In Vivo Bioconcentration of 10 Anionic Surfactants in Rainbow Trout Explained by In Vitro Data on Partitioning and S9 Clearance

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ABSTRACT: Bioconcentration factors (BCFs) in rainbow trout were measured for 10 anionic surfactants with a range of alkyl chain lengths and different polar head groups. The BCFs ranged from 0.04 L kg⁻¹ ww (for C₁₀SO₃) to 1370 L kg⁻¹ ww (C₁₆SO₃). There was a strong correlation between the log BCF and log membrane lipid–water distribution ratio (D_MLW; r² = 0.96), and biotransformation was identified as the dominant elimination mechanism. The strong positive influence of D_MLW on BCF was attributed to two phenomena: (i) increased partitioning from water into the epithelial membrane of the gill, leading to more rapid diffusion across this barrier and more rapid uptake, and (ii) increased sequestration of the surfactant body burden into membranes and other body tissues, resulting in lower freely dissolved concentrations available for biotransformation. Estimated whole-body in vivo biotransformation rate constants k_B-BCF are within a factor three of rate constants estimated from S9 in vitro assays for six of the eight test chemicals for which k_B-BCF could be determined. A model-based assessment indicated that the hepatic clearance rate of freely dissolved chemicals was similar for the studied surfactants. The dataset will be useful for evaluation of in silico and in vitro methods to assess bioaccumulation.

KEYWORDS: BCF, kinetics, biotransformation, membrane lipid, IVIVE

INTRODUCTION

Anionic surfactants are widely used in products such as cleaning agents, detergents, and personal care products. Usage is high, with widespread professional and consumer use in Europe amounting to 97 889 tonnes year⁻¹ in 2016 for alkyl sulfate surfactants alone. Anionic surfactants consist of a negatively charged functional group, frequently a sulfate or sulfonate, coupled with one or several linear or branched alkyl chains, which can also contain other groups. The range of structures is large; 45 anionic surfactants with production volumes >100 tonnes year⁻¹ (of which 7 substances with 10 000–100 000 tonnes year⁻¹) have been registered under the REACH legislation in the European Union (Table S1, Supporting Information). Important groups of anionic surfactants include alkyl sulfates (primarily used in detergents, production in North America 56 000 tonnes in 2006), alkylethoxysulfates (cleaning products, production in NA 229 000 tonnes in 2008), and linear alkyl benzene sulfonates (LASs) (detergents, production in NA 269 000 tonnes in 2008). Due to their extensive usage in down-the-drain products, emissions to surface water can be significant. Hence, careful evaluation of the potential environmental impact of anionic surfactants is required.

One important component of the regulatory assessment of the environmental safety of chemicals is bioaccumulation. Bioaccumulation is commonly evaluated using the bioconcentration factor [BCF, L kg⁻¹ wet weight (ww)] in fish, which is derived from an experiment in which fish are exposed to the chemical via water, and defined as the quotient of the chemical’s concentration in fish and in water at steady state. Despite their low pKₐ (typically < 0), anionic surfactants are taken up systemically by fish to a much greater extent than permanently charged cationic surfactants. BCFs were measured for a range of surfactants in the 1970s and 1980s. However, a review of the available data in 1994 concluded “Most of these data are inappropriate to quantitatively describe the bioconcentration of surfactants because the most frequently used analytical method, LSC without prior chromatographic separation of radiolabeled compounds, does not allow to distinguish between the parent compound and
metabolites. In a second review from 2007, 38 measured BCF values for anionic surfactants representing 10 C_{10}–C_{13} LASs and 7 perfluoroalkyl acids were judged to be quantitatively reliable. There have since been several other BCF values published for LASs. Furthermore, BCF values from several unpublished industry studies have been reported, whereby few details are available on the methodology employed. Among the 45 anionic surfactants with a production volume >100 tonnes year^{-1} registered under REACH, only seven report surfactants are largely restricted to LAS; there is no consistent elimination pathway in which the dominant elimination pathway will be tested against BCF data.

BCF tests require many animals. To reduce the usage of experimental animals, there are ongoing efforts to develop methods that can reliably predict BCF from in vitro tests and in silico models. In vitro tests are used to measure biotransformation rates in cells or cell extracts, in vitro extrapolation (IVIVE) with in silico tools convert the in vitro data to hepatic and whole-body biotransformation clearance rates, and in silico bioconcentration models employ this information combined with the chemical’s partitioning properties to estimate the BCF. Anionic surfactants may be particularly amenable to this alternative approach because there are good methods for precise determination of the membrane lipid–water distribution ratio (D_{MLW}) as the most relevant partitioning property, and there is evidence that their elimination is dominated by biotransformation, which would mean that only this elimination pathway would have to be considered. However, before an alternative approach for determining BCF can be implemented, it must be tested against in vivo BCF data.

The goal of this study was to determine in vivo BCFs of anionic surfactants that could be used to evaluate and further develop alternative approaches to estimate this metric for this class of chemicals. Considering this goal, particular attention was directed to selecting test chemicals that could provide insight into how molecular structure influences BCFs and to optimizing the experimental protocol to allow the maximum number of chemicals to be studied while ensuring that the BCF data were of sufficient quality for the stated purpose. For this, we drew on experience from earlier experiments on the tissue distribution and bioconcentration of cationic surfactants in rainbow trout. Furthermore, to supplement the interpretation of the in vivo data, a rainbow trout liver S9 substrate depletion assay (RT-S9) was used to measure in vitro intrinsic clearance rates of the same set of anionic surfactants.

