients (r^2) for all analytes were ≥ 0.98. Quality assurance and quality
304 control measures included running a continued calibration verification (CCV) sample every
305 five samples to check calibration validity during the run, with an acceptability criterion of ±
306 20%. One blank and duplicate matrix spikes were included in each analytical sample batch.
307 This isotope dilution calibration approach resulted in all matrix spike recoveries between
308 80% and 120%.

309 ***Predictive model***

310 Concentrations of 18 APIs and 3 human tracers were predicted for plasma of 40 d old
311 osprey nestlings (to approximate age of blood sampled nestlings). Initially, daily intake was
312 calculated based of dietary requirements (ingestion of 312 g fish/d) and a body weight of
313 1568 g average for an adult female (Nagy 1987, USEPA 1993) (see Figure S2 in
314 Supplementary Material 1). For each of the 21 analytes, daily intake was calculated by
315 multiplying estimated concentrations in fish tissue by the mass of fish eaten per kg BW
316 (equations previously presented in Lazarus et al. 2015). The cumulative body burden over 40
317 d was calculated using elimination half-lives for humans or where available laboratory
318 mammals, livestock and birds (Table S3, Supplementary Material 1) as summarized in Bean
319 et al. (2017) (Bean et al. 2017). Elimination half-lives have been defined for relatively few
320 APIs in birds (e.g., mainly NSAIDS Rattner et al. 2008, Goessens et al. 2016, Naidoo et al.
321 2010a and b). The API concentration in fish (and subsequent osprey daily API intake) was
322 estimated using measured surface water concentrations defined in this study, and multiplied
323 by bioconcentration factors (BCF, using log Dow at pH 8 (ACS 2017) as used and discussed
324 in Lazarus et al 2015). Osprey plasma concentration was predicted using measured internal

concentrations in fish (plasma concentration used as a proxy for concentration/g fish) and pharmacokinetic data on absorption (bioavailability), distribution (volume of distribution [Vd] L/kg body weight) and elimination (half-life, $t_{1/2}$) from the published literature following the framework previously described for fluoxetine where model assumptions, uncertainty and sensitivity analyses are presented (Bean et al. 2017). The pharmacokinetic parameters for each API are presented in Table S3 in Supplementary Material 1, while Supplementary Material 2 contains an editable version of the framework (active excel worksheet) that can be used to visualize and conduct calculations. To evaluate the suitability of the model, predicted osprey plasma concentrations were compared with measured osprey plasma concentrations (i.e., would detection above MDL be expected? and is accumulation approaching HTC plausible?).

Statistical Analysis

Pearson's correlation coefficients were calculated for water-fish and fish-osprey in each region. Due to the small number of non-detects, the following values were used: when not detected, 0, when <MDL, used the MDL, and when >MDL= the maximum detected concentration in that matrix in that region was used.

The frequency of detectable peaks in chromatograms and the frequency of quantifiable detects (i.e., >MDL) were calculated for each matrix in various study regions. For water, the sample size was too small to permit statistical comparisons. For fish and osprey plasma samples, detection frequency was generally too low to permit statistical comparisons. However for 1 analyte in osprey plasma (acetaminophen), detection frequency permitted an estimate of extremes of the mean by the Kaplan-Meier method (KM) (Helsel 2005). In addition, comparisons of acetaminophen detection frequency (tests of independence, Sokal and Rohlf 1973) and its concentrations (Kruskal-Wallis tests with Bonferroni correction) were made among regions.

Results and Discussion

API concentrations in water, fish and osprey plasma

Pharmaceuticals were detected at the ng/L level in water and the ng/mL level in fish and osprey plasma. Table 1 summarizes the range and detection frequency by study region. Across all three regions, 8 of 21 analytes exceeded the MDL in water, 7 exceeded MDL in fish plasma but only 2 in osprey plasma. For all matrices, there were numerous other analytes that were detected below quantifiable levels (i.e., trace concentrations; 6 for water, 10 for fish plasma and 7 for osprey plasma), and several that were not detected altogether (7 for water, 3 for fish plasma and 12 for osprey plasma). Promethazine was not detected in any samples.

In water, API concentrations and frequency of detection were typically greatest in the North and lowest in the South. Concentrations were typically <50% of those found in water, fish and osprey plasma from Chesapeake Bay (Lazarus et al. 2015). A human would have to drink 10^6 to 10^9 liters of water to obtain a single therapeutic dose. Exposure of ospreys to APIs via consumption of water is not germane as they receive their water requirement from forage fish (Grove et al. 2009).

For fish plasma (n=56 samples), frequency of detection above MDL was low (only 20 of 1176 chromatograms exceeded the MDL, too few detects to calculate means at the level of species or region). Surprisingly, there were no detects above MDL in the North where API concentrations in water were greatest. Detection of APIs only occurred in the Central (10 detects in gizzard shad, 2 in menhaden, and 1 in white perch) and South regions (6 in flounder, 1 in menhaden). One fish sample was analyzed in duplicate; diclofenac was detected just above MDL in one replicate, but below even trace levels in the other replicate.

