Arch Environ Contam Toxicol (2012) 62:306–313
DOI 10.1007/s00244-011-9691-x

Tissue-Specific Uptake and Bioconcentration of the Oral Contraceptive Norethindrone in Two Freshwater Fishes

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Received: 20 December 2010 / Accepted: 6 June 2011 / Published online: 28 June 2011

Abstract The environmental presence of the oral contraceptive norethindrone (NET) has been reported and shown to have reproductive effects in fish at environmentally realistic exposure levels. The current study examined bioconcentration potential of NET in fathead minnow (*Pimephales promelas*) and channel catfish (*Ictalurus punctatus*). Fathead minnows were exposed to 50 µg/l NET for 28 days and allowed to depurate in clean water for 14 days. In a minimized 14-day test design, catfish were exposed to 100 µg/l NET for 7 days followed by 7-day depuration. In the fathead test, tissues (muscle, liver, and kidneys) were sampled during the uptake (days 1, 3, 7, 14, and 28) and depuration (days 35 and 42) phases. In the catfish test, muscle, liver, gill, brain, and plasma were collected during the uptake (days 1, 3, and 7) and depuration (day 14) stages. NET tissue levels were determined by gas chromatography–mass spectrometry (GC–MS). Accumulation of NET in tissues was greatest in liver followed by plasma, gill, brain, and muscle. Tissue-specific bioconcentration factors (BCFs) ranged from 2.6 to 40.8. Although NET has been reported to elicit reproductive effects in fish, the present study indicated a low potential to bioconcentrate in aquatic biota.

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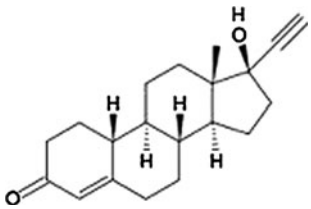
Steroidal sex hormones (estrogens, progestins, and androgens) have been detected in effluents from sewage treatment plants (STPs) and surface waters, often in the low nanograms-per-liter range (Desbrow et al. 1998; Ternes et al. 1999; Labadie and Budzinski 2005). Environmental presence of these compounds, albeit at low levels, is of great concern due to their endocrine-disruptive effects on non-target organisms (Purdom et al. 1994; Sumpter and Jobling 1995; Kidd et al. 2007). For example, 17-α-ethinylestradiol (EE2) exposure at 100 pg/l in male rainbow trout induced the synthesis of vitellogenin, an egg yolk-precursor protein that normally occurs in female fish (Purdom et al. 1994). In addition to this well-documented biomarker of estrogen exposure in wild fish (Tyler et al. 1996), other reproductive effects, such as changes in sexual maturation, decreased sperm counts, intersexual condition, or altered sex ratios, have been reported in fish exposed to estrogenic chemicals (Jobling et al. 1998; Tyler et al. 1998). Due to high sorption efficiencies ($\log K_d \geq 3.0$), most steroid hormones have a tendency to partition to sludge and sediments and hence are predicted to bioaccumulate in aquatic and terrestrial species (Ternes et al. 2002; Kuster et al. 2004; Andersen et al. 2005; Markman et al. 2007).

Norethindrone (NET; 19-nor-17-alpha-ethinyltestosterone) is a synthetic progestational hormone widely used as an oral contraceptive and for the treatment of endometriosis, various types of menstrual disorders, premenopausal and postmenopausal syndromes, and breast and ovarian cancers. Although the therapeutic doses of NET for contraceptive use are in the rage of micrograms to low milligrams, higher dosages are prescribed for treating the conditions listed previously. NET is a potent inhibitor of ovulation that exerts its effects by binding to the progesterone receptor in the target cells (e.g., reproductive), leading to altered responses in gonadotropin-releasing hormone from the hypothalamus. In addition, NET has weak androgenic and estrogenic properties. The use of NET has been extensive due to its availability as a non-prescription drug. For example, in 2002, 34 products containing NET were approved in Sweden, and approximately 30 million doses (containing this ingredient) were sold in the same year. Based on these sales volumes, NET was prioritized as being "dangerous for the environment (R 51/53)" (Carlsson et al. 2006).