### METHODS

#### Overview

The bioconcentration of a mixture of 10 anionic surfactants was measured in rainbow trout in water at pH 7.8. The S9 assays were conducted using both individual compounds and a mixture comparable to that used in the BCF experiment to explore the influence of co-solutes on biotransformation.

#### Test Chemicals and Reagents

The test chemicals for the in vivo experiment were selected with a view to evaluating the influence of the alkyl chain length and the polar head group on the anionic surfactant BCF. Widespread use in household cleaning products and detergents was an exclusion criterion to minimize the risk of background contamination. The chemicals selected consisted of five linear alkyl sulfonates with chain lengths ranging from 5 to 16, four other linear alkyl surfactants with different anionic head groups [two sulfates, one LAS, one tetraethoxy sulfate (SLES)], and two more complex structures with two branched alkyl chains [bis(2-ethylhexyl)phosphoric acid phosphate (BEHP) and bis(2-ethylhexyl)-sulfosuccinate (DOSS)]. BEHP could not be reliably quantified in the BCF experiment, leaving 10 test chemicals in the study. The names and abbreviations of the test chemicals are provided in Table 1, while further information including CAS® and supplier are found in Table S2. Analytical standards were prepared in methanol and stored in glass. Polypropylene vials were employed for storing all extracts and solutions. The solvents employed are detailed in Table S3.

#### Fish Exposure and Sampling

The experiment was conducted in flow-through aquaria with juvenile rainbow trout at 10 °C. Ethical approval for the experiments was obtained from Stockholms djurförsöketiska nämnd (decision 9967-2017). The fish, fish housing, fish handling, and procedure for introducing the mixture of 11 chemicals were very similar to our previous study with cationic surfactants, details are provided in Text S1. The experiment consisted of a 4-day exposure phase and a 7-day depuration phase. The nominal concentrations of the chemicals in water were chosen to minimize exposure while ensuring quantifiable concentrations in the fish based on the results of a pre-experiment and ranged from 5.6 to 52 μg L^{-1} (Table 1). Water samples were collected and fish were sacrificed according to the schedule in Table S4.

The water samples were collected just prior to the daily removal of feces from the aquarium. Triplicate samples were taken at each time point. An autopipette with a polypropylene tip was pumped 7 times with aquarium water, and then 400 μL of aquarium water was sampled and transferred to a 1.5 mL sample vial.
polypropylene vial containing 600 μL of methanol and isotope-labeled standards of BEHP and sodium dodecylsulfate (C₁₂SO₄). The water/methanol mixture was analyzed using liquid chromatography–mass spectrometry (LC–MS)/MS as described below for fish.

At each fish sampling, five fish were sacrificed. Following stunning and severance of the spinal cord, the whole fish were placed in a polyethylene bag, weighed, and immediately frozen at −20 °C. The weight of the individual fish at sacrifice was 24.0 ± 5.0 g.

**Sample Analysis.** Three of the five fish from each time point were prioritized for analysis based on proximity to the median fish weight. Each fish was semihawed and homogenized with an Ultra-Turrax dispersing device. For extraction, 0.15 mL of internal standard solution [isotope-labeled standards of BEHP (D₁₄) and C₁₂SO₄ (D₂₅)], 3 mL of methanol, and 3 steel balls (3.2 mm diameter) were added to 220 mg of the fish homogenate. This mixture was homogenized in a bullet blender for 5 min and then placed in an ultrasonic bath at 45 °C for 15 min. Following centrifugation for 15 min@3200 RCF, the supernatant was decanted and the extraction was repeated. The extracts were combined.

For instrumental analysis, 5 μL of the fish extract or 60 μL of the water/methanol mixture (water samples) was separated on an Acquity UPLC BEH C18 column fitted with a BEH C18 pre-column and analyzed on a TSQ Quantiva triple quadrupole mass spectrometer. The mobile phase was methanol (10 mM ammonium acetate) and Milli-Q-filtered water (10 mM ammonium acetate) (see Table S5 for the liquid chromatography program). To avoid detergent blank peaks originating from cleaning mobile phase bottles, the methanol was prepared directly in the solvent bottle as delivered and Milli-Q was tapped into an empty methanol bottle. Furthermore, a guard column was fitted between the mobile phase and the autosampler to prevent background contamination from disturbing the analysis. All analytes were quantified against the BEHP internal standard, as it gave better reproducibility. The MS parameters are provided in Table S6. A 10-point calibration curve consisting of matrix-matched standards covering a concentration range of 3 orders of magnitude was used.

**Statistical Analysis.** We assumed that the surfactant concentrations in fish over time can be described by a one-compartment fish model with first-order kinetics. The governing equation was

\[
\frac{dC_F}{dt} = k_U C_W(t) - k_B F
\]

(1)

where \(C_F\) is the surfactant concentration in fish (μg kg⁻¹ ww), \(t\) is the time (h), \(C_W(t)\) is the surfactant concentration in water (μg L⁻¹) at time \(t\), and \(k_U\) and \(k_B\) are the uptake and overall elimination rate constants (in units of L kg⁻¹ h⁻¹ and h⁻¹, respectively). Table 2 lists the symbols used in the paper. The central goal of the analysis was to estimate \(k_B\) and \(k_U\) as well as the BCF (\(k_B / k_U\)) from measurements of surfactant concentration in fish and water samples collected at different time points of the experiment. For the fish, only those time points were included for which \(C_F(\text{measured})\) was above the limit of quantitation (LOQ) in all three fish (data below the LOQ will have a positive bias; including them would result in a negative bias in \(k_B\)). In this analysis, there exist two sources of uncertainty: first, uncertainty in the actual concentration in water \(C_W\) at the time of sample collection and second, variability in the concentration in fish around the (expected) surfactant concentrations in fish assumed to follow model (1). We therefore set up a model in which we considered uncertainty in both water and fish measurements simultaneously by assuming a parametric model for the surfactant concentration in the water over time (either linear or quadratic) and accounted for the uncertainty in the corresponding parameters of the water model during the estimation of \(k_U\) and \(k_B\). This was done in a joint Bayesian model that we estimated via Hamiltonian Monte Carlo.

