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Uptake and Tissue Distribution of Pharmaceuticals and Personal Care Products in Wild Fish from Treated-Wastewater-Impacted Streams

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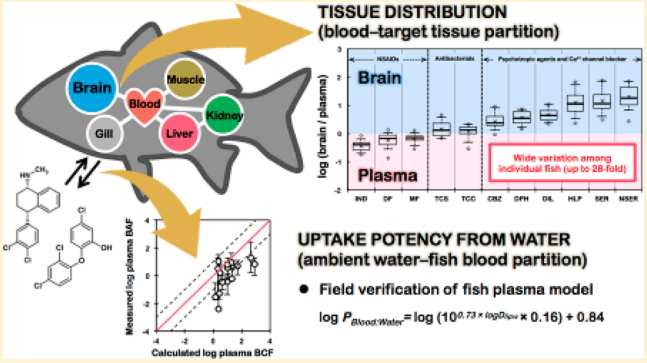
Supporting Information

ABSTRACT: A fish plasma model (FPM) has been proposed as a screening technique to prioritize potential hazardous pharmaceuticals to wild fish. However, this approach does not account for inter- or intraspecies variability of pharmacokinetic and pharmacodynamic parameters. The present study elucidated the uptake potency (from ambient water), tissue distribution, and biological risk of 20 pharmaceutical and personal care product (PPCP) residues in wild cyprinoid fish inhabiting treated-wastewater-impacted streams. In order to clarify the uncertainty of the FPM for PPCPs, we compared the plasma bioaccumulation factor in the field (BAF_{plasma} = measured fish plasma/ambient water concentration ratio) with the predicted plasma bioconcentration factor (BCF_{plasma} = fish plasma predicted by use of theoretical partition coefficients/ambient water concentration ratio) in the actual environment. As a result, the measured maximum BAF_{plasma} of inflammatory agents was up to 17 times higher than theoretical BCF_{plasma} values, leading to possible underestimation of toxicological risk on wild fish. When the tissue−blood partition coefficients (tissue/blood concentration ratios) of PPCPs were estimated, higher transportability into tissues, especially the brain, was found for psychotropic agents, but brain/plasma ratios widely varied among individual fish (up to 28-fold). In the present study, we provide a valuable data set on the intraspecies variability of PPCP pharmacokinetics, and our results emphasize the importance of determining PPCP concentrations in possible target organs as well as in the blood to assess the risk of PPCPs on wild fish.



INTRODUCTION

Pharmaceuticals and personal care products (PPCPs) are considered "pseudo-persistent" contaminants because of their continuous loading into the aquatic environment through effluent discharge from wastewater treatment plants.[1] As a result, aquatic organisms are chronically exposed throughout the life cycle to a large number of PPCP residues in their natural habitats. Various PPCPs have been found in aquatic wildlife inhabiting treated-wastewater-impacted streams.[2−5]

Pharmaceutical chemicals are designed to affect biological targets such as receptors and enzymes that are often evolutionally conserved among vertebrates.[6] Recent laboratory studies have demonstrated that some pharmaceuticals including psychotropic agents displayed mode of action (MOA)-driven effects on fish (e.g., abnormality of feeding activity and predator avoidance, schooling, and courtship behaviors) at exposure doses close to concentrations detected in the natural aquatic environment.[7−10] Such effects on behavioral or physiological actions have a potential impact on fish survival and fitness. Although MOA-related chronic responses have been observed at much lower doses of chemicals[8,11] when compared to those on traditional acute and subchronic end points (standard end points),[1] the nonstandard end points, that is, MOA-related chronic responses, have not been fully validated.[12] Difficulty in selecting species and the growth stage of fish for testing as well as suitable end points and quantification methods makes nonstandardized toxicological studies challenging.[12] Hence, MOA-related ecological effects of PPCPs on wild fish remain to be elucidated.

A fish plasma model (FPM) has been proposed as a screening technique to prioritize pharmaceuticals with higher

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Table 1. Physicochemical Properties of Selected Pharmaceuticals and Personal Care Products[a]

compd	abbr	therapeutic class	formula	MW	pK_a[b]	log K_{ow}[c]	log D_{ow} (pH 7.4)	log D_{lipw} (pH 7.4)
			Pharmaceuticals					
diclofenac	DF	NSAID	$C_{14}H_{11}Cl_2NO_2$	295.0	4.00	4.02	0.87	1.00
indomethacin	IND	NSAID	$C_{19}H_{16}ClNO_4$	357.1	3.80	4.23	0.98	1.11
mefenamic acid	MF	NSAID	$C_{15}H_{15}NO_2$	241.1	3.89	5.28	2.08	2.22
bezafibrate	BZF	antihyperlipidemic	$C_{19}H_{20}ClNO_4$	361.1	3.83	4.25	1.02	1.15
fenofibric acid	FA	fenofibrate metabolite	$C_{17}H_{15}ClO_4$	318.1	3.10	4.00	0.56	0.69
sertraline	SER	antidepressant	$C_{17}H_{17}Cl_2N$	305.1	9.85	5.29	2.88	3.02
norsertaline	NSER	sertraline metabolite	$C_{16}H_{15}Cl_2N$	291.1	9.73	4.82	2.52	2.66
diphenhydramine	DPH	antihistamine	$C_{17}H_{21}NO$	255.2	8.87	3.11	1.63	1.77
carbamazepine	CBZ	antiepileptic	$C_{15}H_{12}N_2O$	236.1	15.96	2.25	2.25	2.39
haloperidol	HLP	antipsychotic	$C_{21}H_{23}ClFNO_2$	375.1	8.05	4.20	3.46	3.62
crotamiton	CTM	anti-itch	$C_{13}H_{17}NO$	203.1	−0.60	2.73	2.73	2.88
diltiazem	DIL	hypertensive	$C_{22}H_{26}N_2O_4S$	414.2	8.18	2.79	1.94	2.08
losartan	LS	hypertensive	$C_{22}H_{23}ClN_6O$	422.2	4.29, 4.07	4.01	1.05	1.18
			Personal Care Products					
triclosan	TCS	antibacterial	$C_{12}H_7Cl_3O_2$	288.0	7.68	4.66	4.48	4.64
triclocarban	TCC	antibacterial	$C_{13}H_9Cl_3N_2O$	314.0	11.4	4.90	4.90	5.07
methyl paraben	MeP	preservative	$C_8H_8O_3$	152.0	8.50	2.00	1.97	2.11
ethyl paraben	EtP	preservative	$C_9H_{10}O_3$	166.1	8.50	2.49	2.46	2.60
propyl paraben	PrP	preservative	$C_{10}H_{12}O_3$	180.1	8.50	2.98	2.95	3.10
butyl paraben	BuP	preservative	$C_{11}H_{14}O_3$	194.1	8.50	3.47	3.44	3.59
N,*N*-diethyl-3-toluamide	DEET	insect repellent	$C_{12}H_{17}NO$	191.1	−0.95	2.26	2.26	2.40