The physicochemical properties of NET (Table 1) indicate its potential to partition into organic-rich fractions. Previous studies have reported accumulation of NET in river sediments (Alda et al. 2002). Synthetic hormones, including NET, are designed to resist structural changes to enhance their persistence in the body. Once excreted, this pharmacologically desirable drug design becomes deleterious to the environment because they resist natural degradation processes. Although NET undergoes considerable metabolism in mammals (only approximately 5% is excreted unchanged), the excreted glucuronidated products could be converted back (through action of β-glucuronidase or aromatase) to active hormonal substances by microorganisms (Labadie and Budzinski 2005; Carlsson et al. 2006). These findings underscore the need for understanding the bioaccumulative potential of NET in aquatic organisms, fish in particular, due to highly conserved progesterone receptors (Huggett et al. 2004).

A recent study from our laboratory indicated complete shutdown of egg production in fathead minnow and Japanese medaka exposed to NET at 25 ng/l (Paulos et al. 2010). Occurrence of reproductive or other chronic effects in aquatic species are frequently attributed to an organism's ability to accumulate xenobiotics because bioaccumulation over a period of time results in the critical tissue concentrations that may trigger certain toxicological responses. Therefore, the current study was aimed at determining the tissue-specific uptake and bioconcentration of NET in two freshwater fishes: fathead minnow and channel catfish. The studies on bioconcentration of pharmaceuticals are significant because current regulatory efforts to understand the environmental and human risks of chemicals are based on bioaccumulation assessments (Arnot and Gobas 2006). In addition, a chemical's ability to bioaccumulate can be used to predict long-term adverse effects that are not always addressed by acute toxicity and short-term exposure tests.

Table 1 Physicochemical characteristics of NET (retrieved from EPISUITE [USEPA 2009])

Property	Description
CAS no.	68-22-4
Molecular weight	298.43
Partition coefficient (log K_{ow})	2.97
Log D_{ow} (log P @ pH 7.0)	3.15[a]
Water solubility @ 25°C (mg l^{-1})	7.04
Vapor pressure @ 25°C (mm Hg)	7.31E−09
Henry's law constant @ 25°C (atm-m^3 mol^{-1})	5.8E−10
Estimated half-lives (h)	
Water	1.44E+3
Soil	2.88E+3
Sediment	1.3E+4
Environmental persistence[b] (h)	2.1E+3
Structure	

[a] See http://www.cerep.com

[b] Using emission rates of 1000 kg/h

Experimental

Chemicals and Reagents

Norethindrone (NET; (17α)-17-hydroxy-19-norpreg-4-en-20-yn-3-one, CAS no. 68-22-4) and norethindrone-d6 (NET-d6; 4-estren-17α-ethynyl-17β-ol-3-one-d6) were obtained from Toronto Research Chemicals (North York, ON, Canada). High-pressure liquid chromatography (HPLC)-grade solvents acetonitrile, methanol, *n*-hexane, ethyl acetate, dichloromethane, dimethylformamide, and acetone were procured from Fisher Scientific (Houston, TX). *N*-methyl-*N*-(trimethyhlsilyl) trilfluoroacetamide (MSTFA; CAS no. 24589-74-4) derivatization reagent was purchased from Thermo Scientific (Rockford, IL). Tricainemesylate (MS-222; ethyl 3-aminobenzoate methanesulfonic acid (CAS no. 886-86-2) was obtained from Sigma-Aldrich (St. Louis, MO). Milli-Q water (18.3 M Ω cm) was obtained in the laboratory from Milli-Q Water System (Millipore, Billerica, MA). Dilution water in this study was City of Denton dechlorinated tap water.