In all three regions, there was no significant correlation between API concentrations in water and fish (df=19 for all, South: r=-0.12, t=-0.53, P= 0.60; Central: r=-0.13, t=-0.55, P=0.59; North: r=-0.10, t=-0.44, P=0.67). However, there were significant correlations

between fish and osprey plasma concentrations (df=19 for all, South: r=-0.88, t=8.14, P<0.001; Central: r=-0.68, t=4.01, P<0.001; North: r=0.92, t=10.45, P<0.001). These data suggest that API concentrations in the plasma of forage fish species eaten by osprey have potential for predicting exposure of ospreys.

The failure to detect APIs in fish plasma above MDL in the North suggests that these samples not have been representative. Although ospreys were nesting and feeding in the North, we, and many recreational anglers had great difficulty catching fish during the July-August 2015 collection interval. This may have resulted in ospreys extending their foraging ranges. Concurrent collection and analysis of additional fish and osprey samples in the North deserves further study. Pharmaceutical concentrations detected in fish were low, typically 1-7 orders of magnitude below HTC.

Unlike Chesapeake Bay osprey plasma samples (Lazarus et al. 2015), diltiazem was not detected >MDL in DRB. However, two APIs were detected in DRB osprey plasma, the pain relievers acetaminophen (>MDL in 22 of 29) and diclofenac (>MDL in 2 of 29). For acetaminophen, in the South region 5 of 10 samples exceeded MDL (extremes of mean 1151-1856 ng/L), in the Central region 8 of 9 exceeded MDL (extremes of mean 1816-1972 ng/L) and in the North 9 of 10 exceeded MDL (extremes of mean 2463-2604 ng/L). While there was a difference in detection frequency across regions (R x C Test of independence: df = 2, G=11.914, p<0.01), pairwise comparisons were not significant (p>0.05). Furthermore, acetaminophen concentration did not differ among regions (p>0.05), and was 3 orders of magnitude below HTC. Diclofenac detects (2330 and 3730 ng/L) were 2 orders of magnitude below HTC.

Seven other analytes were detected at trace levels in osprey plasma, giving a total of 9 APIs (mean ± standard deviation, 5.6 ± 0.74 APIs/osprey). There is concern regarding the

399 hazard and risk of pharmaceutical mixtures to non-target organisms (Backhaus 2014). To
400 provide an estimate of what the most extreme risk from mixture toxicity could be for ospreys,
401 we assumed additive toxicity and summed the fractions that each detect was of its respective
402 HTC. Where only trace levels were present, the calculation was performed using 10% of
403 MDL and then repeated with 100% MDL. For the 21 analytes (mostly highly used and potent
404 compounds) included in this study, ospreys would still only be exposed to between
405 0.0073±0.00623 and 0.073±0.00594 of a HTC, and this level would only be relevant if all
406 compounds had shared mechanisms of action. In reality, only 4 of these analytes (two
407 selective serotonin reuptake inhibitor antidepressants and 2 of their active metabolites) have
408 shared mechanisms of action. If this additive approach was used to extrapolate from 21
409 pharmaceuticals to all 1453 FDA-approved drugs (Kinch et al 2014), then ospreys could be
410 exposed daily to 0.5-5 times the HTC. We believe this extrapolation would be misguided and
411 alarmist as, i) not all APIs are equally used or equally potent; ii) only a fraction of the 1453
412 are likely to be in use in a particular geographic area; iii) not all interactions are additive,
413 particularly for mixtures with different mechanisms of action and iv) many mixture
414 interactions are antagonistic, particularly at low levels typical of environmental exposure
415 (Cedergreen 2014). Therefore, we believe the API exposure levels detected in ospreys are
416 unlikely to be of biological concern, although understanding of internal pharmaceutical doses
417 and effects thresholds in birds and other wildlife is largely unknown.

418 ***Evaluation of API concentrations in surface water to predict levels in fish***

419 To predict the worst case scenario, API levels in fish were estimated from their
420 concentration in water by using either (i) the greatest detected concentration, or (ii) the MDL
421 when only trace levels were present (Table 2). This value was multiplied by a
422 bioconcentration factor at pH 8 (Table S3 Supplementary Material 1, ACS 2017). The
423 predicted concentrations were compared with measured concentrations in fish in each study

region (Table 2). Comparison of predicted and measured API concentrations in fish showed poor agreement. The main discrepancy was the model predicts highest concentrations in the North but we failed to detect any APIs >MDL in fish from this region. Aside from previously discussed difficulties in fish collections in the North, other potential explanations for this discrepancy include (i) the small number of surface water samples, (ii) use of plasma (rather than whole fish homogenates), and (iii) the BCFs used may not accurately reflect uptake kinetics for the targeted fish species.