[a]MW, monoisotopic mass; NSAID, nonsteroidal anti-inflammatory drug; log K_{ow}, logarithm of octanol−water partition coefficient; log D_{ow} (pH), logarithm of octanol−water partition coefficient at a given pH value; log D_{lipw} (pH), logarithm of liposome−water distribution coefficient at a given pH value. [b]From Chemicalize.org, http://www.chemicalize.org [c]Estimated values from database of ChemSpider (EPISuite), http://www.chemspider.com.

potential hazards to wild fish,[13−17] since there are thousands of pharmaceutical ingredients in the market.[18] In this FPM, drug plasma concentration is calculated (from the drug concentration in ambient water) by use of the theoretical partition coefficient between water and fish plasma based on chemical lipophilicity.[19] The predicted fish plasma concentration is then compared with the human therapeutic plasma concentration. The advantage of this approach is that the screening can be easily performed because of readily available physical/chemical and mammalian pharmacological data to assess the potential risk. However, the FPM does not account for inter- or intraspecies variability of pharmacokinetic and pharmacodynamic parameters. In addition, verification and validation of FPM are lacking for pharmaceuticals, as the theoretical partition coefficients between water and fish plasma were developed for organochlorine compounds such as polychlorinated biphenyls (PCBs).[19]

MOA-driven responses of toxicants in organisms are triggered by interactions with specific sites such as transporters and receptors at the target organs.[20] The toxicokinetics of toxicants is partially controlled by ATP-binding cassette transporters that exist in the cell membranes of organs, which play a defensive role as barriers against the penetration of certain toxicants into sensitive organs such as the central nervous system.[21] Inhibition of the efflux transporter activities can enhance retention of toxicants in target organs and subsequent toxic effects. Recent in vitro and in vivo studies indicated that such sensitization (enhanced sensitivity to toxicants) could be caused by a variety of environmental pollutants such as polycyclic musks[21] and antidepressants.[22] In the actual environment, aquatic organisms are exposed to a cocktail of PPCPs and other contaminants, which can have interactive effects on toxicokinetic processes. It is an open question whether blood PPCP concentrations properly reflect the potential hazard of these chemicals retained in target organs of wild fish. Therefore, PPCP concentrations in target tissues might be more informative, compared with those in blood, for better understanding and assessment of the physiological/biological adverse effects of PPCPs in terms of toxicokinetic and toxicodynamic processes. However, only a few studies have focused on tissue distribution of PPCPs in wild fish.[2,23,24] Until now, no information is available in the literature for the relationship between individual blood and tissue concentrations of PPCPs in fish.

We recently developed a sensitive and robust isotope dilution method for the simultaneous determination of PPCPs with the range of 1.40−5.74 for log octanol−water partition coefficient (log K_{ow}) in various tissues including the liver, kidney, brain, and blood.[25] The purpose of the present study elucidated the uptake potency, tissue distribution, and biological risk of 20 PPCP residues in wild cyprinoid fish (n = 24) inhabiting treated wastewater-impacted streams by measuring the PPCP concentrations in six tissues (plasma, brain, liver, kidney, muscle, and gills), possible fish prey (periphytons),[26] and ambient water. In order to clarify uncertainty of the FPM for PPCPs, we compared measured plasma bioaccumulation factor (concentration ratios of fish plasma to ambient water) with predicted plasma bioconcentration factor (value calculated from ambient water concentration by use of theoretical partition coefficients) in the actual environment. We further examined the relationship between the concentration of each target compound in plasma and tissues. To the best of our knowledge, this is the first study to provide comprehensive data on the relationships between individual concentrations in blood and possible target tissues of PPCPs in wild fish.

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MATERIALS AND METHODS

Chemicals and Materials. Physicochemical properties as well as abbreviations of PPCPs targeted in this study are shown in Table 1. Pharmacological parameters (in mammals) and literature toxicity values (in fish or mammal) are shown in Supporting Information (Table S1). Information on preparation for native standard solutions and internal standard (IS) solutions of target PPCPs is described in the previous study[25] and in Supporting Information.