Fish Exposures

After a week's acclimation to the standard laboratory conditions of temperature 25 ± 2°C and a 16:8-h light-to-dark cycle, adult male fathead minnow (source: UNT aquatic toxicology laboratory; 2.9 ± 0.9 g) and juvenile male channel catfish (source: Pond King, Gainesville, TX; 49.9 ± 9.7 g) were exposed to 50 or 100 μg/l NET (5–10% 48-h LC_{50} [unpublished data]) using the following experimental designs: In the fathead minnow test, fish ($n = 50$) were randomly distributed into two 20-l tanks and exposed to 50 μg NET/l (in dimethylformamide [DMF] < 0.003% in exposure tanks) for 28 days in a continuous flow-through system. The fish were then allowed to depurate in clean water for 14 days (Organization for Economic Co-operation and Development [OECD] 1996). For the catfish study, 20 fish were randomly distributed into two 60-l glass tanks and exposed to 100 μg NET/l under similar flow-through conditions. This test consisted of 7 days of exposure and depuration for 1 week in dilution water. To account for possible effects of carrier solvent (DMF), a solvent control ($n = 10$) was included in both of the tests. Minnows and catfish were fed brine shrimp flakes or trout chow, respectively, to satiation twice daily during the course of the experiments.

Sample Collection

For the fathead study, at the end of days 1, 3, 7, 14, 28, 35, and 42, fish ($n = 4$ to 6) were killed in a solution of MS-222 (100 mg/l), and wet weight and length were recorded. Muscle, liver, and kidney tissues were excised to record weights (liver and kidney) and later frozen for analysis. In the catfish test, fish ($n = 4$) were sampled after days 1, 3, 7, and 14 and anaesthetized to collect blood and tissues (muscle, brain, gill, and liver). Blood was collected from the caudal vein using heparinized syringes into test tubes with heparin, and plasma was later separated by centrifugation (8000 rpm for 5 min). Liver and brain weights were recorded, and all of the collected tissues were stored at −20°C for further processing. Water samples in the exposure tanks were collected 4 times (days 0, 1, 3, and 7) in the catfish test and 7 times (days 0, 1, 3, 7, 14, 21, and 28) in fathead minnow test to determine the measured exposure concentration.

NET Extraction and Cleanup

All extraction procedures on the test samples were preceded by the addition of 5 ng d6-NET. NET was extracted from water samples (1 after 2 ml) after liquid–liquid extraction with 1 + 1 (v/v) hexane/ethyl acetate (approximately 5 ml), hereafter referred to as "sample solvent." Plasma samples (approximately 200 μl) were treated with 5 ml ice-cold acetone, evaporated, and back-extracted into the sample solvent. The extracted contents from water and plasma samples were dried under nitrogen for further processing. Approximately 0.1 to 0.2 g blot-dried tissue was homogenized in 4 ml sample solvent using a Mini-Beadbeater (Biospec Products, Bartlesville, OK). The extracted contents were filtered into preweighed vials using 0.45-μm polytetrafluoroethylene filters (Whatman, Sanford, ME). The tissue extracts needed a cleanup method. Briefly, organic extracts were dried, and lipid weights (catfish test only) were determined gravimetrically. The dried residues were resolubilized in acetonitrile (1 ml) and stored overnight at 4°C followed by centrifugation and separation of the supernatant. The resultant "clean" extract was later evaporated to dryness.

GC/MS Analysis

Dried organic extracts were subjected to derivatization with MSTFA according to methods described elsewhere (Shareef et al. 2006). The derivatized residues were resolubilized in 100 μl DCM and analyzed using GC–MS. An Agilent gas chromatographer 6890N (Palo Alto, CA) connected to a mass selective detector (MSD 5973; Agilent) was used for quantification. Briefly, samples were autoinjected (2 μl) in pulsed splitless mode at 260°C onto a 30 m × 0.25 mm × 0.25 μm EC-5 capillary column (Alltech, Deerfield, IL). Ultrapure helium served as a carrier gas, and separation was achieved in the column using the following temperature program for the GC oven: initially at 40°C for 3 min, increased to 200°C at 15°C/min with no hold time, followed by final ramp to 300°C at 15°C/min and held at this temperature for 10 min for a total run time of 36.17 min. The MS quadrupole, source, and transfer line temperatures were set at 150, 230, and 280°C, respectively. MSD was operated under selected ion monitoring mode with a dwell time of 50 msec for the following ions: NET-d6-TMS (309, 361, and 376) and NET-TMS (303, 355, and 370; underlined ions used in quantification). NET quantification was achieved using an eight-point calibration curve (4000 to 31 pg/μl).