Notably, fish species collected in this study have diverse life histories, foraging ranges and feed at different trophic levels. For example, summer flounder are primarily a marine, predatory species eating lower trophic fish and crustaceans. They are generally found in high salinity areas, but may move around with the tide to forage. Channel catfish are omnivores typically found in freshwater (limited to salinities <18ppt); tidal movements are also likely dictated by foraging. Atlantic menhaden and Gizzard shad feed on plankton and algae, they move in schools (Menhaden will school by age class) (Maryland Department of Natural Resources 2017). Gizzard shad are generally found in areas of lower salinity in spring during the spawning season, but can be found in salinities >20 ppt in autumn and winter (Chesapeake Bay Program 1987). White perch generally live in a limited area. They spawn in freshwater but only migrate downstream during the summer into lower portions of rivers but may extend into the bay or coastal waters. Given the movements of the fish, plasma concentrations of APIs likely only reflects current exposure, whereas if we had measured concentrations in specific organs or whole fish then detection may have been more likely (e.g., see BCFs at pH 8 in Table S3), but attributing exposure to a spatial area more challenging for the majority of fish sampled.

The study of bioconcentration and bioaccumulation of ionizable chemicals across environmentally relevant pH gradients, including the pharmaceuticals examined in the

449 present study, is considered a major research need (Rudd et al. 2014, Boxall et al. 2012).
450 Previous efforts identified trophic dilution, not trophic magnification, for ionizable
451 pharmaceuticals in urban freshwater (Du et al. 2014b) and estuarine systems (Du et al. 2016).
452 Haddad et al. (2017) further observed that accumulation of the model ionizable
453 pharmaceutical diphenhydramine did not differ with age of mullet (*Mugil cephalus*), a
454 species which display age-related feeding shifts. Such observations are in contrast to
455 biomagnification of legacy nonionizable contaminants (e.g., PCBs). Collectively these
456 observations from the field, when coupled with recent gill inhalational uptake studies across
457 pH gradients by Nichols et al. (2015), suggests that diet is less important than inhalational for
458 uptake of ionizable chemicals by fish. However, empirical bioconcentration data for ionizable
459 contaminants and predictive gill uptake models are lacking for estuarine fish across pH and
460 salinity gradients.

461 Bioaccumulation of APIs represents an important future research need given recent
462 observations of the base diltiazem accumulating above HTC in plasma of several fish species
463 from urban estuaries of the Gulf of Mexico in Texas (Scott et al. 2016). In the present study
464 detected levels of human pharmaceuticals in fish plasma (e.g., sertraline, diclofenac) were
465 typically 2 orders of magnitude bellows HTCs (Table 2), perhaps due to greater WWTP
466 effluent dilution in DRB than in effluent dependent and dominated instream flows to
467 estuaries along the Texas coastline. However, if the safety factor of 1,000, as previously
468 recommended by the pharmaceutical industry (Huggett et al. 2003), is considered, then fish
469 plasma levels of sertraline, diltiazem, diphenhydramine and diclofenac were exceeded in the
470 present study (Table 2).

471 ***Evaluation of measured API concentrations in fish plasma to predict residues in osprey***
472 ***plasma***

473 The greatest measured concentration of each API in fish plasma was used to predict
474 API concentrations in osprey plasma at its potential peak (just after a meal) and trough (just
475 before a meal) (Table 3, as described by Bean et al. 2017). Where only trace levels were
476 detected (i.e., <MDL) the MDL was used to be conservative. Analytes that were not detected
477 in fish were not modeled (i.e., promethazine and sucralose). Additionally, there were no data
478 available on absorption for the three analytes that are metabolites produced in vivo
479 (benzoylecgonine, desmethylsertraline and norfluoxetine).

480 With the exception of diclofenac (15,455 ng/L osprey plasma), the maximum
481 predicted concentration in osprey plasma (just after a meal, using the pharmacokinetic data
482 most favorable for detection in plasma) was below 1 µg/L (i.e., 1 ng/mL Table 3). These
483 values are typically at least 2 orders of magnitude below HTC in plasma (e.g., Schulz et al.
484 2012, Berninger et al. 2016, Fick et al. 2010b), indicating risk of effects (therapeutic or side
485 effects) in osprey are likely to be low. Furthermore, comparison of predicted osprey plasma
486 concentrations against MDLs indicated low frequency of detection above MDL for all
487 analytes. Diclofenac was the only API predicted to exceed MDL (7.36 × of MDL, Table 3),
488 and acetaminophen was the only analyte to approach the MDL (0.66 × MDL).

489 The 2 APIs detected in osprey plasma were the 2 analytes the model predicted were
490 most likely to exceed the MDL. Frequency of detection above MDL of acetaminophen (22 of
491 29), was much greater than for diclofenac (2 of 29), while the predictive model suggested
492 diclofenac would be the analyte most likely to be detected above MDL. Frequency of
493 detection based on visible peaks in chromatograms (i.e., trace quantities) was 100% for both
494 analytes. However, in terms of risk, the difference between a plasma concentration of 1.41
495 µg/L (MDL for acetaminophen) and 3.95 µg/L (highest measured concentration) are likely to
496 be negligible (HTC = 10,000 µg/L, i.e., 0.00014 and 0.000395 times of HTC). For diclofenac,
497 the two concentrations detected above MDL were 0.0047 and 0.0075 of HTC.