Sample Collection. Wild cyprinoid fish crucian carp (*Carassius carassius*) and common carp (*Cyprinus carpio*) were collected from two treated-wastewater-impacted streams in Kumamoto (November 2011, n = 6) and Ehime (August 2013, n = 7; December 2013, n = 5; April 2014, n = 6), Japan. These wastewater treatment plants used primary settlement and secondary treatment with conventional activated sludge. General characteristics of the treated-wastewater receiving stream are shown in Figure S1. The wastewater receiving stream in Ehime was selected as a main sampling location in this study, because this stream is characterized by a small width (less than 10 m), shallow depth, low flow rate, and it often dries up during rainless periods. These characteristics make for treated-wastewater-dominated conditions.[27] In a previous study, we found many PPCPs about 1 km downstream from the effluent discharge point, at concentrations similar to those measured in the effluents.[28] The cyprinoid fish were collected after at least three consecutive days without rain. Biological characteristics of the collected fish are shown in Table S2. All the fish were stunned by blows to the head, and blood was individually taken from the caudal vein with a heparinized syringe. After blood centrifugation (5 min at 3000g), the plasma was transferred to a polypropylene tube. All the fish from Ehime were then dissected and brain, liver, kidney, muscle, and gills were collected. The collected gills were rinsed three times with Milli-Q water and towel-dried. All tissue samples were stored at −30 °C until chemical analysis. Ambient water samples were collected in receiving areas of wastewater treatment plant discharge at 6-h intervals before the fish were collected. Periphyton communities on rocks were collected from the same site at Ehime on August 2014 and were kept on ice during transport to the laboratory. The periphyton communities were transferred to 50 mL polypropylene centrifuge tubes. Milli-Q water (30 mL) was added to each tube, and they were then centrifuged. The supernatant was discarded and the rinsing procedure with Milli-Q water was repeated five times. The rinsed periphyton residue was transferred to a polypropylene cryovial and stored at −30 °C until analysis.

Sample Preparation. Water samples were filtered through glass-fiber filters (GF/F, pore size 0.7 μm; Whatman, Maidstone, ME) to remove suspended solid. The filtrate (50 mL) was spiked with IS solution (100 μL) and then loaded onto an Oasis HLB cartridge (200 mg; Waters, Milford, MA) preconditioned with methyl *tert*-butyl ether (MTBE) (5 mL), followed by methanol (5 mL) and Milli-Q water (5 mL). The cartridge was washed with 20% methanol in Milli-Q water (5 mL) and then vacuum-dried for 20 min. The analytes retained in the cartridge were eluted with methanol/MTBE (5 mL, 7:3 v/v), and the eluate was concentrated to 0.3 mL under N_2 flow. The residue was reconstituted in acetonitrile/methanol/Milli-Q water (1 mL, 3:3:4 v/v/v) and filtered through a cellulose membrane syringe filter (0.2 μm). Final solution was diluted up to 10-fold prior to instrumental analysis, because of high concentrations of some compounds.

PPCPs in fish tissues and periphytons were analyzed according to the method developed recently[25] with slight modifications. Detailed information on the preparation of fish tissues and periphytons for analysis is given in Supporting Information. Briefly, homogenized sample (0.5 g) was spiked with IS solution and extracted by ultrasonication with acetate buffer and acetonitrile. After centrifugation, the supernatant was transferred to a glass tube and evaporated to less than 6 mL under N_2 flow. The extracted solvent was diluted with 5% NaCl (60 mL) and liquid−liquid-extracted with MTBE. The partition was performed with both acidic and basic condition. Organic phases were combined and subjected to a cleanup protocol with silica gel chromatography, gel-permeation chromatography, and Oasis HLB cartridge. The cleanup extract was reconstituted in acetonitrile/methanol/Milli-Q water (1 mL, 3:3:4 v/v/v) for liquid chromatography−tandem mass spectrometry (LC−MS/MS) analysis.

Instrumentation. Identification and quantification of the analytes were performed on an ultra-high-performance liquid chromatograph system (Shimadzu, Japan) coupled to an AB Sciex Qtrap 5500 mass spectrometer (Applied Biosystems Sciex, Tokyo, Japan) operating in electrospray ionization (ESI) positive and negative modes with multiple reaction monitoring (MRM). Information on MRM transitions, chromatographic separation, and ion source parameters have been described in our previous study.[25]

Quality Assurance and Control. Target compounds in the samples were identified by comparing retention times with those of native standards (within ±0.01 min) and confirmed by comparing the peak area ratio for the two product ions in the samples with native standards (accepted when within ±20%). Target compound concentrations were determined by an isotope-dilution method.[25] One procedural blank was run for each batch of eight samples to check for contamination during sample preparation. The concentrations in the blank sample were subtracted from those of the corresponding biota samples for the same batch.

Reproducibility and recoveries of target compounds in water samples were determined by triplicate analyses of ambient water either spiked (n = 3, spiking level 250 ng·L^{-1}) or unspiked (n = 3) with standards including target PPCPs. Relative standard deviations were less than 10%, and the relative recovery rates were between 77% and 130% (Table S3). For fish samples, reproducibility and relative recoveries were determined by triplicate analyses of target compound-free fish tissues spiked with target compounds (spiked amount depended on the compound).[25] Method detection limits (MDLs) were calculated from the standard deviation (SD) of seven replicate injections of fortified tissue extracts. When target compounds are frequently detected in procedural blank samples, MDLs were determined by use of SD derived from 10 replicates of the method blank procedure. Relative recoveries were between 91% and 120% (Table S4) with relative standard deviations less than 10%, and the MDLs ranged from 0.0051 to 3.3 ng·g^{-1} wet weight (Table S5).

Statistical Analysis. Normal distribution and homogeneity of variance were tested with Shapiro−Wilk and Levene's tests, respectively. For data with normal distribution and variance homogeneity, parametric tests were applied. If the data did not show a normal distribution, nonparametric tests were applied. To assess the relationship between the log-transformed ($\log_{10}$)

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concentration of each target compound in plasma and tissues, nonparametric Spearman's rank correlation coefficients or parametric Pearson correlation coefficients were calculated, depending on data distribution. Friedman test followed by a posthoc Nemenyi test were performed to compare the PPCP levels in different tissues. A p-value of <0.05 was considered statistically significant. All statistical analyses were carried out with XLStat-Pro (Addinsoft, Paris).