Condition Factor and Somatic Indices

Fish weight (g) and caudal length (cm) were used to calculate Fulton's condition factor ($K = \text{weight}/(\text{length})^3 \times 100$). Liver, kidney, and brain weights were used to determine the corresponding somatic indices (tissue weights expressed as % body weights) *viz.*, hepatic somatic index, nephritic somatic index, and brain somatic index (BSI).

BCF Estimation

Tissue-specific BCFs were estimated using two approaches: (1) proportional BCF (BCF_p) calculated as the ratio between the mean concentration of NET in a tissue and the time-weighted measured-exposure concentration and (2) kinetic BCF (BCF_k) determined as the ratio between uptake and depuration rate constants. Assuming first-order kinetics, uptake (k1) and depuration (k2) rate constants were determined using a sequential method that combines linear and nonlinear regression models (Newman 1995) using the following equation (Eq. 1):

$$C_f = k1/k2\ C_w[1 - \exp(-k2t)],$$

where C_f and C_w correspond to the chemical concentrations in fish and water, respectively, and t is the time (days). k2 was first computed using a simple linear curve fit model as follows (Eq. 2):

$$\ln Cf = a(t) + b.$$

Data Analysis

Data analysis was performed using SAS (version 9.1; Cary, NC). In all of the statistical tests, $p < 0.05$ was considered significant. The differences in the condition factor and somatic indices of the control and NET-exposed fish were determined using Student *t* test and Kruskal–Wallis analysis of variance (ANOVA), respectively. One-way ANOVA, followed by SNK multiple range testing, showed differences in tissue uptake levels in the catfish. All of the results were expressed as means ± SEMs (for measured concentration and quality-control results, SDs were used to indicate the variation).

Results

Water Quality and Measured Test Concentrations

Weekly measured water-quality parameters (means ± SDs [$n = 5$]) temperature, pH, and dissolved oxygen in exposure tanks were 21.3 ± 0.6°C, 7.4 ± 0.3, and 7.9 ± 0.7 mg/l, respectively. The data indicated fairly constant levels of oxygen saturation and pH in the continuous flow-through exposure system. During the 28-day exposure, the mean (±SD) time-weighted measured NET water concentration (µg/l) was 35.4 (± 8.78 [$n = 7$]), which is 71% of the nominal exposure level (50 µg/l). In the catfish test, the average measured exposure concentration during the 7-day period was approximately 82% (82.5 ± 8.5 [$n = 4$]) of the nominal exposure level of 100 µg/l. NET was not detected (<8 µg/l) in the solvent control.

Condition Factor and Tissue Somatic Indices

The mean condition factors of the NET-exposed fathead minnow and catfish were not significantly different ($p = 0.45$ and $p = 0.16$, respectively) from that of the control fish (data not shown), indicating the absence of chemical stress in the exposed fish. Additional information on the physiological status of the fishes was obtained from tissue somatic indices (data not shown). For the fathead minnows, relative liver (except for fish sampled at day 1) and kidney weights of NET-exposed fish were larger than those of the control fish; however, these were not statistically significant ($p = 0.32$ and $p = 0.06$, respectively). BSIs of catfish in the control and exposed groups (both uptake and depuration) did not differ significantly ($p = 0.18$). Significantly higher ($p = 0.01$) liver weights were observed in the NET-exposed catfish compared with those of the control fish. The relative liver weights of the depurated fish reverted to weights comparable with those of the control fish, indicating that the observed increase was due to NET exposure.

Tissue-Specific NET Concentrations and BCFs

Fathead Minnow

NET concentration (ng/g wet weight [ww]) in muscle, liver, and kidney tissues of fathead minnow sampled during exposure and depuration are presented in Fig. 1. The uptake levels by muscle, liver, and kidneys ranged from 47 to 167, 75 to 411, and 576 to 1445 ng/g ww, respectively. Overall, accumulation levels in various tissues had the following trend: muscle < liver < kidney. There were no detectable levels (<25 ppb) of NET in tissues of fish from the solvent control as well as depurated fish.

