Environmental Toxicology

Dietary Bioaccumulation and Biotransformation of Hydrophobic Organic Sunscreen Agents in Rainbow Trout

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Abstract: The present study investigated the dietary bioaccumulation and biotransformation of hydrophobic organic sunscreen agents, 2-ethylhexyl-4-methoxycinnamate (EHMC) and octocrylene (OCT), in rainbow trout using a modified Organisation for Economic Co-operation and Development 305 dietary bioaccumulation test that incorporated non-biotransformed reference chemicals. Trout were exposed to 3 dietary concentrations of each chemical to investigate the relationship between dietary exposure concentration and observed accumulation and depuration. Both EHMC and OCT were significantly biotransformed, resulting in mean in vivo whole-body biotransformation rate constants (k_{MET}) of 0.54 ± 0.06 and 0.09 ± 0.01 d⁻¹, respectively. The k_{MET} values generated for both chemicals did not differ between dietary exposure concentrations in the fish were not high enough to saturate biotransformation enzymes. Both somatic and luminal biotransformation factors (BCFs) of EHMC averaged 0.0035 kg lipid kg lipid⁻¹ and 396 L kg⁻¹, respectively, whereas those of OCT averaged 0.0084 kg lipid kg lipid⁻¹ and 1267 L kg⁻¹. These values are 1 to 2 orders of magnitude lower than the BMFs and BCFs generated for reference chemicals of similar log K_{OW} . In addition, for both chemicals, derived BMFs and BCFs fell below established bioaccumulation criteria (1.0 kg lipid kg lipid⁻¹ and 2000 L kg⁻¹, respectively), suggesting that EHMC ad OCT are unlikely to bioaccumulate to a high degree in aquatic biota. *Environ Toxicol Chem* 2020;00:1–13. © 2019 SETAC

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INTRODUCTION

Organic ultraviolet filters (UVFs) are used in personal care products, including sunscreens and cosmetics, to protect the skin from negative effects of UV exposure. In addition, UVFs are used in paints, plastics, and textiles to prevent UV-induced degradation (Tang et al. 2019). Depending on their usage, individual UVFs can enter the aquatic environment via wastewater-treatment plant effluents or by loss from skin during swimming and other recreational activities. Measurable concentrations of UVFs have been reported in surface waters, sediments, sewage sludge, and aquatic biota (Nagtegaal et al. 1997; Balmer et al. 2005; Buser et al. 2006; Zenker et al. 2012, 2013; Picot Groz et al. 2014). Some UVFs are hydrophobic (log $K_{OW} > 4$), which

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has led to concern that they may bioaccumulate in aquatic organisms (Balmer et al. 2005).

2-Ethylhexyl-4-methoxycinnamate (EHMC) and octocrylene (OCT; Figure 1), 2 of the most widely used UVFs, enter the environment primarily via their application in cosmetic products (Christen et al. 2011; Blüthgen et al. 2014). Both chemicals are very hydrophobic (log K_{OW} of 5.80 and 6.88 for EHMC and OCT, respectively), and both have been detected in piscivorous birds (Fent et al. 2010) and marine mammals (Gago-Ferrero et al. 2013; Alonso et al. 2015). Field-derived biomagnification factors (BMFs) for EHMC, obtained by comparing measured concentrations in freshwater fish and their invertebrate prey, ranged from 0.6 to 1.5 kg lipid kg lipid⁻¹ (Fent et al. 2010). A field-derived BMF of 1.1 kg lipid kg lipid⁻¹ was reported for OCT, based on the ratio of measured concentrations in 2 species of marine fish and a smaller prey fish species (Peng et al. 2017). In contrast, Pawlowski et al. (2019) reported a laboratory-derived BMF of 0.034 kg lipid kg lipid⁻¹ for OCT. Presently, there are no established regulatory criteria for chemical BMFs in fish; however, a BMF \geq 1.0 is generally



FIGURE 1: Structures, names, abbreviations, and Chemical Abstracts Service registry numbers of 2-ethylhexyl-4-methoxycinnamate (EHMC) and octocrylene (OCT).

interpreted as evidence of probable bioaccumulation potential (Gobas et al. 2009).

Although several field studies have reported concentrations of EHMC and OCT in fish and other higher-trophic level organisms, empirical bioconcentration factors (BCFs; US National Library of Medicine 2006; Blüthgen et al. 2014; Sigma-Aldrich 2014; Pawlowski et al. 2019), bioaccumulation factors (Fent et al. 2010), and biota-sediment accumulation factors (Gago-Ferrero et al. 2015; Tang et al. 2019) in fish tend to fall well below established bioaccumulation criteria (Table 1). These findings suggest that some fish species have the capacity to biotransform EHMC and OCT. Specifically, ester groups present on both chemicals (Figure 1) may provide a target for esterases and/or cytochrome P450 (CYP) enzymes. Both EHMC and OCT were significantly biotransformed in vitro by rainbow trout liver S9 fractions (Saunders et al. 2019). Incubations performed with and without added NADPH (a reduced form of nicotinamide adenine dinucleotide phosphate) suggested that hydrolysis by carboxylesterases and CYP-mediated

TABLE 1: Bioaccumulation metrics reported for 2-ethylhexyl-4methoxycinnamate and octocrylene

Metric	EHMC	OCT	Bioaccumulation criteria	Regulatory program
Log K _{OW} BCF	5.80 ^ª 175–433 ^b	6.88 ^a 41–972 ^{c,d,e}	≥4.50 ≥2000 "B" ≥5000 "very B"	REACH REACH
BAF	12–970 ^{f,g}	16–125 ^f	≥5000	CEPA
BMF, lab derived	N/A	0.034 ^e	≥1.0	NL
BMF, field derived	0.6–1.5 ^g	1.1 ^h		
BSAF	0.04–0.3 ⁱ	0.04–0.3 ⁱ	≥1.0	NL
^a Gago-Ferrero ^b US National L ^c Blüthgen et al ^d Sigma-Aldrich	et al. (2012). ibrary of Medi . (2014). (2014)	cine (2006).		

^ePawlowski et al. (2019).

^fTang et al. (2019).

⁹Fent et al. (2010).

^hPeng et al. (2017).

Gago-Ferrero et al. (2015).

B = bioaccumulative; BAF = bioaccumulation factor (liters per kilogram); BCF = bioconcentration factor (liters per kilogram); BMF = biomagnification factor (kilograms of lipid per kilogram of lipid); BSAF = biota-sediment accumulation factor (kilograms of organic carbon per kilogram of lipid); CEPA = Canadian Environmental Protection Act; EHMC = 2-ethylhexyl-4-methoxycinnamate; OCT = octocrylene; REACH = European Union Registration, Evaluation, Authorization, and Restriction of Chemicals; N/A = not available; NL = not listed under a regulatory program.

biotransformation are important metabolic routes for both chemicals (Saunders et al. 2019).

The rate of biotransformation is a key parameter in computational models used to predict chemical bioaccumulation in fish (Arnot and Gobas 2003, 2004). Presently, information on UVF biotransformation in fish is limited. In vivo biotransformation rates for EHMC and OCT, expressed as apparent whole-body biotransformation rate constants (k_{MET} ; per day), can be estimated by in vitro-in vivo extrapolation of measured in vitro activity (Saunders et al. 2019). Alternatively, these rates may be predicted using existing quantitative structure-activity relationship models (EPISuite; US Environmental Protection Agency 2012). In vivo data are needed, however, to evaluate the accuracy of biotransformation rates predicted using either approach.

The present study investigated the in vivo biotransformation and bioaccumulation of EHMC and OCT in rainbow trout using a modified Organisation for Economic Co-operation and Development (OECD) 305 dietary bioaccumulation test that incorporates nonbiotransformed reference chemicals (Lo et al. 2015b). The main objective of the study was to generate wholebody biotransformation rate constants for EHMC and OCT at 3 different dosing levels. Three dosing levels were selected to evaluate the potential concentration dependence of in vivo biotransformation because the concentration dependence of in vitro biotransformation for these 2 chemicals was recently demonstrated (Saunders et al. 2019). Measured BMFs for EHMC and OCT were obtained directly from the resulting data sets. A model-based approach was then employed to estimate BCFs (Gobas and Lo 2016) and rates of biotransformation in the lumen of the gastrointestinal tract (Lo et al. 2015b).