RESULTS AND DISCUSSION

Uptake Potency: Comparison between Fish Plasma and Ambient Water. Concentrations of the target PPCPs in wild fish analyzed are shown in Table S6, and PPCP concentrations in ambient water and periphytons are given in Table S7. Nonsteroidal anti-inflammatory drugs (NSAIDs), psychotropic agents (SER, DPH, CBZ, and HLP), the hypertensive agent DIL, and antibacterial agents were frequently (>79%) found in fish plasma. The highest mean plasma concentration was found for TCS, followed by IND and DPH. Individual variations (differences between minimum and maximum values) of plasma PPCP concentrations in the fish collected at the same sampling site and period ranged from 1.3- to 9.9-fold, except for TCS at 19-fold. Although concentrations of BZF and CTM were relatively high in ambient water, these compounds were present at trace or undetectable levels in fish plasma. To evaluate the fish uptake potencies of PPCPs, plasma bioaccumulation factors (BAF_{plasma}) were calculated as follows:

$$BAF_{plasma} = \frac{C_{plasma}}{C_{ambient\ water}} \quad (1)$$

where C_{plasma} and $C_{ambient\ water}$ are the chemical concentrations measured in plasma of fish from the field and in ambient water, respectively. If a compound was not detected in plasma or water samples, half the value of MDL was used as a substitute value, although it can produce a potential bias. BAF_{plasma} values of PPCPs are shown in Table S8. No significant interspecies differences (between common and crucian carp) and no relationships with fish body weight/length were observed for the BAF_{plasma} of all target PPCP compounds. As seen in Table S8, BAF_{plasma} of each compound was approximately similar regardless of the sampling site and period. Median BAF_{plasma} values were the highest for TCS (BAF, 19), followed by IND (11) and SER (8.9). When BAF_{plasma} values measured in cyprinoid fish from Ehime, Japan, were compared with data previously reported in other countries (Table S9),[14,29−31] the values of DF were similar among the present and previous studies. In contrast, BAF_{plasma} values of CBZ in cyprinoid fish ranging from 0.22 to 0.70 were approximately 10 times lower than those previously measured in channel catfish of 7.1,[23] in Nile tilapia of 2.5,[23] in rainbow trout of 0.8−4.2,[14] and in longear sunfish of 11.[32] This difference might be due to interspecies variations in uptake, elimination, and metabolic capacity of CBZ. Median BAF_{plasma} values of basic pharmaceuticals (i.e., SER, 11; DPH, 1.4; and DIL, 0.32) were 10−100 times lower than those previously measured in rainbow trout from Sweden[14] and in longear sunfish from Texas.[32] This discrepancy may be explained not only by interspecies variations in kinetic parameters but also by differences in ambient water pH, because the uptake and bioconcentration potential of basic compounds increases with external pH.[33,34] The range of ambient water pH (6.8−7.4) measured in this study showed lower values compared with those reported in Sweden (7.5−8.0)[14] and Texas (7.87−7.93).[32]

The BAF_{plasma} value in the field is a consequence of both dietary uptake and gill uptake (branchial respiration). When the relationship between log PPCP concentrations in plasma and respiratory organ gills, which play an important role in uptake of waterborne environmental pollutants, was examined, significant positive correlations were found for IND, TCS, and TCC (r = 0.67−0.76, p < 0.02; Figure S2). Also, for MF, SER, and DPH, plasma concentrations displayed a tendency to increase with increase in gill concentrations, but the correlations did not reach statistical significance (r = 0.41−0.46, p = 0.056−0.091; Figure S2). In almost all the fish analyzed, gill concentrations were higher than plasma concentrations for all target PPCPs; the ratios of concentrations in plasma to those in gills ranged from 0.11 (HLP) to 0.74 (MF). These results indicate that uptake and elimination processes of PPCPs are principally through gill membranes. A lower bioaccumulation potential for all PPCPs, except for IND and DF, was found in fish plasma (BAF_{plasma} in Table S8) compared with fish prey periphytons (BAFs in Table S7). This is in agreement with a previous study showing that the trophic magnification factors of CBZ and DPH in aquatic organisms decreased with an increase in the trophic level, which is referred to as trophic dilution.[32] Taken together, our findings suggest a greater contribution of uptake and elimination via the gills to BAF_{plasma} than for dietary exposure routes. If it is assumed that gill uptake (branchial respiration) is the primary exposure route of chemicals, the plasma bioconcentration factor (BCF_{plasma}), equivalent to the chemical partitioning between the aqueous phase and arterial blood via the gills, can be estimated by[19]

$$BCF_{plasma} = \log[(10^{0.73 \log K_{ow}})(0.16) + 0.84] \quad (2)$$

where K_{ow} is the pH-independent octanol−water partition coefficient. Recently, pH-dependent octanol−water partition coefficient (D_{ow}) and pH-dependent liposome−water partition coefficient (D_{lipw}) were proposed as alternatives to K_{ow} for ionizable pharmaceuticals (eqs 3 and 4, respectively):[8,16,35,36]

$$BCF_{plasma} = \log[(10^{0.73 \log D_{ow}})(0.16) + 0.84] \quad (3)$$

$$BCF_{plasma} = \log[(10^{0.73 \log D_{lipw}})(0.16) + 0.84] \quad (4)$$

In this study, BCF_{plasma} values were calculated from all three chemical lipophilic parameters (D_{ow}, D_{lipw}, and K_{ow}) according to eqs 2, 3, and 4, respectively.

The pH-dependent D_{ow} was calculated by

$$D_{ow} = f_{neutral} K_{ow(neutral)} + f_{ion} K_{ow(ion)} \quad (5)$$

where $f_{neutral}$ is the fraction of neutral species at study pH, f_{ion} is the fraction of ionic species at study pH, and $K_{ow\ (neutral)}$ and $K_{ow\ (ion)}$ are the K_{ow} values for neutral and ionic species, respectively. The relationship between f_{ion} and $f_{neutral}$ is defined by[35]

$$f_{ion} = f_{neutral} \times 10^{i(pH - pK_a)} \quad (6)$$

It was assumed that log K_{ow} of ionic species was 3.5 log units lower than that of neutral species.[37] The pH-dependent log D_{lipw} was calculated by[38]

$$\log D_{lipw} = 1.01 \log D_{ow} + 0.12 \quad (7)$$

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Log BCF_{plasma} values calculated from different chemical lipophilic parameters (log D_{ow}, log D_{lipw}, and log K_{ow}) were compared with measured log BAF_{plasma} values, and the results are shown in Table S8 and Figure 1. For approximately half of