Looking beyond acetaminophen and diclofenac, 7 other analytes were found in osprey plasma at trace concentrations, which is in agreement with the predictive model that forecast these compounds to be below the MDL. Caffeine concentration in osprey plasma was ranked tied 3rd based on frequency of detection at trace concentrations (29/29) and 4th in the predictive model (based on concentration). Erythromycin also ranked tied 3rd based on frequency of detection at trace levels and 3rd by the predictive model. Compounds that the measured data ranked 5th (amplodipine), 6th (aripiprazole) and 7th (amitriptyline) were ranked 7th, 5th and 6th using the predictive models. Benzoylecogonine was also present at trace levels in 26 of 29 osprey samples, but could not be modeled due to absence of bioavailabilty data for this metabolite. Of the remaining 9 analytes that could be modeled, none were detected at any level in any of the 29 osprey plasma samples. The model did not predict any of these would be present in osprey at concentrations exceeding their MDLs. Indeed, 4 of these APIs were not predicted to exceed 0.01 × of their respective MDLs (buprenorphine, fluoxetine, sulfamethoxazole, trimethoprim); 3 were predicted not to exceed 0.05 × MDL (methylphenidate, sertraline, diltiazem). The remaining 2 APIs, carbamazepine and diphenhydramine, had low MDLs compared to other analytes (200 and 30 ng/L respectively) but were still only predicted to be, at the most extreme, 0.21 × MDL and 0.39 × MDL. Thus, these 9 analytes that were not found at any level in osprey plasma would not have been expected to be detected based on model predictions. Based on these data, use of the framework represents a useful screening tool for predicting which APIs are most likely to show up in osprey plasma.

Both empirical and modeled data indicate that exposure of ospreys to APIs is at worst only going to be at the periphery of the safety factors (0.01 and 0.001 of effects concentration) applied in risk assessment (EC 2003). While toxicity thresholds and potential mixture effects remain uncertain in osprey, our data suggest that controlled exposure studies

523 to derive such values are not warranted. Based on measurements of oxidative DNA damage,
524 body condition, eggshell thinning, and productivity, we found no evidence that APIs are
525 adversely affecting ospreys (Rattner et al. 2016). While we cannot rule out subtle effects at
526 lower levels of biological organization, our reproductive data suggest that ospreys are able to
527 tolerate current levels of APIs as no effects were observed at the individual- or population-
528 level (Rattner et al. 2016). Indeed, our field monitoring efforts suggest that APIs are unlikely
529 to be as significant a threat as legacy contaminants (e.g., *p,p'*-DDE and other organochlorine
530 pesticides, PCBs), (Rattner and Ackerson 2008, Rattner et al. 2016) and other non-chemical
531 stressors (e.g., habitat destruction, prey availability, toxic algal blooms, nest predation) to
532 ospreys. This is the second study in the past 5 years to find limited exposure of ospreys to
533 APIs in a northeast US estuary (Lazarus et al. 2015). Across both studies, only 3 analytes
534 have been detected above MDL, with >90% falling below this level. No analyte exceeded
535 28% of the HTC in either study (and not greater than 1% in DRB) which should be
536 considered good news. For analytes monitored herein, it is possible to get a reasonable
537 exposure estimate by collecting and analyzing fish.

538 There are a number of uncertainties in the model largely relating to use of human
539 pharmacokinetic data to fill gaps for missing values for birds. However, as pointed out in
540 Bean et al. (2017), use of human values is only likely to cause an over estimation of internal
541 concentrations for birds. Thus, if the null hypothesis is that wildlife are not adversely affected
542 by pharmaceuticals in the environment, risk of Type I errors are likely to be low using our
543 model. While it is possible that our 21 analytes were not the highest priority APIs for DRB,
544 (e.g., metformin [see note in Supplemental Material 1 for details on efforts to detect
545 metformin in osprey plasma] and the chlorination transformation product N-nitroso-
546 dimethylamine have been suggested by others as priority compounds in DRB) (MacGillivray
547 2014, USEPA 2014, DHSS 2017), they do represent a broad spectrum of commonly used

548 drugs. Thus, it would be surprising if a different suite of APIs would cast a significantly
549 greater level of risk to ospreys. The predictive model suggests that collection of avian
550 samples for assessment of API exposure in these estuaries may not be warranted unless, (i)
551 concentrations in fish are approaching HTC, or (ii) pharmacokinetic properties for the API in
552 other species suggest that bioaccumulation is likely.

