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Screening the baseline fish bioconcentration factor of various types of surfactants using phospholipid binding data

Steven T.J. Droge[1*], Peter Scherpenisse,[2] Jon A. Arnot[3], James M. Armitage[4], Michael McLachlan[5], Peter von der Ohe[6], Geoff Hodges[7]

[1] Institute for Biodiversity and Ecosystem Dynamics, Department Freshwater and Marine Ecology, University of Amsterdam, The Netherlands
[2] Institute for Risk Assessment Sciences, Utrecht University, The Netherlands
[3] ARC Arnot Research and Consulting, Toronto, Ontario, Canada
[4] AES Armitage Environmental Sciences, Ottawa, Ontario, Canada
[5] Department of Environmental Science, Stockholm University, Sweden
[6] Umweltbundesamt, Dessau-Roßlau, Germany
[7] Safety and Environmental Assurance Centre, Unilever, Colworth Science Park, Sharnbrook, Bedfordshire, United Kingdom
*Corresponding author; E-mail: steven.droge@gmail.com; Tel: +31 205257437

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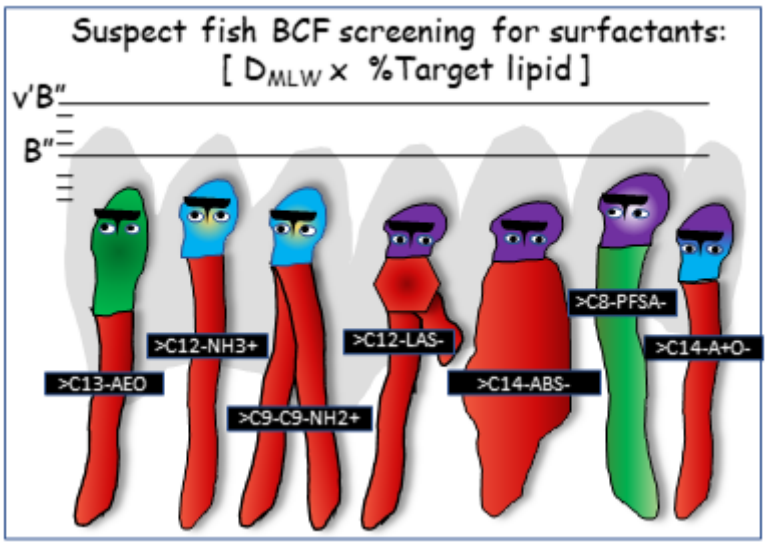
Environmental significance statement

Surfactants can enter aquatic environments via wastewater streams, spray-drift, run-off or accidental spills. The fish bioconcentration factor (BCF) is part of the chemical risk assessment but for many surfactant types the BCF is not tested, while octanol-water partition ratios for ionic surfactants are unreliable and inadequate for BCF assessment. Experimentally consistent phospholipid-water distribution ratios (D_{MLW}) are now available for 26 types of surfactants. As demonstrated for non-biotransformed perfluorinated surfactants, multiplication of D_{MLW} with the membrane lipid fraction of fish can be used as a first tier BCF screening value. This screening step selects surfactants for which further refinement of the BCF estimate would be required, such as weight of evidence on biotransformation rates.

TOC Graph



ABSTRACT

Fish bioconcentration factors (BCFs) are commonly used in chemical hazard and risk assessment. For neutral organic chemicals BCFs are positively correlated with the octanol-water partition ratio (K_{OW}), but K_{OW} is not a reliable parameter for surfactants. Membrane lipid-water distribution ratios (D_{MLW}) can be accurately measured for all kinds of surfactants, using phospholipid-based sorbents. This study first demonstrates that D_{MLW} values for ionic surfactants are more than 100,000 times higher than the partition ratio to fish-oil, representing neutral storage lipid. A non-ionic alcohol ethoxylate surfactant showed almost equal affinity for both lipid types. Accordingly, a baseline screening BCF value for surfactants ($BCF_{baseline}$) can be approximated for ionic surfactants by multiplying D_{MLW} by the phospholipid fraction in tissue, and for non-ionic surfactants by multiplying D_{MLW} by the total lipid fraction. We measured D_{MLW} values for surfactant structures, including linear and branched alkylbenzenesulfonates, an alkylsulfoacetate and an alkylethersulfate, bis(2-ethylhexyl)-surfactants (e.g., docusate), zwitterionic alkylbetaines and alkylamine-oxides, and a polyprotic diamine. Together with sixty previously published D_{MLW} values for surfactants, structure-activity relationships were derived to elucidate the influence of surfactant specific molecular features on D_{MLW}. For 23 surfactant types, we established the

alkyl chain length at which $BCF_{baseline}$ would exceed the EU REACH bioaccumulation (B) threshold of 2000 L kg^{-1}, and would therefore require higher tier assessments to further refine the BCF estimate. Finally, the derived $BCF_{baseline}$ are compared with measured literature *in vivo* BCF data where available, suggesting that refinements, most notably reliable estimates of biotransformation rates, are needed for most surfactant types.