From the governing equation for the fish model (1), we can derive (see Text S2) the expected surfactant concentration in fish at a specific time point \(t\) as

\[
C_F(t) = e^{-k_B t} \int_0^t k_U C_W(t)e^{k_B dt}
\]

(2)

This quantity depends on the surfactant concentration in the water over time, \(C_W(t)\), that we modeled either based on a linear or a quadratic model, \(C_W(t) = b_0 + b_1 t\) or \(C_W(t) = b_0 + b_1 t + b_2 t^2\), respectively. It is then possible to solve the integral in (2) analytically and use the results for deriving the expected likelihood of the observed fish samples collected at different time points in terms of the parameters \(k_B\), \(k_U\), \(b_0\), \(b_1\), and \(b_2\) as well as two variance parameters for the measured concentrations in water and fish, respectively (see Text S2 for a detailed description of the model). We chose between the linear and quadratic model for the concentration in water in the first step by estimating a standard linear model with linear or quadratic time effect to the observed concentration data and selected the model with a lower Bayesian information criterion (BIC). In the full Bayesian model based on eq 2, we used uninformative priors for all parameters (independent of the first analysis step for the model of concentration in water) and monitored convergence and mixing of the chains based on

**Table 2. Symbols Used in the Paper**

| Symbol | Explanation | Unit |
|--------|-------------|------|
| A | surface area of the epithelial membrane in the gills over which transport occurs | m² |
| Cₚ | chemical concentration in fish | μg kg⁻¹ ww<sup>4</sup> |
| Cₘ | chemical concentration in water | μg L⁻¹ |
| D | diffusion coefficient of the chemical in the epithelial membrane in the gills | m h⁻¹ |
| D<sub>FW</sub> | fish–water equilibrium distribution ratio | L kg⁻¹ ww |
| D<sub>MLW</sub> | membrane lipid–water distribution ratio | L kg⁻¹ |
| f<sub>MLF</sub> | mass-to-volume fraction of membrane lipid-like sorbent in the blood | kg⁻¹ ww |
| k<sub>ᵦ</sub> | elimination rate constant due to biotransformation | h⁻¹ |
| k<sub>ᵦ,BF</sub> | estimated from the in vivo BCF experiment | h⁻¹ |
| k<sub>ᵦ,SV</sub> | estimated from the in vitro RT-S9 assay | h⁻¹ |
| k<sub>ᵦ</sub> | uptake rate constant | L kg⁻¹ ww h⁻¹ |
| k<sub>ₑ</sub> | overall elimination rate constant | h⁻¹ |
| k<sub>ₑ</sub> | elimination rate constant due to gill respiration | h⁻¹ |
| M | mass of the fish | kg ww |
| ρ<sub>M</sub> | density of the epithelial membrane of the gills | kg L⁻¹ |
| Q<sub>B</sub> | rate of blood flow through the liver | L h⁻¹ |
| Q<sub>LW</sub> | clearance rate due to transformation of freely dissolved chemicals in the liver | L h⁻¹ |
| t | time | h |
| V<sub>D</sub> | volume of distribution | L |

<sup>4</sup>Wet weight.
trace plots and the R-hat statistic, as implemented in Stan.\textsuperscript{22} Uncertainty quantification for the model parameters and derived quantities was obtained from the posterior samples following standard practice in Bayesian inference. The analysis was performed in R\textsuperscript{23} using the cmdstanr package\textsuperscript{24} for implementation and estimation of the model. Text S3 describes how the uncertainty of several other derivative parameters was estimated and presents a simpler approach for estimating the BCF model based on a stepwise application of parameters.\textsuperscript{25}

In Vitro Estimation of Biotransformation. The in vitro\textsuperscript{26} intrinsic clearance by the S9 fraction of rainbow trout liver was measured for the same 11 anionic surfactants used in the BCF experiment as separate compounds. A mixture of 9 of these surfactants, without C\textsubscript{17}SO\textsubscript{3} and C\textsubscript{18}SO\textsubscript{3}, was tested as well for a preliminary comparison of potential inhibition of transformation due to the presence of co-solutes. An established RT-S9 substrate depletion assay was employed,\textsuperscript{27} predating the currently available OECD guideline 319B.\textsuperscript{28} A single batch of the characterized liver homogenate material was prepared at the US EPA lab in Duluth and shipped on dry ice to the testing facilities at the University of Amsterdam. Properties and measured activity levels are presented in Table S7. The test was conducted using the same method applied for a series of cationic surfactants in Droge et al.\textsuperscript{29} and is summarized together with the in vitro—in vivo extrapolation in Text S4.

### RESULTS AND DISCUSSION

Quality Assurance of the Analytical Methods. For the water method, the repeatability, quantified as the average relative standard deviation of the 14 sets of triplicate samples collected during the exposure phase, ranged between 3 and 25% (Table S8). Repeatability was better for the shorter-chain compounds. Testing in a pre-experiment had shown better repeatability (generally <5%) in aquarium water, but the aquarium contained far fewer fish than during the BCF exposure experiment.

