Determining potential adverse effects in marine fish exposed to pharmaceuticals and personal care products with the fish plasma model and whole-body tissue concentrations

James P. Meador[1,2*], Andrew Yeh[2], and Evan P. Gallagher[2]

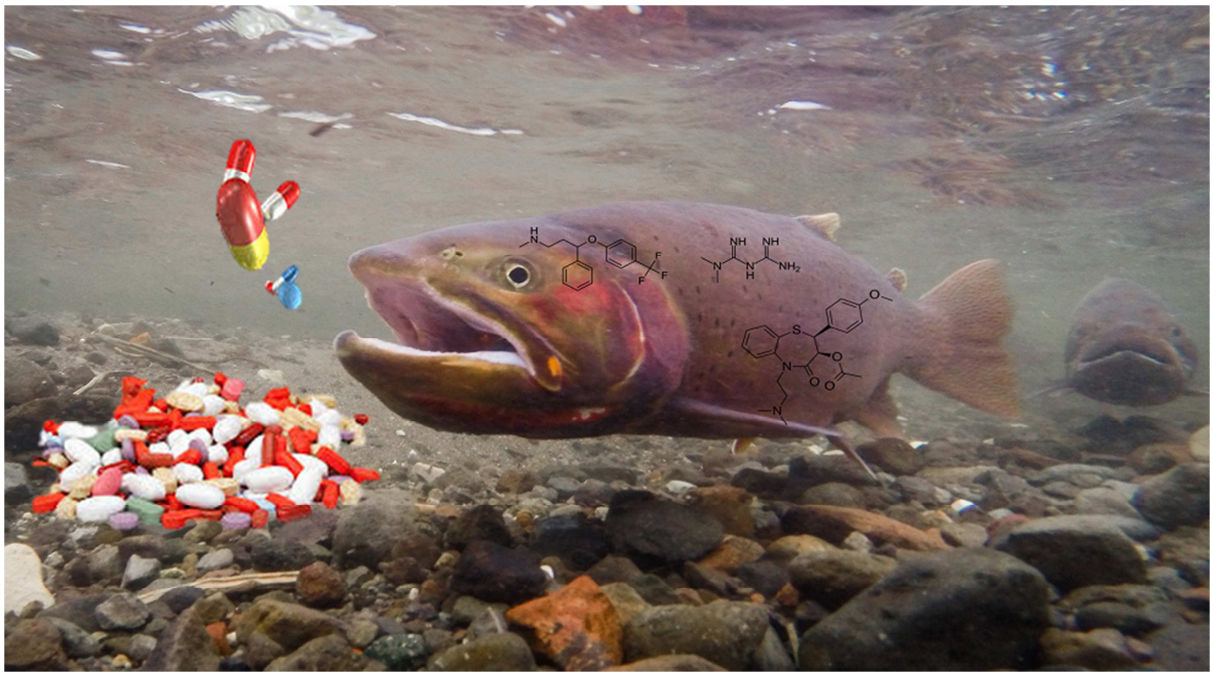
[1]Environmental and Fisheries Sciences Division, Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, 2725 Montlake Blvd. East, Seattle, Washington 98112.

[2]Department of Environmental and Occupational Health Sciences, School of Public Health, University of Washington, 4225 Roosevelt Way, Seattle, Washington 98195.

*Author for correspondence:
James Meador
NOAA Fisheries
2725 Montlake Blvd. East
Seattle, WA. USA 98112
206 860-3321
james.meador@noaa.gov

Andrew Yeh - ayeh3@uw.edu
Evan Gallagher - evang3@u.washington.edu

Andrew Yeh and Evan Gallagher. 4225 Roosevelt Way NE, Suite 100, Seattle, WA 98105–6099 USA



Abstract

The Fish Plasma Model (FPM) was applied to water exposure and tissue concentrations in fish collected from two wastewater treatment plant impacted estuarine sites. In this study we compared predicted fish plasma concentrations to Cmax values for humans, which represents the maximum plasma concentration for the minimum therapeutic dose. The results of this study show that predictions of plasma concentrations for a variety of pharmaceutical and personal care products (PPCPs) from effluent concentrations resulted in 37 compounds (54%) exceeding the response ratio (RR = Fish [Plasma] / 1%$Cmax_{total}$) of 1 compared to 3 compounds (14%) detected with values generated with estuarine receiving water concentrations. When plasma concentrations were modeled from observed whole-body tissue residues, 16 compounds out of 24 detected for Chinook (67%) and 7 of 14 (50%) for sculpin resulted in an RR_{tissue} value greater than 1, which highlights the importance of this dose metric over that using estuarine water. Because the tissue residue approach resulted in a high percentage of compounds with calculated response ratios exceeding a value of unity, we believe this is a more accurate representation for exposure in the field. Predicting plasma concentrations from tissue residues improves our ability to assess the potential for adverse effects in fish because exposure from all sources is captured. Tissue residues are also more likely to represent steady-state conditions compared to those from water exposure because of the inherent reduction in variability usually observed for field data and the time course for bioaccumulation. We also examined the RR in a toxic unit approach to highlight the importance of considering multiple compounds exhibiting a similar mechanism of action.

Keywords: Fish Plasma Model, pharmaceuticals, internal dose, bioaccumulation, Response Ratio.

Capsule: Predicting pharmaceutical concentrations in fish plasma with tissue residues enhances our ability to characterize exposure and assess potential effects for feral fish.

Introduction

The fish plasma model (FPM) as described by Huggett et al. (2003) and explored by several researchers was developed to assess the potential for adverse effects in fish based on observed or predicted plasma concentrations of pharmaceuticals and personal care products (PPCPs). Comparing therapeutic levels in human plasma to fish plasma concentrations provides a basis to determine the relative risk to fish based on the similarity of those levels. Although extrapolating the relationship among chemical exposures and pharmacological responses across species is largely theoretical, studies have emerged that provide supporting experimental data to suggest that in some cases it is reasonable to use a relationship among drug concentrations in human targets to predict the likelihood of adverse effects in nontarget species. Theoretically, this approach may not be limited to PPCPs and may include any compound with an available plasma effect concentration. Several authors have utilized observed concentrations of PPCPs in fish plasma as the dose metric for assessing potential toxicity, including Brown et al. (2007); Owen et al. (2007); Fick et al. (2010a,b); Schreiber et al. (2011). Others have compared human Cmax values with environmental water exposure concentrations (Kostich et al. 2014; Batt et al. 2016).

Many studies have compared observed and predicted plasma concentrations in laboratory and field studies. For example, Winter et al. (2008) found that the measured and predicted plasma concentrations for atenolol in fathead minnow based on water exposure in the laboratory were within a factor of 2 using the same equation as used in the present study. Other studies showing close agreement between observed and predicted plasma concentrations from water exposure in the laboratory include Garcia et al. (2012), Valenti et al. (2012), Nichols et al. (2015), and Nallani et al. (2016b). In some cases predicted values were far less than those observed for fish collected in the field. Du et al. (2014) found higher than predicted

plasma concentrations for diphenhydramine and carbamazepine in field-collected Longear sunfish (*Lepomis megalotis*), which was also noted by Scott et al. (2016) for diphenhydramine and diltiazem in several species of fish.

Most of the available studies that predicted plasma levels in fish are based on water exposure concentrations assuming that uptake via water is the only relevant route of exposure for fish. Based on observed bioaccumulation factors for potential invertebrate and fish prey species, it is clear that excluding dietary uptake would underestimate the potential uptake for fish in the field. One study (Meredith-Williams et al. 2012) reported relatively high bioconcentration factors (BCFs) for carbamazepine, carvedilol, diazepam, and fluoxetine in 3 different invertebrate species exhibiting high variability among species for a given compound. In the aforementioned study, the authors observed a fluoxetine BCF of 185,900 for the amphipod (*Gammarus pulex*). Most of these differences are due to highly variable uptake and elimination rates that were highlighted by Meredith-Williams et al. (2012). Toxicokinetics can be highly variable among apparently similar invertebrate species under identical environmental factors as noted for the ionizable compound tributyltin (Meador 1997) leading to the conclusion that predicting bioaccumulation and toxicity can be difficult for these types of compounds.

Fish plasma concentrations as well as whole-body tissue concentrations can be predicted with simple Quantitative Structure–Activity Relationship Models (QSARs) and water exposure concentrations. A more direct approach would be to measure whole-body tissue concentrations and then predict plasma concentrations for assessment of potential adverse effects. Recently, Tanoue et al. (2015) proposed that PPCP concentrations in target tissues could be more informative for predicting adverse effects than those in blood. Similarly, we propose that tissue concentrations are a better representation of the integrated exposure that fish experience in the field, and that

predicted plasma concentrations when based on those whole-body concentrations would more accurately reflect plasma levels of these compounds under exposure conditions experienced by feral fish. Tissue concentrations are one important step closer in the progression from ambient exposure to concentrations in whole body, organs, and at the receptor. Using tissue concentrations as the dose metric generally provides an important reduction in variability compared to modeling toxicity from water exposure to compound concentrations at the site of the molecular initiating event (Meador et al. 2008; McCarty et al. 2011). As with any aquatic toxicity assay, it is assumed that the ambient exposure concentration is proportional to the tissue concentration, which is in turn proportional to the concentration at the receptor (McCarty et al. 2011). This concept can be applied here and in this case we are using the fish plasma concentration as a surrogate for the internal concentration that can be related to human therapeutic concentrations as measured in plasma. Another important advantage for using tissue residues concerns the formation of metabolites within the body that can be as physiologically active (or potentially more reactive) as the parent compound. Many of these compounds will not be included with water exposure assessments or may occur at far lower concentrations than those produced internally.

One study estimated that 63 – 95% of the commercially available drugs are ionizable (Manallack 2007). Consequently, this is an important factor for QSARs when predicting bioaccumulation and hence toxicity. Standard bioaccumulation QSARs use the octanol-water partition coefficient (K_{ow}) for predicting whole-body tissue concentrations at steady state; however, this parameter can vary widely for ionizable compounds. In the present study, pH specific K_{ow} values (D_{ow}) were generated for use in bioaccumulation models. A number of authors have highlighted the importance of pH in characterizing bioaccumulation and toxicity for ionizable pharmaceuticals.

Rendal et al. (2011) found that fluoxetine, norfluoxetine, propranolol, lidocaine, sertraline, and trimipramine all exhibited increasing toxicity for algae and fish as pH increased from 6.5 to 8.5, which are common values for aquatic ecosystems. This is supported by Nakamura et al. (2008) who also demonstrated large increases in the fluoxetine BCF and toxicity for fish as pH increased from 7 to 9.

A key assumption of the FPM is the degree to which drug targets are conserved between fish species and humans, but also the hypothesis that human pharmaceuticals will interact with such targets to cause a similar target-mediated pharmacological response as observed in humans. In this regard, the evolutionary conservation of a number of structurally- and functionally-conserved protein targets of drugs has been demonstrated in zebrafish (*Danio rerio*) (Gunnarsson et al. 2008) and other aquatic species (Brown et al. 2014). Gunnarsson et al. (2008) examined 1,318 human drug targets and found that 86% were conserved in zebrafish; however, species differences in protein structure associated with amino acid conservation as well as tissue-specific protein expression indicate that commonality in orthologs does not unequivocally assure common functionality in all cases. Despite these caveats, the FPM does provide a reasonable assumption for qualitatively predicting effects in fish using human pharmaceutical data.

While specific sequence information is limited for Chinook salmon and staghorn sculpin, the high degree of similarity among protein drug targets between humans and fish as reported by Gunnarsson et al. (2008) supports the careful application of cross-species extrapolations or the "Read-Across Hypothesis" for assessing potential adverse effects in fish. Additional discussion and supporting information for the read-across hypothesis can be found in Rand-Weaver et al. (2013). It must be noted, that biological drug targets in humans vary markedly and are dependent on the class of compounds as well as individual drugs, and that drug biotransformation and

protein binding is a key component of drug therapy as well as the potential for side effects and toxicity.