INTRODUCTION

A wide variety of surfactants are used in household products and industrial processes, for diverse purposes such as detergents, fabric softeners, emulsifiers, dispersants, biocides, ionic liquids, firefighting foams, and pesticide adjuvants. Most surfactant types used as detergents have been designed to readily degrade during wastewater treatment and upon release to the environment,[1-5] for example, by replacing recalcitrant branched alkyl chains with linear alkyl chains, and by inclusion of ester or ether bonds between hydrophilic headgroups and lipophilic alkyl chains. However, continuous low level emissions via wastewater streams can still result in potential exposure to receiving environments. Some unique industrial uses may lead to different emission scenarios and environmental exposure patterns, for example for surfactants used during pesticide spraying, oil drilling processes or fracking. Some surfactants, such as perfluorinated surfactants, are designed to have high thermal and chemical stability, and are known to be persistent in the receiving environment. Overall, widespread occurrence of many types of surfactants has been reported in samples from sewage treatment plant effluent, natural surface water systems, and coastal regions.[6-15] Adequate aquatic hazard and risk assessment is thus required, and has been reported on for many surfactant types.[16-21] Besides toxicological information to derive safety thresholds, risk assessment also requires exposure estimates based on actual measurements or model predictions. The bioconcentration factor (BCF) from water into organisms is of relevance for predicting potentially toxic internal levels, but also for food chain transfer. Typically, neutral hydrophobic and persistent chemicals have a high potential to bioconcentrate into the lipid fraction of aquatic organisms.[22,23] The degree of actual bioconcentration depends on multiple factors, including tissue composition, exposure scenario, exposure duration vs. time to attain steady state, biotransformation, and external factors such as pH and bioavailability.[23-29] Many surfactants are manufactured or imported in volumes of >100 tonnes/year in the EU, and for that tonnage band information on bioaccumulation is required under the EU 'REACH' regulation (EC 1907/2006: Registration, Evaluation, Authorisation and restriction of CHemicals). Within EU REACH, the threshold for hazard classification as bioaccumulative ('B') is a BCF of 2000 L kg^{-1} wet weight, and 5000 L kg^{-1} wet weight as very bioaccumulative ('vB'). For the Globally Harmonized System of Classification and Labelling of Chemicals (GHS),[30,31] a lower BCF threshold of 500 L kg^{-1} is used, while the US EPA Toxic Substances Control Act (TSCA) considers 1000 L kg^{-1} for 'B' and 5000 L kg^{-1} for

'vB'.[32,33] For surfactants used and discharged from offshore oil and gas operations in the OSPAR regulated region of the North East Atlantic the BCF threshold is even lower.[34,35] set at a value of 100 L kg^{-1}.

Since all surfactants have a hydrophobic 'tail', detailed insight into bioaccumulation potential is relevant. For several types of surfactants *in vivo* BCF data are available in the literature for fish.[23,36] Although BCF data for common detergent ingredients (non-ionic alcohol ethoxylates and anionic LAS) indicate relatively low BCFs, likely due to rapid biotransformation,[37-41] surfactant BCF values in excess of 1000 L kg^{-1} wet weight have also been reported, specifically for several perfluorinated acids such as perfluorooctanesulfonate (PFOS)[42,43] and for protonated alkylamines with chain lengths $\geq C_{12}$.[44,45] The high accumulation of some PFAS chemicals in fish is notable because, unlike many other surfactants, they are not amenable to biotransformation. There is also evidence that perfluorinated surfactants exhibit 'unique' behaviours such as high affinity for certain proteins (e.g., serum albumin, liver fatty acid binding proteins) and interactions with membrane transporters (e.g., organic anion transporters, OATs).[46] PFOA is predominantly eliminated from rainbow trout through renal clearance which appears to be facilitated by active secretion into urine.[47] In contrast, branchial clearance was found to dominate for PFOS and there was no evidence to suggest an important role for active transport processes in the kidney.[48] For many surfactant types, fish BCF data are unavailable or were measured with radiolabeled material[36]; however, new experimental BCF data for some surfactants are available.[44,45] The current study aims to progress towards a scientifically defensible initial BCF screening method based only on a chemical property that adequately describes the ability of surfactants to be stored in fish. This first screening step would serve to identify surfactants for which bioaccumulation is unlikely to be above the BCF trigger value, and those for which refined bioaccumulation assessment is needed, e.g., by models that include biotransformation rates in a weight of evidence approach and avoiding the need for unnecessary *in vivo* testing.

Modeling accumulation of organic micropollutants in fish relies heavily on measured or predicted octanol-water partition coefficients (logK_{OW}, or logP). K_{OW} is used as a key indicator of hydrophobicity that relates to sorption to biotic tissue components (lipids, proteins, carbohydrates).[49,50] However, standardized guidelines to measure K_{OW} state that the method should not be applied to "surface active materials" (OECD 107)[51] and "surface active agents" (OECD 117).[52] The 'slow-stirring' method (OECD 123) indicates that it may also be applied to ionizable chemicals,[53] but for "surfactants" this method may also be problematic.[54] The applicability domains of most K_{OW} predictive tools do not include, or poorly address, specific surfactant moieties (e.g., ionic groups, repetitive ethoxylate units).[54] A recent study that aimed to derive experimental octanol-water distribution ratios (D_{OW}) for different types of surfactants using different methods demonstrated poor correlation between (i) different experimental assays, (ii) homologues of the same surfactant type, and, (iii) experimental values and predictions from common computational approaches, particularly for ionic surfactants.[54] The Supporting Information (SI)

presents an example of the wide range of K_{OW} and D_{OW} values derived experimentally and *in silico* for the anionic surfactant sodium dodecyl sulfate ("SDS", CAS number: 151-21-3, see Text S1 and Table S1). Clearly, surfactant assessments could be improved by progressing towards more appropriate distribution descriptors than K_{OW}, and by attaining greater insight into processes that control the bioconcentration of surfactants.