553 **Conclusions**

554 Exposure of ospreys to pharmaceuticals in two large US estuaries appears to be very low
555 (Lazarus et al. 2015, Rattner et al. 2016). Based on the data from these studies, there is no
556 evidence to support an alternative hypothesis (i.e., null hypothesis must be no effect of
557 pharmaceuticals at environmental concentrations unless evidence suggests otherwise) that
558 these 21 analytes, and presumably many other similar APIs (based on usage and or
559 mechanism of action), represent a significant risk to fish-eating birds. There are thousands of
560 other APIs that we did not quantify, as well as new drugs, and changing prescription and
561 usage patterns, that could cause this seemingly benign situation to change over time. To
562 evaluate the need for avian sample collection and analysis, we suggest that potential wildlife
563 exposure be first modeled using fish API residue data to populate the predictive framework
564 for estimating internal concentrations in birds (Bean et al. 2017). Investigation of API
565 exposure of wildlife by other pathways (e.g., direct uptake from WWTPs, dumps, landfills
566 and sludge amended agricultural lands) and in other geographical locations (e.g., developing
567 countries where environmental regulations may be limited (Kookana et al. 2014)) is
568 warranted. Despite findings of low risk of APIs to ospreys nesting in Delaware and
569 Chesapeake Bays and low frequency of detects above MDL in fish (i.e., a relatively “clean
570 bill of health”) there still remain many compounds and exposure pathways to evaluate before
571 global risk of APIs to wildlife can be completely understood.

572

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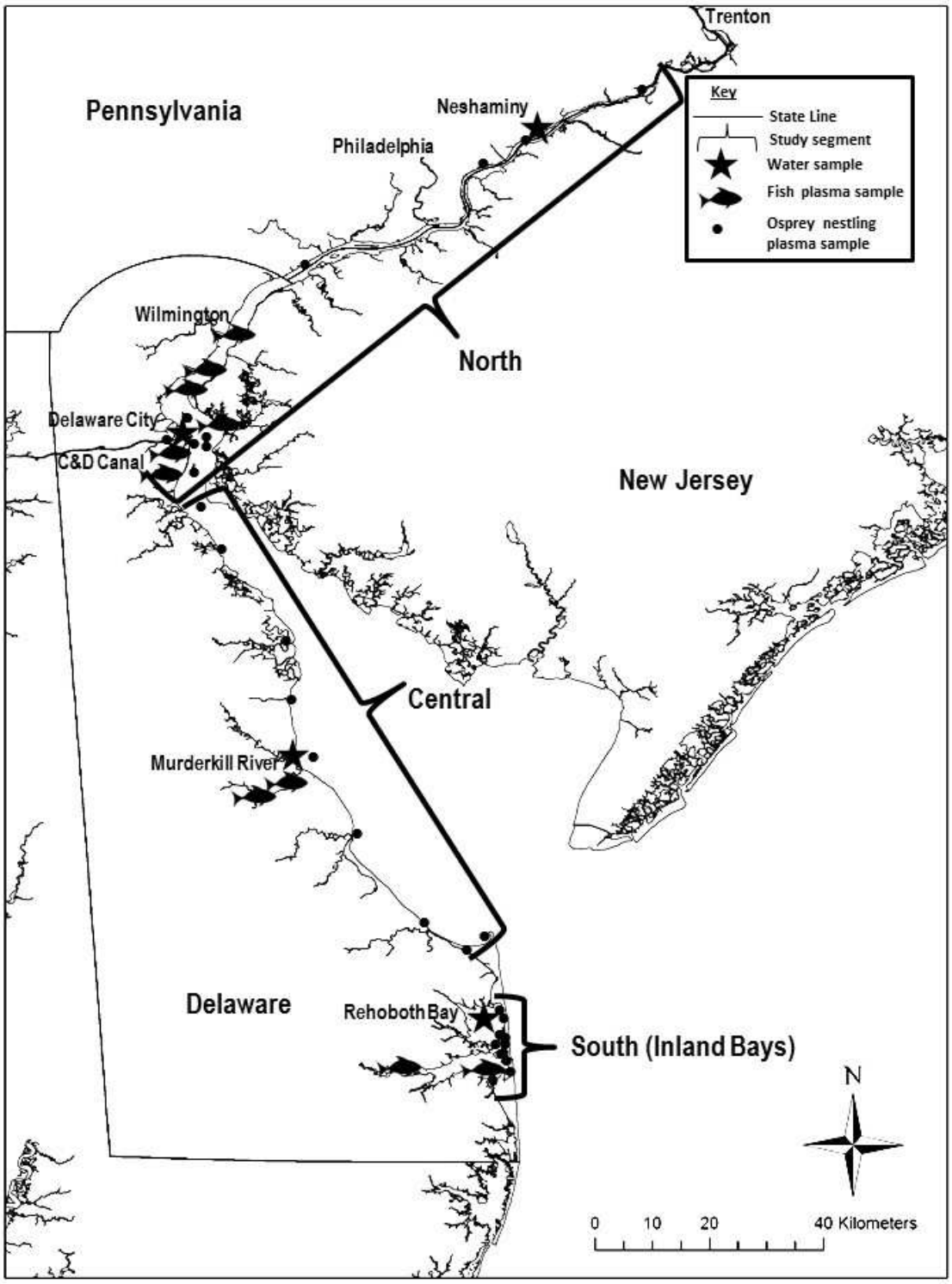

893

894 **Figure 1:** Delaware River and Bay study regions (North, Central and the South). Sample collection
895 sites indicted by symbols for water ★, fish and osprey ●.