Our goal for this study was to examine the FPM in terms of water-only exposure and whole-body tissue concentrations as the dose metric to predict plasma concentrations and assess potential adverse effects in feral fish exposed to PPCPs and other compounds such as triclosan, caffeine, and amphetamine. Additional data on bioaccumulation is included for bisphenol A, nonylphenols, phthalates, perfluorinated compounds, and hexabromocyclododecanes (HBCDDs). Our hypothesis was that plasma concentrations predicted from tissue concentrations observed in field collected fish were likely a better surrogate for dose at the target site and a more accurate representation of the true exposure dose experienced by feral fish as compared to values predicted with ambient water concentrations. To our knowledge, the present study is the first to use whole-body tissue concentrations to predict plasma levels and compare these to human Cmax values in the FPM to assess the potential for toxic effects in fish.

Methods

Water and fish sampling

Three sites in Puget Sound were sampled for wastewater treatment plant (WWTP) effluent, estuarine water, and whole-body tissue concentrations in 2 species of fish. This included 2 effluent-impacted sites and one reference site. Details of site location, sampling procedures, sample preparation, and analytical evaluation were described by Meador et al. (2016). Site location data, water quality parameters, and chemistry composite sample details are listed in Table S1. Briefly, effluent from Bremerton West WWTP and Tacoma Central WWTP was collected in September 2014. At each WWTP a total of 11 one-liter amber glass bottles were filled with effluent sampled at the final stage of processing, just before discharge into the outfall leading to the

estuary. Similarly, at each field site a total of 11 one-liter amber glass bottles were filled with estuarine water at a depth of 2 m below the surface. Estuary water quality parameters were also measured at a depth of 2 m. Two fish species were collected, including Pacific staghorn sculpin (*Leptocottus armatus*), a widely dispersed demersal species in Puget Sound and the U.S. west coast and juvenile ocean-type Chinook salmon (*Oncorhynchus tshawytscha*), a species that resides in local estuaries for several weeks where contaminants are concentrated (Meador 2014). Fish were collected under a Washington State Scientific Collection Permit 13—046 and ESA Section 10(a)(1)(A) permit 17798. All methods for obtaining, transporting, and tissue sampling of fish were approved by the University of Washington Institutional Animal Care and Use Committee (protocol number 4096-01). Fish were collected with a beach seine and transported live to the laboratory for processing. The alimentary canal was opened and cleaned of its contents by flushing with distilled water. The entire fish with all organs was wrapped in aluminum foil and frozen at -80 oC until analyzed. Details of all sampling methods used in this study were reported separately (Meador et al. 2016).

Concentrations for the chemicals of emerging concern (CECs) were determined by AXYS Analytical, Ltd. (Sidney, British Columbia, Canada) using LC/MS/MS techniques. Meador et al. (2016) provides a complete list of the 150 different CEC analytes with their analytical methods and reporting limits. Of the 150 analytes, 147 were analyzed in water samples and 122 were analyzed in whole-body fish tissue. Of the 150 compounds, 92 were detected in fish, effluent, or estuarine receiving waters. Analytes were measured in water and tissue, except hormones, hexabromocyclododecanes (HBCDDs), and phthalate esters. No corrections were applied to the analytical values (e.g. percent recovery or blank correction). Concentrations just above the RL were observed in lab blanks for 3 compounds not detected

232 in fish tissue (albuterol, atenolol, and ofloxacin) and two compounds that
233 were detected in tissue (DEET and nonylphenol). No Cmax value was
234 available for nonylphenol and therefore was not used in the toxicity
235 evaluation.

236 *Bioaccumulation modeling*

237 For estimation of the steady-state whole-body BCF based on effluent or
238 estuary water concentrations we used the equation of Veith et al. (1979) as
239 described by Fu et al. (2009) and Schreiber et al. (2011) for
240 pharmaceuticals. Instead of K_{ow}, we used D_{ow} to more accurately reflect
241 partitioning for ionizable compounds. The BCF was set to 1.41 for all log D_{ow}
242 values below 1 (Log BCF = 0.15 for all Log D_{ow} <1) (Fu et al. 2009).

243 $$\text{Log BCF} = 0.85 \log D_{OW} - 0.70 \quad (1)$$

244 The D_{ow} can be considered as an overall pH-specific octanol-water partition
245 coefficient and it represents the ratio between the concentration in octanol
246 to that in water (Turner and Williamson 2005). Values for D_{ow} were obtained
247 with the plugin LogD within the program Instant JChem (ChemAxon 2016).
248 The D_{ow} was calculated for pH values ranging from 5 to 10 by 0.2 unit
249 increments using the ChemAxon method. Ionic strength was set to 0.25 M
250 for both Na and Cl approximating a salinity of 15 ppt, which is common in
251 estuaries. For most chemicals, structures from DrugBank were imported to
252 InstantJChem for Log D calculations, except for a few that were directly
253 imported from Pubchem as structure files (SDFs). The D_{ow} for pH 8 was
254 used for all calculations, which was the mean value for pH in estuarine water
255 from the 3 sites examined in this study (Table S1). The pH for a given
256 estuary will vary over time supporting the selection of an average value.
257 The K_{ow} for a given compound can be determined by several methods and
258 variability ≤ 0.5 log units (3.1x) among values is considered low (Finizio et
259 al. 1997). The difference in D_{ow} values for the 2 WWTP impacted sites based

on measured pH (8.0 and 8.5) was relatively low with only 1 value (of 83) exhibiting a difference of more than 0.4 log units. Each predicted whole-body concentration (ng/g) was determined by multiplying the predicted BCF (equation 1) by the observed water concentration in effluent or estuarine water (ng/L). The result was divided by 1000 to convert to ng/g.

Predicting blood:water partitioning (Pbw), or the plasma BCF, was accomplished using the equation originally developed by Nichols et al. (1991) and modified by Fitzsimmons et al. (2001). Several authors have utilized this equation for ionizable pharmaceuticals (Du et al. 2014; Tanoue et al. 2015; Nichols et al. 2015), which was developed in the laboratory using water-only *in vivo* exposures and *in vitro* equilibrium after injection. A factor of 0.16 accounts for the fraction of organic material in trout blood (Nichols et al. 2015), which we assumed was similar to that for Chinook salmon.

$$\text{Log } P_{bw} = \log((10^{0.73 logDow} * 0.16) + 0.84) \quad (2)$$

In the same fashion as described by Fu et al. (2009) for the whole-body BCF, the Pbw was set to 1.70 for all log D_{ow} <1, which was the result of equation 2 when log D_{ow}=1. Because arterial blood pH for juvenile Chinook is approximately 7.9 (Clark et al. 2008), no adjustments to D_{ow} were made for equation 2. Predicted plasma concentrations were determined by multiplying P_{bw} by water concentration in ng/L.

We also used equations 1 and 2 to determine the ratio between the whole-body BCF and Pbw (BCF/Pbw), which is equivalent to the volume of distribution, or ratio of total whole-body concentration to plasma concentration. This value was then used to predict plasma concentrations using our observed whole-body concentrations.

$$\text{Plasma concentration (ng/L)} = \frac{tissue}{Vd} * 1000 \quad (3)$$

Where tissue is the observed concentration in ng/g wet weight and Vd is the volume of distribution, which is estimated by BCF/Pbw (equation 1 / equation 2) (reducing to whole-body concentration / plasma concentration by cancellation of water concentration in each term).

These QSAR-generated BCF and plasma concentration values assume steady state, which may or may not occur in fish exposed in these local estuaries. If steady state, or at least 80% of this value as defined by Arnot and Gobas (2006), is not achieved then the results for both equations 1 and 2 would be reduced by the same proportion. Such a reduction due to non-steady state conditions would also cancel out for the prediction of Vd indicating that this parameter is not dependent on steady-state conditions. Another consideration for these compounds is the half-life ($t_{1/2}$). The rate of elimination (k_2) indicates how fast steady-state tissue concentrations will occur (Meador 1997) (approximately $2.5 * t_{1/2}$ for 80% steady state) and the faster the elimination rate the less time is required to achieve steady-state tissue concentrations (Meador et al. 1995). Because the half-life for many of these CECs is relatively short in humans and fish (Figure S1 and Meador et al. 2016), steady-state bioaccumulation (80% or greater) is expected to occur relatively quickly, except for the perfluorinated compounds and nonylphenols. Even though most of these compounds exhibit relatively short half-lives, they can be considered as pseudo-persistent (Daughton 2002) in the environment because of their continuous input from WWTPs.

With these data, we could then compare predicted plasma concentrations based on water exposure concentrations to those predicted with whole-body concentrations. Because whole-body concentrations provide a more accurate representation of field exposure for these fish, the predicted plasma concentrations were more likely to reflect a higher certainty for assessing risk of potential adverse effects.

Effects assessment

We used the human therapeutic dose Cmax ($Cmax_{tot}$) values for assessing the potential for adverse effects, which were obtained from Schulz et al. (2012), Moffat et al. (2011), or a few research studies as noted in Table S2. Plasma concentrations were used in lieu of standard aquatic toxicity metrics (e.g. lowest observed concentration; LOEC or effective concentration based on a proportion of the population responding; ECp) because such data for PPCPs are generally not available for fish. Unfortunately, Cmax values or plasma-effect concentrations were not available for many of the ubiquitous and abundant CECs in this study such BPA, nonylphenols, phthalates, or perfluorinated compounds. Such data for these compounds would greatly enhance our ability to adequately characterize potential toxic effects when assessed with the FPM.

The most common approach for assessing potential adverse impacts is the *Effect Ratio* (ER), which is the Cmax divided by the fish plasma concentration (Huggett et al. 2003). In this study we used the inverse of this ratio, which we call the Response Ratio (RR) (equation 4).

$$\text{Response ratio} = FPCss/HtPC \quad (4)$$

Where the FPCss is the predicted or observed plasma concentration in fish at steady state and the HtPC is the human therapeutic plasma concentration, which is most cases is Cmax, or some fraction of that (e.g., 1% Cmax). For our values of RR in this study we used $1\%Cmax_{total}$ for the denominator, which is the maximal plasma or serum concentration for the minimum therapeutic dose. This concentration is the total amount of chemical bound and unbound (free) in plasma. The bound fraction in plasma can exceed 90% in many cases for pharmaceuticals (Moffat et al. 2011).

We used RR values instead of ER values because it is more intuitive to equate ratios less than 1 for a determination of no adverse effects compared to ratios greater than 1 that would indicate likely adverse effects. At a glance, the reader can tell if an observed or predictive fish plasma concentration is likely to result in physiological effects in fish. This type of ratio also has greater utility for assessing mixtures and is more amenable to a toxic unit approach when adding ratios to determine the probability of adverse effects. Summed values that approach or exceed unity give the reader an easy way to quickly assess potential toxicity.

Mixtures

As discussed by Backhaus (2014) there are two approaches for considering the combined effects of pharmaceutical mixtures, which have been applied to numerous compounds since these approaches were described almost 80 years ago by Bliss (1939). For those compounds exhibiting a common mechanism of action (MeOA), an accepted approach is dose addition (DA), (also called concentration addition) as described by equation 5 and used here. The other approach is response addition, which was not considered. As noted by Backhaus (2014) a number of studies have found high predictive value with DA, even when mixtures contain compounds that are not acting by the same MeOA. Several studies are cited within Backhaus (2014) showing such results.

In the present study we used the following equation to highlight a few examples for mixture toxicity based on SSRIs, beta-blockers, and calcium channel blockers detected in effluent, estuary water, and tissue.