Measured membrane lipid-water distribution ratios (D_{MLW}) circumvent many of the issues related to deriving the K_{OW} for surfactants. More importantly, D_{MLW} directly relates to a relevant tissue component, which is not a bulk neutral solvent but an anisotropic organic structure. Phospholipid bilayers have both a hydrophobic core and a (mostly zwitterionic and polar) hydrophilic head group domain, consequently, they may sorb amphiphilic organic chemicals in a uniquely intercalated position that allows for both favorable hydrophobic and electrostatic/polar interactions.[55] Thus, D_{MLW} values improve insight into the actual distribution within cells of organisms, and therefore are important additional parameters for surfactant assessments. Hampering inclusion as a data requirement, though, is the lack of standardized guidelines to determine D_{MLW} values, which are well established for K_{OW}. It is very promising, therefore, that several studies have shown close correspondence between D_{MLW} values for surfactants derived with the three most common experimental set-ups using different phospholipid materials, i.e., dissolved unilamellar liposomes,[56,57] lipid bilayers non-covalently coated on macroporous silica,[55,58,59] and covalently linked phospholipid monolayers on HPLC grade silica.[55,59] Moreover, the accuracy of measured D_{MLW} values is further demonstrated by consistent increments between series of homologue and analogue surfactant structures in these studies. D_{MLW} values have already been published for sixty pure surfactant structures, covering non-ionic surfactants,[56] cationic surfactants,[55] anionic surfactants,[59] perfluorinated surfactants,[57,59] and ionic liquids.[58] D_{MLW} values relate directly to a target tissue site for both toxicity assessment (i.e., membranes) and bioaccumulation assessment. Cell membrane binding of surfactants is of high toxicological relevance since most surfactants are expected to act only by baseline toxicity ('narcosis'), which impairs basic cell membrane functioning at a critical sorbate concentration in the phospholipids making up the bilayer. The simple ratio between the critical cell membrane burden at which narcosis occurs (~20-200 mmol/kg phospholipid) and the D_{MLW} (in kg phospholipid/ L water) has been shown to be predictive of the aquatic toxicity (e.g. LC_{50} in mmol/L) of various anionic surfactants[59] (Text S1 provides an example for SDS).

The traditional focus of bioconcentration assessment has been on predicting the partitioning of chemicals into the total lipid pool in tissue. However, ionized organic chemicals have a relatively low affinity for octanol, representing neutral storage lipid, but much higher affinity for phospholipid partly due to favorable electrostatic interactions with zwitterionic headgroups.[60-65] For ionic surfactants, membrane (phospho)lipids may thus be the dominant component driving the overall affinity to sorb to tissue. D_{MLW} values can thus predict the baseline bioconcentration factor ($BCF_{baseline}$), assuming only equilibrium partitioning between the water and the fish tissue (neglecting active elimination processes

such as biotransformation). For non-ionic surfactants, measured D_{MLW} values may also provide insight into the sorption affinity to the overall lipid content. To the best of our knowledge, no systematic data are available for partitioning of surfactants to neutral storage lipids, although for perfluorinated surfactants it is widely recognized that these chemicals do not accumulate preferentially in blubber of marine mammals because of their ionized form and propensity for interaction with proteins as well as membrane lipids.[66]

The first objective of this study was to confirm the greater affinity of ionic surfactants for the zwitterionic phospholipids relative to that for neutral storage lipids such as triglyceride esters. In order to mimic the neutral storage lipids of fish as closely as possible with a readily available reference material, we selected commercial fish oil. Tests were done in customized 'slow stirring' dual-vial systems. Experimentally, the oily phase is less problematic than octanol as it is less readily emulsified.

The currently available D_{MLW} data set contains only linear alkyl structures, no zwitterionic surfactants, and almost no examples of how additional polar functionalities in a surfactant structure contribute to the D_{MLW}. Therefore, the second objective of this study was to extend the D_{MLW} data matrix with other common ionic surfactant types, including zwitterionic surfactants, branched structures and several ester- and ether-based surfactants, and a polyprotic diamine. The same sorbent dilution series with solid-supported lipid membranes (SSLM) were used as applied for cationic and anionic surfactants. As a third objective, the total dataset was used to explore how structural moieties contribute to the D_{MLW}. Our goal was to derive a model to make extrapolative predictions of D_{MLW} for structural analogs of those surfactants for which D_{MLW} have been measured (e.g., longer alkyl chain structures), and for which the determination of D_{MLW} is not experimentally feasible with the SSLM approach.

Based on $BCF_{baseline}$ values derived for homologues with different alkyl chain lengths, using the extrapolative D_{MLW} predictions if required, the fourth objective was to derive the critical alkyl chain length that will surpass regulatory BCF thresholds when assuming negligible biotransformation for all surfactant types for which D_{MLW} values are available. The process should facilitate the classification of a surfactant as potentially bioaccumulative at the screening level, based on adequate parameterization of the sorption affinity to tissue by D_{MLW}. Finally, the derived $BCF_{baseline}$ are compared with literature *in vivo* BCF data, to explore to what extent toxicokinetic refinements (preferably *in vitro* and *in silico*) are likely required for surfactants.