For fish analysis, method precision was assessed via triplicate injections of one extract (instrumental precision) and triplicate extraction of the same homogenate (extraction + instrumental precision). The instrumental precision ranged from 3 to 15%, and the extraction + instrumental precision ranged from 0.4 to 23%, whereby the longer-chain compounds showed better precision (Table S9). One reason for the poorer precision of the shorter-chain compounds, in particular, C\textsubscript{9}SO\textsubscript{3} and C\textsubscript{17}SO\textsubscript{3}, could be the proximity of the concentrations to the LOQ. In general, the extraction + instrumental precision was poorer than the instrumental precision by itself, indicating that the extraction step made a significant contribution to the extraction + instrumental precision. DOSS was a notable exception, as it had the poorest instrumental precision (15%) and the best extraction + instrumental precision (0.4%). This is likely a reflection of the limited quantity of data for this assessment. The precision was judged to be satisfactory for direct injection of a complex matrix and sufficient for the purpose of this study.

The test chemical concentrations in the control fish and the fish method blanks were <1 ng g\textsuperscript{-1} ww in most cases. The LOQ of the method (calculated as mean + 10\times standard deviation of the control fish) ranged from 0.2 to 8 ng g\textsuperscript{-1} ww (Table S10).

Concentrations in Water. The surfactant concentrations in the aquarium water during the exposure phase showed a decreasing trend for some substances (Figure S1). Over the 4-day period, the concentrations decreased by a factor of \textasciitilde ~2 for C\textsubscript{17}SO\textsubscript{3}, C\textsubscript{18}SO\textsubscript{3}, and C\textsubscript{19}SO\textsubscript{4}, and a factor of 4 for C\textsubscript{14}SO\textsubscript{4}. The concentrations of these substances were relatively constant for the first 24 h. A possible explanation for this behavior is that a microbial population developed in the aquarium that biodegraded these substances.

Considering the decreasing time trend for some substances, the agreement with the nominal concentrations was evaluated using the data from the first 6 h of exposure. The relative standard deviation of the mean concentrations from the seven time points sampled ranged from 7 to 22% (Table 1). It was correlated with the precision of the analytical method (see Table S8), which suggests that the method uncertainty contributed significantly to the measured variability in the water concentrations. There was good agreement between the mean measured concentration and the nominal concentration for C\textsubscript{10}SO\textsubscript{4}, C\textsubscript{15}SO\textsubscript{3}, C\textsubscript{14}SO\textsubscript{3}, C\textsubscript{14}SO\textsubscript{4}, C\textsubscript{17}SO\textsubscript{4}, and C\textsubscript{17}-1-LAS (Table 1). The mean measured concentration was a factor of 1.7–2.0 higher than the nominal for C\textsubscript{14}SO\textsubscript{4}, C\textsubscript{15}-EO\textsubscript{4}, and DOSS. These were the analytes with the poorest precision in the analytical method. Although the method precision could not explain the discrepancy between the observed and nominal concentration, the correlation between precision and over-estimation could be an indication that there were problems with the water method, for instance, during sampling, that led to a positive bias in the results. In contrast to these analytes, the mean measured concentration of C\textsubscript{16}SO\textsubscript{3} was lower than the nominal concentration (a factor of 0.6). Given that C\textsubscript{16}SO\textsubscript{3} was the most hydrophobic analyte, one possible explanation is sorption of C\textsubscript{16}SO\textsubscript{3} to surfaces in the aquarium.

The concentration of all test chemicals was at least 100 times lower during the elimination phase than during the exposure phase (Table S11). The one exception was C\textsubscript{16}SO\textsubscript{3}, for which the concentration 1 h after the beginning of the elimination phase was just a factor of 30 lower, but 6 h later, the concentration had decreased to >100 times lower than at the end of the exposure phase.

In summary, two requirements for a BCF experiment were fulfilled, namely, measurable exposure and a strong gradient in exposure between the exposure phase and the elimination phase. The drift in water concentrations for some analytes shows that a dynamic kinetic model that accounts for changing exposure concentrations must be used to evaluate the data.

Concentrations in Fish. All test chemicals were above the LOQ in the fish throughout most of the exposure phase except for C\textsubscript{10}SO\textsubscript{3} (Figure S2). During the elimination phase, the concentrations of all test substances with alkyl chain lengths <14 eventually fell below the LOQ because of their rapid elimination.

For the experiment as a whole, the variability in concentrations between fish at a given time point ranged from 19 to 38% (mean RSD, Table S12). When a data point exceeded the mean of the concentration at the preceding and succeeding time points by \textasciitilde 10 \times mean RSD, it was treated as an outlier. This affected 19 of the \textasciitilde 450 data points above the LOQ (Table S13). When they were removed, mean RSD decreased for several of the substances, and the range was lowered to 18–26% (Table S12). This was still considerably
for C10SO3 and C11SO3, for which elimination was very rapid. Elimination kinetics were obtained for all test chemicals except C16SO3 (Table S14). The total elimination half-lives were <24 h for most of the test chemicals, which led to the employment of the one-compartment inference method as described in the Statistical Analysis section to determine the BCF of the remaining test chemicals while accounting for the variability in intervals for the model parameters.

There was a pronounced response of the modeled concentration in water during the first 6 h (Figure S2), indicating that there were significant interindividual differences in accumulation. However, due to the possibility of a positive bias in the measured concentrations in water for some substances discussed above, we also provide BCFs calculated using the nominal concentrations in water for all test chemicals. There was generally a good fit between the modeled and observed concentrations of the BCF estimates (Figure S2) and the posterior distribution of the BCF estimates was quite narrow (Figure S3). The one-compartment fish model provided a good description of the elimination behavior in fish during the second half of the exposure phase, confirming the need for a dynamic kinetic model to describe the experimental observations. The simpler model parameter estimation method provided results for mixture BCFs that were similar to those obtained using the Bayesian method (compare Tables 3 and S5), indicating that the simpler approach is also valid. Its use is recommended as bioaccumulative.