Sum of toxic units (ΣTU_{rr}) = $\sum_{i=1}^{n} \frac{[FPCss]_i}{HtPC_i}$ (5)

Where ∑TUrr is the sum of individual toxic units based on the response ratio (RR) for each compound exhibiting the same MeOA or interaction with the same receptor. FPCss is the predicted or observed plasma concentration in fish at steady state and the HtPC is the human therapeutic plasma concentration, which was selected as 1%$Cmax_{tot}$. The degree to which adverse effects are expected would increase as TU values approach unity, and exceed this value. Values much less than 1 would be considered unlikely to cause adverse effects in fish.

Results

Water and fish tissue concentrations are reported in detail in Meador et al. (2016) and represent a comprehensive analysis of whole-body tissue concentrations for a large number of contaminants of emerging concern. Table S2 lists all 92 detected analytes in water or fish along with each log D_{ow} (pH 8) value, and results of the bioaccumulation models (equations 1 and 2). Additional data in Table S2 includes the predicted volume of distribution (ratio between BCF and Pbw) and the 70 available Cmax values.

For each observed effluent or estuarine water concentration, an expected plasma concentration was determined with equation 2. All predicted plasma concentrations from observed water exposure concentrations are listed in Table S3. The aforementioned value was then used in equation 4 to determine the response ratios, as based on partitioning between water and plasma, which are shown in Figures 1a.b and Table S4. Pharmaceutical and personal care products (PPCPs) from effluent concentrations resulted in 37 compounds (54%) exceeding the response ratio (RR = Fish Plasma / 1%$Cmax_{tot}$) of 1. In comparison, only 3 compounds (14%) with modeled plasma levels from estuarine water concentrations exceeded the threshold value of 1.

All values of predicted plasma concentrations for those fish samples with detectable whole-body tissue concentrations are highlighted in Table S5. These values were generated with the maximum observed tissue concentration among replicates for a given species and site using equation 3. The predicted plasma values were compared to their respective 1%$Cmax_{tot}$ value for determination of the RR_{tissue} value with equation 4 (Figure 2, Table S6). Of the 27 total chemicals with RR_{tissue} values based on whole-body concentrations, 67% exhibited RR values >1 (16 of 24 chemicals) for Chinook and for sculpin, 50% of the RR values exceeded unity (7 of 14 chemicals). Cmax values were not available for many of the hydrophobic CECs (e.g., BPA, perfluorinated compounds, phthalates, nonylphenols, and HBCDDs) occurring at elevated concentrations precluding calculation of the RR value and thus were not included in Tables S4 or S6.

The maximum RR_{tissue} value for each species was compared to the maximum effluent or estuary water value (RR_{water}) for a given site for those chemicals with both values (Tables S4 and S6). This ratio (RR_{tissue}/RR_{water}) was generated for all plasma RR values based on QSAR modeling for all observed whole-body tissue and aqueous concentrations (Table S7). In most cases the ratio was greater than 1, indicating that the RR value was higher for tissue-based ratios compared to those based on water exposure. For RR_{tissue}, 56% (n=25 compounds) of compounds were higher compared to values determined with effluent exposure concentrations, which was similar to that for estuary water based values (73%, n=11). Several of the RR_{tissue}/RR_{water} ratios were between 0.1 and 1 (32%) for effluent based values indicating that RR_{tissue} values were not substantially different compared to those based on effluent (88% RR_{tissue}/RR_{water} >0.1).

Table S8 presents the data for all detected analyte concentrations observed in whole-body fish and the expected whole-body concentrations that were based on equation 1 and the observed water concentration for effluent or

estuarine water. The expected tissue concentration as based on the steady-state BCF was valid for either species and the result was compared to the analytical reporting limit (RL). Chemicals for which a ratio of observed to predicted tissue concentrations could be generated are shown in Figure 3 and most of these ratios ranged between 0.1 and 100. The lowest values were for the nonylphenols, which were likely not at steady state. Of the 92 detected analytes in water or tissue, 14 were either detected in tissue and not water (n=7), or were not analyzed in one of the matrices (n=7) (Table S8). A large percentage of predicted whole-body tissue concentrations were below their respective RL value for tissue, indicating that a chemical may have been present in fish tissue at that predicted level, but below the analytical level for quantification. A total of 47 compounds were predicted based on observed water concentrations that were not detected in fish from the field and only 3 of these were above their respective tissue RL (clarithromycin, PFNA, and simvastatin). For those 44 compounds without observed but predictable tissue concentrations that were below their respective RL, we generated predictions of plasma concentrations based on equation 3 (Table S9). Of those 44 compounds originally modeled from effluent exposure, 13 exhibited predicted RR_{tissue} values that exceeded a value of 1. The notable compounds with elevated RR values in this category include atenolol, atorvastatin, albuterol, dimethylxanthine, hydrochlorothiazide, metoprolol, oxycodone, promethazine, triamterene (Table S9). No RR_{tissue} values generated from estuarine water for non-detected tissue concentrations exceeded a value of 1. As a result of the symmetry for predicting plasma concentrations with observed water or tissue concentrations for this exercise, the RR values in Table S9 predicted from plasma with expected whole-body concentrations are the same as those generated directly with water exposure concentrations (Table S4). The utility of this exercise was to demonstrate that observed water

concentrations may have resulted in bioaccumulated tissue levels but were not detected during analysis and many of those may have exhibited plasma levels exceeding the threshold RR value of 1. This is best shown with tissue concentrations that were below their respective analytical reporting limit.

Based on equation 5 for toxic units using the response ratio, three examples are shown for effluent, estuary water, and tissue generated plasma RR values (Table 1) and includes selective serotonin reuptake inhibitors (SSRIs), β blockers, and calcium channel blockers. Chemicals within each group were assumed to affect the same protein target in fish. For SSRIs the $\sum TU_{rr}$ value was much greater than 1 for both effluent and tissue based values, which was also the case for calcium channel blockers. The ∑TUrr for β blockers was elevated (= 8.6) only for effluent based values and there were no observed tissue concentrations for this class of compounds. When RR_{tissue} values were modeled for those concentrations that may have been present but below the analytical reporting limit (Tables S8 and S9), the predicted $\sum TU_{rr}$ based on expected tissue concentrations was 8.5 (Table 1).

One final analysis is provided to highlight the advantages of using whole-body tissue concentrations. There are few studies reporting fish plasma RL values or method detection limits (MDLs), which are lower than RLs and less reliable metrics for quantitation. We compared our RL values (Meador et al. 2016) for 18 compounds in common with the reported MDLs found in Lazarus et al. (2015) (Table S10). The comparison of fish plasma MDLs and whole-body RLs indicates that on average, plasma MDLs are higher (median ratio = 2.6) indicating greater sensitivity for quantifying whole-body concentrations. Several noteworthy values are seen for fluoxetine, gemfibrozil, and sertraline. Overall, analyzing whole-body fish will likely result in more detected analytes compared to analyses of plasma only.

Discussion

The Fish Plasma Model (FPM) was applied to water exposure and tissue concentrations in fish collected from two wastewater effluent-impacted estuarine sites and we used the therapeutic human dose level in plasma (Cmax) to determine potentially undesirable changes in fish physiology. The present study identifies a large number of PPCPs that exceeded the 1%$Cmax_{tot}$ benchmark value.

Cmax and Safety factors

While Cmax has become the comparative standard, therapeutic effects can occur between this value and the minimum plasma concentration (Cmin) for the lowest therapeutic dose. One noteworthy assumption for the fish plasma model is that human therapeutic effect concentrations are generally considered as adverse physiological levels for fish, which is likely the case for many drugs that can alter behavior, metabolism, endocrine systems, and other physiological functions. These effects may be beneficial for humans, but deleterious for fish that rely on normal lipid metabolism, behavioral cues, and hormone levels to successfully complete their life cycle.

When comparing toxicity values in ecological risk assessment among disparate species, such as humans and fish, safety factors (SF, also known as uncertainty factors and assessment factors) are usually applied. These SF have been discussed by several authors (Chapman et al. 1998; Duke and Taggart 2000; Huggett et al. 2003). Safety factors, or adjustments to Cmax, are usually applied for expected differences in toxicokinetics, pharmacodynamics, inter- and intraspecific differences (e.g., human to fish), internal partitioning, multi-drug interaction, drug sensitivity, temporal sampling bias, and adjustments for converting low- to no- effect concentrations. Route of exposure, which is addressed in the present study should also be considered as a contributor to variance. Although the magnitude of these factors can vary widely, a value of 1000 is often

recommended in regulatory contexts (Huggett et al. 2003; Tanoue et al. 2015). Similarly, an analysis of uncertainty associated with interspecies differences in susceptibility by Brown et al. (2014) supports an application factor of at least 10-fold. Another important aspect of uncertainty concerns the variability around predicted plasma and tissue concentrations. One noteworthy study reported observed water-plasma bioconcentration factors (Pbw) for goldfish ranging from 52 to 113 for gemfibrozil depending on the exposure concentration (Mimeault et al. 2005). If a pH of 7 is assumed for ambient water these values would be 11 to 23 times higher than predicted using log D_{ow} and equation 2. If pH was >7, the difference between observed and predicted BCFs would be even greater. Using 1%$Cmax_{tot}$ as the reference level for effects adds a reasonable level of conservatism to the model for all these abovementioned uncertainties.

The selection of this benchmark value is supported by studies demonstrating effects in fish at observed or predicted plasma concentrations below human therapeutic Cmax values. One study reported various behavioral effects for fluoxetine at 16 ng/L in water exposures (Saaristo et al 2017). The predicted plasma concentration would be in the range of 200 ng/L, which is 0.16% of the Cmax value. Cuklev et al. (2011) reported differential gene expression in rainbow trout with plasma concentrations of diclofenac as low at 1.5% of the Cmax. Another study (Huerta et al. 2016) found effects in fish exposed to oxazepam when plasma concentrations were about one third of the Cmax value. Other studies reported significant effects on growth, fertility, and behavioral effects in fish with observed or predicted plasma concentrations similar to the human Cmax value (Niemuth and Klaper 2015; Valenti et al. 2012).

An important parameter supporting a safety factor for these extrapolations includes species differences in toxicokinetics. A plot of previously reported half-lives for fish and humans shows that for almost all pharmaceuticals

plotted, the fish half-life is substantially longer in duration compared to that for human (Fig. S1), which may lead to an extended time for Cmax and physiological responses. Because most half-life values for drugs in humans are based on plasma concentrations and many of those for fish are whole-body values, a chemical-by-chemical evaluation would be needed to determine persistence across species. Plasma half-life is generally proportional to whole-body half-life and such assumptions are used to predict the terminal volume of distribution (ratio of total quantity of drug in the body / total concentration in plasma) for humans (Berezhkovskiy 2013; Benet and Zia-Amirhosseini 1995), which may support comparability among species. Support for this in fish can be found in Nallani et al. (2016a) who report essentially identical half-lives for plasma and various tissues (muscle, heart, liver, gill, kidney) in catfish exposed separately to verapamil and clozapine. In a comparison of plasma half-lives between fish and humans for 5 different pharmaceuticals, Nallani (2010) reported longer half-lives in fish for each compound ranging from 2 to 7.5 times longer. Additional support comes from the study by Connors et al. (2013) who demonstrated very low liver metabolism for a variety of pharmaceuticals in rainbow trout liver S9 subcellular fractions compared to rates observed using human hepatocytes and microsomes, suggesting the potential for higher bioaccumulation and longer half-lives compared to humans. Approximately 50% of the fish half-lives presented in Table S1 are based on plasma concentrations and therefore directly comparable to human values.