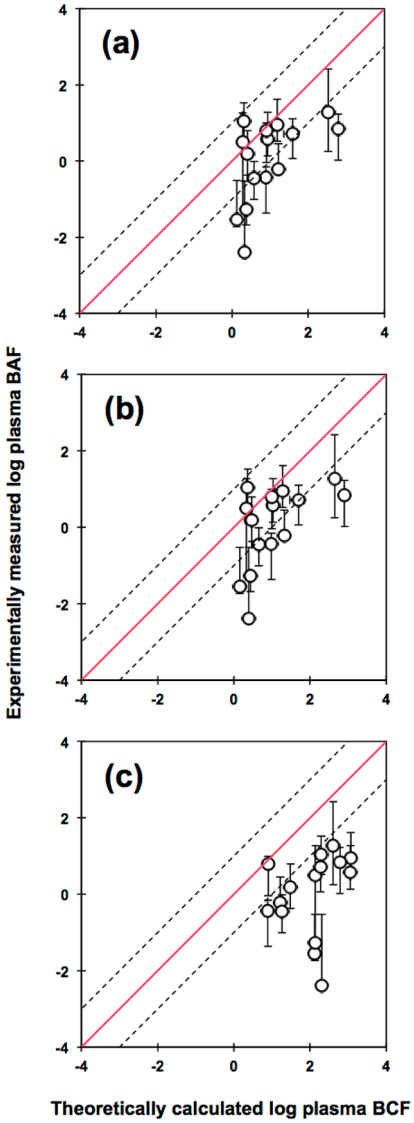

Figure 1. Comparison of measured log plasma BAFs with calculated log plasma BCFs based on chemical lipophilic parameters: (a) log D_{ow} at pH 6.8−7.4, (b) log D_{lipw} at pH 6.8−7.4, and (c) log K_{ow}. The solid red line represents an exact match between measured and predicted values. The dashed line denotes log unit deviations.

target PPCPs, the measured BAF_{plasma} values were within a log unit deviation of those theoretically calculated by use of pH-dependent log D_{ow} (Figure 1a) or log D_{lipw} (Figure 1b). However, when pH-independent log K_{ow} was used, the measured plasma BAFs for most compounds were 1−5 orders of magnitude lower than the calculated values (Figure 1c). These results support previous studies highlighting the importance of taking the water pH influence into account when BCFs of PPCPs are estimated.[8,16,35] Interestingly, measured BAF_{plasma} values of BZF, FA, CBZ, CTM, LS, TCS, and TCC were 1−3 orders of magnitude lower than theoretical values calculated by log D_{ow} and log D_{lipw}. There are some possible reasons for these discrepancies between measured BAFs and calculated BCFs, but it is unlikely that variations in exposure levels or nonequilibrium conditions of the above PPCP compounds is involved. As shown in Table S7, variations in the concentrations of PPCPs in ambient water were much smaller (CV < 30%). Additionally, rapid attainment of equilibrium concentrations in the plasma of rainbow trout (within 2 days) has been demonstrated for several pharmaceuticals,[31] and steady state of 17α-ethynylestradiol levels among water, plasma, and liver samples of rainbow trout has been reached within 16 h.[39] Possibly, the observations between measured BAFs and calculated BCFs might be attributed to differences in uptake/elimination mechanisms at gill membranes, plasma protein binding, and/or hepatic clearances among PPCPs. For example, TCC had a very short elimination half-life of 1 h in an experimental study of Japanese medaka,[40] despite the relatively high log K_{ow} of 4.9. It has been previously reported that fish have a deficiency of 2B, 2C, and 2D homologues of cytochrome P450 enzymes (CYPs),[41] which are primarily responsible for drug metabolism in humans, hence ≥40% of human pharmaceuticals are assumed to be metabolized by nonorthologous CYPs in fish.[42] Unfortunately, studies on PPCP metabolism in fish are very limited[43,44] and CYPs responsible for the metabolism of PPCPs remain largely unknown.

Even though log K_{lipw} has been proposed as a more accurate descriptor to estimate BCF of chemicals in aquatic organisms than log K_{ow},[45] there is a smaller difference between the two experimental pH-adjusted partition coefficients, as is clear from eq 7, than the differences between measured BAFs and calculated BCFs in our study. Thus, on the basis of our results only, we cannot judge and discuss utility between log D_{ow} and log D_{lipw}.

The present study illustrates the uncertainties associated with predicting plasma PPCP levels, that is, discrepancies between measured BAFs and theoretically calculated BCFs, in wild fish under natural conditions. When FPM is used as a screening tool for the risk assessment of PPCPs, an overestimated prediction would not be serious from the viewpoint of precautionary principle. In contrast, calculation of theoretical BCF values lower than actual BAF_{plasma} may lead to an underestimation of toxicological risk on wild fish. In this study, it is noteworthy that the measured maximum BAF_{plasma} of IND was approximately 17 times higher than the theoretical value (Table S8). In a recent study using FPM, it has been reported that the theoretical value of DPH calculated for wild fish plasma from Texas was underestimated by 10 times.[32] In this regard, a recent laboratory study using fathead minnows[46] has demonstrated good agreement between measured and model-derived BCF_{plasma} for DPH, taking into account the plasma binding parameter for the ionic species of the compound.