Table 3. Uptake Rate Constant ($k_U$), Elimination Rate Constant ($k_e$), and Bioconcentration Factor (BCF) of the Test Chemicals Determined from Measured Concentrations in Water, Together with the Membrane Lipid–Water Distribution Ratio ($D_{MLW}$), the Diffusion Coefficient ($D$), Gill Elimination Rate Constant ($k_r$), Biotransformation Rate Constant ($k_{BCF}$) Estimated from the In Vivo kinetic Data, and the Biotransformation Rate Constant Estimated from the In Vitro RT-S9 Assay ($k_{BCF,i}^{RT-S9}$).

| Chemical       | $k_U$ (L kg$^{-1}$ ww h$^{-1}$) | $k_e$ (h$^{-1}$) | $D_{MLW}$ (L kg$^{-1}$) | $D$ (m$^{-2}$ s$^{-1}$) | $k_r$ (h$^{-1}$) | $k_{BCF}$ (h$^{-1}$) | $k_{BCF,i}^{RT-S9}$ (h$^{-1}$) |
|----------------|-------------------------------|-----------------|-------------------------|-------------------------|-----------------|-----------------------|-------------------------------|
| C10SO3        | 0.034 (0.030–0.039)           | 0.165 (0.149–0.187) | 0.030 (0.026–0.034)  | 0.010 (0.008–0.012) | 0.176 (0.160–0.196) | 0.096 (0.089–0.103)  | 0.034 (0.030–0.039)           |
| C11SO3        | 0.025 (0.022–0.029)           | 0.057 (0.052–0.062) | 0.029 (0.026–0.032)  | 0.011 (0.009–0.013) | 0.113 (0.105–0.122) | 0.061 (0.054–0.068)  | 0.025 (0.022–0.029)           |
| C12SO4        | 0.64 (0.56–0.72)              | 0.0047 (0.0042–0.0052) | 0.0041 (0.0039–0.0045) | 0.0037 (0.0035–0.0039) | 0.0032 (0.0028–0.0035) | 0.0030 (0.0026–0.0032) | 0.0037 (0.0035–0.0039)         |
| C13SO3        | 0.136 (0.121–0.163)           | 0.017 (0.016–0.020) | 0.041 (0.039–0.045)  | 0.0038 (0.0035–0.0041) | 0.0034 (0.0030–0.0037) | 0.0033 (0.0029–0.0036) | 0.0035 (0.0032–0.0038)         |
| C14SO3        | 1.62 (1.38–1.91)              | 0.048 (0.043–0.053) | 0.09 (0.08–0.10)     | 0.006 (0.005–0.006)   | 0.005 (0.004–0.006)   | 0.0048 (0.0045–0.0051) | 0.006 (0.005–0.006)           |
| C16SO3        | 0.77 (0.67–0.87)              | 0.044 (0.040–0.050) | 0.08 (0.07–0.09)     | 0.008 (0.007–0.009)   | 0.007 (0.006–0.008)   | 0.0077 (0.0072–0.0082) | 0.008 (0.007–0.009)           |
| C13EO2SO4     | 0.090 (0.074–0.109)           | 0.100 (0.083–0.120) | 0.08 (0.07–0.09)     | 0.008 (0.007–0.009)   | 0.007 (0.006–0.008)   | 0.0080 (0.0075–0.0085) | 0.008 (0.007–0.009)           |
| D0S3          | 0.009 (0.007–0.110)           | 0.063 (0.056–0.072) | 0.06 (0.05–0.07)     | 0.008 (0.007–0.009)   | 0.007 (0.006–0.008)   | 0.0073 (0.0068–0.0079) | 0.008 (0.007–0.009)           |

The 95% credible confidence interval is provided in brackets. *Not quantifiable. **Steady-state BCFs (as opposed to kinetic BCFs for the other test chemicals). Measurement from Droge. *Measurement from Droge et al. Estimated using QSAR from Droge et al. Lower limit of the RT-S9 assay to detect clearance (LLS9), the value reported is LLS9/3.
Despite the limited high-quality measurements of the BCF of anionic surfactants in the literature, several comparisons
with our data are possible. Our result for C_{10}-1-LAS can be
compared with the pioneering work of Johannes Tolls for C_{11}-
2-LAS, a molecule with a similar structure. Tolls’ measure-
ments were also conducted in rainbow trout and at a similar
water hardness.\textsuperscript{4} Our value for C_{10}-1-LAS, 16.3 L kg\textsuperscript{−1},
is similar to the value of 17.2 L kg\textsuperscript{−1} reported for seven of the test chemicals (Table 3). For the
remaining three test chemicals, anionic surfactants than the neutral lipid
expected to play a greater role in the bioconcentration of
membrane lipid

Anionic surfactants partition more than 100 000 times more strongly into membrane lipids than into neutral lipids.\textsuperscript{37} The
membrane lipid—water distribution ratio (D_{MLW}) is therefore
expected to play a greater role in the bioconcentration of anionic surfactants than the neutral lipid—water distribution ratio D_{OW}). Measured values of D_{MLW} have been
reported for seven of the test chemicals (Table 3). For the
remaining three test chemicals, D_{MLW} has been estimated using
QSARs built from D_{MLW} measurements of structurally similar
anionic surfactants.\textsuperscript{19} We found an excellent correlation
between log BCF and log D_{MLW} (\textit{r} = 0.96, Figure 1, upper
panel). D_{MLW} had a very strong influence on BCF; an increase in D_{MLW} by a factor of 10 corresponded to an increase in BCF by a factor of 25. BCFs determined for 4 LAS isomers in rainbow trout held at 14 °C are also strongly correlated with D_{MLW} and lie close to the regression line obtained from our
data.\textsuperscript{4} However, two persistent perfluorinated anionic
surfactants, PFOA and PFOS,\textsuperscript{32} lay approximately two log
units above the regression line (Figure 1, upper panel). To
understand how D_{MLW} influences BCF, it is necessary to study
the kinetic parameters for uptake and elimination.