Predicting concentrations

The results of this study show that predictions of plasma concentrations for a variety of PPCPs from effluent concentrations resulted in 37 compounds (54%) exceeding the response ratio (RR) of 1, which is far greater compared with the number of values estimated with estuarine water as the dose metric. Indeed, Figures 1a,b indicate that only 3 chemicals resulted in an

565 RR_{water} >1 when based on estuarine water exposure and BCF modeling with
566 equation 1.

567 Our values for predicting plasma concentrations, which utilized well-
568 established QSARs for whole-body BCF and Pbw were expected to be a
569 reasonable approximation for observed values. One study that measured
570 these parameters directly for fathead minnows exposed to the
571 pharmaceutical diphenhydramine (log_{10} Kow = 3.3) supports this approach
572 (Nichols et al. 2015). Their observed BCF and Pbw values for
573 diphenhydramine at pH 7.7 were 26.6 and 9.2, respectively. Using the
574 ChemAxon modeled D_{ow} for pH 7.7 we obtained a predicted BCF of 22.8 for
575 diphenhydramine and a Pbw value of 10.2. These values resulted in similar
576 BCF/Pbw ratios of 2.5 and 2.9 reported in the present study and by Nichols
577 et al. (2015), respectively.

578 For tissue concentrations predicted with effluent concentrations, the
579 observed-to-predicted tissue residue ratios exceeded parity or were within a
580 factor of 10 for a high percentage of compounds (Figures 3a, b). These data
581 indicate that bioaccumulation modeled with effluent concentrations were
582 similar to observed whole-body concentrations. This may have resulted via
583 several factors, including uptake via multiple routes of exposure and
584 facilitated via membrane associated drug transporters, or exposure to high
585 aqueous concentrations that were expected to be diluted in the estuary.
586 Based on these very high ratios we can conclude that diluted estuary
587 concentrations will underestimate the bioaccumulation potential for fish
588 nearby these outfalls. Therefore predicted plasma concentrations based on
589 observed tissue concentrations or modeled plasma values using effluent
590 concentrations would be a better representation of exposure and lead to a
591 more accurate assessment of risk.

Unfortunately, there are few data quantifying the volume of distribution (Vd) for pharmaceuticals in fish. Using human values to predict tissue-plasma partitioning in fish will likely result in erroneous values. For example, Tanoue et al. (2015) provided tissue-plasma partition coefficients for brain, liver, kidney, and muscle for a variety of pharmaceuticals each exhibiting large variation. The median tissue-specific partition coefficients for sertraline ranged from 11 – 17 for these organs, except for muscle, which exhibited a median value of 1.2. For a one-year-old rainbow trout, these organs comprise a low percentage of total body weight with brain approximately 0.2% and liver around 2% (Denton and Yousef 1976). Based on a mass balance approach, the whole body-plasma partition coefficient (Vd) is expected to be far lower than the human value (20 L/Kg) and closer to the predicted whole-body Vd of 3.2 in the present study. Tanoue et al. (2015) provide similar data for other compounds many of which appear to be similar to our predicted values. Another example is found in Nichols et al. (2015) for diphenhydramine who observed a Vd of 3.0 for the fathead minnow, which is lower than the human Vd ranging from 4.5 – 8 L/Kg. It is important to note that the lower the Vd the higher the plasma concentration in relation to the whole-body concentration. Because plasma concentration is the metric for comparison to human therapeutic levels, accurate predictions are critical for protecting fish against adverse effects.

Exposure

These results highlight the importance of predicting plasma concentrations based on whole-body tissue concentrations for comparison to Cmax values and potential toxic effects. Because the frequency of exceeding an RR value for tissue is closer to that observed for effluent over that for estuary water, the assumption that estuary water should be the preferred exposure metric is not supported. Even though detection limits are much lower for water compared to tissue, plasma concentrations predicted with ambient water

621 concentrations are often insufficient for characterizing actual internal
622 concentrations and therefore would underestimate risk.

623 Analyzing whole-body concentrations and predicting plasma concentrations
624 is a reasonable approach for assessing potentially toxic effects in fish, which
625 provides a more accurate representation of the exposure for fish collected in
626 these estuaries. Measuring plasma directly is also desirable as long as
627 reasonable analytical reporting limits (RL) can be achieved with small
628 volumes. As RL values decline with improved methodology, whole-body
629 concentrations and direct plasma determinations should be the preferred
630 matrices for characterizing exposure and internal concentrations.

631 Many studies have reported relatively high bioaccumulation factors for fish in
632 the field for several CECs supporting the hypothesis that prey species for fish
633 may also contribute to body burden and thus plasma levels. Tanoue et al.
634 (2015) noted that field-based values for BAF_{plasma} (measured plasma /
635 ambient water) were several times higher than those for a predicted water-
636 only BCF_{plasma} value, which were obtained with modeled values (equation 2
637 above) based on ambient water concentrations indicating that
638 bioaccumulation of these compounds is generally higher than predictions
639 based on water-only exposure. Other studies have observed plasma
640 concentrations that are higher than those predicted using basic
641 bioaccumulation equations with log D_{ow} and water concentrations (Du et al.
642 2014; Scott et al. 2016) indicating that aqueous uptake may underestimate
643 the amount bioaccumulated in the environment. The data of Du et al.
644 (2014) indicated that concentrations for many of these pharmaceuticals
645 were substantially higher in invertebrates as compared to fish, sometimes by
646 an order of magnitude. Noteworthy differences included diphenhydramine,
647 sertraline, fluoxetine, desmethylsertraline, celecoxib, and diclofenac.
648 Observations of high accumulation factors in fish prey have also been
649 observed by Meredith-Williams et al. (2012) and Lagesson et al. (2016) who

reported higher BAFs in several invertebrates (snail, insect larvae, isopod) compared to a fish (*Perca fluviatilis*) exposed to diphenhydramine, hydroxyzine, and oxazepam in a pond. Importantly, one study reported a dietary uptake efficiency of 46% for oxazepam from exposed damselfly larvae fed to fish (Brodin et al. 2014). The dietary route of uptake is expected to contribute to overall tissue levels in fish; however, an understanding of the amount assimilated from diet is generally poorly known. Additional efforts to quantify dietary uptake and assimilation that could be used to develop QSAR models would certainly further our understanding for this important route.

The fish plasma model is generally based on water exposure and relates the ambient concentrations to those in plasma, which does not consider additional sources such as dietary exposure. Additionally, no corrections are made for possible uptake from drug transporters that may substantially enhance bioaccumulation (Kostich and Lazorchak 2008). Membrane drug transporters may enhance accumulation of environmental contaminants in fish tissue over that predicted with QSAR modeling and should be considered when assessing uptake. One study (Popovic et al. 2014) examined the organic anion transporting polypeptide Oatp1d1 and demonstrated that a number of CECs including PFOS, nonylphenol, gemfibrozil, and caffeine exhibited high affinity for this transporter whereas others such as PFOA, diethyl phthalate, and bisphenol A were inhibitory. Oatp1d1 orthologs are present in a variety of fish species and are therefore considered important transporters for a range of compounds. It is unknown if membrane transporters are relevant for ambient exposure (water ventilation or dietary uptake) or would interact with these compounds at low environmental concentrations.

Effects Assessment

It may be argued that estuary water is the more appropriate exposure matrix for fish because effluent concentrations are a point source that are rapidly diffused and not likely to result in elevated tissue concentrations. When the response ratio was determined with predicted plasma values from whole-body concentrations for fish collected in our local estuaries, 16 chemicals of the 24 detected (67%) exceeded an RR value of 1 for juvenile Chinook. This observation highlights the importance of using whole-body tissue concentrations over that using estuarine water concentrations in such calculations of risk for fish in WWTP effluent-impacted estuaries. Noteworthy are an additional 13 compounds without detectable whole-body concentrations that may have accumulated in fish, but were below their analytical reporting limits and were predicted to have plasma levels producing an RR_{tissue} value >1. This conclusion regarding the importance of using tissue concentrations is supported by the ratios for RR_{tissue}/RR_{water}, which were always >1 for estuary values and mostly so for the effluent generated values (Table S7) indicating that modeling plasma levels with these aqueous concentrations frequently underestimates potential adverse biological effects. Those RR_{tissue}/RR_{water} ratios > 1 along with others that are only 5 or 10 times lower (0.1 – 0.2x) provides strong evidence that plasma modeled from whole-body tissue concentrations can be substantially higher than expected for free-ranging fish collected several hundred meters from the WWTP outfall pipe.

Mixtures

Selective serotonin reuptake inhibitors (SSRIs) can regulate the activity of several neurotransmitters via modulation of receptors, including the main target 5-hydroxytryptamine transporter (5-HTT). The 5-HTT and serotonergic system in general are similar among mammals and fish (Kreke and Dietrich 2008) implying the potential for effects due to environmental exposure and additivity from multiple SSRIs. Behavioral effects resulting

from low dose exposure to SSRIs have been demonstrated in fish by several authors (Painter et al. 2009; Schultz et al. 2011; Saaristo et al. 2017). Similarly, β adrenergic receptors in fish share structural similarity to those in mammals (Owen et al. 2007) and are potentially susceptible to multiple β blocker drugs (antagonists). A recent review by Godoy et al. (2015) highlights a number of studies with these compounds showing reproductive and metabolic effects in a variety of species. A third example is calcium channel blockers, such as amlodipine, diltiazem, and verapamil. There are few studies testing for effects in fish for these compounds; however, Nallani et al. (2016a) reported an increased condition factor and reduced liver and kidney weights in fish exposed to verapamil. Voltage-gated calcium channels, which are highly conserved among vertebrates and may act by the same mechanism in humans and fish. The implication is that multiple drugs within these classes may act on these receptors potentially leading to additive effects in fish. Given the purported conserved nature of many receptors that modulate drug action between fish and humans, and the potential for additive effects resulting from exposure to these pharmaceutical classes, the toxic unit approach may be a reasonable approach for assessing mixture effects for these and other classes of compounds. As noted by Backhaus (2014) many studies show that dose addition may be predictive for mixtures containing compounds acting by different MeOAs and some of these RR values may be additive, indicating a higher potential for adverse effects compared to the usual evaluation of one chemical at a time.

Conclusions and further considerations

Our study examined 92 CECs that were detected in water or whole-body fish with Cmax values for 70 of these, the majority being pharmaceuticals. Collectively, this represents a small percentage of the more than 4,000 available pharmaceuticals (approximately 1,000 that are unique) and unknown numbers of chemicals used in personal care products. A variety of

CECs and other contaminants may contribute and potentially impair normal physiological function in feral fish. Even if many of those compounds occur at relatively low concentrations, their additive contribution to toxicity could be important. Based on the results presented here, we conclude that a number of compounds occur at concentrations that may result in adverse effects for fish, especially when occurring in mixtures. Furthermore, these data counter the assumption that estuarine water would be the more realistic exposure scenario compared to worst-case exposure from point source effluent and are counter to the popular notion that the "solution to pollution is dilution".

We propose that the best dose metric for assessing adverse effects for these chemicals from WWTP is predicted plasma levels based on observed tissue concentrations or directly quantified plasma concentrations. The combination of generally lower detection limits for solid tissue and higher whole-body concentrations compared to the observed values for plasma indicates that quantifiable detections are more likely for whole-body analysis. Predictions for plasma concentrations based on tissue-plasma partitioning will likely result in a greater number of compounds that can be used to assess potential adverse effects for fish exposed to CECs. Future studies to characterize fish-specific volume of distribution values for CECs will enhance our ability to detect these compounds and provide more accurate assessments based on plasma levels. If limited to water concentrations, it appears that predicting plasma concentrations with effluent-exposure modeling would be the conservative choice for assessing potential toxic effects for feral fish, which is supported by the data presented here. It is clear that additional data focused on accurately characterizing tissue-plasma and water-plasma partitioning for fish is required to advance our use of the FPM, especially with whole-body tissue residues. Continued evaluation of the read-across hypothesis for relating human therapeutic and

765 adverse effects in fish for individual compounds is also critical. Careful
766 consideration of the dose metric and the potential for adverse effects for fish
767 exposed to these contaminants is essential for ensuring that ecosystems are
768 not compromised, especially in light of suboptimal environmental conditions
769 that may be present.