MATERIALS AND METHODS

Fish

Rainbow trout (Oncorhynchus mykiss, Erwin strain) were obtained as eggs from the US Geological Survey Upper Midwest Environmental Sciences Center in LaCrosse, Wisconsin, and reared to desired size (~35 g) at the US Environmental Protection Agency laboratory in Duluth, Minnesota. The study was performed in 40-gallon fiberglass tanks (Dura-Tech Industrial and Marine) supplied with 0.5 L min⁻¹ Lake Superior water (singlepass, sand-filtered, and UV-treated). Fish were fed commercial fish chow (3.0 mm Skretting sinking chow) at a target rate of 1.3% body weight per day. Mean (±standard deviation [SD]) water characteristics were temperature 11 ± 0.5 °C, pH 7.4 ± 0.02 , total organic carbon 1.60 ± 0.29 mg L⁻¹, total ammonia $0.07\pm0.01\,\text{mg}\,\text{L}^{-1},$ and dissolved oxygen $90\pm0.02\%$ of saturation. The study was performed under a 12:12-h light:dark schedule. Fish were acclimatized for 2 wk to the experimental conditions before initiating chemical exposures.

Chemicals

The test chemicals EHMC (Chemical Abstracts Service [CAS] no. 5466-77-3) and OCT (CAS no. 6197-30-4) were purchased from Sigma-Aldrich. 1,3,5-Trichlorobenzene (3TCBz), 1,2,4,5tetrachlorobenzene (4TCBz), pentachlorobenzene (PCBz), hexachlorobenzene (HCBz), d⁸-naphthalene, and d¹²-chrysene were obtained from Sigma-Aldrich. 2,2',5,5'-Tetrachlorobiphenyl (PCB 52), 2,3,4,4',6-pentachlorobiphenyl (PCB 115), and 2,2',4,4',6,6'-hexachlorobiphenyl (PCB 155) were purchased from AccuStandard. ¹³C-Hexachlorobenzene (¹³C-HCBz) was obtained from MSD Isotopes (now Cambridge Isotopes). Solvents were purchased from Fisher Chemical. Bondesil-C18, and primary secondary amine (PSA) silica bulk sorbents were purchased from Agilent Technologies. All chemicals were reagent-grade or higher in quality, with purities >97%.

Study design

Fish were fed a control diet or a contaminated diet containing EHMC or OCT and 6 reference chemicals (i.e., 3TCBz, 4TCBz, PCBz, HCBz, PCB 52, PCB 155) at a target daily feeding rate of 1.3% body weight per day. The reference chemicals were selected because of their recognized persistence and resistance to biotransformation. Although biotransformation of some reference chemicals (e.g., PCB 52) has been observed in fish (Koenig et al. 2012), biotransformation rates were found to be too low to have a significant effect on the derivation of whole-body biotransformation rate constants in fish (Lo et al. 2015b).

Dietary concentrations of EHMC and OCT were varied by approximately 2 orders of magnitude to represent low, medium, and high treatment levels. Measured concentrations of EHMC averaged 0.004, 0.038, and 0.318 mmol kg⁻¹ for the low, medium, and high treatment levels, respectively, whereas those of OCT averaged 0.003, 0.086, and 1.05 mmol kg⁻¹ (Supplemental Data, Table S1). Dietary concentrations were below chronic toxicity thresholds (no- and lowest-observed-effect levels) reported in ECOTOX (US Environmental Protection Agency 2018). Measured concentrations of reference chemicals in food ranged from approximately 0.01 to 0.35 mmol kg⁻¹ (Supplemental Data, Table S1).

Dietary exposures were conducted in 7 tanks (6 test and 1 control), each of which contained 21 fish to start. Fish in treatment tanks were exposed to a contaminated diet for 14 d, followed by a 14-d depuration period when fish were fed the control diet. All fish were fed daily at 3:00 PM. On sampling days, fish were collected by 9:00 AM. Three fish were collected from each treatment tank on days 7, 14, 15, 17, 19, 22, and 28 of the experiment and analyzed independently. Four or 5 fish from the control tank were collected on days 7, 14, 17, 22, and 28 and analyzed independently to test for sample background contamination and toxicity.

Fish were euthanized with an overdose of buffered ethyl 3-aminobenzoate methanesulfonate (Finquel; Argent Laboratories). Sampled fish were separated into liver, gastro-intestinal tract (minus the pyloric ceca, stomach, and gut contents), and carcass. The anterior intestine was combined with the carcass samples for analysis. Samples were frozen at -80 °C until processing and extraction.

Food preparation

Test and reference chemicals were dissolved in 15 mL acetone containing 0.875 g corn oil. This spiking solution was slowly added to 175 g of fish food and left to mechanically stir in an open system overnight. The spiked diets were then stored at $-20 \,^{\circ}$ C in sealed containers. The control diet was prepared in a similar manner but without test or reference chemicals. All fish received the control diet during a 2-wk acclimation period prior to the experiment. Measured concentrations of EHMC, OCT, and reference chemicals in the control diet were below their method detection limit (MDL; Supplemental Data, Table S2).

Sample preparation and extraction

Samples were extracted using a modified quick, easy, cheap, effective, rugged, and safe (Quechers) method. These procedures were based on those used to extract UVFs in marine mussels (Picot Groz et al. 2014) and are similar to methods for extracting polychlorinated biphenyls from fish tissues (Chamkasem et al. 2016). Whole-liver (283-966 mg) samples were processed in their entirety. Carcass samples (35.98-56.57 g) were homogenized with 2 volumes of Milli-Q (MQ) water and extracted in 6-g batches. Fish food was subsampled on days 0, 7, and 14 of the exposure and extracted in 1-g batches. Each sample was placed in a 50-mL polypropylene centrifuge tube and spiked with $50\,\mu\text{L}$ of a 10-ppm internal standard solution prepared in acetone. A volume of MQ water was added, followed by 30s of vortexing. Acetonitrile (ACN) was added, and the tube was vigorously shaken by hand for 2 min. A salt mixture containing 8 parts anhydrous Na₂SO₄, 2 parts NaCl, 2 parts sodium citrate dihydrate, and 1 part sodium citrate dibasic sesquihydrate was added. The tube was then shaken by hand for 1 min and centrifuged at 3500 g for 5 min. Following centrifugation, the upper ACN layer was transferred to a 15-mL polypropylene tube containing 1 g of bulk sorbents (3 parts Na₂SO₄, 1 part Bondesil-C18, and 1 part PSA silica) for dispersive solid-phase extraction (d-SPE). Formic acid was added, followed by 1 min of vortexing and 5 min of centrifugation at 5000 g. The exact amounts of added water, ACN, salts, sorbents, and formic acid were adjusted to each type of sample and are given in Supplemental Data, Table S3.

Following d-SPE, the ACN supernatant was transferred to a 4-mL amber glass vial, placed under a gentle stream of N₂, and evaporated to approximately 0.5 mL at 35 °C. N-Hexane (0.5 mL) was added to the vial, and the sample was vortexed for 1 min to extract analytes and internal standards. The combined hexane–ACN extract was transferred quantitatively to a second 2-mL amber vial (2 rinses with 250 μ L n-hexane). The vial was then centrifuged at 7000 g for 10 min. Finally, the extract was filtered through a Pasteur pipette containing 0.25 g of hexane-washed d-SPE sorbents to eliminate sample lipids. Filtered extracts that were below the MDL were pooled and reanalyzed. Extraction recoveries and MDLs of UVFs and reference chemicals in fish tissues and food are presented in Supplemental Data, Table S2.

