Environmental Science & Technology

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In Vivo Biotransformation Rates of Organic Chemicals in Fish: Relationship with Bioconcentration and Biomagnification Factors

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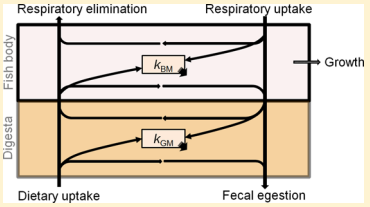
Supporting Information

ABSTRACT: In vivo dietary bioaccumulation experiments for 85 hydrophobic organic substances were conducted to derive the in vivo gastrointestinal biotransformation rates, somatic biotransformation rates, bioconcentration factors (BCF), and biomagnification factors (BMF) for improving methods for bioaccumulation assessment and to develop an in vivo biotransformation rate database for QSAR development and in vitro to in vivo biotransformation rate extrapolation. The capacity of chemicals to be biotransformed in fish was found to be highly dependent on the route of exposure. Somatic biotransformation was the dominant pathway for most chemicals absorbed via the respiratory route. Intestinal biotransformation was the dominant metabolic pathway for most chemicals absorbed via the diet. For substances not biotransformed or transformed exclusively in the body of the fish, the BCF and BMF appeared to be closely correlated. For substances subject to intestinal biotransformation, the same correlation did not apply. We conclude that intestinal biotransformation and bioavailability in water can modulate the relationship between the BCF and BMF. This study also supports a fairly simple rule of thumb that may be useful in the interpretation of dietary bioaccumulation tests; i.e., chemicals with a BMF_L of <1 tend to exhibit BCFs based on either the freely dissolved ($BCF_{WW,fd}$) or the total concentration ($BCF_{WW,t}$) of the chemical in the water that is less than 5000.



INTRODUCTION

[29]The capacity of chemicals to bioaccumulate in biota is recognized as an important property that contributes to a substances' potential to harm wildlife. Bioaccumulation is therefore widely considered in international and national chemical management programs.[1−5] The bioconcentration factor (BCF) is a common metric used in regulations to express the extent of chemical bioaccumulation. The chemical's octanol−water partition coefficient (K_{OW}; $C_{Octanol}/C_{Water}$) is a surrogate used to predict the extent of bioaccumulation. The field-derived bioaccumulation factor (BAF; $C_{Organism}/C_{Water}$) may also be used. Recent guidance also includes the biomagnification factor (BMF; $C_{Organism}/C_{Diet}$) and the trophic magnification factor (TMF; the antilog of the log−linear regression slope of $C_{Organism}$ versus trophic level) and recommends a weight of evidence approach in bioaccumulation assessments.[6−8] However, to date, the BCF often remains the preferred metric used in regulatory evaluations. The BCF is typically measured in laboratory bioconcentration tests, in which organisms (e.g., fish) are exposed to the chemical via water. The preferred method for the determination of the BCF conforms with guidelines developed by the OECD.[9] Current OECD protocols for bioaccumulation testing provide options for tests involving both aqueous and dietary exposure. Bioaccumulation tests are typically costly, time-consuming, and require substantial animal use. An alternative to such testing is the use of bioaccumulation models. These models have shown to be successful at estimating the BCF and BAF for chemicals that are not biotransformed considerably in the organism[10,11] but overestimate the extent of bioaccumulation for chemicals that are biotransformed.[12] This bias is due to the fact that the models do not a priori incorporate predictions of the biotransformation rates of chemicals in organisms. To develop methods for improving BCF estimates of the many thousands of chemicals in commerce requiring evaluation, several research initiatives have developed. One initiative involves the back-calculation of biotransformation rates from BCFs using the AQUAWEB model[10] and the subsequent development of a quantitative structure activity relationship (QSAR) for biotransformation that is incorporated in the U.S. Environmental Protection Agency EPI Suite program for

Received: July 18, 2016
Revised: October 2, 2016
Accepted: November 11, 2016
Published: November 11, 2016

DOI: 10.1021/acs.est.6b03602
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

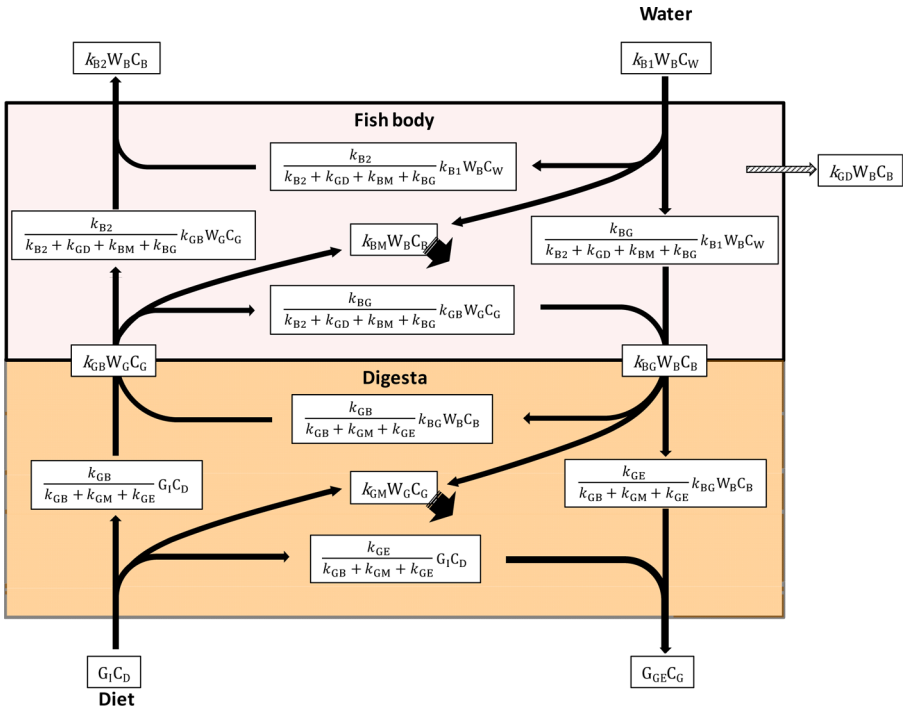


Figure 1. Detailed schematic diagram of the chemical fluxes in a two-compartment model separating the fish body from the contents of the digestive tract illustrating the role of biotransformation (represented by the black arrow) in the body (k_{BM}) and the gastrointestinal tract (k_{GM}) of the fish.

estimating BCFs.[13−16] A second initiative uses in vitro measurements of chemical depletion rates in liver homogenates and hepatocytes, which are then extrapolated to make estimates of whole-organism biotransformation rates and then used as input to extrapolation models to estimate BCF values.[17−23] This initiative aims to make BCF determinations less labor-intensive, cheaper, and less animal-use intensive. A third initiative, explored in this study, involves the development of simplified test designs (involving fewer animals and costs and less labor than typical OECD bioconcentration tests) to measure in vivo biotransformation rates and corresponding BCFs of chemicals. This research serves to fill an important data gap for biotransformable substances because it provides actual measurements of biotransformation rates of chemicals in whole animals. The biotransformation rate data can be used to test the ability of in vitro and QSAR-based methods to reliably estimate in vivo biotransformation rates and BCFs.