770 **Acknowledgements**

771 This work was supported in part by a grant from the Washington
772 Department of Ecology (G1300089), as well as the National Institute of
773 Environmental Health Sciences Superfund Research Program [P42-
774 ES004696], and the University of Washington Sea Grant Program, pursuant
775 to National Oceanic and Atmospheric Administration Project R/OCEH-8
776 [NA10OAR4170057]. Andrew Yeh was supported in part by the UWNIEHS
777 training grant in Environmental Pathology/Toxicology (T32ES007032). The
778 authors gratefully acknowledge insightful review comments from Dr. Bowen
779 Du (University of Washington) and 3 anonymous reviewers. We also
780 appreciate the technical assistance in the laboratory and field provided by
781 University of Washington personnel Richard Ramsden, Christopher Monson,
782 Stuart Munsch, Yasushi Shibata, Anna Bute, Chase R. Williams, Monica
783 Chang, Nancy Huizar, Margaret G. Mills, Jeff Cordell, Michael Caputo, and
784 Jason Toft. Additional thanks to Georgina Brooks, Candice Navaroli, Cynthia
785 Tomey, Sean Campbell, Richard Grace, and additional laboratory staff at
786 AXYS Analytical Services, Ltd, for their excellent analytical skills.

787 **Citations**

788 Arnot, J.A., Gobas, F.A., 2006. A review of bioconcentration factor (BCF) and
789 bioaccumulation factor (BAF) assessments for organic chemicals in aquatic
790 organisms. Environ. Rev. 14, 257–297. doi:10.1139/a06-005

791 Backhaus, T., 2014. Medicines, shaken and stirred: a critical review on the
792 ecotoxicology of pharmaceutical mixtures. Philos. Trans. R. Soc. B Biol. Sci.
793 369. doi:10.1098/rstb.2013.0585

794 Batt, A.L., Kincaid, T.M., Kostich, M.S., Lazorchak, J.M., Olsen, A.R., 2016.
795 Evaluating the extent of pharmaceuticals in surface waters of the United
796 States using a National-scale Rivers and Streams Assessment survey.
797 Environ. Toxicol. Chem. 35, 874–881. doi:10.1002/etc.3161

798 Benet, L.Z., Zia-Amirhosseini, P., 1995. Basic principles of pharmacokinetics.
799 Toxicol. Pathol. 23, 115–23. doi:10.1177/019262339502300203

800 Berezhkovskiy, L.M., 2013. Prediction of drug terminal half-life and terminal
801 volume of distribution after intravenous dosing based on drug clearance,
802 steady-state volume of distribution, and physiological parameters of the
803 body. J. Pharm. Sci. 102, 761–771. doi:10.1002/jps

804 Bliss, C., 1939. The toxicity of poisons applied jointly. Ann. Appl. Biol. 26,
805 585–615. doi:10.1111/j.1744-7348.1939.tb06990.x

806 Brodin, T., Piovano, S., Fick, J., Klaminder, J., Heynen, M., Jonsson, M.
807 2014. Ecological effects of pharmaceuticals in aquatic systems—impacts
808 through behavioural alterations. Phil. Trans. R. Soc. B 369: 20130580.
809 Doi:10.1098/rstb.2013.0580

810 Brown, A.R., Gunnarsson, L., Kristiansson, E., Tyler, C.R., 2014. Assessing
811 variation in the potential susceptibility of fish to pharmaceuticals,
812 considering evolutionary differences in their physiology and ecology. Philos.
813 Trans. R. Soc. B Biol. Sci. 369, 20130576–20130576.
814 doi:10.1098/rstb.2013.0576

815 Brown, J.N., Paxéus, N., Förlin, L., Larsson, D.G.J., 2007. Variations in
816 bioconcentration of human pharmaceuticals from sewage effluents into fish
817 blood plasma. Environ. Toxicol. Pharmacol. 24, 267–274.
818 doi:10.1016/j.etap.2007.06.005

819 Chapman, P.M., Fairbrother, A., Brown, D., 1998. A critical evaluation of
820 safety (uncertainty) factors for ecological risk assessment. Environ. Toxicol.
821 Chem. 17, 99–108.

822 ChemAxon, 2016. Instant JChem, version 16.5.23.0.
823 (http://www.chemaxon.com).

824 Clark, T.D., Sandblom, E., Cox, G.K., Hinch, S.G., Farrell, A.P., 2008.
825 Circulatory limits to oxygen supply during an acute temperature increase in

826 the Chinook salmon (Oncorhynchus tshawytscha). Am. J. Physiol. Regul.
827 Integr. Comp. Physiol. 295, R1631–R1639. doi:10.1152/ajpregu.90461.2008

828 Cuklev, F., Kristiansson, E., Fick, J., Asker, N., Förlin, L., Larsson, D.G.,
829 2011. Diclofenac in fish: blood plasma levels similar to human therapeutic
830 levels affect global hepatic gene expression. Environ. Toxicol. Chem. 30,
831 2126-2134.

832 Connors, K.A., Du, B., Fitzsimmons, P.N., Hoffman, A.D., Chambliss, C.K.,
833 Nichols, J.W., Brooks, B.W., 2013. Comparative pharmaceutical metabolism
834 by rainbow trout (*Oncorhynchus mykiss*) liver S9 fractions. Environ. Toxicol.
835 Chem. 32, 1810–1818. doi:10.1002/etc.2240

836 Daughton, C.G., 2002. Environmental stewardship and drugs as pollutants.
837 Lancet 360, 1035-1036.

838 Denton, J.E., Yousef, M.K., 1976. Body composition and organ weights of
839 rainbow trout (*Salmo gairdneri*). Jour Fish Biol 8, 489-499.

840 Du, B., Haddad, S.P., Luek, A., Scott, W.C., Saari, G.N., Kristofco, L.A.,
841 Connors, K.A., Rash, C., Rasmussen, J.B., Chambliss, C.K., Brooks, B.W.,
842 2014. Bioaccumulation and trophic dilution of human pharmaceuticals across
843 trophic positions of an effluent-dependent wadeable stream. Philos. Trans. R.
844 Soc. B Biol. Sci. 369, 20140058. doi:10.1098/rstb.2014.0058

845 Duke, L.D., Taggart, M., 2000. Uncertainty Factors In Screening Ecological
846 Risk Assessments. Environ. Toxicol. Chem. 19, 1668–1680.

847 Fick, J., Lindberg, R.H., Parkkonen, J., Arvidsson, B., Tysklind, M., Joakim
848 Larsson, D.G., 2010a. Therapeutic levels of levonorgestrel detected in blood
849 plasma of fish: results from screening rainbow trout exposed to treated
850 sewage effluents. Environ. Sci. Technol. 44, 2661–2666.
851 doi:10.1021/es903440m

852 Fick, J., Lindberg, R.H., Tysklind, M., Larsson, D.G.J., 2010b. Predicted
853 critical environmental concentrations for 500 pharmaceuticals. Regul.
854 Toxicol. Pharmacol. 58, 516–523. doi:10.1016/j.yrtph.2010.08.025

855 Finizio, A., Vighi, M., Sandroni, D., 1997. Determination of N-octanol/water
856 parition coefficient (Kow) of pesticide critical review and comparison of
857 methods. Chemosphere 34, 131–161.

858 Fitzsimmons, P.N., Fernandez, J.D., Hoffman, A.D., Butterworth, B.C.,
859 Nichols, J.W., 2001. Branchial elimination of superhydrophobic organic
860 compounds by rainbow trout (Oncorhynchus mykiss). Aquat. Toxicol. 55,
861 23–34. doi:10.1016/S0166-445X(01)00174-6

862 Fu, W., Franco, A., Trapp, S., 2009. Methods for estimating the
863 bioconcentration factor of ionizable organic chemicals. Env. Toxicol Chem 28,
864 1372–1379. doi:08-233 [pii]\r10.1897/08-233.1

865 Garcia, S.N., Foster, M., Constantine, L.A., Huggett, D.B., 2012. Field and
866 laboratory fish tissue accumulation of the anti-convulsant drug
867 carbamazepine. Ecotoxicol. Environ. Saf. 84, 207–211.
868 doi:10.1016/j.ecoenv.2012.07.013

869 Godoy, A.A., Kummrow, F., Pamplin, P.A.Z., 2015. Occurrence,
870 ecotoxicological effects and risk assessment of antihypertensive
871 pharmaceutical residues in the aquatic environment - A review.
872 Chemosphere 138, 281–291. doi:10.1016/j.chemosphere.2015.06.024

873 Gunnarsson, L., Jauhiainen, A., Kristiansson, E., Nerman, O., Larsson,
874 D.G.J., 2008. Evolutionary conservation of human drug targets in organisms
875 used for environmental risk assessments. Environ. Sci. Technol. 42, 5807–
876 5813. doi:10.1021/es8005173

877 Huerta, B., Margiotta-Casaluci, L., Rodriguez-Mozaz, S., Scholze, M., Winter,
878 M.J., Barceló, D., Sumpter, J.P. 2016. Anti-anxiety drugs and fish behavior:
879 establishing the link between internal concentrations of oxazepam and
880 behavioral effects. Environ. Toxicol. Chem. 35, 2782–2790

881 Huggett, D.B., Cook, J.C., Ericson, J.F., Williams, R.T., 2003. Theoretical
882 model for prioritizing potential impacts of human pharmaceuticals to fish.
883 Hum. Ecol. risk Assess. 9, 1789–1799. doi:10.1080/10807030390260498

884 Kostich, M.S., Batt, A.L., Lazorchak, J.M., 2014. Concentrations of prioritized
885 pharmaceuticals in effluents from 50 large wastewater treatment plants in
886 the US and implications for risk estimation. Environ. Pollut. 184, 354–359.
887 doi:10.1016/j.envpol.2013.09.013

888 Kostich, M.S., Lazorchak, J.M., 2008. Risks to aquatic organisms posed by
889 human pharmaceutical use. Sci. Total Environ. 389, 329–339.
890 doi:10.1016/j.scitotenv.2007.09.008

891 Kreke, N., Dietrich, D.R., 2008. Physiological endpoints for potential SSRI
892 interactions in fish. Crit. Rev. Toxicol. 38, 215–247.
893 doi:10.1080/10408440801891057

894 Lagesson, A., Fahlman, J., Brodin, T., Fick, J., Jonsson, M., Byström, P.,
895 Klaminder, J., 2016. Bioaccumulation of five pharmaceuticals at multiple
896 trophic levels in an aquatic food web - Insights from a field experiment. Sci.
897 Total Environ. 568, 208–215. doi:10.1016/j.scitotenv.2016.05.206

898 Lazarus, R.S., Rattner, B.A., Brooks, B.W., Du, B., McGowan, P.C., Blazer,
899 V.S., Ottinger, M.A., 2015. Exposure and food web transfer of
900 pharmaceuticals in ospreys (*Pandion haliaetus*): predictive model and
901 empirical data. Integr. Environ. Assess. Manag. 11, 118-129. doi:
902 10.1002/ieam.1570.