Gas chromatography-mass spectrometry

Sample extracts were analyzed using an Agilent 6890 N gas chromatograph coupled to an Agilent 5975C mass spectrometer. Separations were performed on a DB-1HT $15 \text{ m} \times 320 \mu \text{m}$, $0.25 \mu \text{m}$ film column (Agilent). The oven temperature was 45 °C for 1.5 min, increasing to 150 °C at 15 °C min⁻¹, finally increasing 10 °C min⁻¹ to 285 °C, and held for 5 min. The injection port and ion source temperatures were 45 and 230 °C, respectively. The carrier gas was helium flowing at 1 mLmin⁻¹. The mass spectrometry data were acquired in selected ion monitoring mode (136 for d⁸-naphthalene, 180 for 3TCBz, 216 for 4TCBz, 240 for d¹²-chrysene, 250 for PCBz, 284 for HCBz, 290 for ¹³C-HCBz, 292 for PCB 52, 326 for PCB 115, and 360 for PCB 155). For EHMC, the quantification and identification ions were 178 and 161, respectively. For OCT, the quantification ion was 249, and the identification ions were 232 and 204. A 1- $\!\mu\text{L}$ sample of extract was injected into the column using a 5-µL gas-tight glass syringe (Agilent). Peak areas were integrated and used to quantify test chemicals using Chemstation software (Hewlett Packard). Chemical concentrations were calculated using the relative response factor approach.

Lipid content determination

Total lipid content (Bligh and Dyer 1959) was determined for livers from sampled control fish that had not been selected for chemical extraction (days 7, 14, 17, 22, 28). Additional measurements were made for homogenized carcass samples from all control and exposed animals on days 7, 14, 22, and 28. The carcass lipid content averaged across these sampling days provided an overall mean value for each tank. Then, to determine the fractional lipid content of whole fish $(\phi_{\rm BL})$ from treatment and control tanks, the mean mass of lipid in the carcass samples was added to the mean mass of lipid determined for livers from control fish and divided by the combined wet weights of the 2 samples. This approach assumes there were no treatment-related effects on the lipid content of the liver. Fish food containing 0.5% corn oil was also analyzed for total lipids to determine the fractional dietary lipid content (ϕ_{DL}).

Chemical concentrations in the fish

Chemical concentrations in the liver (C_L) were determined by dividing the chemical masses measured in the liver by the wet weights of the liver. Chemical concentrations in the fish soma (C_B) were determined by summing chemical masses measured in liver and carcass samples and then dividing by the combined wet weight of the 2 compartments.

Whole-body depuration rate constants

Whole-body depuration rate constants (k_{BT} ; per day) were derived for EHMC, OCT, and reference chemicals by

linear regression of the natural logarithm of chemical concentrations in the fish soma against time during the depuration phase

$$LnC_{t} = LnC_{B} - k_{BT} \times t$$
⁽¹⁾

where C_B is the chemical concentration (µmol kg⁻¹) at the beginning of the depuration period and C_t is the concentration at time t (days).

Whole-body biotransformation rate constants

To determine whole-body biotransformation rate constants, a linear least squares weighted regression of the measured depuration rate constants of the reference chemicals ($k_{BT,R}$) and the reciprocal of each chemical's K_{OW} (1/ K_{OW}) was performed

$$k_{\rm BT,R} = \frac{1}{\omega} \times \frac{1}{K_{\rm OW}} + \beta \tag{2}$$

where $1/\omega$ and β are regression coefficients in units of days (Gobas and Lo 2016). The slope term $(1/\omega)$ describes the depuration of hydrophobic organic chemicals to water predominantly via the respiratory route (k_2 ; per day), whereas the intercept (β) describes the contribution of other depuration processes, limited in this instance to the growth dilution and fecal egestion. For EHMC and OCT, the $k_{\text{BT,R}}$ was derived using Equation 2 and represents the depuration rate constant of EHMC and OCT in the absence of biotransformation.

Whole-body biotransformation rate constants (k_{MET} ; per day) for EHMC and OCT were then calculated as the difference between the measured whole-body depuration rate constants (k_{BT}) of EHMC and OCT and the corresponding $k_{\text{BT,R}}$ values as (Lo et al. 2015b):

$$k_{\rm MET} = k_{\rm BT} - k_{\rm BT,R} \tag{3}$$

The standard error of k_{MET} (SE_{*k*MET}) was propagated from the standard errors of k_{BT} (SE_{*k*BT}) and $k_{\text{BT,R}}$ (SE_{*k*BT,R}) as (Gobas and Lo 2016):

$$SE_{k_{MET}} = \sqrt{\left(SE_{k_{BT}}^2 + SE_{k_{BT,R}}^2\right)}$$
(4)

Calculation of dietary uptake efficiency

Dietary uptake efficiency ($E_{D,M}$) was determined for the reference chemicals, EHMC, and OCT by fitting chemical concentrations measured in the soma to the integrated form of the kinetic rate equation for constant dietary exposure (Organisation for Economic Co-operation and Development 2012)

$$C_{\rm B} = \left(\frac{E_{\rm D,M} \times I \times C_{\rm D}}{k_{\rm BT}}\right) \times (1 - e^{-k_{\rm BT}t})$$
(5)

where I is the feeding rate, C_B is the concentration in the fish (soma) at the beginning of the depuration period (µmol kg^{-1}),

 C_{D} is the concentration in the diet (µmol kg^{-1}), and t is time (days).

Bioaccumulation potential of EHMC and OCT

Kinetic lipid-normalized BMFs were generated for EHMC, OCT, and reference chemicals according to

$$\mathsf{BMF} = \frac{\mathsf{E}_{\mathsf{D},\mathsf{M}} \times \mathsf{I} \times \phi_{\mathsf{DL}}}{k_{\mathsf{BT}} \times \phi_{\mathsf{BL}}} \tag{6}$$

where $E_{D,M}$ is the dietary uptake efficiency, I is the proportional feeding rate, k_{BT} is the somatic depuration rate constant (per day), ϕ_{DL} is the measured fractional dietary lipid content (0.079 ± 0.003 [SD] kg lipid kg food⁻¹; present study), and ϕ_{BL} is the fractional lipid content of the fish (kg lipid kg fish⁻¹).

Respiratory uptake rate constants (k_1) and BCFs were generated from the dietary bioaccumulation tests according to Gobas and Lo (2016)

$$k_{\rm I} = \left(\frac{1}{\omega}\right) \times \left(\frac{\phi_{\rm BL}}{d_{\rm L}}\right) \tag{7}$$

where $1/\omega$ is the slope term derived from Equation 2, d_L is the density of the fish lipids (assumed to be 0.90 kg L⁻¹), and ϕ_{BL} is the measured lipid content of fish (kg lipid kg fish⁻¹). A detailed derivation of Equation 7 is provided in Gobas and Lo (2016) and is based on the assumptions that it applies to test chemicals with log $K_{OW} \ge 3$ and that chemical partitioning between the fish and water (i.e., k_1/k_2) is represented by $K_{OW} \times \phi_{BL}$.

Bioconcentration factors $(L kg^{-1})$ expressed on a free chemical basis were determined as

BCF =
$$\frac{k_1}{k_{BT}} \times (1/[1 + C_{OC} \times K_{OC}])$$
 (8)

where C_{OC} is the measured total concentration of organic carbon in water (1.60 × 10⁻⁶ kg L⁻¹; present study) and K_{OC} is the octanol-carbon partition coefficient, calculated as log $K_{OC} = 0.97 \times \log K_{OW} - 1.27$ (Burkhard 2000).

Chemical transformation in the gastrointestinal tract

The dietary uptake efficiency for a nonbiotransformed chemical ($E_{D,R}$) was related to K_{OW} by the relationship (Lo et al. 2015b)

$$E_{D,R}^{-1} = \alpha + \beta K_{OW}$$
⁽⁹⁾

where α and β are fitted coefficients determined by a weighted nonlinear regression of empirical E_D observations (E_{D,M}) of the reference chemicals. The parameters α and β characterize organic (i.e., octanol or lipid-like) and aqueous-phase resistances, respectively.