Generally accepted bioassays for the measurement of in vivo biotransformation rates do not exist to date. Previous work on experimentally deriving in vivo biotransformation rates revealed that in vivo biotransformation involves both hepatic and gastrointestinal biotransformation rates and that the contribution of somatic and gastrointestinal biotransformation to bioaccumulation is dependent on the route of chemical exposure.[24] This means that BCFs and BMFs may be affected by biotransformation in different ways. However, in most bioaccumulation models, in vivo biotransformation is viewed as depuration from the body (i.e., somatic) only,[25] hence affecting BCF and BMFs in a similar way.

The objective of the present study is to develop and apply a method for simultaneously determining in vivo gastrointestinal biotransformation rates, somatic biotransformation rates, BCFs, and BMFs. Such a test methodology does currently not exist. A second goal is to provide measurements of in vivo biotransformation rates for a number of structurally diverse chemicals to allow the testing and further development of quantitative structure activity relationships for predicting biotransformation rates and the testing of extrapolation methods for estimating in vivo rates from in vitro biotransformation rate data. Such a biotransformation database is also not available to date. A third objective is to investigate the relationship between the BCFs and BMFs for substances subject to somatic and gastrointestinal biotransformation. This information is also not available. The main purpose of the study is to make bioaccumulation determinations for substances more accurate, efficient, and less costly while reducing animal use.

THEORY

Bioaccumulation Model for in Vivo Biotransformation Studies. To describe the contribution of biotransformation of chemicals in the soma (i.e., somatic biotransformation including hepatic metabolism) and in the gut of the fish (i.e., gastrointestinal biotransformation in the lumen of the intestines due to intestinal microflora and gastric enzymes), the fish is divided into two compartments, i.e., the body (B) and the gastrointestinal content or digesta (G). The following mass balance for the body of the fish describes this process:[24]

$$dC_B/dt = k_{B1}\cdot C_{WD} + (k_{GB}/(k_{GB} + k_{GE} + k_{GM}))\cdot(G_I/W_B)\cdot C_D - (k_{B2} + k_{BG}\cdot((k_{GE} + k_{GM})/(k_{GB} + k_{GE} + k_{GM})) + k_{GD} + k_{BM})\cdot C_B \quad (1)$$

where C_B is the concentration of the chemical in the body of the fish (mol/kg fish); G_I is the food ingestion rate (kg food·day^{-1}); C_{WD} is the freely dissolved concentration of the chemical in the water (mol chemical·L^{-1}), C_D is the concentration of the chemical in ingested diet (mol chemical·kg food^{-1}); W_B is the weight of the fish (kg) on a wet-weight (ww) basis; k_{B1} is the uptake clearance rate for respiratory uptake (L water·kg ww fish^{-1}·day^{-1}); k_{B2}, k_{GB}, k_{BG}, k_{GD}, k_{BM},

DOI: 10.1021/acs.est.6b03602
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k_{GE}, and k_{GM} are the rate constants (day^{-1}) for respiratory elimination, chemical transfer from the gastrointestinal content to the fish body, chemical transfer from the fish body to the gastrointestinal content, growth dilution, biotransformation of the chemical in the body of the fish (i.e., somatic biotransformation), fecal egestion of the gastrointestinal content, and biotransformation of the chemical in the gastrointestinal content, respectively; and t is time (day).

This equation can be simplified by recognizing that $k_{GB}/(k_{GB} + k_{GE} + k_{GM})$ in eq 1 is the dietary uptake efficiency for a substance that is biotransformed in the gastrointestinal tract ($E_{D,M}$) and that (G_I/W_B) is the proportional feeding rate F_D expressed as the fraction of the fish's body weight consumed in food per day:

$$dC_B/dt = k_{B1}\cdot C_{WD} + E_{D,M}\cdot F_D\cdot C_D - (k_{B2} + k_{BG}\cdot(1 - E_{D,M}) + k_{GD} + k_{BM})\cdot C_B \quad (2)$$

A detailed derivation of the model can be found in Lo et al.[24] Figure 1 shows that intestinal biotransformation includes both (i) chemical transformation upon ingestion and (ii) chemical transformation upon chemical elimination from the body of the fish into the lumen. Likewise, the chemical flux biotransformed in the soma also consists of dual contributions, i.e., (i) chemical transformation upon respiratory uptake and (ii) chemical transformation upon chemical transport from the lumen into the fish body. The total chemical flux biotransformed due to gastrointestinal biotransformation (i.e., $k_{GM}\cdot M_G$) or somatic biotransformation (i.e., $k_{BM}\cdot M_B$) is therefore dependent on the route of chemical intake.

Somatic Biotransformation Rate Constant. The application of a mass balance approach to determine biotransformation rates is a frequently used strategy in biotransformation research. It is based on the assumption that loss of mass of the test chemicals relative to nonbiotransformable reference chemicals is due to biotransformation. This research strategy can derive overall biotransformation rates of chemicals but lacks the capacity to detect individual biotransformation products. It complements research focused on detection of specific metabolites. Under conditions of first-order kinetics of biotransformation and transport kinetics, the somatic biotransformation rate constant (k_{BM}) can be determined from measurements of the depuration rate constants when nonbiotransformable reference chemicals[24] are used because, for biotransformed chemicals, the total elimination rate constant in the body (k_{BT}) is

$$k_{BT} = k_{B2} + k_{BG}\cdot(1 - E_{D,M}) + k_{GD} + k_{BM} \quad (3)$$

while for the nonbiotransformed reference chemicals, the total depuration rate constant of the chemical from the body of the fish ($k_{BT,R}$) is

$$k_{BT,R} = k_{B2} + k_{BG}\cdot(1 - E_{D,N}) + k_{GD} \quad (4)$$

where $E_{D,N}$ is the dietary uptake efficiency for a nonbiotransformed substance (i.e., $E_{D,M}$ but with a k_{BM} of 0). The somatic biotransformation rate constant in the body of the fish can therefore be determined as

$$k_{BM} = k_{BT,R} - k_{BT} \quad (5)$$

where $k_{BT,R}$ is depuration rate constant of a reference chemical with the same K_{OW} as that of the test chemical. For substances with a log K_{OW} of >3, the following linear regression model[26] can be used to determine $k_{BT,R}$:

$$k_{BT,R} = (1/\omega)\cdot(1/K_{OW}) + \beta \quad (6)$$

where $1/\omega$ and β are regression coefficients in units of $days^{-1}$. The intercept β represents the $k_{BT,R}$ for a substance with an infinite K_{OW} and, hence, can be approximated with k_{GD}. As described in Gobas and Lo,[26] $1/\omega$ represents the increase in resistance to chemical transport from the fish to the water with increasing K_{OW} and is a function of the lipid content fish body Φ_{BL} and the body weight of the fish.[27] To derive a relationship between $k_{BT,R}$ and $1/K_{OW}$ that can account for the differences in growth rates, lipid contents, and body weights between the multiple bioaccumulation tests of the present study, eq 6 was rewritten as

$$k_{BT,R} = (\alpha\cdot {W_B}^b/\Phi_{BL})\cdot(1/K_{OW}) + k_{GD} \quad (7)$$

where α and b are allometric coefficients, describing the fish's body weight dependence of the water-phase transport parameter. It should be stressed that when following this method for deriving biotransformation using structurally different test and reference chemicals, it is inherently assumed that K_{OW} is the most important chemical-specific factor controlling the nonbiotransformation-related depuration kinetics of nonionic hydrophobic substances.

Respiratory Uptake and Elimination Rate Constants and the Bioconcentration Factor. As detailed in the Supporting Information, the wet-weight-based BCF based on the freely dissolved concentration of the chemical in the water ($BCF_{WW,fd}$) can be derived as

$$BCF_{WW,fd} = k_{B1}/k_{BT} = (\alpha\cdot {W_B}^b/d_L)/k_{BT} \quad (8)$$

BCFs calculated in this fashion are kinetic BCFs at steady-state-based on freely dissolved concentrations of the chemical. BCFs based on the total concentration of the chemical in the water ($BCF_{WW,t}$) measured in OECD 305-style aqueous exposure tests and considered in most regulations are based on a total chemical concentration in the water and are lower than those calculated here, especially for very hydrophobic chemicals due to their high binding affinity to organic matter in the water. The $BCF_{WW,fd}$ based on freely dissolved concentrations of the chemical in the water can be converted into the $BCF_{WW,t}$ following equations by Burkhard[28] or Arnot and Gobas[10] based on equilibrium partitioning of the chemical between the water and dissolved organic matter:

$$BCF_{ww,t} = BCF_{ww,fd}\cdot(1 + \chi_{OC}\cdot K_{OC})^{-1} \quad (9)$$

where χ_{OC} is the concentration of organic carbon in the water (kg/L). K_{OC} is the equilibrium partition coefficient of the chemical between organic carbon and water. The $BCF_{ww,t}$ and $BCF_{ww,fd}$ can be expressed on a lipid normalized basis as $BCF_{L,t}$, i.e., $BCF_{L,t} = BCF_{WW,t}/\Phi_{BL}$ and $BCF_{L,fd} = BCF_{WW,fd}/\Phi_{BL}$ respectively, or expressed as a BCF for fish with a lipid content of 5%, i.e., $BCF_{5\%,t} = 0.05\cdot BCF_{L,t}$ and $BCF_{5\%,fd} = 0.05\cdot BCF_{L,fd}$, respectively, if the BCF follows a linear relationship with the lipid content of the fish (e.g., lipophilic chemicals).

Gastrointestinal Biotransformation. Under conditions of first order kinetics of biotransformation and transport kinetics, the intestinal biotransformation rate constant (k_{GM}) can be determined from measurements of the dietary uptake efficiencies for biotransformable test chemicals ($E_{D,M}$) and nonbiotransformable reference ($E_{D,N}$) chemicals[24] as

DOI: 10.1021/acs.est.6b03602
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$$k_{GM} = (E_{D,M}{}^{-1} - E_{D,N}{}^{-1})\cdot(E_{D,N}/(1 - E_{D,N}))\cdot(G_{GE}/W_G) \quad (10)$$

where G_{GE} (kg digesta·day^{-1}) is the fecal egestion rate, and W_G (kg) is the steady state amount of digesta in the gastrointestinal tract. As described in Lo et al.,[24] G_{GE} can be estimated from the dietary ingestion rate G_I, i.e., the product of the proportional feeding rate F_D and the weight of the body of the fish W_B, and the food assimilation efficiency γ_{GI} as $\gamma_{GI}\cdot G_I$. W_G can be estimated as the ratio G_I/δ, where δ is the digesta evacuation rate constant (day^{-1}), which can be approximated by the 95% digesta evacuation time ($t_{E,95}$) as $3/t_{E,95}$, as explained in the Supporting Information.

Biomagnification Factors. BMFs can be determined from the dietary uptake efficiency and the depuration rate constant as

$$BMF_{WW} = k_{BD}/k_{BT} = E_{D,M}\cdot F_D/k_{BT} \quad (11)$$

The BMF_{WW} can be expressed on a lipid normalized basis as BMF_L, i.e., $BMF_L = BMF_{WW}\cdot(\Phi_{DL}/\Phi_{BL})$. The BMF_L expresses true chemical magnification, i.e., an increase in chemical potential (or activity) that occurs as a result of dietary bioaccumulation.

EXPERIMENTAL SECTION

General. To measure somatic and intestinal biotransformation rates, dietary uptake efficiencies, BCFs, and BMFs of a range of neutral hydrophobic organic chemicals in rainbow trout, 10 dietary bioaccumulation tests (i.e., nine studies performed at Exxon Mobil Biomedical Sciences. Inc. (EMBSI) and one study[24] conducted at Simon Fraser University (SFU)) following a similar methodology were carried out. The SFU study complements the EMBSI study by providing reference chemicals that cover the range of log K_{OW} of the test chemicals in the EMBSI study. Details of the study at SFU can be found in ref 24. The tests performed at EMBSI are described below. Methods for chemical and lipid content analyses are in the Supporting Information.

Fish. Juvenile rainbow trout (*Oncorhynchus mykiss*) were obtained from Thomas Fish Company. Fish were kept in 31 L flow-through aquaria, with a flow-through rate of approximately five to seven replacement volumes per day. An average of 53 (standard error (SE): 5) test fish were used in each test. Water temperatures were kept at 13.6 (standard devidation (SD): 0.3) °C, approximately the same to the 12.6 °C in Lo et al.[24] Water contained a mean dissolved oxygen content of 9.0 (SD 0.2) mg/L, and the pH was 7.7 (SD: 0.2). The mean fish weight from all experiments was 1.5 (SD: 0.5; range 0.9−2.3) g, and the mean fish lipid content was 3.6 (SD: 0.8; range 2.4−5.6) % wet weight (Table S1). Fish were fed Finfish Starter, no. 1 crumble (Zeigler Bros., Inc., Gardners, PA) an average of 3 (SD 1) % body weight·day^{-1}. The dietary lipid content in the studies ranged between 15 and 15.6% and was slightly lower than the value of 18.6% used in Lo et al.[24] In each feeding study, a control fish group was present to monitor for effects and to account for potential background concentrations of the test substances in fish tissues. Effects monitored in both the test and the control fish groups included mortality, growth rate constants, changes in feeding behavior (any deviations from rapid feeding), and other adverse effects including physical attributes (e.g., pigmentation, etc.), lethargy, and swimming behavior.