METHODS

Materials

Surfactants were obtained from various suppliers at the highest purity possible, structures are listed in SI Table S2. To facilitate reading and grouping, the chain length of surfactants is indicated by subscript x in 'C_x', an abbreviated name code is used, and the charge of surfactants is indicated by superscripts $^-$ and $^+$. Sodium dodecylsulfonate ($C_{12}SO_3^-.Na^+$), Sodium dodecylsulfate ($C_{12}SO_4^-.Na^+$; 'SDS'), and *N,N,N*-trimethyldodecyl-ammonium chloride ($C_{12}N(CH_3)_3^+.Cl^-$), and tetraethylene glycol monododecyl ether ($C_{12}EO_4$) were from Sigma-Aldrich. Pure linear alkylbenzenesulfonate compounds (C_x-'y'-LAS$^-$), varying in linear chain length and position ('y') of the benzosulfonate moiety on the alkyl chain, were from batches synthesized by Tolls *et al.*,[67] kindly provided by Utrecht University. A sample of the product Witconate P-1059, containing branched alkylbenzenesulfonates (ABS$^-$) alongside a variety of LAS components, was kindly supplied by Akzo-Nobel (Arnhem, NL). Procter & Gamble (Brussels, Belgium) supplied the pure anionic surfactant $C_{12}EO_4S^-$, a component of the technical ethoxymer mixture sodium laurylethersulfate (SLES$^-$.Na$^+$). Sodium bis(2-ethylhexyl)sulfosuccinate (DOSS$^-$.Na$^+$), bis(2-ethylhexyl)amine (BEHN$^+$), and bis(2-ethylhexyl)phosphate (BEHP$^-$) were from Sigma-Aldrich, as well as the two ester-based surfactants 2-aminoethyl laurate ($C_{12}AcN^+$) and sodium lauryl sulfoacetate ($C_{12}AcS^-.Na^+$), and the four zwitterions (lauryldimethylammonio)acetate ($C_{12}A^+Ac^-$), *N*-Dodecyl-*N,N*-(dimethylammonio)butyrate ($C_{12}A^+Bu^-$), *N,N*-dimethyldecylamine *N*-oxide ($C_{10}A^+O^-$), and *N,N*-dimethyldodecylamine *N*-oxide ($C_{12}A^+O^-$). *N*-dodecylpropane-1,3-diamine ($C_{12}N^+PN^+$) was obtained by AK Scientific.

Fish oil was purchased as 'Solgar Omega-3 Triple Strength' capsules from a pharmacy. The label indicated that the oil contained high concentrations of methyl esters of DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid) 'from deep-sea, cold-water fish'. Two types of solid-supported lipid membrane (SSLM) assay plates were purchased from Sovicell GmbH (Leipzig, Germany) as TRANSIL^{XL} Intestinal Absorption kits (lipid content 0.048-0.9 µL), and TRANSIL^{XL} Intestinal Absorption kits for low affinity compounds (lipid content 0.884-16.7 µL). TRANSIL^{XL} phospholipids are characterized as Egg-PC.

Fish oil – water distribution assays

Slow-stirring distribution experiments with fish-oil were performed with $C_{12}SO_3^-$, $C_{12}SO_4^-$, C_{11}-2-LAS$^-$, $C_{12}N(CH_3)_3^+$, and $C_{12}EO_4$. Customized 10 mL glass twin-vial systems were used, connected at the bottom with glass tubing, and with PTFE stir bars in both vials (see Figure S1). Aqueous buffer of 0.1 M ammonium acetate (pH 7) was added first and fish oil was added only in one of the vials. This system allowed for sampling from oil and water from the different vials separately. When stirring is too strong, the fish oil forms a milky dispersion, and care was taken to fully avoid this during testing. Water samples from the vial without oil were therefore expected to represent freely dissolved surfactant concentrations. For $C_{12}SO_3^-$, $C_{12}SO_4^-$, C_{11}-2-LAS$^-$, and $C_{12}N(CH_3)_3^+$, the ratio oil:water was 1:10 and both oil spiked systems (10 or 100 mg/L) and water spiked systems (1, 10 or 100 mg/L) were tested with at least three

replicates. Pilot tests indicated equilibrium was reached within 24 hours. For $C_{12}EO_4$, the ratio oil:water was 1:25 and only spiking of the oil phase resulted in detectable equilibrated aqueous concentrations. In a first test with 16.3 g/L of $C_{12}EO_4$ in oil a low surfactant recovery was found compared to nominal values. Additional tests were run with $C_{12}EO_4$ spiked in oil at 1.3 g/L, 4 g/L, and 13 g/L. Distribution ratios were determined as the average ratio of the measured concentrations in all replicates per spiking treatment. More experimental details are presented in Table 1 and SI Text S2.