Intestinal (luminal) biotransformation rate constants (k_{lumen}) for EHMC and OCT were calculated from $E_{D,M}$ and from $E_{D,R}$

determined for a nonbiotransformed reference chemical of equivalent K_{OW} (Equation 9; Lo et al. 2015b)

$$k_{\text{lumen}} = \left(E_{\text{D},\text{R}}^{-1} - E_{\text{D},\text{M}}^{-1}\right) \times \left(\frac{E_{\text{D},\text{R}}}{1 - E_{\text{D},\text{R}}}\right) \times (G_{\text{GE}}/W_{\text{G}})$$
 (10)

where G_{GE} (kg digesta d⁻¹) is the fecal egestion rate and W_G (kg) is the steady-state amount of digesta in the entire intestinal tract (including stomach, pyloric ceca, and anterior intestine). The G_{GE} was estimated from the administered food ingestion rate (G1; kg food d^{-1} [or $I \times W_B$]) and the food assimilation efficiency (γ_{GI} ; unitless) as $G_I \times \gamma_{GI}$. The γ_{GI} was estimated from the diet composition and the assimilation efficiencies of the diet constituents using values for the assimilation efficiencies of various food constituents (Supplemental Data, Table S5). The γ_{GI} was approximately 0.59 and is similar to the value of 0.52 measured in rainbow trout using chromic oxide (Gobas et al. 1999). The $\rm W_{G}$ was estimated as the ratio of G_1 to δ , where δ is the digesta evacuation rate constant (2.07 d^{-1}), which is approximated by the 95% digesta evacuation time ($t_{E,95}$; 1.45 d) as $3/t_{E,95}$ (Lo et al. 2015b). Parameters and equations used in this analysis are provided in Supplemental Data, Table S5.

Contribution of luminal and somatic biotransformation

To describe the contribution of somatic (whole-body) and luminal biotransformation, the fish is divided into 2 compartments: the soma (B) and the gastrointestinal content or digesta in the lumen (G). A detailed derivation of the model is described elsewhere (Lo et al. 2015b, 2016). The relative contributions of somatic (Φ_{soma}) and luminal (Φ_{lumen}) biotransformation to total chemical biotransformation in fish can be calculated as

$$\Phi_{\text{soma}} = k_{\text{MET}} M_{\text{B},X} / (k_{\text{MET}} M_{\text{B},X} + k_{\text{lumen}} M_{\text{G},X})$$
(11)

$$\Phi_{\text{lumen}} = k_{\text{lumen}} M_{\text{G},\text{X}} / (k_{\text{MET}} M_{\text{B},\text{X}} + k_{\text{lumen}} M_{\text{G},\text{X}})$$
(12)

where M_B and M_G are the masses of chemical in the fish soma and lumen, respectively. The subscript "X" denotes whether the exposure was through a dietary (D) or aqueous (AQ) route.

For the dietary exposure, $M_{B,D}$ is the mass of chemical $(C_B \times W_B)$ in the soma measured in the present study on day 14, whereas $M_{G,D}$ was estimated as in Lo et al. (2015b)

$$M_{G,D} = \frac{G_{I}C_{D} + k_{BG}M_{B,D}}{k_{GB} + k_{GE} + k_{lumen}}$$
(13)

where k_{BG} is the rate constant for chemical transfer from fish soma to lumen (per day), k_{GB} is the rate constant for chemical transfer from lumen to fish soma (per day), and k_{GE} is the rate constant for fecal egestion (per day).

Using rate constants generated here for respiratory uptake (k_1) and whole-body depuration $(k_{\rm BT})$, the steady-state mass of

chemical in the fish soma following an aqueous exposure $(M_{\text{B},\text{AQ}})$ can be estimated as

$$M_{B,AQ} = \frac{k_1}{k_{BT}} \times C_{WT} \phi \times W_B$$
(14)

where C_{WT} is the total concentration of chemical in the water (µmol L⁻¹) and ϕ is the bioavailable solute fraction (unitless), which is equal to $1/(1 + C_{OC} \times K_{OC})$ (Equation 8).

Using Equation 13, the $M_{G,AQ}$ is calculated by replacing $M_{B,D}$ with $M_{B,AQ}$ and setting C_D equal to $0\,\mu mol\,kg^{-1}$. Parameters and equations used in this analysis are provided in Supplemental Data, Table S5.

Statistical analyses

All statistical analyses were performed in R (Ver 3.3.3). An analysis of variance followed by a Tukey's honestly significant difference test was used to evaluate differences in the hepatosomatic index (HSI; liver mass/body mass × 100) and in carcass lipid content among the treatment and control tanks. A multiple regression model was used to test for differences in the slopes (i.e., $k_{\rm BT}$) of the depuration curves (Equation 1) to evaluate whether EHMC or OCT depuration rate constants differed with respect to dietary exposure concentration. Differences in $k_{\rm MET}$ and $E_{\rm D,M}$ with respect to dietary exposure concentration. Standard errors of $E_{\rm D,M}$, BMF, k_1 , BCF, and $k_{\rm lumen}$ were propagated according to Gobas et al. (2019). For all analyses, p < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Fish

Three of 126 fish exposed to UVFs died: one each in the OCT low, EHMC low, and EHMC high tanks on days 23, 25, and 28, respectively. No fish died in the control population. Growth rate constants (k_G ; per day) determined for each tank did not differ statistically from 0, indicating negligible growth throughout the 28-d study period (Table 2; Supplemental Data, Figure S1). Mean HSI values (Table 2) and carcass lipid contents (Supplemental Data, Table S4) did not differ between control

and treatment tanks (p = 0.4609 and p = 0.3606, respectively). The lipid contents of livers sampled from control fish averaged $3.5 \pm 0.2\%$. The soma lipid content (ϕ_{BL}), determined from the mass of lipid in the liver and carcass samples (Supplemental Data, Table S4), ranged between 3.5 and 4.6% across control and treatment tanks (Table 2).

Whole-body depuration rate constants of EHMC, OCT, and reference chemicals

Measured concentrations of EHMC and OCT in fish on day 14 were similar to those on day 7, suggesting that the fish were approaching steady state (Figure 2). After day 14, on initiation of the depuration phase, concentrations of EHMC and OCT in the fish declined in a log-linear manner. Measured concentrations of EHMC and OCT in the soma of control fish were below their MDL. The whole-body depuration rate constants ($k_{\rm BT}$; Equation 1) calculated for fish exposed to EHMC were $(\text{mean} \pm \text{SE}) 0.473 \pm 0.078 \text{ d}^{-1}$ (low), 0.680 ± 0.195 d⁻¹ (medium), and $0.532 \pm 0.138 d^{-1}$ (high). The $k_{\rm BT}$ values for fish exposed to OCT were $0.102 \pm 0.023 d^{-1}$ (low), $0.134 \pm 0.020 d^{-1}$ (medium), and $0.114 \pm 0.035 d^{-1}$ (high). These fitted rate constants did not differ significantly among treatment groups, indicating that for both chemicals the kinetics of depuration were independent of dietary exposure concentration (p = 0.7872 [EHMC] and 0.6700 [OCT]).

Measured concentrations of the 6 reference chemicals in fish soma increased throughout the 14-d exposure, declining thereafter during the depuration phase (Supplemental Data, Figure S2). For the most hydrophobic reference chemicals (HCBz, PCB52, and PCB155) concentrations measured at 14 d were substantially higher than those measured at 7 d, suggesting that fish were far from steady state. By comparison, concentrations measured at 7 and 14 d for 3TCBz, 4TCBz, and PCBz were relatively similar. Measured concentrations of reference chemicals in the soma of control fish were below their MDL. Calculated whole-body depuration rate constants ($k_{\rm BT}$) for the 6 reference chemicals exhibited an inverse relationship with chemical log K_{OW} (Supplemental Data, Table S6). Averaged across all 6 treatment tanks, the $k_{\rm BT}$ values were (mean \pm SE) 0.344 ± 0.019 , 0.181 ± 0.042 , 0.069 ± 0.022 , 0.026 ± 0.012 , 0.015 ± 0.012 , and $0.0004 \pm 0.013 d^{-1}$ for 3TCBz, 4TCBz, PCBz,

TABLE 2: General parameters for the treatment and control tanks including mean weights of fish, mean hepatosomatic indices, mean lipid content of the fish soma, and growth rate constants

Treatment	W _B ^{a,b}	HSI ^{a,b}	$\phi_{\sf BL}{}^{\sf b}$	k _G ^c
Control	37.24 ± 7.64	1.23 ± 0.24	0.035 ± 0.004	0.0099 ± 0.0053
OCT Low	32.94 ± 5.75	1.18 ± 0.13	0.036 ± 0.007	0.0028 ± 0.0057
OCT Medium	37.04 ± 7.38	1.27 ± 0.20	0.038 ± 0.006	0.0004 ± 0.0063
OCT High	36.58 ± 9.11	1.21 ± 0.23	0.038 ± 0.006	0.0080 ± 0.0068
EHMC Low	38.56 ± 5.81	1.17 ± 0.33	0.042 ± 0.006	0.0035 ± 0.0047
EHMC Medium	36.62 ± 5.73	1.24 ± 0.18	0.046 ± 0.007	0.0018 ± 0.0052
EHMC High	39.92 ± 9.20	1.13 ± 0.22	0.038 ± 0.010	0.0024 ± 0.0073

^aMean value for all sampled animals during the 28-d exposure.