**Uptake Rate Constant.** The uptake rate constant, \( k_U \),
ranged from 0.090 L kg\textsuperscript{−1} h\textsuperscript{−1} for DOSS to 6.3 L kg\textsuperscript{−1} h\textsuperscript{−1} for
C_{16}SO_{3} (it could not be measured for C_{14}SO_{4} and C_{13}SO_{4}, Table 3). For all of the chemicals except C_{16}SO_{3}, \( k_U \) was
markedly lower than the median \( k_U \) of 266 L kg\textsuperscript{−1} day\textsuperscript{−1} (11.1 L kg\textsuperscript{−1} h\textsuperscript{−1}) that we measured for 10 neutral chemicals in
somewhat larger rainbow trout.\textsuperscript{35} This indicates that the
primary resistance for uptake does not lie in transport through
water to the surface of the gill membranes, as in this case \( k_U \)
would be similar for all of these compounds. Instead, transport
through the membranes into blood must be rate limiting. This
is consistent with the passive diffusion of small organic
molecules through membranes being much slower for ions
than for comparable neutral structures.\textsuperscript{36}

Like the BCF, \( k_U \) was positively correlated with D_{MLW}. When
the bulky structure with two branched chains, DOSS, was
excluded, then the slope of the regression of log \( k_U \) and log D_{MLW} became 0.89 with a 95% confidence interval that
intersected 1 (Figure 1, middle panel). This means that the
relationship between \( k_U \) and D_{MLW} was also approximately
linear. \( k_U \) measured for seven LAS isomers in fathead minnows
held at an average temperature of 21.4 °C show a similar

**Figure 1.** BCF and kinetic parameters from the bioconcentration experiment plotted against the log membrane lipid—water distribution ratio (D_{MLW}). Upper panel: BCF; the line shows the linear regression of the two variables for the data from this study. Middle panel: Uptake rate constant (\( k_U \)); the line shows the linear regression of the blue data points. The data from Consoer et al.\textsuperscript{33,34} were size-corrected to 24 g using the algorithm provided in their paper.\textsuperscript{34} Lower panel: Overall elimination rate constant (\( k_E \)) and biotransformation rate constant determined using an \textit{in vitro} RT-S9 assay (\( k_{RS9} \); the line shows the model described in the text.
dependence on $D_{MLW}$ and lie close to the regression line determined from our data.\textsuperscript{4} $k_1$ reported for PFOA in rainbow trout in respirometer-metabolism chambers at 11 °C when size-corrected to 24 g fish agree well with the regression,\textsuperscript{34} while PFOS falls a factor 4 below the regression line (Figure 1, middle panel).\textsuperscript{33}

Presuming that uptake occurs primarily via diffusion across the gills and that the primary resistance to diffusive mass transport is the epithelial membrane of the gills, then $k_U$ (L kg\textsuperscript{-1} ww h\textsuperscript{-1}) can be expressed as follows (derivation provided in Text S5):

$$k_U = 1000D\rho_MA/MD_{MLW}$$  \hspace{1cm} (3)

where $D$ is the diffusion coefficient of the chemical in the membrane (m h\textsuperscript{-1}), $\rho_M$ is the membrane density (kg L\textsuperscript{-1}), $A$ is the surface area over which diffusive transport occurs ($m^2$), $M$ is the fish mass (kg ww), and 1000 is a unit conversion factor (L m\textsuperscript{-3}). Measured values of $D_{MLW}$ were available (Table 3), $A$ was estimated from the average fish weight (0.024 kg) according to the equation specific for estimating the gill surface area of rainbow trout from Hughes et al.\textsuperscript{33} corrected for the units used here, and $\rho_M$ was set to 1 kg L\textsuperscript{-1}, allowing eq 3 to be solved for $D$. While the magnitude of $D$ is sensitive to errors in the estimates of $\rho_M A$, and $M$, the relative differences in $D$ between chemicals are not affected by such errors. The calculated $D$ varied between $9.3 \times 10^{-9}$ and $3.9 \times 10^{-8}$ m h\textsuperscript{-1} (Table 3), indicating that diffusive transport across the membrane is influenced by other molecular properties besides $D_{MLW}$. The diffusion cross section of the molecule is one property that can be expected to affect $D$. With the exception of $C_{16}SO_3$ similar values of $D$ were obtained for the sulfates and sulfonates, which are consistent with them having similar head groups and a single unbranched alkyl tail that would not impede passage into the membrane. Somewhat smaller $D$ values were obtained for $C_{10}$-LAS and $C_{12}$-EO$_4$SO$_4$, suggesting that the head groups of these molecules may have a somewhat larger diffusion cross section. A markedly smaller $D$ value was obtained for DOSS (a factor of 4 less than the sulfates and sulfonates). DOSS has a dialkyl structure with branched chains, which one would expect to make the diffusion cross section larger.

In summary, the variability in $k_U$ was primarily driven by the extent to which the chemical partitions from water into the gill membranes. For the bulkier substance DOSS, there was a substantial additional effect on the diffusion coefficient arising from its larger diffusion cross section.