SSLM sorbent dilution assays

The same sorbent dilution series approach with commercial SSLM material was applied as described previously for cationic[55] and anionic surfactants.[59] The TRANSIL bead suspensions were transferred from the well plate vials to pre-weighed glass vials (see Figure S2), and the original PBS solution was thereby exchanged for a 0.1 M pH 7.0 buffer solution of ammonium acetate (Sigma-Aldrich) in all SSLM experiments. This buffer also resembles the pH and high salinity of physiological solution, but the absence of involatile salts in this buffer allows for direct injections of samples for LC-MS/MS analysis. For the multiprotic diamine surfactant $C_{12}N^+PN^+$, the SSLM assay was performed in 4 different buffers: pH 4 with ammonium formate/formic acid, pH 5 with ammonium acetate/acetic acid, and ammonium bicarbonate/ammonia set to pH 7 and 8.5. Further details on the SSLM assay are presented in Table 2 and SI Text S2. For most surfactants, one or two TRANSIL dilution series with 6 sorbent dosages were tested at the same spiked concentration of surfactant of ~1 µM. Average concentrations in samples without sorbent were used as reference levels in the mass balance approach. Given analytical uncertainties, the reduction of the dissolved concentration (by the SSLM sorbed fraction) should account for at least 25% in order for a sample to be included in the isotherm fitting. Additional samples without sorbent in which surfactants were spiked in 50% methanol confirmed that losses to glass surfaces were minimal. The measured aqueous concentration (C_{aq}) and calculated phospholipid concentration ($C_{phospholipid}$) in each spiked sample is treated as a single data point for fitting a sorption isotherm. The sorption process to phospholipid has been shown to be a linear process for all surfactants thus far[55,59]. Since the sorbent dilution series do not need to cover orders of magnitude to investigate nonlinearity, the $\log D_{MLW}$ is derived by the Y-axis off-set for the linear line with a slope of 1 in a plot of $\log C_{phospholipid}$ versus $\log C_{aq}$.

Chemical analysis

For the fish oil experiments, concentrations were measured using a MDS Sciex API 3000 LC-MS/MS System (Applied Biosystems). External calibration standards and internal standards in all samples were used for quantification: $C_{13}SO_3^-$ for $C_{12}SO_3^-$, $C_{14}SO_3^-$ for $C_{12}SO_4^-$, C_{13}-2-LAS$^-$ for C_{11}-2-LAS$^-$, $C_{16}N(CH_3)_3^+$ for $C_{12}N(CH_3)_3^+$, and $C_{13}EO_8$ for $C_{12}EO_4$ (all from Sigma-Aldrich except for C_{13}-2-LAS$^-$ which was custom synthesized[67]). Further details on separation and eluents are presented in SI Text S2.

For the SSLM assay, concentrations were measured using a HPLC system (Prominence UFLC-XR, Shimadzu) coupled to a tandem mass spectrometer (QTRAP 4000, Applied Biosystems) with electrospray injection, and quantified using external calibration standards prepared in the same medium. Further details on chromatographic separation are provided in SI Text S2; MS-detection signals of each compound are listed in Table S2.

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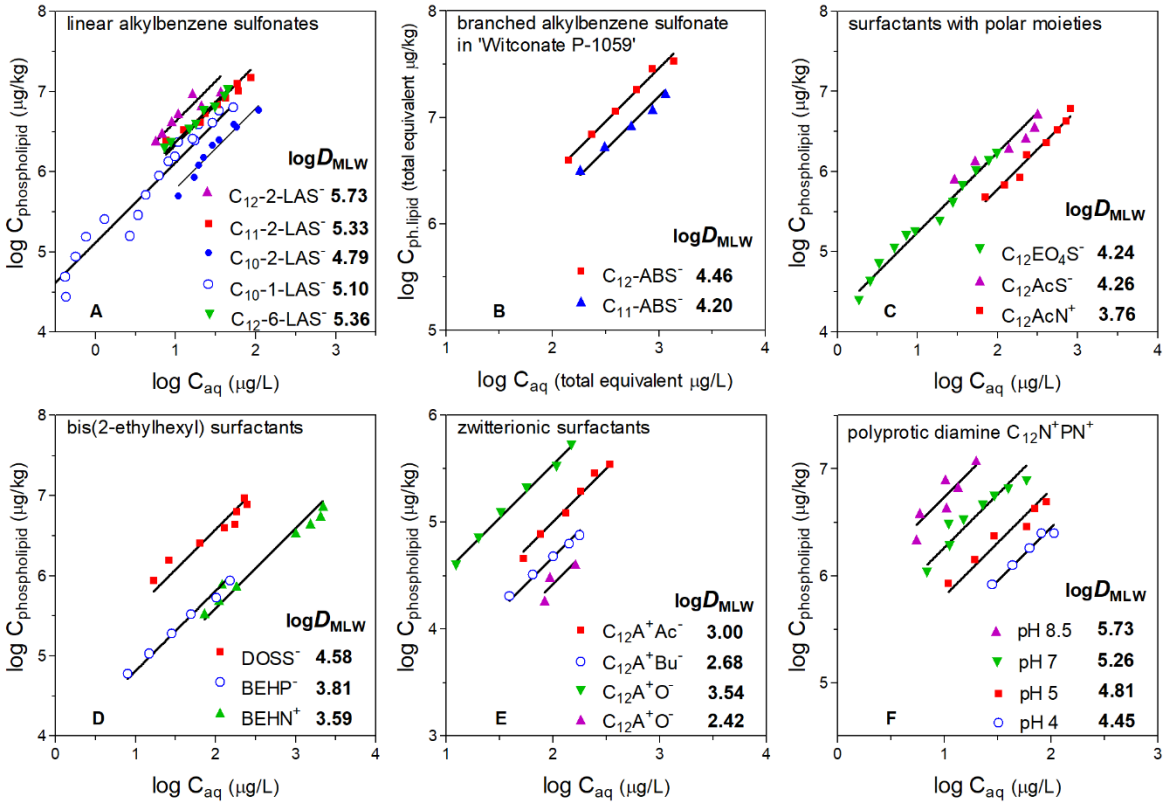