^bError values represent the standard deviation of the mean.

^cError values represent the standard error of the estimate.

EHMC = 2-ethylhexyl-4-methoxycinnamate; ϕ_{BL} = mean lipid content of the fish soma (grams of lipid per gram of fish); HSI = hepatosomatic index (grams of liver per gram of fish × 100); k_G = growth rate constant (per day); OCT = octocrylene; W_B = mean weight of fish (grams).



FIGURE 2: Natural logarithm-transformed concentrations of 2-ethylhexyl-4-methoxycinnamate (A) and octocrylene (B) in the fish soma throughout the dietary bioaccumulation experiment following exposure to "high" (filled squares), "medium" (filled circles), and "low" (filled triangles) doses. The vertical dotted line represents the beginning of the depuration phase of the experiment. The horizontal dashed line represents the method detection limit (Supplemental Data, Table S1). EHMC = 2-ethylhexyl-4-methoxycinnamate; OCT = octocrylene.

HCBz, PCB 52, and PCB 155, respectively. Measured k_{BT} values for the reference chemicals determined in each tank were plotted against the reciprocal of chemical K_{OW} to obtain a set of tank-specific linear relationships that describe the K_{OW} dependence of chemical depuration that occurs by all nonmetabolic pathways ($k_{BT,R}$; Figure 3).

Measured $k_{\rm BT}$ values for EHMC and OCT were greater than their corresponding $k_{\rm BT,R}$ values, suggesting a significant contribution of biotransformation to chemical depuration from the fish soma (Figure 3). The $k_{\rm BT,R}$ values determined for a hypothetical reference chemical with a $K_{\rm OW}$ equivalent to that of EHMC were (mean ± SE) $0.006 \pm 0.009 \, d^{-1}$ (low), $0.027 \pm 0.013 \, d^{-1}$ (medium), and $0.030 \pm 0.013 \, d^{-1}$ (high). Calculated in the same manner, the $k_{\rm BT,R}$ values for OCT were $0.025 \pm 0.020 \, d^{-1}$ (low), $0.030 \pm 0.016 \, d^{-1}$ (medium), and $0.017 \pm 0.006 \, d^{-1}$ (high). For EHMC, measured $k_{\rm BT}$ values were approximately 17- to 80-fold greater than corresponding $k_{\rm BT,R}$ values (depending on the tank). The measured $k_{\rm BT}$ values for OCT were approximately 4- to 6-fold greater than corresponding $k_{\rm BT,R}$ values.

Whole-body biotransformation rate constants of EHMC and OCT

The whole-body biotransformation rate constants (k_{MET}) for EHMC, calculated as the difference between $k_{\text{BT,R}}$ and k_{BT} were (mean ± SE) 0.467 ± 0.078 d⁻¹ (low), 0.653 ± 0.197 d⁻¹ (medium), and 0.502 ± 0.141 d⁻¹ (high; Figure 4A). For OCT, the calculated k_{MET} values were 0.077 ± 0.034 d⁻¹ (low),

 $0.104 \pm 0.036 d^{-1}$ (medium), and $0.097 \pm 0.039 d^{-1}$ (high; Figure 4B). Overall mean (±SE) whole-body biotransformation half-lives (i.e., ln[2]/ $k_{\rm MET}$) for EHMC and OCT were 1.31 ± 0.13 and 7.60 ± 0.71 d, respectively. There was no significant relationship between k_{MET} and dietary exposure concentrations of EHMC (p = 0.8429) or OCT (p = 0.7820), suggesting that UVF concentrations in the fish were not high enough to saturate biotransformation enzymes. Previously, Saunders et al. (2019) determined $K_{\rm M}$ values for EHMC and OCT using a trout liver S9 system and expressed these values on a gram per lipid basis. For EHMC, the lipid-normalized $K_{\rm M}$ was 0.69 μ mol g lipid⁻¹, whereas that determined for OCT was 7.41 μ mol g lipid⁻¹. In either case, these lipid-normalized $K_{\rm M}$ values are substantially higher than lipid-normalized chemical concentrations measured in the present study in fish soma or liver (Figure 4C and D). Taken together, these findings suggest that biotransformation of EHMC and OCT at all dietary dosing levels was occurring under near first-order conditions (i.e., C_B or $C_L \ll K_M$).

The assumption that chemical uptake and elimination processes exhibit first-order kinetics is thought to be appropriate to describe accumulation of neutral organic chemicals in animals exposed to the relatively low concentrations in most field scenarios (Kim et al. 2016). The highest dietary concentrations of EHMC and OCT evaluated in the present study (95 and 380 mg kg food⁻¹, respectively) are substantially higher than measured concentrations in field-collected aquatic biota (Gago-Ferrero et al. 2012) but within the range of spiking concentrations recommended in the OECD 305 protocol (1–1000 mg kg food⁻¹; Organisation for Economic Co-operation and Development 2012). For EHMC and OCT,



FIGURE 3: Mean (±standard error) depuration rate constants for the reference chemicals (filled round circles), octocrylene (OCT; red squares), and 2-ethylhexyl-4-methoxycinnamate (EHMC; purple triangles) in the fish soma versus K_{OW}^{-1} . Data generated for OCT are presented in panels **A** (high), **C** (medium), and **E** (low), whereas data generated for EHMC are presented in panels **B** (high), **D** (medium), and **F** (low). The solid line represents the model used to fit the depuration rate constant data for the nonbiotransformed reference chemicals. The dashed lines represent the 95% confidence intervals for the predicted model values. k_{BT} = whole-body depuration rate constant.

current OECD guidelines appear to provide recommended test concentrations that avoid saturation of biotransformation enzymes in vivo.

In other cases, however, saturation of biotransformation enzymes in laboratory exposures and field settings remains a possibility. For example, previously reported $K_{\rm M}$ values for several polycyclic aromatic hydrocarbons are up to an order of magnitude lower (Lo et al. 2015a; Nichols et al. 2018) than the $K_{\rm M}$ values generated for EHMC and OCT (Saunders et al. 2019). Nichols et al. (2018) compared the measured $K_{\rm M}$ for pyrene, expressed on a free chemical basis, to aqueous chemical concentrations commonly employed in standardized BCF testing



FIGURE 4: Whole-body biotransformation rate constants for 2-ethylhexyl-4-methoxycinnamate (EHMC; **A**) and octocrylene (OCT; **B**) as a function of dietary exposure concentration. Mean (\pm standard deviation) lipid-normalized concentrations of EHMC (**C**) and OCT (**D**) that accumulated in the fish soma (circles) and liver (diamonds) following dietary exposure. The horizontal solid lines represents the Michaelis-Menten constant (K_{M}) previously measured in liver S9 fractions by Saunders et al. (2019). $k_{MET} =$ whole-body biotransformation rate constant.

efforts (i.e., 1/100 lethal levels). The results of this analysis suggested that pyrene concentrations in fish during in vivo testing may approach levels associated with enzyme saturation, potentially resulting in concentration-dependent accumulation. For such chemicals, it may be necessary to perform in vivo exposures at concentrations close to environmental concentrations so that the laboratory data can be extrapolated to field scenarios with greater confidence (Oliver and Niimi 1985).