Chemicals. Test chemicals included parent and alkylated aromatic hydrocarbons, cycloalkanes, and linear and branched aliphatic hydrocarbons, musk xylene, and methoxychlor. The log K_{OW} of the test chemicals were obtained from EpiSuite 4.11 and varied between 3.3 and 8.9 (Table S2). All nine dietary bioaccumulation tests included the reference chemical hexachlorobenzene, which was assumed to undergo no or negligible biotransformation. The test chemical *trans*-decalin was also considered a reference chemical because previous work found *trans*-decalin to resist somatic biotransformation in rainbow trout.[24] In each of the 9 tests, 5−14 test and reference chemicals (Table S2) were dissolved in corn oil and added to the feed. Individual chemicals in the test mixture were selected to provide diverse hydrocarbon structures of varying hydrophobicity and facilitate use of a common analytical method while avoiding toxicity (assessed following methods described by McGrath and DiToro[29] and included in the Supporting Information) to exposed fish. Motivations for investigating multiple test compound exposures rather than individual chemicals were to reduce vertebrate animal use, testing costs, and time required for in vivo data collection. The content of corn oil spiked to diet was 0.5%. The chemical concentrations in the diet were measured in triplicate at the beginning and end of the uptake period to confirm the stability of the chemical in the food, as described in the Supporting Information. The mean and standard deviation of dietary exposure concentrations are reported in Table S2.

Dietary Bioaccumulation Studies and Kinetic Analysis. Fish were fed a contaminated diet for 10 to 14 days, followed by a 3−24 day depuration phase with no chemical exposure. Diets contained an average of 11 (range of 5−14) chemicals per test (Table S2). Fish were sampled throughout the uptake and depuration phase, with 3−10 fish sampled for each time point. The whole fish were homogenized and used for chemical extraction. The methodology for the kinetic analysis of the data is included in the Supporting Information.

RESULTS AND DISCUSSION

Diet. Measured concentrations of the various test chemicals in the diet ranged from 370 to 1171 μg/g (Table S2). Concentrations of the test and reference chemicals in the diet did not appear to change significantly throughout the exposure period, as evidenced by the low standard deviations of the dietary concentration measured throughout the exposure period and the associated low coefficient of variation for concentrations in fish foods from the beginning and end of the exposure period.

Fish. No fish mortalities, changes in feeding behavior, or other apparent adverse effects were observed in the exposure and control groups of all experiments. Also, there was no evidence of a difference in growth rates (k_{GD}) between control and test groups (Table S1). Mean fish body weights increased over time. The growth rates, calculated as the slope of the natural logarithm of the fish weight versus time for each of the nine experiments, varied from 0.027 to 0.047 day^{-1} with a mean of 0.040 (SD: 0.010) day^{-1}. Starting fish weights among nine experiments ranged between 0.88 and 2.3 g with a mean value of 1.5 (SD: 0.5) g (Table S1). The lipid content of the fish body among the tests varied between 2.4 and 5.6%. The mean lipid content of the fish's diet was 15.5% (range 15−15.6%) (Table S1).

Concentrations of Chemicals in the Fish Body. Concentrations of the test and reference chemicals in the control fish groups were below limits of quantitation. In all cases, the mean concentration of the chemicals in the test fish

DOI: 10.1021/acs.est.6b03602
Environ. Sci. Technol. XXXX, XXX, XXX−XXX

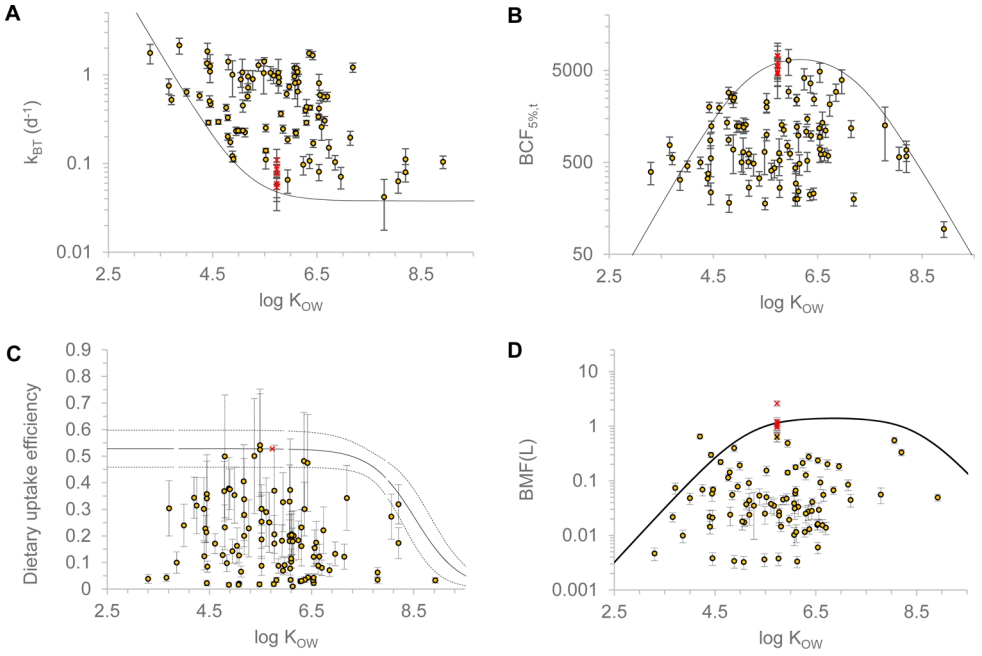

Figure 2. (A) The total depuration rate constant from the fish body, k_{BT}. (B) The bioavailability-corrected bioconcentration factor normalized to 5% lipid, $BCF_{5\%,t}$. (C) The dietary uptake efficiency ($E_{D,M}$ for test chemicals and $E_{D,N}$ for reference chemicals) normalized to hexachlorobenzene in each test. Solid and dashed lines represent predicted 95% confidence intervals of the predicted $E_{D,N}$ (eq 13). (D) The lipid normalized biomagnification factor BMF_L (bottom right) versus log K_{OW}. Solid lines in Figure 2A–D are parametrized to fish with experiment specific values of W_B = 1.5 g, Φ_{BL} = 5%, k_{GD} = 3.7%·day^{-1}, Φ_{DL} = 15%, and F_D = 3.5% kg·kg^{-1}·day^{-1}. Error bars represent the standard error of the mean values. The red "X" represent the reference chemicals in each test.

body increased throughout the dietary exposure phase, with certain chemicals approaching an apparent steady state before the end of the exposure period. During the depuration phase, mean concentrations in the fish body decreased in an apparent log–linear fashion, with concentrations of certain chemicals decreasing below detectable levels before the end of the depuration period (Figure S1).