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259 **Figure 1.** Measured aqueous surfactant concentrations in SSLM assays plotted against mass-balance
260 calculated concentration in phospholipid. All fitted curves have a slope of 1, and are used to calculate
261 the logD_{MLW}. Branched alkylbenzene sulfonates in graph B are based on the total peak area of the
262 clusters of C_{11}-ABS$^-$ and C_{12}-ABS$^-$ isomers as components in the technical product Witconate P-1059,
263 as presented in SI-Figure S5.

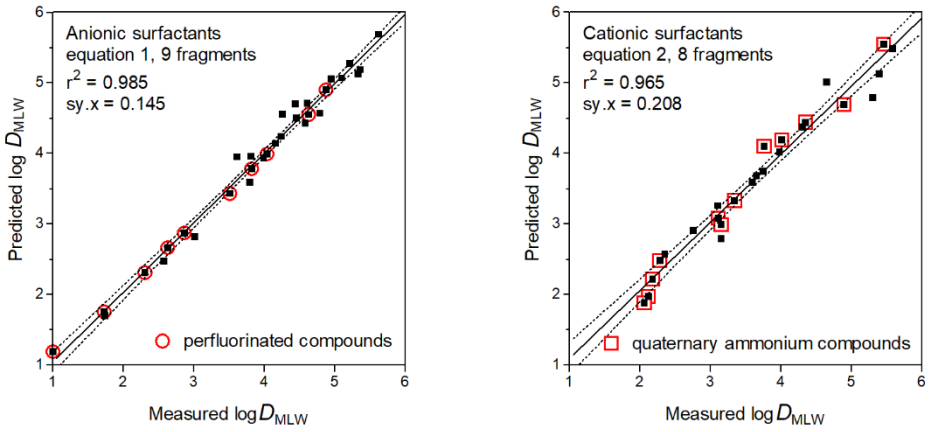

Figure 2. Measured logD_{MLW} values plotted against predicted logD_{MLW} values for (left graph) anionic surfactants using the fragment-activity eq.1, and (right graph) cationic surfactants using fragment-activity eq.2. Perfluorinated anionic compounds are highlighted with a red circle, quaternary ammonium cationic compounds with a red square.

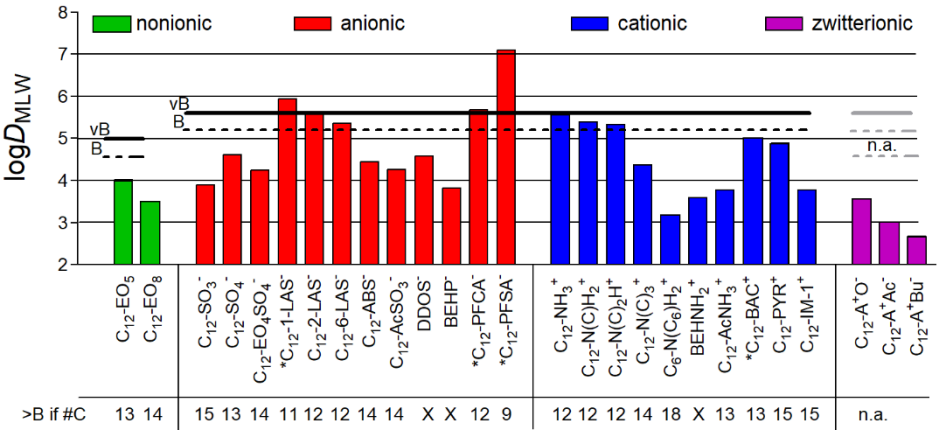

Figure 3. LogD_{MLW} values for different surfactants with a C_{12}-chain (except for the two C_8-chains of di-ethylhexyl-surfactants $DOSS^-$, $BEHP^-$ and $BEHN^+$, and the two C_6-chains of dihexylamine C_6-$N(C_6)H_2^+$). Surfactants marked by * have D_{MLW} values extrapolated from shorter chain homologues. The broken and solid lines show the logD_{MLW} that in a screening step would correspond to a $BCF_{baseline}$ = 2000 and 5000 L kg^{-1} (thresholds for B and vB classification under EU REACH). For non-ionic surfactants this is based on 5% total lipid, for cationic and anionic surfactants this is based on 1.25% phospholipid. For zwitterionic compounds the relevance of storage lipids is yet unknown and setting the critical chain length is thus not applicable (n.a., indicated by two grey broken lines). The row below the figure shows for each surfactant type the number of carbon atoms required in the alkyl chain for $BCF_{baseline}$ = 2000 L kg^{-1} to be exceeded, and for which further refinement of the BCF estimate would be required. For the dialkylamines, that number represents the total C in both chains, for di-ethylhexyl-surfactants extrapolation to analogues is not possible (X).