Luminal biotransformation rate constants of EHMC and OCT

Measured dietary uptake efficiencies for 5 of the 6 reference chemicals were used to develop a set of weighted nonlinear regressions that relate estimated dietary uptake efficiencies for nonbiotransformed reference chemicals ($E_{D,R}$) to chemical log K_{OW} (Figure 5; Supplemental Data, Table S7). The reference chemical 3TCBz was excluded from this analysis because the mean (\pm SE) $E_{D,M}$ for all tanks ($19 \pm 3.2\%$; n = 6) was

substantially lower than that determined for the other reference chemicals. This lower-than-expected $E_{D,M}$ may have been attributable to biotransformation of 3TCBz in the intestinal lumen of fish. If this was the case, 3TCBz may be a poor reference chemical to include in future investigations. The resulting nonlinear regressions plateaued at maximal $E_{D,R}$ values ranging from approximately 44 to 69% for nonbiotransformed reference chemicals with log K_{OW} values between 4 and 7 (Figure 5).

For all of the treatment tanks, the mean $E_{D,R}$ for PCB155 (log $K_{OW} = 7.55$) was lower than that determined for the other 4 reference chemicals (Figure 5). This finding is consistent with previous data indicating that $E_{D,R}$ values for fish decline with increasing log K_{OW} at log K_{OW} values >7 (Gobas et al. 1988; Lo et al. 2015b; Arnot and Mackay 2018). However, the extent of this decline varied among the treatment tanks. It is possible that variability in the measurement of C_D and C_B could contribute to a higher estimate of $E_{D,R}$ for PCB155. Also, the method used to calculate $E_{D,R}$ requires an estimate of k_{BT} (Equation 5). For very hydrophobic chemicals such as PCB155, k_{BT} is difficult to estimate because the rate of elimination is very



FIGURE 5: Mean (\pm standard error) dietary uptake efficiencies of the reference chemicals (black data points), octocrylene (OCT; red squares), and 2ethylhexyl-4-methoxycinnamate (EHMC, purple triangles) versus log K_{OW} (error bars represent the standard error of the estimate). Data generated for OCT are presented in panels **A** (high), **C** (medium), and **E** (low), whereas data generated for EHMC are in panels **B** (high), **D** (medium), and **F** (low). The solid line represents nonlinear regression fit (Equation 9) to the dietary uptake efficiency data for 5 of 6 reference chemicals (black circles) and excludes 1,3,5-trichlorobenzene (black square). $E_D =$ dietary uptake efficiency.

slow. Extending the depuration period beyond 14 d would have addressed this issue, but there is a limit to which this can be done given the need to simultaneously measure $k_{\rm BT}$ values for lower log $K_{\rm OW}$ reference chemicals and for test chemicals that undergo biotransformation.

The $E_{D,M}$ values generated for EHMC and OCT fell well below the nonlinear regression fit of $E_{D,R}$ and ranged between 2.7 and

14% for EHMC and between 2.4 and 5.2% for OCT (Figure 5 and Table 3). These low $E_{D,M}$ values may reflect significant biotransformation of EHMC and OCT in the lumen of the gastro-intestinal tract (Lo et al. 2015b, 2016). There was no significant relationship between $E_{D,M}$ and dietary exposure concentration for EHMC (p=0.2261) or OCT (p=0.4856). Modeled luminal biotransformation rate constants (k_{lumen}) ranged between 10 and

TABLE 3: Empirical dietary uptake efficiencies, estimated dietary uptake efficiencies of a nonbiotransformed chemical of equivalent K_{OW} , luminal biotransformation rate constants, and proportions of total mass transformed in the lumen and the fish soma for 2-ethylhexyl-4-methoxycinnamate and octocrylene following dietary exposure to low-, medium-, and high-dose treatments^a

Treatment	E _{D,M}	E _{D,R}	k _{lumen}	Φ_{lumen}	Φ_{soma}
OCT					
Low	0.052 ± 0.030	0.450 ± 0.057	17 ± 12	0.97	0.02
Medium	0.024 ± 0.008	0.633 ± 0.041	86 ± 33	0.99	0.01
High	0.016 ± 0.007	0.446 ± 0.049	59 ± 30	0.99	0.01
EHMC					
Low	0.027 ± 0.011	0.452 ± 0.072	35 ± 19	0.97	0.03
Medium	0.078 ± 0.034	0.489 ± 0.130	13 ± 10	0.91	0.09
High	0.143 <u>+</u> 0.078	0.608 ± 0.052	10 ± 8	0.85	0.15

^aError values represent the standard error.

 $\begin{array}{l} {\sf E}_{{\sf D},{\sf M}}={\sf empirical} \mbox{ dietary uptake efficiency (unitless); } {\sf E}_{{\sf D},{\sf R}}={\sf estimated dietary uptake efficiency of a nonbiotransformed chemical of equivalent } {\sf K}_{{\sf OW}} \mbox{ (unitless); } \\ {\sf EHMC}=2{\sf -ethylhexyl-4-methoxycinnamate; } \Phi_{{\sf lumen}}={\sf proportion of total mass transformed in the lumen; } \Phi_{{\sf soma}}={\sf proportion of total mass transformed in the fish soma (unitless); } \\ {\sf k}_{{\sf lumen}}={\sf luminal biotransformation rate constant (per day); } \\ {\sf OCT}={\sf octocrylene.} \end{array}$

35 d⁻¹ for EHMC and between 17 and 86 d⁻¹ for OCT (Table 3). For EHMC and OCT, the rates of biotransformation, expressed in units of μ mol d⁻¹ in the lumen (i.e., $k_{lumen} \times M_G$) were up to 110-fold greater than biotransformation rates determined in the fish soma (i.e., $k_{MET} \times M_B$; Supplemental Data, Table S8). Following dietary exposure, the relative contribution of luminal biotransformation (Φ_{lumen}) to total biotransformation was as high as 97 and 99% for EHMC and OCT, respectively (Equation 12 and Table 3). One possible explanation for this apparent high level of biotransformation in the lumen is that gut microflora hydrolyze ester groups present on EHMC and OCT.

Chemical biotransformation processes in the lumen and epithelial tissues of the gastrointestinal tract of fish have been shown to reduce chemical uptake from the diet (Van Veld et al. 1988; Kleinow et al. 1998) and may substantially reduce chemical bioaccumulation in fish (Lo et al. 2015b, 2016; Arnot and Mackay 2018). Luminal biotransformation rate constants derived in the present study and elsewhere (Lo et al. 2015b, 2016) suggest that biotransformation in the gut lumen may contribute more to the overall biotransformation of some dietary contaminants than does somatic biotransformation. In combination with hepatic in vitro bioassays for estimating whole-body biotransformation rate constants, the development of in vitro assays for estimating intestinal biotransformation rates may provide additional screening tools needed to improve chemical bioaccumulation assessments. This could include in vitro assays performed using collected gut contents and/or cultured gut microflora, as well as assays that employ cultured epithelial cells and/or epithelial subcellular fractions.

Bioaccumulation potential of EHMC and OCT

Calculated respiratory uptake rate constants (k_1), BCFs, and lipid-normalized BMFs for the reference chemicals, EHMC, and OCT are provided in Supplemental Data, Table S7. The BMF values obtained for the 6 reference chemicals increased with increasing log K_{OW} . A BMF exceeding 1.0 kg lipid kg lipid⁻¹ is indicative of probable bioaccumulation potential (Gobas et al. 2009). The average BMF for PCB155, calculated across all tanks was (mean ± SE, n = 6) 14 ± 10 kg lipid kg lipid⁻¹. Calculated BMF values exceeding 1.0 kg lipid kg lipid⁻¹ were noted for HCBz and PCB52 in at least 1 of the 6 treatment tanks.

The calculated BMF values for EHMC were (mean \pm SE) 0.0013 \pm 0.0041 kg lipid kg lipid⁻¹ (low), 0.0026 \pm 0.040 kg lipid kg lipid⁻¹ (medium), and 0.0067 \pm 0.0275 kg lipid kg lipid⁻¹ (high), whereas those determined for OCT were 0.0167 \pm 0.004 kg lipid kg lipid⁻¹ (low), 0.0048 \pm 0.001 kg lipid kg lipid kg lipid⁻¹ (medium), and 0.0038 \pm 0.001 kg lipid kg lipid⁻¹ (high). All BMF values generated for EHMC and OCT were approximately 2 orders of magnitude lower than those obtained for reference chemicals with similar log K_{OW} values (Figure 6A) and in each case were far below 1.0. This result illustrates how somatic biotransformation and luminal biotransformation can act to prevent biomagnification of chemicals taken up from the diet.