Depuration Rate Constants. Total depuration rate constants from the fish body for the reference ($k_{BT,R}$) and test (k_{BT}) chemicals (Table S2) decreased with increasing K_{OW} (Figure 2A). This is due in part to the decrease in respiratory elimination with increasing hydrophobicity. A weighted multiple linear regression (regression weights = SE^{-1}) of the depuration rate constants of the reference chemicals ($k_{BT,R}$) in the study by Lo et al.[24] (n = 8) and in the present study (n = 10) using eq 7 for the juvenile rainbow trout produced the following relationship:

$$k_{BT,R} = [291(SE34)W_B^{[-0.19(SE0.02)]}/\Phi_{BL}]\cdot(1/K_{OW}) + k_{GD}$$
$$n = 18;\ \text{RMSE} = 0.15 \qquad (12)$$

where fish body lipid content (Φ_{BL}), fish body weight (W_B), and the growth rate (k_{GD}) of the test fish were specific to each experiment (Table S1). Empirical $k_{BT,R}$ values for each test chemical are reported in Table S2. Figure S3 shows that all test chemicals exhibited depuration rate constants that were equal to or greater than the $k_{BT,R}$, with the exception of naphthalene, 1,3,5-trimethylbenzene, and cis-bicyclo(4,3,0)nonane, which exhibit some of the lowest reported K_{OW} values of the chemicals in the present study. These chemicals may illustrate the limits of the current study design for determining somatic biotransformation rates. For these low K_{OW} chemicals, respiratory elimination rate constants are high, making it difficult to obtain reliable values of relatively low biotransformation rate constants, which are derived as the difference between the high depuration rates of both test and reference chemicals.

Somatic Biotransformation Rate Constants. The somatic biotransformation rate constant (k_{BM}) estimates for the test chemicals are listed in Table S2. Somatic biotransformation rate constants of six of the test chemicals in the EMBSI studies were in good agreement with those in Lo et al.[24] after the somatic biotransformation rate constants are normalized to the same size fish following Arnot et al.[14] (Figure S4). A comparison of empirical k_{BM} and body-weight normalized k_M values estimated by the BCFBAF QSAR (EpiSuite 4.11) illustrates some agreement between k_{BM} and k_M values (Figure 3). A regression analysis of the empirical log k_{BM} data and BCFBAF QSAR log k_M estimates indicates a correlation coefficient (r^2) of 0.23 and that k_M estimates are approximately within 2 orders of magnitude of the empirical k_{BM} data in 95% of cases. It should be stressed that because fish were exposed to multiple chemicals at a single concentration for each chemical, there is the potential that both competitive inhibition and enzyme saturation effects affect the measured biotransformation rates. The in vivo biotransformation rate constants reported in this study may therefore be more conservative (i.e., lower) than in single-compound experiments in which dietary chemical concentrations are lower than those used in this study and in which competing substrates are absent.

Dietary Uptake Efficiency. The following relationship between $E_{D,N}$ and K_{OW} (Figure S5) observed for the reference chemicals from Lo et al.[24] (n = 7) was used to determine intestinal biotransformation rate constants:

DOI: 10.1021/acs.est.6b03602
Environ. Sci. Technol. XXXX, XXX, XXX–XXX

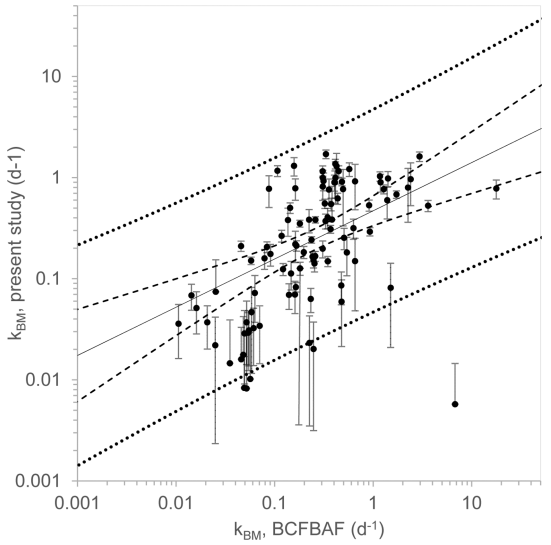

Figure 3. Observed somatic biotransformation rate constants (k_{BM}) from the present study as a function of BCFBAF QSAR (EPI Suite v. 4.11)-predicted biotransformation rate constants, normalized to the same fish weights as those corresponding to the observed values. The solid line represents the mean regression fit, dashed lines represent the 95% confidence intervals of the predicted mean, and the dotted lines represent the 95% prediction interval of individual chemicals.

$$E_{D,N}^{-1} = 5.6 \times 10^{-9}\ (\text{SE}\ 1.8 \times 10^{-9}) \times K_{OW} + 1.9\ (\text{SE}\ 0.1) \quad (13)$$

In all tests, the dietary assimilation efficiencies of test chemicals were either equal to or less than the dietary assimilation efficiencies of the corresponding reference chemicals (Figure 2C). This indicates that the $E_{D,N}$–K_{OW} relationship serves as a reasonable reference point for deriving intestinal biotransformation rates in fish species.

Gastrointestinal Biotransformation Rate Constants. Estimates of k_{GM} are listed in Table S4. For the majority of chemicals tested in this study, there are no gastrointestinal biotransformation rate data that can be used for comparison. However, benzo[*a*]pyrene and related polycyclic aromatic hydrocarbons (PAHs) have shown low dietary uptake efficiencies in rainbow trout[30−33] and are significantly biotransformed into water-soluble metabolites in the intestines of fish, with a 50% (for uninduced animals) and 90% (for induced animals) recovery of benzo[*a*]pyrene from the portal vein in the form of metabolites.[34] These findings are in agreement with the findings from this study and further indicate the importance of gastrointestinal biotransformation on bioaccumulation of many substances in fish.