896

Table 1: APIs detected in water, fish plasma and osprey nestling plasma (ng/L) from Delaware River and Bay (DRB) study regions South, Central and North

Therapeutic Use Class	API	South (Inland Bays) Water	South (Inland Bays) Fish plasma	South (Inland Bays) Osprey plasma	Central Water	Central Fish plasma	Central Osprey plasma	North Water	North Fish plasma	North Osprey plasma
		Extremes (ng/L) Frequency of detectable peaks			Extremes (ng/L) Frequency of detectable peaks			Extremes (ng/L) Frequency of detectable peaks		
Psychoactive drugs										
Anticonvulsant	Carbamazepine	0.94-1.04 2/2	ND-<MDL 1/18	ND 0/10	6.80-7.17 2/2	ND-230 7/19 (3 of 7 detects >MDL)	ND 0/9	2.35-9.39 4/4	ND 0/18	ND 0/10
Antidepressant	Amitriptyline	ND 0/2	ND 0/18	ND-<MDL 1/10	<MDL 2/2	ND-<MDL 2/19	ND 0/9	ND-<MDL ¾	ND 0/18	ND-<MDL 1/10
	Fluoxetine	<MDL 2/2	ND-<MDL 1/18	ND 0/10	<MDL 2/2	ND-<MDL 8/19	ND 0/9	<MDL 4/4	ND-<MDL 3/18	ND 0/10
	Sertraline	<MDL 2/2	ND 0/18	ND 0/10	<MDL 2/2	ND-1130 7/19 (1 of 7 detects >MDL)	ND 0/9	<MDL 4/4	ND 0.18	ND 0/10
Antipsychotic	Aripiprazole	ND 0/2	ND 0/18	ND-<MDL 2/10	ND 0/2	ND 0/19	ND-<MDL 3/9	ND 0/4	ND-<MDL 4/18	ND-<MDL 2/10
Narcotic	Burprenorphine	ND 0/2	ND-<MDL 3/18	ND 0/10	ND 0/2	ND 0/19	ND 0/9	ND 0/4	ND 0/18	ND 0/10
Sedative	Promethazine	ND 0/2	ND 0/18	ND 0/10	ND 0/2	ND 0/19	ND 0/9	ND 0/4	ND 0/18	ND 0/10
Stimulant	Methylphenidate	ND 0/2	ND-150 1/18	ND 0/10	ND 0/2	ND 0/19	ND 0/9	ND 0/4	ND 0/18	ND 0/10
Active metabolites	Norfluoxetine (of fluoxetine)	ND 0/2	ND-<MDL 1/18	ND-<MDL 1/10	ND 0/2	ND-<MDL 13/19	ND 0/9	ND 0/4	ND-<MDL 2/18	ND 0/10
	Desmethylsertraline (of sertraline)	ND 0/2	ND 0/18	ND 0/10	ND 0/2	ND 0/19	ND 0/9	ND 0/4	ND 0/18	ND 0/10
Allergy relief										
Antihistamine	Diphenhydramine	0.18 - 0.24 2/2	ND – 220 7/18 (1 of 7 detects >MDL)	ND 0/10	1.38-1.41 2/2	ND-390 8/19 (3 of 8 detects>MDL)	ND 0/9	0.28-1.71 4/4	ND 0/18	ND 0/10

Antimicrobials										
Antibiotics	Erythromycin	ND 0/2	<MDL 18/18	<MDL 10/10	ND 0/2	<MDL 19/19	<MDL 9/9	ND 0/4	<MDL 18/18	<MDL 10/10
	Sulfamethoxazole	<MDL 2/2	ND-<MDL 1/18	ND 0/10	8.09-8.34 2/2	ND 0/19	ND 0/9	<MDL-14.73 4/4	ND 0/18	ND 0/10
	Trimethoprim	ND 0/2	ND-<MDL 1/18	ND 0/10	ND 0/2	ND 0/19	ND 0/9	1.45-2.29 4/4	ND 0/18	ND 0/10
Blood Pressure medications										
Calcium channel blocker	Amlodipine	ND 0/2	<MDL 18/18	ND-<MDL 4/10	ND 0/2	<MDL 19/19	ND-<MDL 1/9	ND 0/4	<MDL 18/18	ND-<MDL 5/10
	Diltiazem	ND 0/2	ND-160 1/18	ND 0/10	ND 0/2	ND-260 5/19 (4 of 5 detects >MDL	ND 0/9	ND-1.11 2/4	ND 0/18	ND 0/10
Pain relief										
Analgesic	Acetaminophen	<MDL 2/2	ND-4660 12/18 (1 of 12 detects >MDL)	<MDL-2910 10/10 (5 of 10 detects>MDL)	<MDL 2/2	ND-<MDL 3/19	<MDL-2850 9/9 8 of 9 detects >MDL	ND-<MDL 2/4	ND 0/18	<MDL-3950 10/10 9 of 10 detects >MDL
NSAID	Diclofenac	ND 0/2	ND-3620 2/18	<MDL 10/10	<MDL 2/2	ND-11930 2/19 (2 of 2 detects >MDL)	<MDL-3730 9/9 (2 of 9 detects>MDL)	<MDL 4/4	ND-<MDL 1/18	<MDL 10/10
Human tracers										
Artificial sweetener	Sucralose	354.39 – 371.43 2/2	ND 0/18	ND 0/10	1341.83-1364.18 2/2	ND 0/19	ND 0/9	483.65-1263.48 4/4	ND 0/18	ND 0/10
Stimulant	Caffeine	8.05 - 8.07 2/2	<MDL 18/18	<MDL 10/10	8.61-9.14 2/2	<MDL 19/19	<MDL 9/9	21.32- 26.92 4/4	<MDL 18/18	<MDL 10/10
Inactive metabolite of cocaine	Benzoylecgonine	0.55 - 0.58 2/2	ND-360 1/18	<MDL 10/10	0.75-0.83 2/2	ND 0/19	ND-<MDL 7/9	1.51-6.88 4/4	ND 0/18	ND-<MDL 9/10