Tissue Distribution in Fish. The present study successfully determined the tissue−blood partition coefficients of 11 PPCPs in fish. Their partition coefficients, which were calculated as the ratio of concentration in tissue (brain, liver, kidney, or muscle) to that in plasma, are shown in Table 2 and Figure S3. The mean tissue/plasma concentration ratios of DF, IND, and CBZ ranged from 0.16 to 3.9, suggesting relatively low transportability of these compounds into tissues from plasma. In contrast, SER, NSER, DPH, HLP, and DIL showed high transportability into tissues with tissue/plasma concentration ratios of 12−160 for liver, 21−76 for kidney, and 4.1−28 for brain. In clinical pharmacology, the volume of distribution (V_d, liters per kilogram) is defined as the ratio of total dose to blood

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Table 2. Tissue−Plasma Partition Coefficients[a] of Pharmaceuticals and Personal Care Products in Wild Fish

	brain			liver			kidney			muscle		
compd	mean	median	5−95[b]	mean	median	5−95[b]	mean	median	5−95[b]	mean	median	5−95[b]
DF	0.66	0.67	0.30−1.1	1.5	0.97	0.19−3.2	0.79	0.40	0.11−2.0	0.30	0.17	0.069−0.76
IND	0.39	0.40	0.20−0.65	1.6	1.6	0.67−2.8	2.3	1.8	0.70−5.8	0.16	0.14	0.054−0.34
MF	0.72	0.70	0.39−1.1	20	6.1	1.4−68	6.7	0.99	0.13−25	0.10	0.092	0.061−0.16
BZF[c]												
FA[c]												
SER	22	12	4.1−74	17	11	3.9−57	43	17	6.2−160	1.6	1.2	0.54−3.3
NSER	28	18	7.1−72	27	21	5.7−78	44	24	6.5−140	5.3	4.2	1.4−13
DPH	4.1	3.5	1.7−7.2	12	8.6	2.6−28	21	16	5.1−49	0.63	0.49	0.27−1.1
CBZ	3.2	2.4	1.3−8.3	3.9	2.7	1.6−8.1	3.4	2.6	1.5−6.9	1.1	0.89	0.47−2.2
HLP	18	12	3.5−54	160	90	34−490	76	45	15−250	*c*	*c*	
CTM[c]												
DIL	5.3	4.4	2.5−11	24	18	4.1−76	41	32	7.4−110	0.78	0.58	0.25−2.0
LS[c]												
TCS	1.8	1.3	0.69−3.9	30	27	6.3−69	4.5	3.3	1.2−13	0.37	0.32	0.066−0.83
TCC	1.4	1.4	0.53−2.2	5.4	5.0	1.6−10	1.9	1.7	0.72−4.2	0.40	0.37	0.17−0.67
MeP[c]												
EtP[c]												
PrP[c]												
BuP[c]												
DEET[c]												

[a]Calculated as the ratio of concentration in tissue to that in plasma. [b]5th−95th percentile range. [c]Not applicable due to no detection or low detection frequency (<50%).

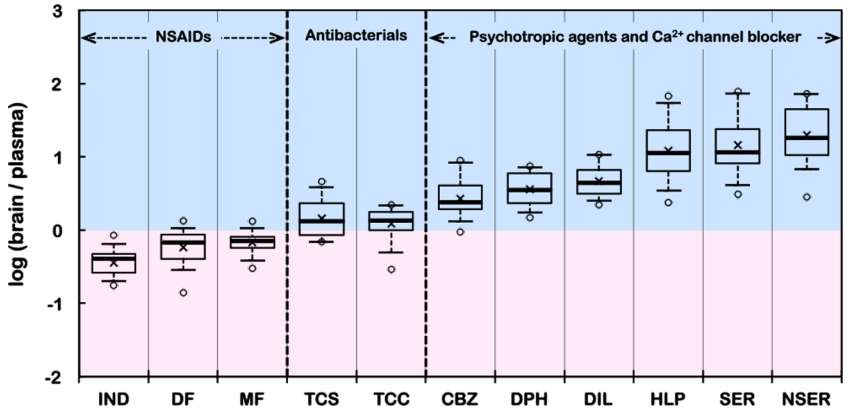

Figure 2. Logarithmic plasma−brain partition coefficients (brain/plasma concentration ratios) estimated in wild fish (n = 18). The box plot shows 5th (lower whisker), 25th (bottom edge of box), 75th (top edge of box), and 95th (upper whisker) percentiles. The horizontal line and cross (×) in the box represent median and mean, respectively. The small dots (○) are outliers representing the 95th and 5th percentiles. Chemical abbreviations are given in Table 1.

concentration of drug. A higher V_d indicates higher transportability into some tissues from blood. Literature V_d values[47] (Table S1) of SER, DPH, HLP, and DIL are approximately 10 times greater than those of DF, IND, and CBZ. By comparing the tissue/plasma concentration ratios determined in this study with the literature V_d values, it can be suggested that PPCP transportability into tissues from plasma is similar in fish and humans. Furthermore, V_d value may be a good indicator to estimate the distribution of drugs in fish, as recently proposed by Nichols et al.[46]

Brain/plasma concentration ratios of PPCPs are shown in Figure 2. The mean brain/plasma ratios of psychotropic agents (3.2−28) were higher than those of NSAIDs (0.39−0.72) and antibacterial agents (1.4−1.8), indicating higher transportability of psychotropic agents to the fish brain. To the best of our knowledge, this is the first report to provide comprehensive data on the brain/plasma ratios of PPCPs in fish. A comparison of brain/plasma concentration ratios of PPCPs observed in this study with the values previously reported (in mammals) is given in Table S10. The mean brain/plasma ratios of NSER (28), SER (22), and HLP (18) observed in this study were comparable to those reported in rodents or humans, whereas the brain/plasma ratios of CBZ (3.2), TCS (1.8), and IND (0.39) in this study were higher than the reported values in mammals. The higher brain/plasma ratios of CBZ observed in this study could be due to the difference in exposure levels of this compound. A significant negative correlation between brain/plasma ratios and plasma levels of CBZ was found in this study (r = −0.54, p < 0.02, Pearson correlation coefficient), and plasma CBZ levels detected in this study were 4−6 orders of magnitude lower than those measured in previous mammalian studies.