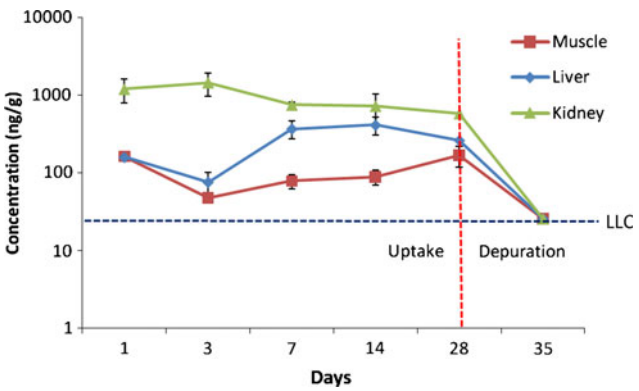


Fig. 1 NET concentration (mean ± SEM ng/g ww, $n = 1$–5) in muscle, liver, and kidney tissues of fathead minnow exposed to 50 µg/l NET. NET concentration was below the lower limit of calibration (LLC; 25 ppb) in tissues of depurated fish

Table 2 Tissue-specific 28-day kinetic and proportional NET BCFs for fathead minnow exposed to 50 µg/l NET

Tissue	BCF_k	BCF_p
Muscle	2.6	4.7
Liver	9.3	7.4
Kidney	26.8	16.3

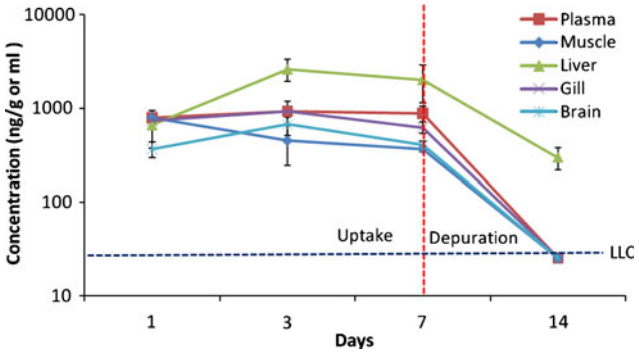


Fig. 2 NET concentration (mean ± SEM ng/g ww or µg/l plasma, $n = 2$ to 5) in different tissues of channel catfish exposed to 100 µg/l NET. NET concentration was below the lower limit of calibration (LLC; 25 ppb) in tissues of depurated fish

Tissue-specific NET kinetic and 28-day proportional BCFs for fathead minnow ranged from 2.9 to 26.8 (Table 2). In accordance with the tissue uptake trend, kidney and muscle tissues had the highest and lowest BCFs, respectively, whereas the BCF for the liver tissue was intermediate. The measured BCFs were well below (<2%) the regulatory trigger value (BCF = 2000), indicating a low bioconcentration potential for NET (European Commission 2003).

Channel Catfish

NET concentrations (ng/g ww) in muscle, brain, gill, liver, and plasma (µg/l) of catfish are presented in Fig. 2. Accumulation in muscle tissue decreased from days 1 through 7 with a 47% decrease in the day-7 tissue concentration. In all of the other tissues, there was an increase in the concentration, with the highest accumulation noticed at day 3 after exposure. Although the increase in NET concentration from days 1 through 3 was approximately 4× for liver tissue, the corresponding increases were smaller (approximately 1.5×) for plasma, gill, and brain tissues.

Tissue-specific kinetic and 7-day proportional NET BCFs for catfish are listed in Table 3. BCFs (ww) ranged from 4.5 to 40 with the following trend: liver > plasma > gill > brain > muscle. The 7-day lipid-normalized BCFs were approximately 1 order of magnitude greater than the corresponding wet-weight BCFs.