MDL = Method detection limit; ND = Not Detected i.e., no detectable peak in chromatograms

MDLs in water, fish plasma and osprey plasma (ng/L): Carbamazepine 0.27, 160, 200; Amitriptyline 5.30, 990, 1990; Fluoxetine 2.39, 850, 1370; Sertraline 1.52, 990, 700; Aripiprazole 2.21, 2280, 3350; Buprenorphine 6.35, 2270, 2160; Promethazine 9.65, 2600, 2330; Methylphenidate 0.14, 60, 110; Norfluoxetine 1.77, 990, 940; Desmethylsertraline 7.16, 2190, 1110; Diphenhydramine 0.11, 130, 30; Erythromycin 8.60, 8600, 14000; Sulfamethoxazole 1.30, 1900, 4000; Trimethoprim 1.30, 2800, 400; Amlodipine 12.03, 2110, 1980; Diltiazem 0.31, 60, 60; Acetaminophen 3.47, 2840, 1410; Diclofenac 4.74, 2310, 2100; Sucralose 2.62, 2910, 640; Caffeine 4.43, 1690, 1440; Benzoylecgonine 0.05, 100, 80.

906

907 **Table 2:** Measured API concentrations in surface water and fish plasma compared with predicted concentrations in fish

Therapeutic use Class	API	Peak human therapeutic plasma concentration (cMax) [a] ng/L	South (Inland Bays) Water	South (Inland Bays) Fish plasma	South (Inland Bays) Predicted fish	Central Water	Central Fish plasma	Central Predicted fish	North Water	North Fish plasma	North Predicted fish
			Greatest measured conc. or MDL* (ng/L)		(ng/kg)	Greatest measured conc. or MDL* (ng/L)		(ng/kg)	Greatest measured conc. or MDL* (ng/L)		(ng/kg)
Psychoactive drugs											
Anticonvulsant	Carbamazepine	2,000,000	1.04	230	16.8	7.17	160*	116.2	9.39	ND	152.1
Antidepressants	Amitriptyline	62,000	ND	990*	ND	5.30*	ND	441.0	5.30*	ND	441.0
	Fluoxetine	12,900	2.39*	850*	13.0	2.39*	850*	13.0	2.39*	850*	13.0
	Sertraline	190,000	1.52*	1,130	215.8	1.52*	ND	215.8	1.52*	ND	215.8
Antipsychotic	Aripiprazole	27,300	ND	ND	ND	ND	ND	ND	ND	2280*	ND
Narcotic	Buprenorphine	500	ND	ND	ND	ND	2270*	ND	ND	ND	ND
Sedative	Promethazine	50,000	ND	ND	ND	ND	ND	ND	ND	ND	ND
Stimulant	Methylphenidate	10,000	ND	ND	ND	ND	150	ND	ND	ND	ND

Active metabolites	Norfluoxetine	NA	ND	990*	ND	ND	990*	ND	ND	990*	ND
	Desmethylsertraline	NA	ND	ND	ND	ND	ND	ND	ND	ND	ND
Allergy Relief											
Antihistamine	Diphenhydramine	50,000	0.24	390	4.0	1.41	220	23.6	1.71	ND	28.6
Antimicrobials											
Antibiotics	Erythromycin	2,000,000	ND	8600*	ND	ND	8600*	ND	ND	8600*	ND
	Sulfamethoxazole	30,000,000	1.30*	ND	1.3	8.34	1900*	8.3	14.73	ND	14.7
	Trimethoprim	1,500,000	ND	ND	ND	ND	2800*	ND	2.29	ND	3.4
Blood Pressure Medicines											
Calcium channel blockers	Amlodipine	23,000	ND	2110*	ND	ND	2110*	ND	ND	2110*	ND
	Diltiazem	30,000	ND	260	ND	ND	160	ND	1.11	ND	380.7
Pain Relievers											
Analgesic	Acetaminophen	10,000,000	3.47*	4660	4.6	3.47*	2840*	4.6	3.47*	ND	4.6
NSAID	Diclofenac	500,000	4.74*	11,930	4.7	4.74*	3620	4.7	4.74*	2310*	4.7
Human Tracers											
Artificial sweetener	Sucralose	NA	371.43	ND	371.4	1364.18	ND	1364.2	1263.48	ND	1263.5
Stimulant	Caffeine	4,000,000	8.07	ND	8.1	9.14	ND	9.1	26.92	ND	26.9