Respiratory Uptake and Elimination Rate Constants and BCF. The bodyweight-scaled respiratory uptake rate constant (k_{B1}) and elimination rate constant (k_{B2}) were derived as $\alpha \cdot W_B^{\ b}/d_L$ and $(\alpha \cdot W_B^{\ b}/\Phi_{BL}) \cdot (1/K_{OW})$ respectively, based on the regression coefficients α of 291 (SE: 34), b of −0.19 (SE: 0.02) (eq 12), the density of lipid d_L of 0.90, and the lipid content of the fish (Φ_{BL}) in each experiment (Table S3). $BCF_{WW,fd}$ derived as k_{B1}/k_{BT} and $BCF_{WW,t}$ and corresponding values that are adjusted to a 5% lipid content ($BCF_{5\%,t}$) are listed in Table S3. The mean $BCF_{WW,fd}$ and a $BCF_{WW,t}$ of all test chemicals were less than 5000. The mean $BCF_{5\%,t}$ was also less than the regulatory criterion of 5000 for all chemicals except hexadecahydropyrene. No test chemical was found to exhibit a $BCF_{WW,fd}$, $BCF_{WW,t}$, or $BCF_{5\%,t}$ significantly ($p = 0.05$) greater than 5000. There appears little correlation between the $BCF_{5\%,t}$ and K_{OW} for the test chemicals (Figure 2B), caused in large part by the fact that the majority of test chemicals are biotransformed at rates that exceed respiratory elimination rates. The lack of correlation between $BCF_{5\%,t}$ and K_{OW} suggests caution in the derivation of the $BCF_{5\%,t}$ from $BCF_{ww,t}$, as linearity between the $BCF_{ww,t}$ and the fish's lipid content may not exist for biotransforming chemicals.

Dietary Uptake and Excretion Rate Constants and BMF. Rate constants for gastrointestinal exchange (k_{GB} and k_{BG}) and fecal egestion (k_{GE}), derived from the experimental observations according to Lo et al.,[24] are listed in Table S5. The BMFs, derived as $(F_D \cdot E_{D,M})/k_{BT}$ are reported in Table S4 for all test chemicals and are also expressed on a lipid-normalized basis (as BMF_L in units of L dietary lipid/L fish body lipids) in Figure 2D. The BMF_L of all test chemicals were less than 1. Only the reference chemical hexachlorobenzene exhibited a BMF_L greater than 1. There appeared to be no relationship between the BMF_L and K_{OW}, likely as a result of the high biotransformation rates of the test chemicals in both the soma and the gastrointestinal contents of the fish.

Internal Distribution. The ability of the test design to derive the various rate constants (Tables S1−S5) in the bioaccumulation model (Figure 1) from the empirical data allows for the evaluation of the internal distribution of the test and reference chemicals in fish and to determine the contribution of transport and transformation processes to the bioaccumulation behavior of the chemical under various exposure scenarios. Figure 4, which shows chemical transport and transformation fluxes as a fraction of the total intake flux from either a dietary or aqueous exposure, illustrates that benzo[*a*]pyrene is biotransformed in both the digesta and the body (including the liver) of the fish. When exposed via the diet, the great majority of benzo[*a*]pyrene (i.e., 98.1%) is biotransformed in the intestinal tract. Vetter et al.[35] also demonstrated rapid benzo[*a*]pyrene metabolism in the intestines of fish. Bock et al.[36] demonstrated that benzo[*a*]-pyrene is extensively metabolized during the passage through the gastrointestinal tract of the rat. Figure 4 also shows that when fish are exposed via the respiratory route, the great majority of benzo[*a*]pyrene (i.e., 98.5%) is biotransformed in the body of the fish. For benzo[*a*]pyrene, both somatic and intestinal biotransformation appear to play an important role in the chemical's bioaccumulation. The relative contribution of the soma and intestines as sites for biotransformation is largely controlled by the relative concentrations of benzo[*a*]pyrene in the diet and water of the fish. Figure 4 shows that this is not the case for triphenylene. Triphenylene appears to be virtually recalcitrant in the intestinal tract while it is quickly biotransformed in the fish body. Upon ingestion, approximately half of the ingested dose of triphenylene is egested in an untransformed state in fecal matter. The other half of the ingested dose is absorbed into the body of the fish and then almost fully biotransformed in fish body. Upon respiratory uptake via water exposure, virtually all triphenylene is biotransformed in the fish body.

Figure 5A illustrates the relative contribution of somatic and gastrointestinal biotransformation for all the test chemicals when exposed only through the diet. It shows that for the majority of the ingested test chemicals, gastrointestinal biotransformation contributes the majority of a substance's biotransformation. Figure 5B shows that test chemicals exposed

DOI: 10.1021/acs.est.6b03602
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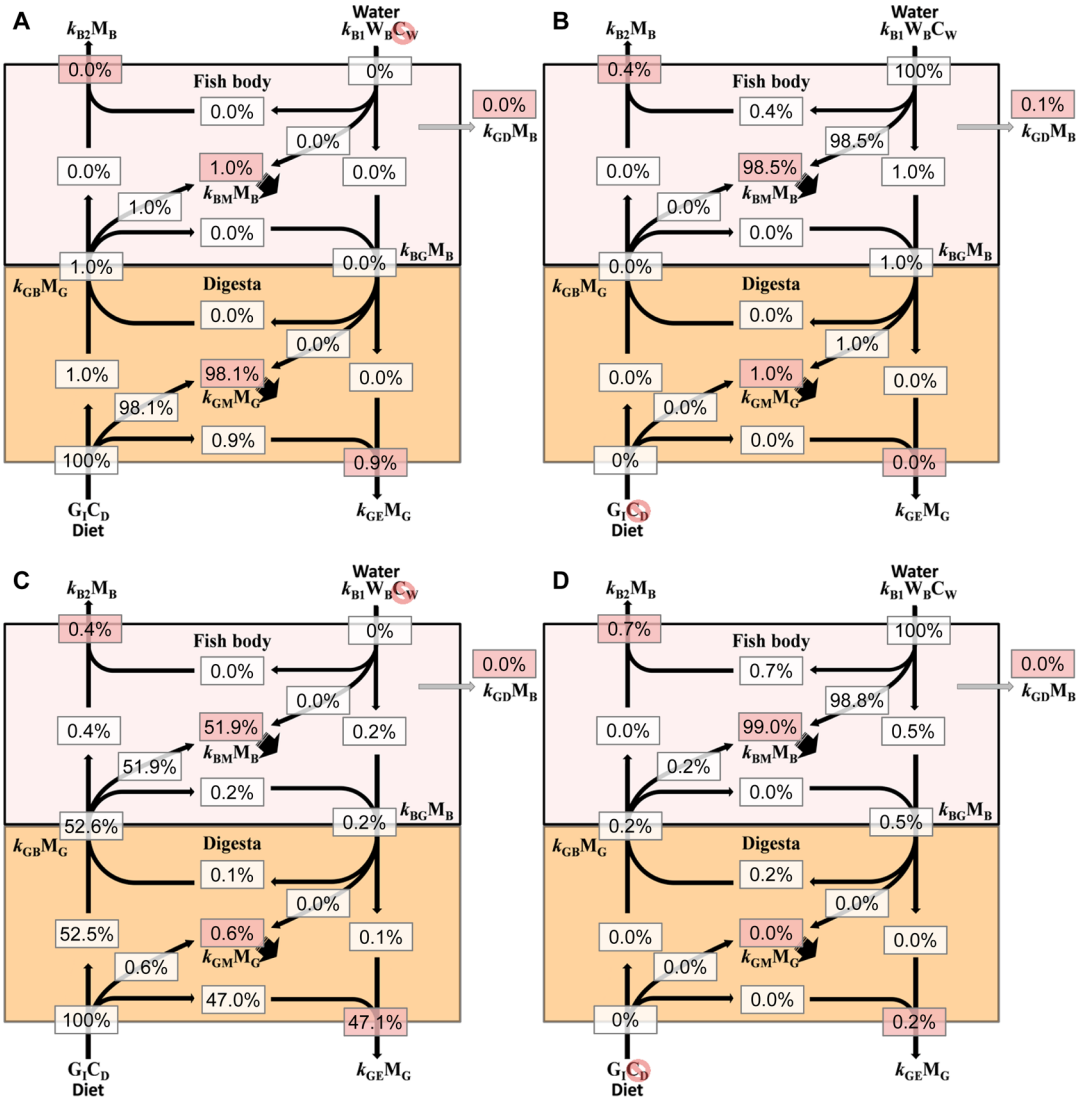