903 Manallack, D.T., 2007. The pK(a) Distribution of Drugs: Application to Drug
904 Discovery. Perspect. Medicin. Chem. 1, 25–38. doi:10.1201/b13128-7

905 McCarty, L.S., Landrum, P.F., Luoma, S.N., Meador, J.P., Merten, A.A.,
906 Shephard, B.K., van Wezel, A.P., 2011. Advancing environmental toxicology
907 through chemical dosimetry: External exposures versus tissue residues.
908 Integr. Environ. Assess. Manag. 7, 7–27. doi:10.1002/ieam.98

909 Meador, J., Stein, J., Reichert, W., Varanasi, U., 1995. Bioaccumulation of
910 polycyclic aromatic hydrocarbons by marine organisms. Rev. Environ.
911 Contam. Toxicol. 143, 79–165. doi:10.1007/978-1-4612-2542-3_4

912 Meador, J.P., 1997. Comparative toxicokinetics of tributyltin in five marine
913 species and its utility in predicting bioaccumulation and acute toxicity.
914 Aquat. Toxicol. 37, 307–326. doi:10.1016/S0166-445X(96)00827-2

915 Meador, J.P., 2014. Do chemically contaminated river estuaries in Puget
916 Sound (Washington, USA) affect the survival rate of hatchery-reared
917 Chinook salmon? Can. J. Fish. Aquat. Sci. 71, 162–180. doi:10.1139/cjfas-
918 2013-0130

919 Meador, J.P., McCarty, L.S., Escher, B.I., Adams, W.J., 2008. The tissue-
920 residue approach for toxicity assessment: concepts, issues, application, and
921 recommendations. J. Environ. Monit. 10, 1486–1498. doi:10.1039/b814041n

922 Meador, J.P., Yeh, A., Young, G., Gallagher, E.P., 2016. Contaminants of
923 emerging concern in a large temperate estuary. Environ. Pollut. 213, 254–
924 267. doi:10.1016/j.envpol.2016.01.088

925 Meredith-Williams, M., Carter, L.J., Fussell, R., Raffaelli, D., Ashauer, R.,
926 Boxall, A.B. a, 2012. Uptake and depuration of pharmaceuticals in aquatic
927 invertebrates. Environ. Pollut. 165, 250–258.
928 doi:10.1016/j.envpol.2011.11.029

929 Mimeault, C., Woodhouse, A.J., Miao, X.S., Metcalfe, C.D., Moon, T.W.,
930 Trudeau, V.L., 2005. The human lipid regulator, gemfibrozil bioconcentrates
931 and reduces testosterone in the goldfish, *Carassius auratus*. Aquat. Toxicol.
932 73, 44–54. doi:10.1016/j.aquatox.2005.01.009

933 Moffat, A.C., Osselton, M.D., Widdop, B. (Eds.), 2011. Clarke's Analysis of
934 Drugs and Poisons, Fourth edition. Pharmaceutical Press, London. 2609 pp.

935 Nakamura, Y., Yamamoto, H., Sekizawa, J., Kondo, T., Hirai, N., Tatarazako,
936 N., 2008. The effects of pH on fluoxetine in Japanese medaka (*Oryzias*
937 *latipes*): Acute toxicity in fish larvae and bioaccumulation in juvenile fish.
938 Chemosphere 70, 865–873. doi:10.1016/j.chemosphere.2007.06.089

939 Nallani, G.C., 2010. Determination of bioconcentration potential of selected
940 pharmaceuticals in fathead minnow, *Pimephales promelas*, and channel
941 catfish, *Ictalurus punctatus*. Dissertation, University of North Texas.
942 (digital.library.unt.edu/ark:/67531/metadc33189/: accessed May 17, 2017),
943 University of North Texas Libraries, Digital Library, digital.library.unt.edu.
944 233 p.

945 Nallani, G.C., Edziyie, R.E., Paulos, P.M., Venables, B.J., Constantine, L.A.,
946 Huggett, D.B., 2016a. Bioconcentration of two basic pharmaceuticals,
947 verapamil and clozapine, in fish. Environ. Toxicol. Chem. 35, 593–603.
948 doi:10.1002/etc.3244

949 Nallani, G.C., Venables, B., Constantine, L., Huggett, D., 2016b. Comparison
950 of measured and predicted bioconcentration estimates of pharmaceuticals in
951 fish plasma and prediction of chronic risk. Bull. Environ. Contam. Toxicol. 96,
952 580–584. doi:10.1007/s00128-016-1782-y

953 Niemuth, N.J., Klaper, R.D., 2015. Emerging wastewater contaminant
954 metformin causes intersex and reduced fecundity in fish. Chemosphere 135,
955 38–45. doi:10.1016/j.chemosphere.2015.03.060

956 Nichols, J.W., McKim, J.M., Lien, G.J., Hoffman, A.D., Bertelsen, S.L., 1991.
957 Physiologically based toxicokinetic modeling of three waterborne
958 chloroethanes in rainbow trout (*Oncorhynchus mykiss*). Toxicol. Appl.
959 Pharmacol. 110, 374–389. doi:10.1016/0041-008X(91)90040-L

960 Nichols, J.W., Du, B., Berninger, J.P., Connors, K.A., Chambliss, C.K.,
961 Erickson, R.J., Hoffman, A.D., Brooks, B.W., 2015. Observed and modeled
962 effects of pH on bioconcentration of diphenhydramine, a weakly basic
963 pharmaceutical, in fathead minnow. Environ. Toxicol. Chem. 34, 1425–1435.
964 DOI: 10.1002/etc.2948

965 Owen, S.F., Giltrow, E., Huggett, D.B., Hutchinson, T.H., Saye, J.A., Winter,
966 M.J., Sumpter, J.P., 2007. Comparative physiology, pharmacology and
967 toxicology of β-blockers: mammals versus fish. Aquat. Toxicol. 82, 145–162.
968 doi:10.1016/j.aquatox.2007.02.007

969 Painter, M.M., Buerkley, M. a, Julius, M.L., Vajda, A.M., Norris, D.O., Barber,
970 L.B., Furlong, E.T., Schultz, M.M., Schoenfuss, H.L., 2009. Antidepressants
971 at environmentally relevant concentrations affect predator avoidance
972 behavior of larval fathead minnows (*Pimephales promelas*). Environ. Toxicol.
973 Chem. 28, 2677–2684. doi:10.1897/08-556.1

974 Popovic, M., Zaja, R., Fent, K., Smital, T., 2014. Interaction of
975 environmental contaminants with zebrafish organic anion transporting
976 polypeptide, Oatp1d1 (Slco1d1). Toxicol. Appl. Pharmacol. 280, 149–158.
977 doi:10.1016/j.taap.2014.07.015

978 Rand-Weaver, M., Margiotta-Casaluci, L., Patel, A., Panter, G.H., Owen, S.F.,
979 Sumpter, J.P., 2013. The read-across hypothesis and environmental risk
980 assessment of pharmaceuticals. Environ. Sci. Technol. 47, 11384–95.
981 doi:10.1021/es402065a

982 Rendal, C., Kusk, K.O., Trapp, S., 2011. Optimal choice of pH for toxicity
983 and bioaccumulation studies of ionizing organic chemicals. Environ. Toxicol.
984 Chem. 30, 2395–2406. doi:10.1002/etc.641

985 Saaristo, M., McLennan, A., Johnstone, C.P., Clarke, B.O., Wong, B.B.M.,
986 2017. Impacts of the antidepressant fluoxetine on the anti-predator
987 behaviours of wild guppies (*Poecilia reticulata*). Aquat. Toxicol. 183, 38–45.
988 doi:10.1016/j.aquatox.2016.12.007

989 Schreiber, R., Gündel, U., Franz, S., Küster, A., Rechenberg, B.,
990 Altenburger, R., 2011. Using the fish plasma model for comparative hazard
991 identification for pharmaceuticals in the environment by extrapolation from
992 human therapeutic data. Regul. Toxicol. Pharmacol. 61, 261–275.
993 doi:10.1016/j.yrtph.2011.08.006

994 Schultz, M.M., Painter, M.M., Bartell, S.E., Logue, A., Furlong, E.T., Werner,
995 S.L., Schoenfuss, H.L., 2011. Selective uptake and biological consequences
996 of environmentally relevant antidepressant pharmaceutical exposures on
997 male fathead minnows. Aquat. Toxicol. 104, 38–47.
998 doi:10.1016/j.aquatox.2011.03.011

999 Schulz, M., Iwersen-Bergmann, S., Andresen, H., Schmoldt, A., 2012.
1000 Therapeutic and toxic blood concentrations of nearly 1,000 drugs and other
1001 xenobiotics. Crit. Care 16, R136. doi:10.1186/cc11441

1002 Scott, W.C., Du, B., Haddad, S.P., Breed, C.S., Saari, G.N., Kelly, M.,
1003 Broach, L., Chambliss, C.K., Brooks, B.W., 2016. Predicted and observed
1004 therapeutic dose exceedances of ionizable pharmaceuticals in fish plasma
1005 from urban coastal systems. Environ. Toxicol. Chem. 35, 983–995.
1006 doi:10.1002/etc.3236

1007 Tanoue, R., Nomiyama, K., Nakamura, H., Kim, J.W., Isobe, T., Shinohara,
1008 R., Kunisue, T., Tanabe, S., 2015. Uptake and tissue distribution of
1009 pharmaceuticals and personal care products in wild fish from treated-
1010 wastewater-impacted streams. Environ. Sci. Technol. 49, 11649–11658.
1011 doi:10.1021/acs.est.5b02478

1012 Turner, A., Williamson, I., 2005. On the relationship between Dow and Kow
1013 in natural waters. Environ. Sci. Technol. 39, 8719–8727.
1014 doi:10.1021/es050135a

1015 Valenti, T.W. Jr., Gould, G.G., Berninger, J.P., Connors, K.A., Keele, N.B.,
1016 Prosser, K.N., Brooks, B.W. 2012. Human therapeutic plasma levels of the
1017 selective serotonin reuptake inhibitor (SSRI) sertraline decrease serotonin
1018 reuptake transporter binding and shelter-seeking behavior in adult male

fathead minnows. Environ. Sci. Technol. 46, 2427−2435
doi:10.1021/es204164b

Veith, G.D., DeFoe, D.L., Bergstedt, B. V., 1979. Measuring and estimating the bioconcentration factor of chemicals in fish. J. Fish. Res. Board Canada 36, 1040–1048. doi:10.1139/f79-146

Winter, M.J., Lillicrap, A.D., Caunter, J.E., Schaffner, C., Alder, A.C., Ramil, M., Ternes, T.A., Giltrow, E., Sumpter, J.P., Hutchinson, T.H., 2008. Defining the chronic impacts of atenolol on embryo-larval development and reproduction in the fathead minnow (*Pimephales promelas*). Aquat. Toxicol. 86, 361–369. doi:10.1016/j.aquatox.2007.11.017

Figure legends

Figure 1. Response Ratio based on plasma concentrations predicted with maximum effluent or estuary water exposure concentrations (equations 2 and 4). Values above 1 indicate a greater potential for adverse effects.

Figure 2. Response Ratio based on plasma concentrations predicted with observed whole-body tissue concentrations for juvenile Chinook salmon and staghorn sculpin as determined with equations 3 and 4. Values above 1 indicate an increasing potential for adverse effects. Values for a given chemical and matrix were determined with the maximum replicate concentration.

Figure 3. Ratios of observed to predicted tissue concentrations shown in Table S8 for juvenile Chinook salmon. Predicted whole-body concentrations based on equation 1 and effluent or estuary water concentrations for a given impacted site. Maximum replicate values used for a given site (observed tissue only). A. Puyallup River estuary, B. Sinclair Inlet.

Table 1. Sum RR represents the sum of toxic units for compounds with a similar mechanism of action.