Inactive metabolite of cocaine	Benzoylecgonine	NA	0.58	100*	0.6	0.83	360	0.8	6.88	ND	6.9

a. cMax values taken from Schulz et al. 2012 and Berninger et al. 2016

*If only trace levels were detected then the MDL was assigned as a conservative estimate for use in the predictive model

917 **Table 3:** Measured API concentration in fish (max) and osprey (range) and predicted osprey plasma concentrations**.**

918

Therapeutic use		**Fish plasma (ng/L)**		**Osprey plasma (ng/L)**		**Osprey plasma (ng/L)**	
Class	API	Greatest concentration detected across all regions * (Detection frequency)	Rank [a]	Measured extremes * (Detection frequency)	Rank [a]	Predicted extremes of peak concentration [b]	Rank [a]
Psychoactive Drugs							
Anticonvulsant	Carbamazepine	230 (8/55 of which 3>MDL)	6	ND (0/29)	=8	41.5	8
Antidepressants	Amitriptyline	990* (2/55 of which 0>MDL)	14	ND-<1990* (2/29 of which 0>MDL)	7	8.99-132.2	6
	Fluoxetine	850* (10/55 of which 0>MDL)	11	ND (0/29)	=8	2.63	14
	Sertraline	1,130 (7/55 of which 1>MDL)	3	ND (0/29)	=8	14.3-17.9	10
Antipsychotic	Aripiprazole	2,280* (4/55 of which 0>MDL)	12	ND-<3350* (7/29 of which 0>MDL)	6	340.6	5
Narcotic	Buprenorphine	2,270 * (3/55 of which 0>MDL)	13	ND (0/29)	=8	0.83-3.57	12
Sedative	Promethazine	ND (0/55)	=17	ND (0/29)	=8	NA	NA
Stimulant	Methylphenidate	150 (1/55 of which 1>MDL)	7	ND (0/29)	=8	2.14	16
Active metabolites	Norfluoxetine	990* (16/55 of which 0>MDL)	ǂ	ND-<940* (1/29 of which 1>MDL)	ǂ	ǂ	ǂ
	Desmethylsertraline	ND	ǂ	ND	=8	ǂ	ǂ

		(0/55)		(0/29)			
Allergy Relief							
Antihistamine	Diphenhydramine	390 (15/55 of which 4>MDL)	4	ND (0/29)	=8	3.88-11.6	11
Antimicrobials							
Antibiotic	Erythromycin	8,600* (55/55 of which 0>MDL)	=8	<14000* (29/29 of which 0>MDL)	=3	183.7-701.9	3
	Sulfamethoxazole	1,900* (1/55 of which 0>MDL)	=15	ND (0/29)	=8	20.0-32.0	9
	Trimethoprim	2,800* (1/55 of which 0>MDL)	=15	ND (0/29)	=8	2.02-2.43	15
Blood Pressure Medications							
Calcium channel blockers	Amlodipine	2,110 * (55/55 of which 0>MDL)	=8	ND-<1980* (10/29 of which 0>MDL)	5	39.0-42.2	7
	Diltiazem	260 (6/55 of which 5>MDL)	5	ND (0/29)	=8	2.93	13
Pain Relievers							
Analgesic	Acetaminophen	4,660 (5/55 of which 1 >MDL)	2	<1410* - 1,420-3,950 (29/29 of which 22>MDL)	1	721.0-927.0	2
NSAID	Diclofenac	11,930 (5/55 of which 4>MDL)	1	<2100* - 2,330-3,730 (29/29 of which 2>MDL)	2	7,490.0-15,454.7	1
Human Tracers							
Artificial sweetener	Sucralose	ND (0/55)	=17	ND (0/29)	=8	NA	NA
Stimulant	Caffeine	1,690* (55/55 of which 0>MDL)	=8	<1440* (29/29 of which 0>MDL)	=3	553.1	4
Inactive metabolite	Benzoylecgonine	360	ǂ	ND-<80*	ǂ	ǂ	ǂ

of cocaine		(1/55 of which 1>MDL)	(26/29 of which 0>MDL)	

a. Measured concentration in fish and osprey plasma ranked on highest concentration and then by frequency of detection above MDL, and then frequency of detection of all visible peaks that were <MDL

b. The extremes are presented for predicted osprey plasma where a range of values were given for pharmacokinetic parameters

*Used MDL as proxy for samples where detects were <MDL

ND, Not detected (i.e., not even detected at trace levels), Analytes that were not detected (ND) in fish were not modelled in osprey (i.e., Not Applicable, NA)

ǂ metabolites produced in vivo, no pharmacokinetic data available for consideration of bioavailability