Relationships between tissue and plasma concentrations of 10 PPCPs frequently detected in all tissues were examined. A

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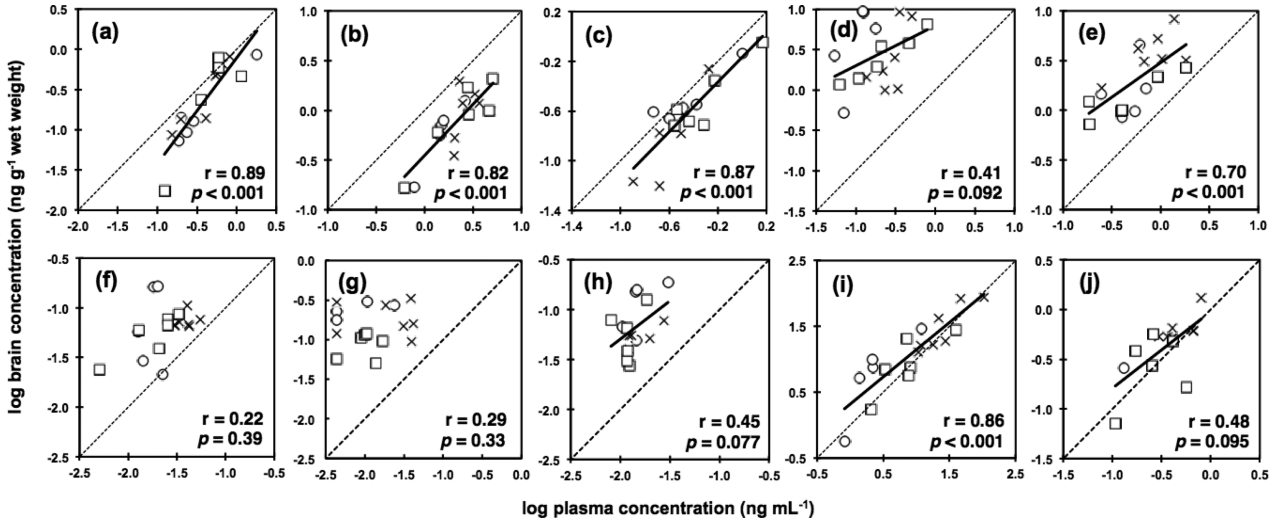

Figure 3. Log-linear correlations between brain and plasma concentrations of PPCPs in wild fish (Pearson correlation coefficient). *y*-axis, logarithmic PPCP levels in the brain; *x*-axis, logarithmic PPCP levels in plasma. The solid line is the linear regression, and the dashed line represents an exact match between PPCP levels in plasma and brain. Sampling periods were (×) Aug 2013, (○) Dec 2013, and (□) Apr 2014. (a) DF, (b) IND, (c) MF, (d) SER, (e) DPH, (f) CBZ, (g) HLP, (h) DIL, (i) TCS, (j) TCC. Chemical abbreviations are given in Table 1.

linear regression equation of log-transformed (log10) tissue and plasma concentrations was determined for PPCPs with normally distributed correlation coefficient more than 0.4 (Table S11). Individual concentrations of SER, DPH, TCS, and TCC were positively correlated between plasma and all tissues, showing that concentrations of these compounds in tissues can be estimated from plasma levels by use of the equations given in Table S11. For PPCPs other than the above compounds, no relationships between several tissue and plasma levels were found, implying tissue-specific distribution of these compounds. For example, no correlation between kidney and plasma concentrations was found for IND, although this compound showed significant positive correlations between plasma and other tissues. NSAIDs, including IND, reduce production of prostaglandins by inhibiting cyclooxygenase (COX): COX genes are widely conserved across vertebrate species.[6] Prostaglandins are involved in regulation of blood circulation, especially in the kidney, as well as inflammation, and thus a reduction in prostaglandin production in fish kidney may lead to renal disorder. In order to appropriately assess the risk on biological effects by IND, measurement of residue levels in the kidney would be necessary, since it is likely that concentrations of this compound in the kidney cannot be predicted from the corresponding plasma concentrations in wild fish, as observed in this study.

Brain is a possible target organ for biological effects by PPCPs frequently detected in fish plasma. When the relationships between brain and plasma concentrations of 10 PPCPs were examined (Figure 3), significant positive correlations were shown for NSAIDs, DPH, and TCS (r = 0.70−0.89, p < 0.001). Also for SER, DIL, and TCC, brain concentrations displayed a tendency to increase with increasing plasma concentrations, but the correlations did not reach statistical significance (r = 0.41−0.48, p = 0.077−0.095). No relationships were found for CBZ and HLP. It is likely that low or no correlations between brain and plasma concentrations of psychotropic agents were caused by large variations in brain/plasma ratios among individual wild fish, as shown in Figure 2. The differences (max/min ratios) between minimum and maximum values of brain/plasma ratios for psychotropic agents were up to 28-fold: the variations were substantially larger than those for NSAIDs and antibacterial agents (max/min ratios 4.4−9.5-fold). The max/min ratios of NSER (26), SER (25), and HLP (28) estimated in wild fish were higher than those (up to 7.9) previously observed in humans[48] (see Table S10). Larger individual variations of brain/plasma ratios found in this study might be due to differences in plasma concentrations of these psychotropic agents, amount of plasma proteins, expression levels of efflux transporters such as p-glycoprotein (Pgp) at the blood−brain barrier, and/or residue levels of contaminants that inhibit efflux transporter's activities.

Positive Pearson correlations (r = 0.46−0.69) were found among the brain/plasma ratios of CBZ, DIL, HLP, SER, and NSER (Figure S4), which suggest similar influx and efflux mechanisms at the blood−brain barrier for these compounds. Recent studies have shown that DIL, HLP, and SER are potential Pgp substrates and/or can inhibit Pgp-mediated transport.[22,49,50] Intercompound positive correlations of brain/plasma ratios observed in this study imply the interaction of these psychotropic agents with specific influx and/or efflux transporters including Pgp in the brains of wild fish.