Table 3 Tissue-specific kinetic and 7-day proportional BCFs for channel catfish exposed to 100 µg/l NET

Tissue	BCF_k	BCF_p	
		Wet-weight basis	Lipid normalized
Muscle	7.1	4.5	186
Brain	7.4	4.9	39.5
Gill	11.1	7.5	73.9
Plasma	13.4	10.6	–
Liver	40.8	24.5	251.7

Expressing BCFs on the basis of lipid weights is a way to normalize the data for better comparison among species. Although the current regulatory criteria are not based on lipid-normalized BCFs, such data are useful for achieving better harmonization (Arnot and Gobas 2006). In addition, lipid-based BCFs can be used to compute whole-body wet-weight BCFs. In this study, the tissue-specific NET BCFs obtained for both the species did not differ significantly (i.e., by orders of magnitude). Both species showed a low tendency (i.e., BCF < 2000) to bioconcentrate NET.

QC Results

The accuracy of the extraction methods was expressed in terms of the percent recoveries obtained by spiking a known concentration of NET (5 ng) to the dilution water, method blank (extraction solvent), control fish plasma, and tissues, with results listed in Table 4. The precision (% RSD) of the instrument was estimated at 2.1% ($n = 11$) using a continuing calibration standard (CONCAL).

Discussion

This article is the first report on the experimentally determined BCFs of NET in fish. Minnows and catfish were exposed to a single concentration of NET because Woodburn and Springer (2004) indicated that the BCF values did not differ when fish were exposed to multiple levels of selected test chemicals. Taking the data from the two species collectively, NET uptake by various tissues had the following rank order: kidney > liver > plasma > gill > brain > muscle. Mammalian pharmacology of NET indicates that it is subjected to first-pass metabolism, approximately 61% bound to albumin, and after extensive biotransformation, >50% is eliminated by way of urine and the remainder through biliary excretion (http://www.rxlist.com). An in vitro study on NET clearance from fish hepatic tissues showed that NET is metabolized in fish (Gomez et al. 2010). Higher accumulation of NET is expected in the tissues with greater perfusion rates because

Table 4 NET % recovery (mean ± SDs) for different sample matrices

Test	Matrix	*n*	% Recovery
Fathead minnow	Water	1	98.7
	Tissue[a]	6	115 ± 21
Channel catfish	Method blank[b]	2	112 ± 13.4
	Plasma	3	112 ± 24
	Tissue	11	120 ± 14.4

[a] Muscle tissue from control fish

[b] Sample solvent

Table 5 Partition coefficient[a] for NET in different compartments based on tissue-specific catfish bioconcentration

Tissue compartments	Partition coefficient
Blood:water	10.7
Blood:muscle	2.4
Blood:liver	0.4
Blood:gill	1.4
Blood:brain	2.2

[a] Calculated from the ratio of concentration of NET in plasma to that in water or tissue

the chemical is fairly lipophilic such that it crosses the endothelial layer lining the capillaries and hence has greater partitioning into liver and kidneys (Table 5). NET was also detected in gills, indicating that this could be an important site for the uptake and clearance. Although NET was not determined in the fish bile in our study, such an analysis could be useful in predicting the magnitude of exposure (in terms of bioaccumulation) in wild animals (Larsson et al. 1999; Pettersson et al. 2006) as well as in metabolite profiling (Kallio et al. 2010). One of the mammalian target tissues for NET is the brain, where this study observed measurable NET residues.

The bioaccumulative potential of estrogenic compounds by algae and aquatic invertebrates has previously been reported (Lai et al. 2002; Gomes et al. 2004; Dussault et al. 2009). However, the potential bioaccumulation of sex hormones in aquatic vertebrates and the resultant altered sexual functions is of concern because of greater homology of steroid receptors between fish and mammals (Gunnarsson et al. 2008; Al-Ansari et al. 2010). For example, estrone, 17 β-estradiol, and EE2 were reported to concentrate in the bile of rainbow trout exposed to an STP effluent. The accumulation levels were at least 4 to 6 orders of magnitude greater than the water concentrations (Larsson et al. 1999). In another study, fathead minnows exposed (for 158–245 days) to EE2 at 12 and 47 ng/l had whole-body BCFs of 660 and 610, respectively (Länge et al. 2001). Bioconcentration of androgens in fish has also been reported. Testosterone exposure at a nominal concentration of 1 μg/l for 6 days in three-spined stickleback resulted in a plasma BCF of 200 (Maunder et al. 2007). Although studies exist to confirm the bioaccumulation of estrogens and androgens in fish, current understanding on the potential toxicological effects, including the bioaccumulative potential of progesterone and/or its synthetic analogues in the aquatic environment, is limited (Besse and Garric 2009).