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284 **Table 1.** Experimental details for fish-oil/water distribution ($D_{oil\text{-}W}$) assays and comparison to
285 phospholipid-water distribution coefficients (D_{MLW}) storage lipid-water.

Surfactant	Spiking medium (concentration in mg/L)	Mass balance (%nom ± s.d)	Final C_{aq} (mg/L)	Final C_{oil} (mg/L)	Log $D_{oil\text{-}W}$ (L/kg)	Log D_{MLW} (L/kg)	Ref
Nonionic alcohol ethoxylate surfactant							
$C_{12}EO_4$	oil (16340, n=3)	68 ± 2	2.58	11175	3.64 ± 0.01	4.13 [a]	56
	oil (13000, n=1)	107	3.16	13917	3.64		
	oil (4000, n=2)	113-119	1.09-1.13	4581	3.60-3.63		
	oil (1350, n=5)	113 ± 7	0.41	1527	3.58 ± 0.04		
Cationic quaternary ammonium surfactant							
$C_{12}N(CH_3)_3^+$	oil (104, n=4)	118 ± 7	9.6-11.3	2.9-5.3	-0.4 ± 0.15	4.35	55
	water (10.7, n=4)	116 ± 5	11.9-13.2	0.9-1.6	-1.0 ± 0.12		
Anionic surfactants							
$C_{12}SO_4^-$	oil (111)	98 ± 14	8	<LOQ	ND	4.61	59
	water (101, n=4)	89 ± 16	75.3-98.3	2.2-4.8	-1.5 ± 0.14		
$C_{12}SO_3^-$	oil (10, n=4)	74 ± 6	0.57-0.68	0.033-0.067	-1.1 ± 0.16	3.99	59
	water (1, n=4)	116 ± 7	1	<0.06	-1.6 ± 0.93		
C_{11}-2-LAS^-	oil (104)	77 ± 3	7.3-7.7	0.6-2.5	-0.8 ± 0.30	5.33	*b*
	water (8.5)	99 ± 10	7.0-8.9	0.65-0.89	-1.1 ± 0.10		

286 s.d. = standard deviation, LOQ = limit of quantification, ND = not possible to determine.
287 [a] extrapolated from experimental result for $C_{12}EO_5$ (D_{MLW} = 4.01) and contribution of -0.12 for each EO-unit.[56]
288 [b] measured in this study.

Table 2. Experimental details for phospholipid membrane/water distribution (D_{MLW}) obtained with the solid-supported lipid membrane (SSLM) assay.

Surfactant	TRANSIL LD or HD (mL medium) [a] %sorbed fraction	Recovery Medium controls	Duplicates Medium control	Log D_{MLW} (SSLM [b])	95% range	N
linear and branched alkylbenzene sulfonates						
C_{10}-1-LAS$^-$	LD (12/12) >39-92%	82/108%	117/104%	5.10	5.01 - 5.18	11
C_{10}-2-LAS$^-$	LD (1.6/1.6) >68-97%	84/68%	104/112%	4.79	4.73 - 4.85	9
C_{11}-2-LAS$^-$	LD (12/12) >35-96%	92/91%	103/102%	5.33	5.27 - 5.40	9
C_{12}-2-LAS$^-$	LD (12/12) >66-97%	99/83%	105/102%	5.62	5.52 - 5.73	7
C_{12}-6-LAS$^-$	LD (12/12) >75-95%	99/94%	103/112%	5.36	5.33 - 5.39	10
C_{11}-ABS$^-$	LD (1.6) >38-76%	82%	105%	4.16	4.06 - 4.26	5
C_{12}-ABS$^-$	LD (1.6) >64-94%	114%	102%	4.46	4.41 - 4.53	5
Bis(2-ethylhexyl) chemicals						
DOSS$^-$	LD (1.6/1.6) >54-97%	77/77%	101/103%	4.58	4.48 - 4.68	8
BEHP$^-$	HD (1.6) >76-99%	94%	102%	3.81	3.75 - 3.88	6
BEHN$^+$	LD (1.6/1.6) >27-72%	86/94%	101/103%	3.59	3.49 - 3.70	8
Other surfactants with polar moieties						
C_{12}AcN$^+$	LD (1.6/1.6) >28-80%	109/92%	102/100%	3.75	3.66 - 3.84	9
C_{12}AcS$^-$	LD (1.6) >35-94%	93%	101%	4.26	4.13 - 4.39	9
$C_{12}EO_4S^-$	LD (1.6/1.6) >34-87%	51/48%	101/109%	4.24	4.19 - 4.29	12
Zwitterionic surfactants						
$C_{10}A^+O^-$	HD (1.6) >25-71%	95%	102%	2.42	2.21 - 2.64	3
$C_{12}A^+O^-$	HD (1.6) >67-97%	121%	102%	3.54	3.50 - 3.58	6
$C_{12}A^+Ac^-$	HD (1.6) >36-90%	77%	n.d.	3.01	2.96 - 3.05	6
$C_{12}A^+Bu^-$	HD (1.6) >25-85%	59%	102%	2.68	2.63 - 2.72	5
Polyprotic diamine surfactant						
$C_{12}N^+PN^+$	LD (7) >23-80%	109%	100%	4.45 (pH 4)	4.39 - 4.51	5
	LD (7) >27-90%	99%	104%	4.81 (pH 5)	4.72 - 4.90	6
	LD (1.6/10) >39-96%	90/110%	103/105%	5.26 (pH 7)	5.18 - 5.35	8
	LD (1.6/10) >23-80%	101/92%	105/102%	5.73 (pH 8.5)	5.61 - 5.86	6

[a] LD indicates the low lipid density series, HD the high lipid density series, transferred from well plates into glass vials with test medium volumes indicated. If two sorbent dilution series were used, information is separated by '/ ', the D_{MLW} is fitted to the total data set.