Figure 4. Detailed schematic diagram of the internal distribution dynamics, expressed in terms of the fraction of the administered chemical intake rate in units of grams per day for benzo[*a*]pyrene from test no. 3 (A,B) and triphenylene in test no. 7 (C,D) in a dietary-only exposure environment (A,C) and in an aqueous-only exposure environment (B,D). Biotransformation (represented by the black arrows) occurs in the body (k_{BM}) and the gastrointestinal tract (k_{GM}) of the fish. Red boxes indicate routes of elimination.

via the respiratory route are in most cases primarily biotransformed in the fish body. In real-world exposure scenarios, where exposure occurs via both the respiratory and the dietary routes, the relative contribution of somatic and intestinal biotransformation and, hence, the rate of biotransformation will depend on the relative concentrations of the chemical in the water and the diet. Figure S6 shows that for the chemicals tested, there is not a general relationship between the somatic and gastrointestinal biotransformation rate constants. This suggests that biotransformation pathways and the associated metabolic stability of a chemical in the liver and the intestinal tract may differ substantially. These findings suggest that while biotransformation rate determinations in hepatocytes, liver tissues, or liver homogenates such as S9 and liver microsomes are useful measures of somatic biotransformation rates, they do not fully characterize the ability of biotransformation processes in the fish to mitigate the bioaccumulation of chemicals. Extrahepatic biotransformation in the intestinal tract due to digestive and intestinal mucosal enzymes and resident bacteria is recognized for many food components and chemicals.[37] The development of in vitro bioassays for gastrointestinal biotransformation may be a useful contribution to ongoing hepatic in vitro to in vivo extrapolation methods for bioaccumulation assessments. Further research is needed to better understand the roles of fish enzymes and microflora in the biotransformation of chemicals in the intestinal tract and to characterize biotransformation pathways in the gut. Further analysis of dietary in vivo bioaccumulation test data in relation to chemical structure may support the development of quantitative structure−activity relationships for both somatic and gastrointestinal biotransformation that are needed to advance in silico methods for improving bioaccumulation model predictions.

Relationship between BCF and BMF. Because the testing methodology explored in this study produces both BCF and BMF estimates, the relationship between the BCF and BMF can be explored. The BCF−BMF relationship is useful in interpreting data from dietary bioaccumulation tests in terms of the BCF required by regulations. Figure 6 illustrates that, within a single test, the freely dissolved wet weight BCF in rainbow

DOI: 10.1021/acs.est.6b03602
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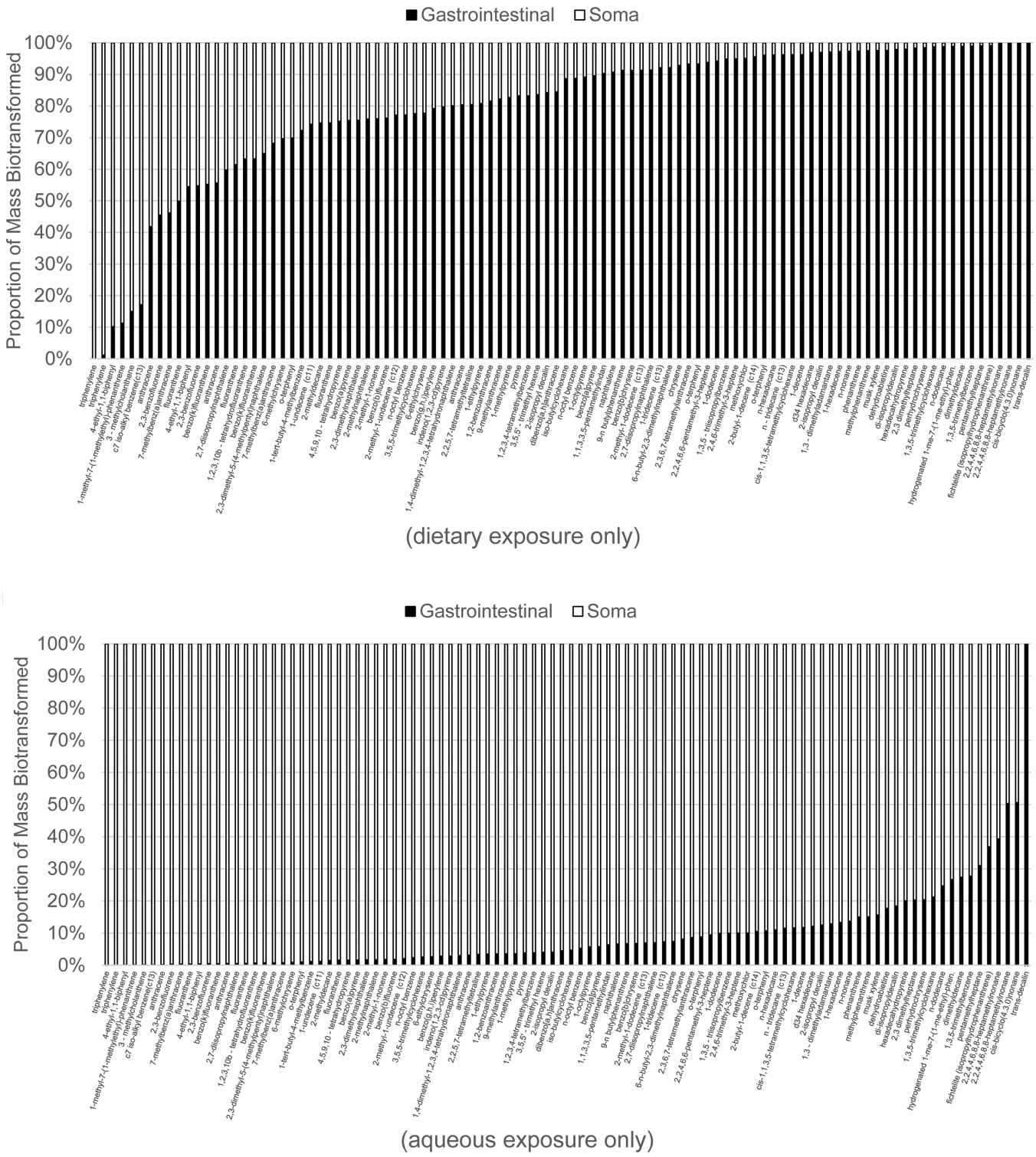