MoA	Receptor	Chemicals	Effluent		Estuary		Tiss salmon		Tiss sculpin	
			RR	Sum RR	RR	Sum RR	RR	Sum RR	RR	Sum RR
SSRI	5-Hydroxy tryptamine transporter (SERT)	Amitriptyline	1.5	66		0.005	0.5	59		1.1
		Fluoxetine	0.5				2		0.7	
		Sertraline	61				54			
		Paroxetine	1.2							
		Amitriptyline 10-OH	1.6		0.005		0.24		0.35	
		Norfluoxetine	0.2				2			
Beta blockers	Beta adrenergic antagonists	Atenolol	4.1	8.6	0.04	0.04	**4.1**	**8.5**		
		Metaprolol	4.1				**4.1**			
		Propanolol	0.4				**0.26**			
Calcium channel blockers	L-type voltage-gated calcium channel	Amlodipine	1.5	19	0.2	0.2	40.2	56.5	0.4	2.8
		Diltiazem	0.3				2.5			
		Diltiazem (desmethyl)	12.6				8.2			
		Nifedipine	1.4							
		Norverapamil	1.4				5.2		2.2	
		Verapamil	1.8				0.4		0.2	

Maximum value for each sample matrix (effluent, estuary water, and whole-body tissue). RR is the response ratio (see equation 4). Bold values taken from Table S9 for compounds without observed tissue concentrations, but based on predicted concentrations.

Figure 1a

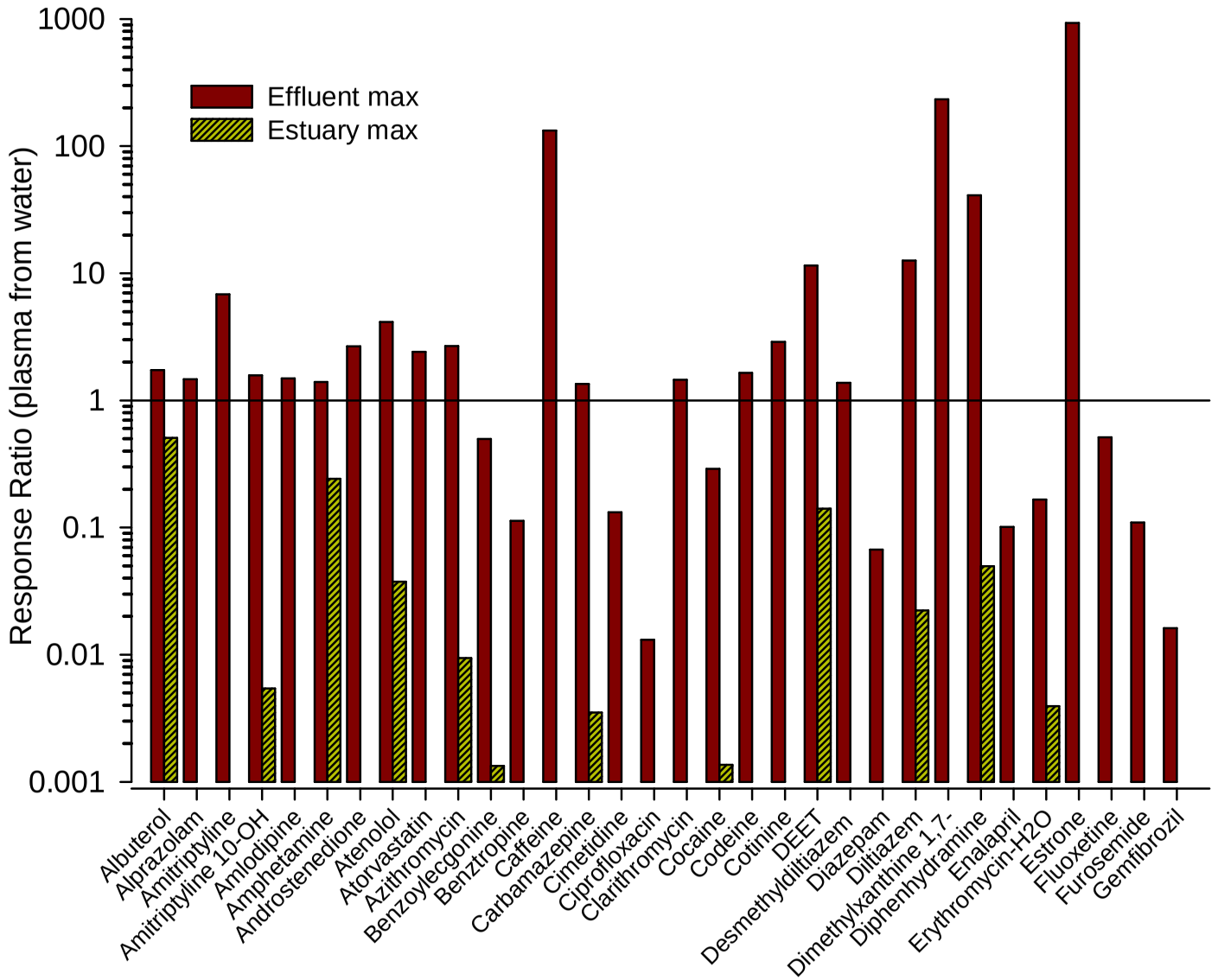

Effluent max
Estuary max
Response Ratio (plasma from water)
1000
100
10
1
0.1
0.01
0.001
Albuterol
Alprazolam
Amitriptyline
Amitriptyline 10-OH
Amlodipine
Amphetamine
Androstenedione
Atenolol
Atorvastatin
Azithromycin
Benzoylecgonine
Benztropine
Caffeine
Carbamazepine
Cimetidine
Ciprofloxacin
Clarithromycin
Cocaine
Codeine
Cotinine
DEET
Desmethyldiltiazem
Diazepam
Diltiazem
Dimethylxanthine 1,7-
Diphenhydramine
Enalapril
Erythromycin-H2O
Estrone
Fluoxetine
Furosemide
Gemfibrozil

Figure 1b

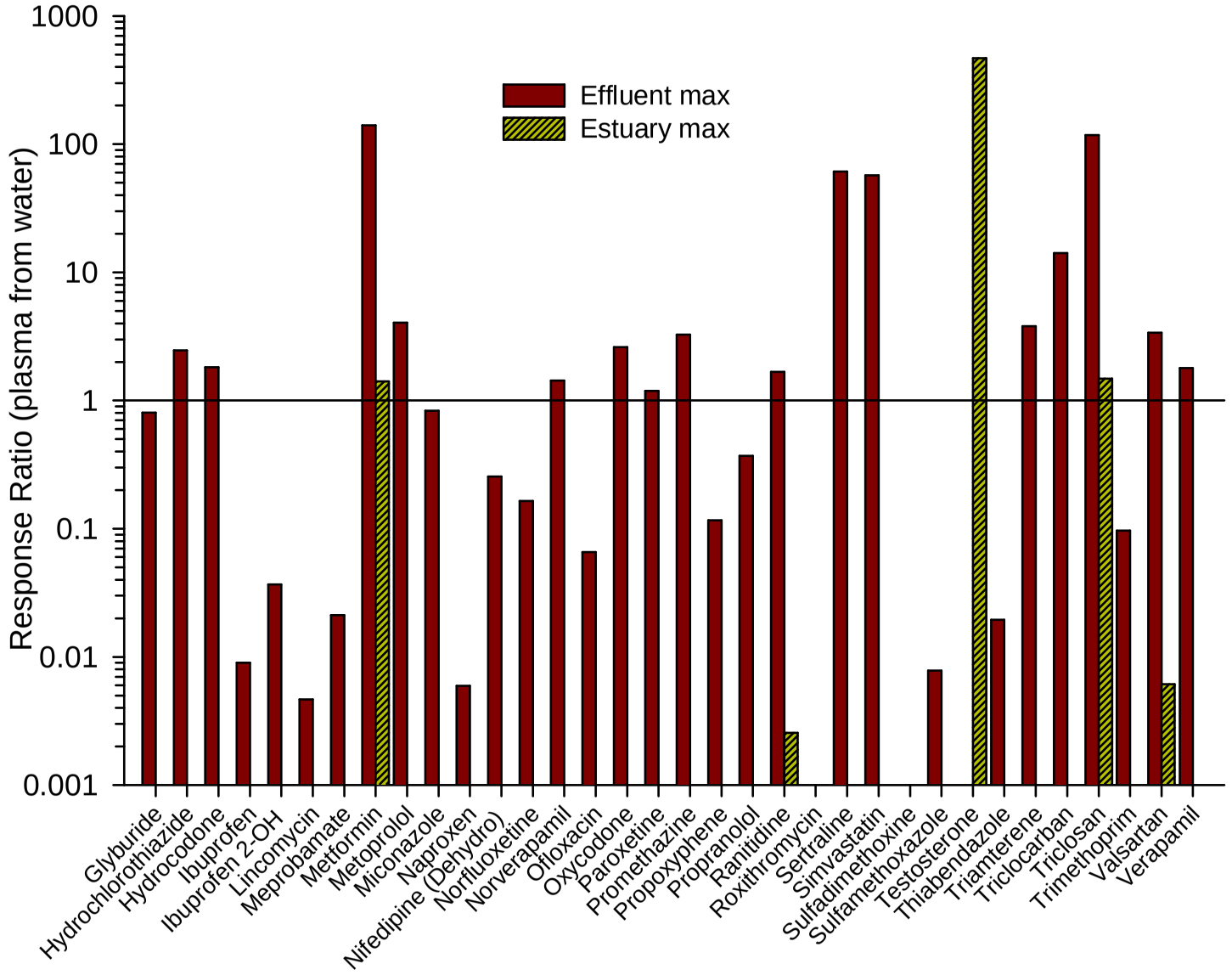

Effluent max
Estuary max
Response Ratio (plasma from water)
1000
100
10
1
0.1
0.01
0.001
Glyburide
Hydrochlorothiazide
Hydrocodone
Ibuprofen
Ibuprofen 2-OH
Lincomycin
Meprobamate
Metformin
Metoprolol
Miconazole
Naproxen
Nifedipine (Dehydro)
Norfluoxetine
Norverapamil
Ofloxacin
Oxycodone
Paroxetine
Promethazine
Propoxyphene
Propranolol
Ranitidine
Roxithromycin
Sertraline
Simvastatin
Sulfadimethoxine
Sulfamethoxazole
Testosterone
Thiabendazole
Triamterene
Triclocarban
Triclosan
Trimethoprim
Valsartan
Verapamil

Figure 2.