Risk Assessment and Prioritization. Comparison of fish plasma levels with human therapeutic plasma concentrations (H_TPCs) has been proposed as a screening technique to estimate the potential hazards of pharmaceuticals in wild fish.[13−17] To estimate the potential hazards of pharmaceuticals detected in plasma of wild fish, the lowest H_TPCs[51] were used as effect concentrations in the present study. As effect concentrations for personal care product ingredients including antimicrobial agents, in vivo or in vitro mammalian toxicity data given in Table S1 were used. Effect ratio (ER) was calculated by[29]

$$\mathrm{ER} = \frac{\text{effect concn}}{\mathrm{FPC}_{\mathrm{measd}}} \quad (8)$$

where $\mathrm{FPC}_{\mathrm{measd}}$ is the fish plasma concentration measured in this study. Because this approach does not consider inter- or intraspecies variability of toxicokinetic and toxicodynamic parameters, the use of an uncertainty factor (UF) is warranted.[52,53] Generally, UF is initially set at 300−1000

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based on three separate UF values: 10 for interspecies variation (extrapolation from animal to human), 10 for interindividual variation,[54] and 3−10 for extrapolation from a lowest-observed-effect level (LOEL) to a no-observed-effect level (NOEL).[55] The UF value of 10 for interindividual variation is further divided into two default subfactors each of $10^{0.5}$ for variation of toxicokinetics and toxicodynamics, respectively.[54] The validity of the default values for subfactors should be further improved when data on toxicokinetics and toxicodynamics are available.

In this study, we estimated the interindividual variations of toxicokinetics as the ratios of PPCP concentrations in possible target tissue to plasma. These ratios varied, depending on compounds and tissues, and generally ranged from $10^{0.3}$- to $10^{1.4}$-fold difference between 95th percentile and 50th percentile measured values, which were larger than the default value of $10^{0.5}$. On the other hand, H_TPC is considered to be the internal effect concentration. We finally concluded that it is reasonable to apply UFs of $10^{2.8}$−$10^{3.9}$ depending on the compounds, based on four separate UF values: (1) interindividual variability of toxicokinetics (UF = $10^{0.3}$−$10^{1.4}$) observed in this study, (2) interindividual variability of toxicodynamics (default UF = $10^{0.5}$), (3) interspecies difference between humans and fish (default UF = 10^{1}), and (4) conversion of H_TPC into NOEL (default UF = 10^{1}).

The ER values of 10 PPCPs frequently detected in fish plasma are shown in Figure S5. Nine compounds showed ER smaller than the corresponding UF values. The median ER values were smallest for TCS (12), followed by SER (71) and DPH (90). It should be noted that the maximum fish plasma TCS concentration (110 ng·mL^{-1}) observed in this study exceeded the effect concentration previously reported in mouse plasma (0.31 ± 0.09 μM = 89 ng·mL^{-1}) exhibiting 25% depression of cardiac hemodynamics.[56] The antibacterial agent TCS is contained in a variety of personal care products, including soaps, deodorants, household cleaners, and dental care products.[57] Both in vivo and in vitro studies revealed that TCS has weak estrogenic and androgenic activities,[58,59] interferes with the thyroid axis,[60] and weakens cardiac and skeletal muscle contractility.[56,61] The TCS concentrations of ambient water measured in this study were comparable to those reported in the United States[62] and the European Union,[63] indicating that the potential risks to wild fish from this antibacterial agent are not unique to this study area. The antidepressant SER, which is a selective serotonin reuptake inhibitor (SSRI), increases serotonin levels at synapses by inhibiting the function of serotonin reuptake transporter (SERT) in vertebrates. Recent studies examining toxicological effects of psychotropic agents on aquatic organisms highlight behavioral anxiety-related end points.[64] For example, exposure to SER for fathead minnows decreased time spent in shelter (place to hide) by interacting with SERT in the brain.[8] DPH is a first-generation antihistamine agent found in a large number of over-the-counter medicines, including antiallergy medicines and sleeping pills. DPH exerts multiple MOAs by interaction with the histamine H1 receptor, SERT, and acetylcholine receptor.[9,65] Although DPH is ubiquitous in surface water, sediments, fish, and aquatic invertebrates,[5,66] studies of adverse effects on aquatic organisms are lacking.[9,65]

Environmental risk assessment of pharmaceuticals by comparing fish plasma concentration with H_TPC is based on the "read-across hypothesis," which assumes that a drug affects a wider range of organisms if the molecular targets are conserved and that plasma drug concentrations eliciting specific pharmacological effects are comparable.[13,67] However, only a limited number of studies have validated this hypothesis,[10] as toxicological thresholds in aquatic organisms have been obtained from measurement of external exposure (in ambient water) rather than internal exposure (in blood and/or target organs). Without data on internal chemical concentrations, it is impossible to determine whether the observed effects are due to high bioconcentration or the organism is more sensitive than other species. Measurement of internal chemical concentrations in aquatic organisms is essential to address this important issue.

The present study illustrates the uncertainties related to the use of FPM as a screening tool for risk assessment of PPCPs in wild fish: (1) prediction of plasma PPCP concentrations, calculated by use of the theoretical partition coefficient between ambient water and fish plasma based on chemical lipophilicity, may lead to underestimated biological risk for some PPCPs; and (2) large variations in possible-target tissue/plasma concentration ratios among individual wild fish for some PPCPs. Our results emphasize that determination of PPCP concentrations in possible target organs as well as blood would provide helpful information for better understanding and assessing the risk of PPCPs on wild fish.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b02478.

Tables listing biological characteristics of fish, quality assurance and control data, concentrations of PPCPs in fish, ambient water, and periphytons, BAF values of target PPCPs in fish, a comparison of BAFs calculated in this study with previously reported values, correlation between plasma/gill ratios of PPCPs, comparison of brain/plasma concentration ratios of PPCPs calculated in this study with previously reported values, linear regression equations of $\log_{10}$ PPCP concentrations between tissues and plasma; figures showing log−linear correlations between plasma and gill concentrations and between PPCP brain/plasma ratios, tissue distribution, and ER values of 10 PPCPs (PDF)

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Notes

The authors declare no competing financial interest.

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