Few experimental BMF data for EHMC and OCT are available in the literature. For OCT, the BMF values calculated in the present study were consistent with a previously reported laboratory-derived BMF of 0.034 kg lipid kg lipid⁻¹ (Pawlowski et al. 2019). However, BMF values calculated in the present study for EHMC and OCT were substantially lower than those



FIGURE 6: Lipid-normalized biomagnification factors (BMF; **A**) and bioconcentration factors (BCF; **B**) for reference chemicals (black circles), 2-ethylhexyl-4-methoxycinnamate (purple triangles), and octocrylene (red squares) compared to log K_{OW} . The horizontal solid lines represent bioaccumulation criteria of BMF of 1.0 kg lipid kg lipid⁻¹ (**A**) and BCF of 2000 L kg⁻¹ (**B**).

determined in field-collected fish (BMF \ge 1.0; Table 1). The higher lipid-normalized BMFs determined in field-collected fish may be attributable to a lower biotransformation capacity in the selected fish species. Other factors, such as inadequate characterization of fish prey items or fish migration patterns, can influence BMF values determined in field studies (Kidd et al. 2018). In addition, spatial and temporal heterogeneity of EHMC and OCT concentrations in water could contribute to overestimation of true BMF values if fish were collected from areas where concentration gradients and/or seasonal fluctuations exist (Kim et al. 2016; Pawlowski et al. 2019). Based on laboratory-collected BMF data presented here and elsewhere (Pawlowski et al. 2019), we conclude that EHMC and OCT have a low potential to biomagnify in fish.

The respiratory uptake constants (k_1 ; Supplemental Data, Table S7) determined for EHMC were (mean ± SE) 231 ± 17 L kg⁻¹ d⁻¹ (low), 240 ± 19 L kg⁻¹ d⁻¹ (medium), and 209 ± 24 L kg⁻¹ d⁻¹ (high), whereas those calculated for OCT were 217 ± 45 L kg⁻¹ d⁻¹ (low), 208 ± 29 L kg⁻¹ d⁻¹ (medium), and 242 ± 14 L kg⁻¹ d⁻¹ (high).

The BCFs for the reference chemicals increased with increasing log K_{OW} (Figure 6B). When the BCFs were averaged across treatment tanks, mean BCFs ranged from 641 ± 45 (SE, n = 6) for 3TCBz to 37 359 ± 15 965 (SE, n = 6) for PCB155. The BCFs calculated for EHMC were (mean \pm SE) $471 \pm 77 \text{ Lkg}^{-1}$ (low), $340 \pm 97 \text{ L kg}^{-1}$ (medium), and $379 \pm 98 \text{ L kg}^{-1}$ (high), whereas those determined for OCT were $1345 \pm 298 \text{ Lkg}^{-1}$ (low), $1105 \pm 163 \text{ L kg}^{-1}$ (medium), and $1350 \pm 419 \text{ L kg}^{-1}$ (high). In each case, the BCFs for EHMC and OCT are approximately 1 to 2 orders of magnitude lower than the BCFs generated for reference chemicals with similar log K_{OW} values. The comparatively lower BCF values generated for EHMC and OCT illustrate the influence of somatic biotransformation in reducing bioconcentration. When fish are exposed via the respiratory route, somatic biotransformation contributes up to 99 and 93% of total biotransformation for EHMC and OCT, respectively (Equation 11; Supplemental Data, Table S8). The results also suggest that whole-body biotransformation rates are sufficient to reduce BCFs for EHMC and OCT below the European Union Registration, Evaluation, Authorisation, and Restriction of Chemicals regulation criterion for bioaccumulative substances (2000 L kg⁻¹; Figure 6B). The BCFs determined for EHMC were in good agreement with the empirical range of 175 to $433 \,\mathrm{Lkg^{-1}}$ measured in rainbow trout (Table 1), whereas those determined for OCT were only marginally higher than the upper range of empirical BCFs reported in zebrafish $(41-972 \text{ Lkg}^{-1}; \text{ Table 1})$.

SUMMARY AND CONCLUSIONS

The hydrophobic organic UVFs EHMC and OCT were biotransformed by rainbow trout following dietary exposure. Estimated whole-body biotransformation rate constants were independent of dietary exposure concentration. Lipidnormalized chemical concentrations in fish soma or liver were also much lower than previously generated $K_{\rm M}$ values (i.e., $C_{\rm B}$ or $C_{\rm L} \ll K_{\rm M}$). Collectively, these observations suggest that somatic biotransformation of EHMC and OCT was occurring under near first-order conditions. In addition to being biotransformed in the soma, a model-based evaluation of dietary uptake data suggested that metabolic activity in the gut lumen contributes substantially to biotransformation of EHMC and OCT. Somatic and luminal biotransformation greatly reduce the potential for bioaccumulation of EHMC and OCT in trout. Modeled BMFs and BCFs generated for both chemicals were 1 to 2 orders of magnitude lower than BMFs and BCFs generated for reference chemicals of similar log K_{OW} . In addition, for both chemicals, BMFs and BCFs fell below established bioaccumulation criteria (1.0 kg lipid kg lipid⁻¹ and 2000 L kg⁻¹, respectively), suggesting that EHMC and OCT are unlikely to pose a bioaccumulation hazard in rainbow trout.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4638.

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REFERENCES

- Alonso MB, Feo ML, Corcellas C, Gago-Ferrero P, Bertozzi CP, Marigo J, Flach L, Meirelles ACO, Carvalho VL, Azevedo AF, Torres JP, Lailson-Brito J, Malm O, Diaz-Cruz MS, Eljarrat E, Barcelo D. 2015. Toxic heritage: Maternal transfer of pyrethroid insecticides and sunscreen agents in dolphins from Brazil. *Environ Pollut* 207:391–402.
- Arnot JA, Gobas F. 2003. A generic QSAR for assessing the bioaccumulation potential of organic chemicals in aquatic food webs. QSAR Comb Sci 22:337–345.
- Arnot JA, Gobas FAPC. 2004. A food web bioaccumulation model for organic chemicals in aquatic ecosystems. *Environ Toxicol Chem* 23:2343–2355.
- Arnot JA, Mackay D. 2018. The influence of chemical degradation during dietary exposures to fish on biomagnification factors and bioaccumulation factors. Environ Sci Process Impacts 20:86–97.
- Bachelot M, Li Z, Munaron D, Le Gall P, Casellas C, Fenet H, Gomez E. 2012. Organic UV filter concentrations in marine mussels from French coastal regions. *Sci Total Environ* 420:273–279.
- Balmer ME, Buser H-R, Müller MD, Poiger T. 2005. Occurrence of some organic UV filters in wastewater, in surface waters, and in fish from Swiss lakes. *Environ Sci Technol* 39:953–962.
- Bligh EG, Dyer WJ. 1959. A rapid method of total lipid extraction and purification. *Can J Biochem Phys* 37:911–917.
- Blüthgen N, Meili N, Chew G, Odermatt A, Fent K. 2014. Accumulation and effects of the UV-filter octocrylene in adult and embryonic zebrafish (Danio rerio). Sci Total Environ 476–477:207–217.
- Burkhard LP. 2000. Estimating dissolved organic carbon partition coefficients for nonionic organic chemicals. *Environ Sci Technol* 34:4663–4668.