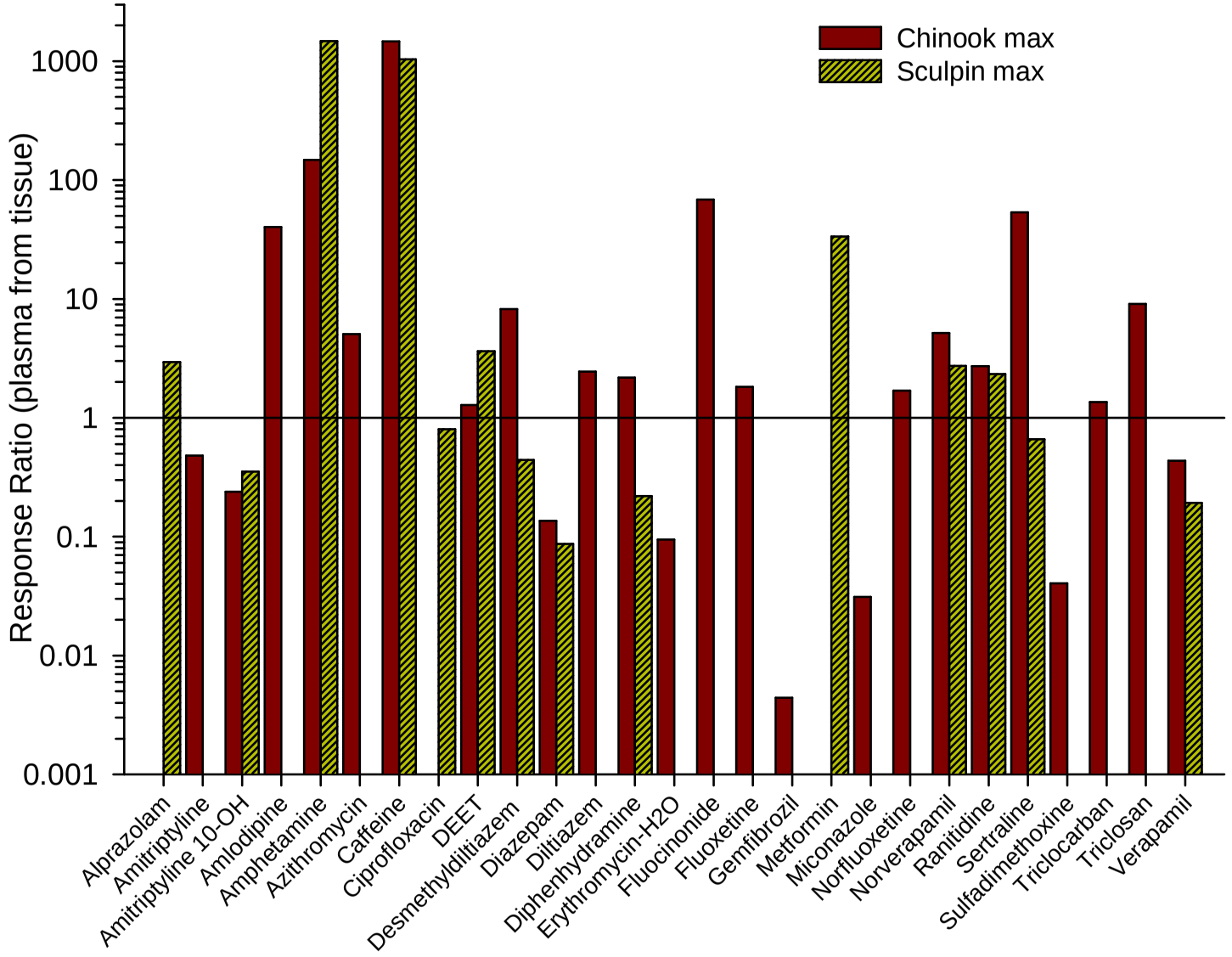

Chinook max
Sculpin max
Response Ratio (plasma from tissue)
1000
100
10
1
0.1
0.01
0.001
Alprazolam
Amitriptyline
Amitriptyline 10-OH
Amlodipine
Amphetamine
Azithromycin
Caffeine
Ciprofloxacin
DEET
Desmethyldiltiazem
Diazepam
Diltiazem
Diphenhydramine
Erythromycin-H2O
Fluocinonide
Fluoxetine
Gemfibrozil
Metformin
Miconazole
Norfluoxetine
Norverapamil
Ranitidine
Sertraline
Sulfadimethoxine
Triclocarban
Triclosan
Verapamil

Figure 3a.

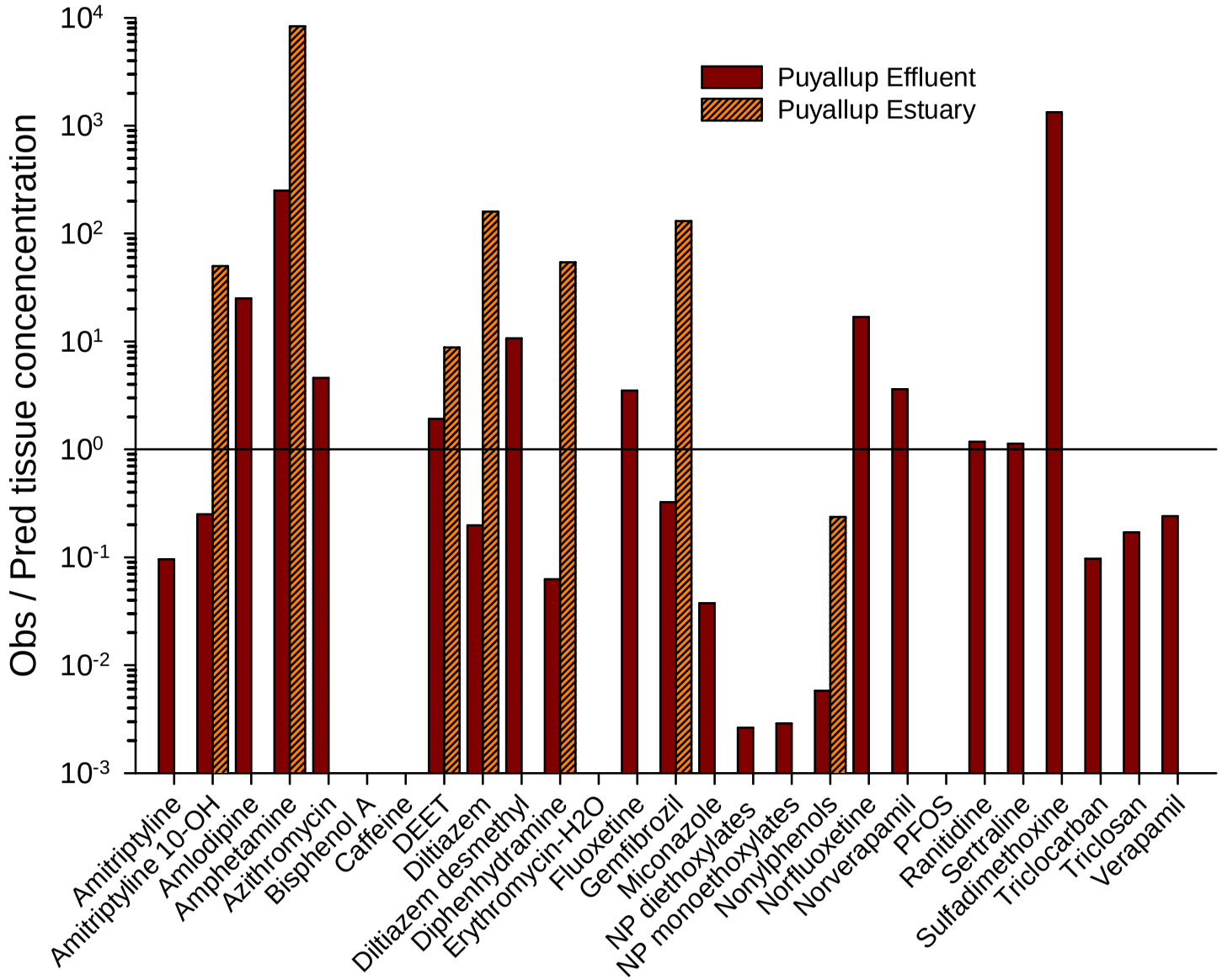
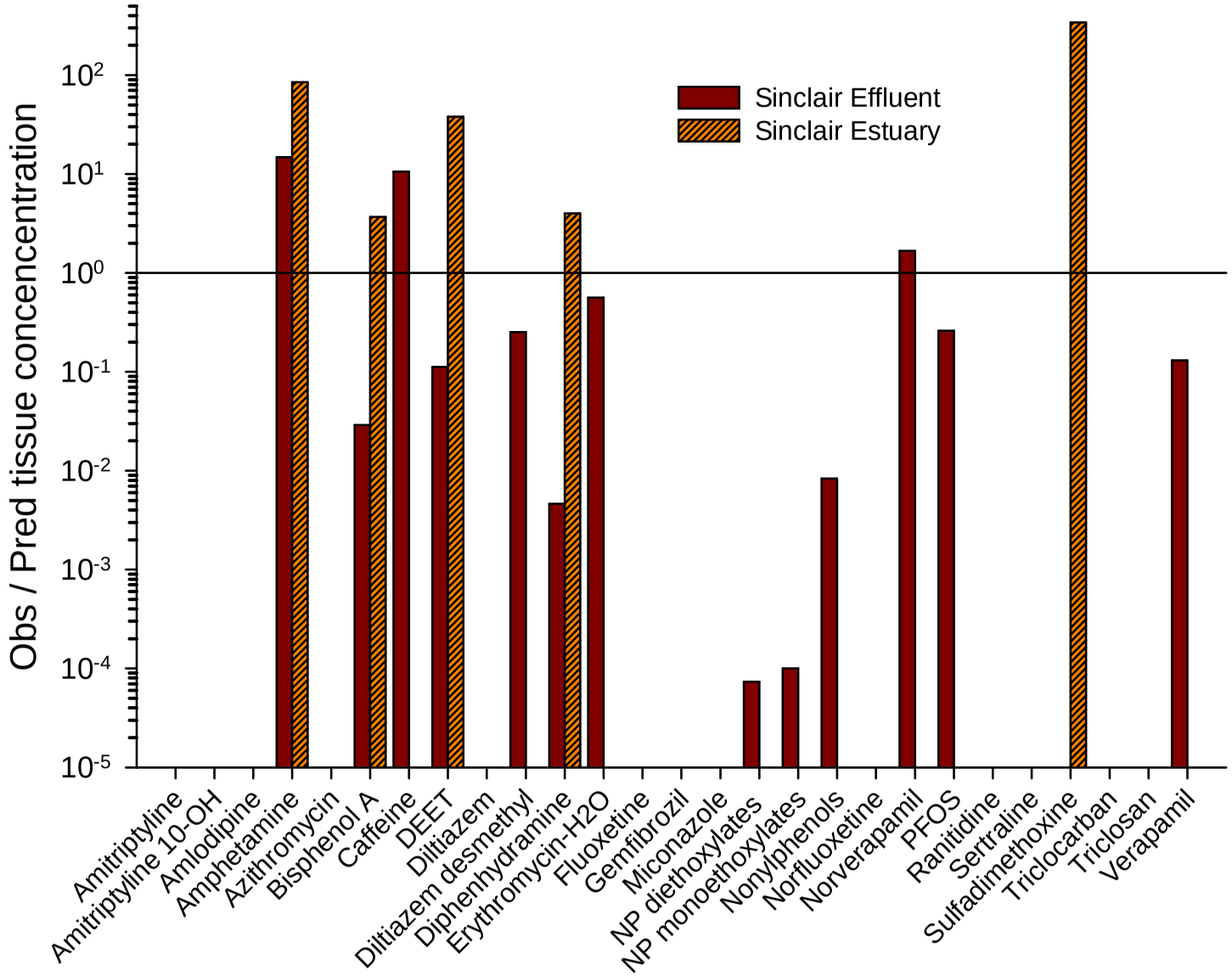

Obs / Pred tissue concencentration
10^4
10^3
10^2
10^1
10^0
10^{-1}
10^{-2}
10^{-3}
Puyallup Effluent
Puyallup Estuary
Amitriptyline
Amitriptyline 10-OH
Amlodipine
Amphetamine
Azithromycin
Bisphenol A
Caffeine
DEET
Diltiazem
Diltiazem desmethyl
Diphenhydramine
Erythromycin-H2O
Fluoxetine
Gemfibrozil
Miconazole
NP diethoxylates
NP monoethoxylates
Nonylphenols
Norfluoxetine
Norverapamil
PFOS
Ranitidine
Sertraline
Sulfadimethoxine
Triclocarban
Triclosan
Verapamil

Figure 3b.


Obs / Pred tissue concencentration
10^{2}
10^{1}
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
Sinclair Effluent
Sinclair Estuary
Amitriptyline
Amitriptyline 10-OH
Amlodipine
Amphetamine
Azithromycin
Bisphenol A
Caffeine
DEET
Diltiazem
Diltiazem desmethyl
Diphenhydramine
Erythromycin-H2O
Fluoxetine
Gemfibrozil
Miconazole
NP diethoxylates
NP monoethoxylates
Nonylphenols
Norfluoxetine
Norverapamil
PFOS
Ranitidine
Sertraline
Sulfadimethoxine
Triclocarban
Triclosan
Verapamil

Highlights for Meador et al.

- Fish Plasma Model (FPM) to assess risk based on water and fish tissue concentrations.
- Plasma levels predicted with receiving water concentrations underestimate exposure for feral fish
- Predicted plasma concentrations from fish tissue captures exposure from all sources
- Response ratio is a useful metric for assessing risk and mixture toxicity