In our study, tissue-specific wet-weight BCFs ranged from 2.6 to 40.8, indicating that NET has a low potential to bioconcentrate in these two fishes. In addition, the BCF levels are well below the current regulatory trigger value of 2000 used in pharmaceutical prioritization approaches (EMEA 2006). In contrast, Environment Canada has no separate ERAs for pharmaceuticals, and hence the medicinal products are also subjected to persistent (P), bioaccumulative (B), and toxic (T) chemical assessments. Under the United States Environmental Protection Agency's "PBT" policy, a compound with BCF value between 100 and 1000 is of "medium concern" in terms of its environmental effects. Based on the aforementioned regulatory guidelines, no environmental risk assessments are necessary for NET. However, it should be noted that the BCF criteria in prioritization schemes apply only when other potential concerns, such as endocrine disruption, genotoxicity, etc., are absent. NET has recently been shown to induce reproductive effects in fish even at concentrations as low as 25 ng/l and hence may require complete ERA assessments if predicted environmental concentrations are high (Paulos et al. 2010).

Laboratory data are essential for determining BCFs, which are used in PBT assessments, but they can also help make better sense of field-collected fish-tissue data. From the catfish studies, in which water, plasma, and selected tissue NET concentrations were determined, partition coefficients can be calculated. Calculated blood:water ($P_{B:W}$), blood:muscle ($P_{B:M}$), blood:liver ($P_{B:L}$), blood:gill ($P_{B:G}$)n and blood:brain ($P_{B:B}$) partition coefficients are listed in Table 5. The importance of these partition coefficients resides in the ability to take a single tissue measurement and calculate additional tissue burdens. For instance, if a plasma concentration is known, then one can calculate muscle, liver, etc., concentrations based on the partition coefficients. In addition, the coefficients can be useful when comparing fish data with mammalian data (Huggett et al. 2004). For example, the fish plasma model, developed by Huggett et al. (2003), compares fish plasma concentrations of pharmaceuticals with corresponding human therapeutic plasma concentrations to predict potential chronic risk to fish.

It is important to emphasize the need for tissue-specific BCF assessments here. For example, in our study, the

highest (liver) and lowest (white muscle) NET wet-weight BCFs differed by 1 order of magnitude. These values are likely not a large difference from a regulatory perspective because fish tissue-specific BCFs have been reported to differ by several orders of magnitude (Schwaiger et al. 2004). In the absence of potential concerns, such as endocrine disruption, bioconcentration becomes an important regulatory end point in prioritizing pharmaceuticals for ERAs. Bioconcentration studies are often focused either on the uptake of a chemical in white muscle or whole-fish BCFs (Paterson and Metcalfe 2008). Therefore, it is essential to generate tissue-specific BCF data at least on a few selected compounds. Tissue-specific accumulation data are useful for developing physiologically based pharmacokinetic models, which are primarily based on partitioning (among different tissue compartments) parameters (Table 5). Tissue-specific BCFs will also be helpful in identifying tissues that may be targets for a chemical: The highest concentration may be at the site (i.e., tissue) of action. It may not be necessary to analyze every single tissue, but the selection could be based on the available mammalian drug target information, metabolically important tissues, etc. This selection process ensures the selection of optimum BCFs in screening approaches.

In summary, NET has a low potential to bioconcentrate in fish. The NET tissue-specific BCF assessments gathered in this study will be useful for future laboratory toxicity and field assessments of NET.

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