- Buser H-R, Balmer ME, Schmid P, Kohler M. 2006. Occurrence of UV filters 4-methylbenzylidene camphor and octocrylene in fish from various Swiss rivers with inputs from wastewater treatment plants. *Environ Sci Technol* 40:1427–1431.
- Chamkasem N, Lee S, Harmon T. 2016. Analysis of 19 PCB congeners in catfish tissue using a modified QuEChERS method with GC–MS/MS. *Food Chem* 192:900–906.
- Christen V, Zucchi S, Fent K. 2011. Effects of the UV-filter 2-ethyl-hexyl-4trimethoxycinnamate (EHMC) on expression of genes involved in hormonal pathways in fathead minnows (*Pimephales promelas*) and link to vitellogenin induction and histology. Aquat Toxicol 102:167–176.
- Fent K, Zenker A, Rapp M. 2010. Widespread occurrence of estrogenic UV-filters in aquatic ecosystems in Switzerland. Environ Pollut 158:1817–1824.
- Gago-Ferrero P, Alonso MB, Bertozzi CP, Marigo J, Barbosa L, Cremer M, Secchi ER, Domit C, Azevedo A, Lailson-Brito J Jr, Torres JP, Malm O, Eljarrat E, Diaz-Cruz MS, Barcelo D. 2013. First determination of UV filters in marine mammals. Octocrylene levels in Franciscana dolphins. *Environ Sci Technol* 47:5619–5625.
- Gago-Ferrero P, Díaz-Cruz MS, Barceló D. 2012. An overview of UVabsorbing compounds (organic UV filters) in aquatic biota. *Anal Bioanal Chem* 404:2597–2610.
- Gago-Ferrero P, Díaz-Cruz MS, Barceló D. 2015. UV filters bioaccumulation in fish from Iberian river basins. *Sci Total Environ* 518–519:518–525.
- Gobas FAPC, de Wolf W, Burkhard LP, Verbruggen E, Plotzke K. 2009. Revisiting bioaccumulation criteria for POPs and PBT assessments. Integr Environ Assess Manag 5:624–637.
- Gobas FAPC, Lee YS, Lo JC, Parkerton TF, Letinski DJ. 2019. A toxicokinetic framework and analysis tool for interpreting OECD-305 dietary bioaccumulation tests. Environ Toxicol Chem, in press. DOI: 10.1002/etc.4599
- Gobas FAPC, Lo JC. 2016. Deriving bioconcentration factors and somatic biotransformation rates from dietary bioaccumulation and depuration tests. *Environ Toxicol Chem* 35:2968–2976.
- Gobas FAPC, Muir DCG, Mackay D. 1988. Dynamics of dietary bioaccumulation and faecal elimination of hydrophobic organic chemicals in fish. *Chemosphere* 17:943–962.
- Gobas FAPC, Wilcockson JWB, Russell RW, Haffner GD. 1999. Mechanism of biomagnification in fish under laboratory and field conditions. *Environ Sci Technol* 33:133–141.
- Kidd KA, Burkhard LP, Babut M, Borgå L, Muir DCG, Perceval O, Ruedel H, Woodburn K, Embry MR. 2018. Practical advice for selecting or determining trophic magnification factors for application under the European Union Water Framework Directive. *Integr Environ Assess Manag* 15:266–277.
- Kim J, Gobas FAPC, Arnot JA, Powell DE, Seston RM, Woodburn KB. 2016. Evaluating the roles of biotransformation, spatial concentration differences, organism home range, and field sampling design on trophic magnification factors. *Sci Total Environ* 551–552:438–451.
- Kleinow KM, James MO, Tong Z, Venugopalan CS. 1998. Bioavailability and biotransformation of benzo[a]pyrene in an isolated perfused in situ catfish intestinal preparation. *Environ Health Perspect* 106:155–166.
- Koenig S, Fernàndez P, Solé M. 2012. Differences in cytochrome P450 enzyme activities between fish and crustacea: Relationship with the bioaccumulation patterns of polychlorobiphenyls (PCBs). *Aquat Toxicol* 108:11–17.
- Lo JC, Allard GN, Otton SV, Campbell DA, Gobas FAPC. 2015a. Concentration dependence of biotransformation in fish liver S9: Optimizing substrate concentrations to estimate hepatic clearance for bioaccumulation assessment. *Environ Toxicol Chem* 34:2782–2790.

- Lo JC, Campbell DA, Kennedy CJ, Gobas FAPC. 2015b. Somatic and gastrointestinal in vivo biotransformation rates of hydrophobic chemicals in fish. *Environ Toxicol Chem* 34:2282–2294.
- Lo JC, Letinski DJ, Parkerton TF, Campbell DA, Gobas FAPC. 2016. In vivo biotransformation rates of organic chemicals in fish: Relationship with bioconcentration and biomagnification factors. *Environ Sci Technol* 50:13299–13308.
- Nagtegaal M, Ternes TA, Baumann W, Nagel R. 1997. UV-filtersubstanzen in wasser und fischen. Umweltwissenschaften und Schadstoff-Forschng 9:79–86.
- Nichols JW, Ladd MA, Fitzsimmons PN. 2018. Measurement of kinetic parameters for biotransformation of polycyclic aromatic hydrocarbons by trout liver S9 fractions: Implications for bioaccumulation assessment. *Appl In Vitro Toxicol* 4:365–378.
- Oliver BG, Niimi AJ. 1985. Bioconcentration factors of some halogenated organics for rainbow trout: Limitations in their use for prediction of environmental resides. *Environ Sci Technol* 19:842–849.
- Organisation for Economic Co-operation and Development. 2012. Test No. 305: Bioaccumulation in fish: Aqueous and dietary exposure. *OECD Guidelines for the Testing of Chemicals*. Paris, France.
- Pawlowski S, Lanzinger AC, Dolich T, Füβl S, Salinas ER, Zok S, Weiss B, Hefner N, Sloun PV, Hombeck H, Klingelmann, Petersen-Thiery M. 2019. Evaluation of the bioaccumulation of octocrylene after dietary and aqueous exposure. *Sci Total Environ* 672:669–679.
- Peng X, Fan Y, Jin J, Xiong S, Liu J, Tang C. 2017. Bioaccumulation and biomagnification of ultraviolet absorbents in marine wildlife of the Pearl River estuarine, South China Sea. *Environ Pollut* 225:55–66.
- Picot Groz M, Martinez Bueno MJ, Rosain D, Fenet H, Casellas C, Pereira C, Maria V, Bebianno MJ, Gomez E. 2014. Detection of emerging contaminants (UV filters, UV stabilizers and musks) in marine mussels from Portuguese coast by QuEChERS extraction and GC-MS/MS. *Sci Total Environ* 493:162–169.
- Saunders LJ, Fontanay S, Nichols JW, Gobas FAPC. 2019. Concentration dependence of in vitro biotransformation rates of hydrophobic organic sunscreen agents in rainbow trout S9 fractions: Implications for bioaccumulation assessment. *Environ Toxicol Chem* 38:548–560.
- Sigma-Aldrich. 2014. Safety data sheet: 2-Ethylhexyl 2-cyano-3,3diphenylacrylate [cited 2017 September 23]. Available from: https:// www.sigmaaldrich.com/catalog/product/aldrich/415820
- Tang Z, Zhong F, Cheng J, Nie Z, Han X, Han Y, Yang Y. 2019. Concentrations and tissue-specific distributions of organic ultraviolet absorbents in wild fish from a large subtropical lake in China. *Sci Total Environ* 647:1305–1313.
- US Environmental Protection Agency. 2012. Estimation Programs Interface Suite for Microsoft Windows, Ver 4.11. Washington, DC [cited 2019 March 7]. Available from: https://www.epa.gov/tsca-screening-tools/episuitetm-estimation-program-interface
- US Environmental Protection Agency. 2018. ECOTOX User Guide: ECO-TOXicology Knowledgebase System, Ver 5.0. [cited 2019 March 15]. Available from: http://www.epa.gov/ecotox
- US National Library of Medicine. 2006. TOXNET Hazardous Substances Data Bank. Washington, DC. [cited 2018 February 30]. Available from: https://toxnet.nlm.nih.gov/newtoxnet/hsdb.htm
- Van Veld PA, Stegeman JJ, Woodin BR, Patton JS, Lee RF. 1988. Induction of monooxygenase activity in the intestine of spot (*Leiostomus xanthurus*), a marine teleost, by dietary polycyclic aromatic hydrocarbons. *Drug Metab Dispos* 16:659–665.
- Zenker A, Schmutz H, Fent K. 2008. Simultaneous trace determination of nine organic UV-absorbing compounds (UV filters) in environmental samples. *J Chromatogr A* 1202:64–74.