**Elimination Rate Constant.** The elimination rate constants $k_T$ varied by a factor of 40, between 0.0047 and 0.178 h\textsuperscript{-1} (Table 3). There was a negative correlation between $k_T$ and $D_{MLW}$ (Figure 1, lower panel).

From eq 3 it follows that $k_T$ can be defined as (derivation provided in Text S5)

$$k_T = \frac{1000D\rho_MA/M}{D_{FW}} + k_B$$  \hspace{1cm} (4)

where $D_{FW}$ is the fish—water equilibrium distribution ratio (L kg\textsuperscript{-1}) and $k_B$ is the whole-body biotransformation rate constant (h\textsuperscript{-1}). Membranes are an important tissue component for the storage of ionic surfactants in fish and a useful model for other tissue components that sorb amphiphilic chemicals.\textsuperscript{19} Therefore, $D_{FW}$ was approximated as

$$D_{FW} = f_{MLF}D_{MLW}$$  \hspace{1cm} (5)

where $f_{MLF}$ is the mass fraction of the fish that is membrane lip or has equivalent partitioning properties. Substituting this into eq 4 yields

$$k_T = \frac{1000D\rho_MA/M}{f_{MLF}} + k_B$$  \hspace{1cm} (6)

The first term on the right-hand side of this equation is the rate constant for gill elimination $k_T$. It was estimated using the estimates of the diffusion coefficient ($D$) from Table 3, the values of $\rho_M A$, and $M$ given in the Uptake Rate Constant section, and assuming a value of 0.0125 for $f_{MLF}$.\textsuperscript{35,39} Note that the latter accounts only for the contribution of phospholipids to the sorption capacity of the fish, thus possibly underestimating $f_{MLF}$ and, consequently, resulting in an overestimation of $k_T$. An estimate of $k_B$ from the BCF experiment ($k_B^{BCF}$) was then determined from the difference between measured $k_T$ and the estimated $k_2$.

$$k_B^{BCF} = k_T - k_2 = k_T - \frac{1000D\rho_MA/M}{f_{MLF}}$$  \hspace{1cm} (7)

$k_B^{BCF}$ was greater than $k_2$ for all chemicals (Table 3), indicating that biotransformation was the primary pathway of elimination. The difference was at least an order of magnitude, meaning that the estimated contribution of respiration to overall elimination was negligible.

Recently, the concept of a baseline screening BCF was introduced for surfactants, based on equilibrium partitioning assuming no biotransformation.\textsuperscript{19} The baseline screening BCF for the study chemicals exceeded the measured BCF by 1–2.5 orders of magnitude (Table S16), providing further evidence that biotransformation is an important factor influencing the elimination—and thereby the BCF—of the anionic surfactants studied here in rainbow trout. The deviation of PFOA and PFOS from the regression between BCF and $D_{MLW}$ (Figure 1, upper panel) is attributable to them not being biotransformed. $k_B$ was also estimated from the RT-S9 assays conducted with individual chemicals ($k_B^{S9}$). The RT-S9 assay yielded statistically significant clearance for eight of the test chemicals (Table S17 and Figure S4), while for two ($C_{14}SO_3$ and $C_{16}SO_3$), no significant clearance was measured and the lower limit of the RT-S9 assay to detect clearance (LL$_{S9}$) was used to estimate $k_B^{S9}$ (see Text S6). There was good agreement (within a factor of 3) between $k_B^{S9}$ and $k_B^{BCF}$ for six of the eight test chemicals for which $k_B^{BCF}$ could be measured (Table 3). This result provides some confidence in the utility of the RT-S9 assay and the IVIVE estimation method. We note that the RT-S9 assay with the anionic surfactant mixture yielded significantly lower $k_B^{S9}$ values compared to the single chemical RT-S9 assay for three out of six chemicals (Table S18, Figure S5, and Text S6). The mixture effect was observed for rapidly transformed shorter-chain analogues and thus mostly for structures with a lower BCF. The influence of co-solutes on the RT-S9 assay and how this might relate to in vivo biotransformation behavior deserve further investigation.\textsuperscript{40}

The substances showing the poorest agreement between $k_B^{BCF}$ and $k_B^{S9}$ were $C_{14}SO_3$ and DOSS. In both cases, $k_B^{S9}$ was less than $k_B^{BCF}$ (by a factor of 11 and 6, respectively). Possible explanations for the discrepancy include an overestimation of $k_T$, an under-estimation of $k_2$, or an under-estimation of $k_B^{S9}$. A significant overestimation of $k_T$ can be
ruled out for C14SO3 for which the 95% credible interval was 0.022–0.026 h⁻¹ (see Table 3). For DOSS, on the other hand, there were few observations during the elimination phase of the experiment and there was considerable variability in the fish concentrations, leading to a broader 95% credible interval of 0.056–0.072 h⁻¹ (see Table 3). A significant under-estimation of k is an unlikely explanation, as we are aware of no plausible hypotheses that could increase k to the extent that it would influence k_B,BCF. An under-estimation of k_B,SS by up to a factor of ~3 is possible for C14SO3 as this chemical was below the LLSS and therefore LLSS/3 was used to estimate k_B,SS. A factor of 3 would be sufficient to explain a significant part of the difference between k_B,BCF and k_B,SS for C14SO3. As reported in more detail in Text S6, the RT-S9 depletion assay performed with the mixture of anions indicated a depletion slope significantly different from 0 for both C14SO3 and C16SO3, though still just at the apparent LLSS. This would result in a higher kB of a factor of 2.7 and 2.5, respectively, compared to the assumed LLS9/3 from the single-compound RT-S9 assay. Another factor that may explain lower kB is the RT-S9 assay. Another factor that may explain lower kB compared to k_B,BCF is that the former only considers biotransformation in the liver, while extra-hepatic biotransformation may be a contributing factor for some chemicals. Identification and comparative study of the toxicokinetics of metabolites in the in vivo and in vitro systems could provide more insight into these questions.