Figure 5. Contribution (%) of somatic (white) and gastrointestinal (black) biotransformation to the overall mass of chemical biotransformed following continuous dietary (top) or aqueous exposure (bottom).

trout, ($BCF_{WW,fd}$) and the lipid-normalized BMF (BMF_L) of nonbiotransformable substances, represented by the reference chemicals, are closely related:

$$\log BCF_{WW,fd} = 1.20\ (SE\ 0.11)\cdot \log BMF_L + 3.72\ (SE\ 0.06)$$
$$n = 16;\ r^2 = 0.90;\ p < 0.001 \qquad (14)$$

The close relationship between the $BCF_{WW,fd}$ and BMF_L is due to the fact that both the BCF and the BMF are a function of the same depuration rate constant (k_{BT}). The theoretical basis for the relationship between the BCF and BMF has been discussed in more detail in Mackay et al.[25] Eq 14 shows that if a nonbiotransformable substance exhibits a $BCF_{WW,fd}$ equal to regulatory criterion of 5000, then the mean predicted BMF_L can be expected to be approximately 1.0 with lower and upper

DOI: 10.1021/acs.est.6b03602
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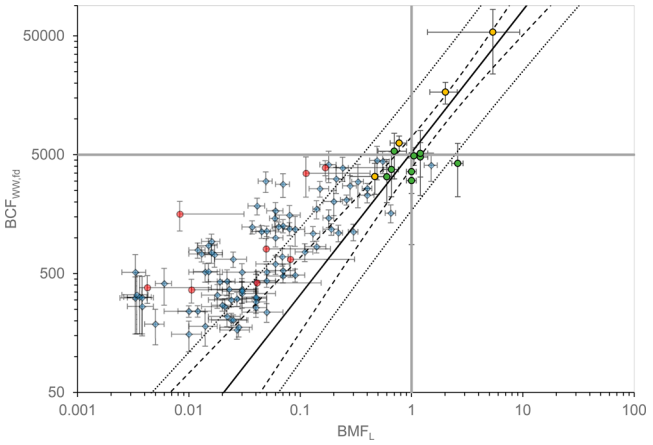

Figure 6. $BCF_{WW,fd}$ vs BMF_L for test chemicals (blue and red circles) and reference chemicals (green and yellow circles) in the present study (green and blue) and in Lo et al. 2015 (yellow and red). The solid black line represents the predicted log BMF_L − log $BCF_{WW,fd}$ linear regression of the reference chemicals. Dotted lines represent the 95% prediction interval. The gray lines represent the regulatory criteria for a BCF of 5000 and a BMF_L of 1, indicating chemical biomagnification.

95% confidence intervals of 0.8 and 1.3, respectively. This confirms that the regulatory criterion for the BCF of 5000 is a reasonable threshold for identifying nonbiotransformable chemicals that have significant biomagnification potential. Nonbiotransformable substances with $BCF_{WW,fd}$ less than the regulatory criterion of 5000 do not show a significant biomagnification potential (Figure 6). Substances that are biotransformed exclusively in the body of the fish also adhere to the same $BCF_{WW,fd}$−BMF_L relationship applicable to non-biotransforming substances because somatic biotransformation contributes to the whole body depuration rate that controls both the biomagnification and bioconcentration factors. However, Figure 6 shows that a loss of the $BCF_{WW,fd}$−BMF_L relationship occurs for substances that are biotransformed in the intestines of the fish. In all cases, intestinal biotransformation produces BMF_L that are less than expected from the $BCF_{WW,fd}$ -BMF_L relationship described by eq 14. Gastrointestinal biotransformation lowers the effective concentration of the chemical in the intestinal tract and reduces the chemical's dietary uptake efficiency. For substances subject to intestinal biotransformation, the BCF has a tendency to overestimate the biomagnification potential of substances. Substances that are significantly transformed in the intestinal tract do not have a biomagnification potential and may be of less concern for bioaccumulation than chemicals that biomagnify (i.e., BMF_L > 1). Bioaccumulation tests using aqueous exposure can only identify the bioconcentration behavior of the test chemical because it is insensitive to the intestinal biotransformation rate. Dietary bioaccumulation tests, which are often less costly, time-involved, and labor-intensive than bioconcentration tests, are more insightful than standard bioconcentration tests because of their ability to provide information on somatic and intestinal biotransformation rates as well as the BMF and BCF. Empirical correlations between the BCF and BMF,[38] which are attractive because of their ability to express data from dietary bioaccumulation tests, in terms of the regulatory required BCF, pose considerable limitations because the correlation is highly sensitive to the inclusion of chemicals subject to high rates of intestinal biotransformation and low bioavailability in water. As this study shows, many hydrophobic organic chemicals are subject to intestinal biotransformation and exhibit a reduced bioavailability in water due to their high sorption potential to organic matter in the water phase. While this study demonstrates that intestinal biotransformation and bioavailability in water can modulate the relationship between the BCF and BMF, this study also supports a fairly simple rule of thumb that may be useful in the interpretation of dietary bioaccumulation tests: namely, that chemicals with a BMF_L of <1 tend to exhibit BCFs (based on either freely dissolved ($BCF_{WW,fd}$) or total concentration ($BCF_{WW,t}$) of the chemicals in the water) that are less than 5000.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b03602.

The derivation of k_{BG} and δ and details on the measurement of chemical concentrations, lipid content, total tissue concentrations, general bioaccumulation and exchange rate parameters, and the estimation of k_{BT}, k_{BM}, $BCF_{WW,fd}$, $E_{D,M}$, lipid body burdens, concentrations of test chemicals through uptake and depuration phases, k_{GM}, and elimination rate constants are described in the Supporting Information. (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors acknowledge the Natural Science & Research Council of Canada for support of this study.

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