The k_B,SS data are also plotted in Figure 1 (lower panel). In addition to visualizing the comparison above (since k is negligible, kT in the figure is approximately equal to k_B,BCF), they provide more insight into the influence of DMLW on kB. In contrast to k_B,BCF, k_B,SS was available for C10SO3 and C12SO3, which have lower DMLW than the other test chemicals. The combined dataset suggests that kB is approximately constant up until log DMLW = 4 and thereafter transitions into a regime where kB is inversely proportional to DMLW.

To explore the influence of DMLW on kB, a simple mathematical model of anionic surfactant elimination via the liver was created. We assumed that the liver behaves like a well-mixed reactor and that the blood/water (dissolved phase) distribution ratio is proportional to DMLW (see Text S7). This assumption is based on partitioning data for alkyl sulfates and sulfonates indicating a >100 000 times larger affinity for phospholipids compared to storage lipids (fish oil) and the expectation that DMLW provides a relative indication of sorption to other amphiphilic blood components such as proteins for these anionic surfactants. The resulting equation for kB was

\[ \frac{1}{kB} = \frac{VD}{kB} \left( \frac{f_{MLW} DMLW}{Q_{MLW}} + 1 \right) Q_{B} \]  

(8)

where VD is the volume of distribution (L), fMLW is the mass-to-volume fraction of membrane lipid-like sorbents in the blood (kg L⁻¹), QMLW is the clearance rate due to transformation of freely dissolved chemicals in the liver (L h⁻¹), and Qb is the rate of blood flow through the liver (L h⁻¹). This model fits the characteristics described above: at low DMLW, kB is constant, and at high DMLW, kB is inversely proportional to DMLW.

Assuming VMLW/QMLW and Qb to be constants, eq 8 was fitted to DMLW and kB for the 10 test chemicals (kB,BCF for the eight chemicals for which it was available, kB,SS for C10SO3 and C11SO3, Table 3), yielding

\[ \frac{1}{kB} = 0.000204 DMLW + 4.07 \]  

(9)

with an RMSE = 0.18 log units (see Figure 1, lower panel).

Given that assuming VMLW/QMLW to be constant gave a good fit to the data, and furthermore that VD and fMLW are expected to be similar for the test chemicals, it follows that QMLW, the clearance rate due to transformation of freely dissolved chemicals in the liver (L h⁻¹), is also quite similar for the test chemicals. This is consistent with the observation that biotransformation of most anionic surfactants that possess linear alkyl chains occurs via a common mechanism involving omega-oxidation followed by β-oxidation.20,41 Our results suggest that it may be possible to predict the kB of some anionic surfactants from DMLW with good accuracy.

Summary of the Chemical Properties Influencing BCF. The BCF of the anionic surfactants studied here is highly correlated with DMLW. DMLW affects bioconcentration in two ways that amplify each other such that BCF increases more rapidly than DMLW. The first is the positive influence of DMLW on the uptake rate constant. We attribute this to the greater partitioning from water into the gill membrane, which in turn amplifies the gradient for diffusive transport of the anionic surfactant across the membrane into the fish. The second is the negative influence of DMLW on the elimination rate constant. We attribute this to the reduced availability of the surfactant for interaction with degrading enzymes because of sequestration of the surfactant from the freely dissolved form into the sorbed form. Since log DMLW is positively correlated with alkyl chain length, the consequence is a pronounced increase in BCF with alkyl chain length.

Two other chemical properties also modulate the BCF. The membrane diffusion coefficient of the chemical decreases as its diffusion cross section gets larger, reducing passive transport across the gills and thereby the uptake rate constant. For the spectrum of chemicals studied here, this effect amounted to a factor of 4. The second modulating property is the inherent reaction rate of the freely dissolved chemical. This appeared to be similar for the surfactants studied.

More information is desirable for other classes of anionic surfactants. Considering the use of multiple types of phosphorus-based anionic surfactants produced in the EU in the range of 100–1000 tonnes year⁻¹ and their apparently low overall biodegradability, particularly for single-chain alklyphosphate surfactants (Table S1), further research on DMLW and RT-S9, and additional validation with limited in vivo BCF studies, would be relevant. We also note that per- and polyfluorinated surfactants behave differently than the alkyl surfactants studied here, particularly with respect to their persistence and other unique molecular interactions in vivo.33,34

The very strong influence of DMLW on BCF and the proximity of the BCF for C10SO3 (log DMLW = 6.2) to the threshold for B classification under REACH suggest that anionic surfactants with higher DMLW may exceed this threshold even though they are readily biotransformed. Thus, B evaluation is a particularly relevant component of chemical safety assessment for these anionic surfactants. The work presented here, as synthesized in eqs 3, 6, and 9 including the estimates for D in Table 3, represents a significant step forward in our ability to assess the BCF of this important class of chemicals without having to resort to in vivo testing.
**ASSOCIATED CONTENT**

**Supporting Information**
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.1c05543.

Text providing details of the methods and models used; tables of anionic surfactants, experimental materials, sampling schedule, analytical parameters, method performance indicators, test chemical concentrations in water and fish, BCF model parameters and S9 assay results; figures illustrating test chemical concentrations in water, fish and S9 assays with model fits (PDF)

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**Notes**
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