[b] for all chemicals tested in 0.1 M ammonium acetate (pH7), except for $C_{12}N^+PN^+$ which was tested at 4 pH with 0.1M buffers: ammonium formate/formic acid (pH4); ammonium acetate/acetic acid (pH5); ammonium bicarbonate/ammonia/acetic acid (pH7 and pH8.5).

Table 3. Summary of logarithmic distribution ratios between membrane lipid-water (D_{MLW}), storage lipid-water (D_{SLW}, using fish oil), and various protein-water (D_{PW}) for pure homologue structures (except C_x-ABS) of different surfactant types from the current study (‘*new*’) and other references (ref).

Structure	logD_{MLW}	ref
Nonionic surfactants		
alcohol ethoxylates		
C_8EO_5	2.24	56
$C_{10}EO_5$	2.97	56
$C_{12}EO_5$	4.01	56
$C_{14}EO_5$	4.86	56
$C_{10}EO_8$	2.55	56
$C_{12}EO_8$	3.42	56
$C_{14}EO_8$	4.45	56
$C_{14}EO_{11}$	4.12	56
Zwitterionic surfactants		
alkyldimethylamine oxides		
$C_{10}N(CH_3)_2{}^+O^-$ ($C_{10}A^+O^-$)	2.42	*new*
$C_{12}N(CH_3)_2{}^+O^-$ ($C_{12}A^+O^-$)	3.54	*new*
alkylbetaines		
$C_{12}N(CH_3)_2{}^+CCO_2^-$ ($C_{12}A^+Ac^-$)	3.01	*new*
$C_{12}N(CH_3)_2{}^+C_3CO_2^-$ ($C_{12}A^+Bu^-$)	2.68	*new*
Polyprotic diamine surfactant		
$C_{12}N^+C_3N^+$		
pH 4.0	4.45	*new*
pH 5.0	4.81	*new*
pH 7.0	5.26	*new*
pH 8.5	5.73	*new*
Storage lipid/water ratios	logD_{SLW}	
$C_{12}EO_4$	3.6	*new*
$C_{12}SO_4^-$	-1.1 to -0.8	*new*
$C_{12}SO_3^-$	-1.1	*new*
C_{11}-2-LAS$^-$	-1.5	*new*
$C_{12}N(CH_3)_3^+$	-1.0 to -0.4	*new*
Protein/water ratios (*Blood protein / Muscle protein*)	logD_{BPW} / logD_{MPW}	
PFOA$^-$	4.20 [68] / n.a.	
PFOS$^-$	4.67 [68] / n.a.	
C_8-1-LAS$^-$	4.84 [69] / 2.8 [70]	
C_8-BAC$^+$	n.a. / 1.4 [70]	

Structure	logD_{MLW}	ref
Anionic surfactants		
1-alkanesulfonates		
$C_8SO_3^-$	1.74	59
$C_{10}SO_3^-$	3.01	59
$C_{12}SO_3^-$	3.99	59
$C_{13}SO_3^-$	4.46	59
$C_{14}SO_3^-$	4.95	59
alkyl sulfates		
$C_8SO_4^-$	2.58	59
$C_{10}SO_4^-$	3.79	59
$C_{12}SO_4^-$	4.61	59
$C_{13}SO_4^-$	5.21	59
perfluorinated carboxylates		
PFBA$^-$	1.0	59
PFPA$^-$	1.73	59
PFHxA$^-$	2.31	59
PFHpA$^-$	2.87	59
PFOA$^-$	3.51	59
PFNA$^-$	4.04	59
PFDA$^-$	4.63	59
perfluorinated sulfonates		
PFBS$^-$	2.63	59
PFHxS$^-$	3.82	59
PFOS$^-$	4.88	59
linear alkylbenzenesulfonates		
C_8-1-LAS$^-$	3.61	65
C_{10}-1-LAS$^-$	5.10	*new*
C_{10}-2-LAS$^-$	4.79	*new*
C_{11}-2-LAS$^-$	5.33	*new*
C_{12}-2-LAS$^-$	5.62	*new*
C_{12}-6-LAS$^-$	5.36	*new*
branched alkylbenzenesulfonates		
C_{11}-ABS$^-$	4.16	*new*
C_{12}-ABS$^-$	4.44	*new*
1-alkylether sulfates		
$C_{12}EO_4S^-$ (pure SLES component) [h]	4.24	*new*
bis(2-ethylhexyl)-anions		
DOSS$^-$ (dioctyl sulfosuccinate)	4.58	*new*
BEHP$^-$ (bis(2-ethyl-hexyl)phosphate)	3.81	*new*
ester-based C_{12}-sulfonate		
$C_{12}AcS^-$ (lauryl sulfoacetate)	4.26	*new*

Structure	logD_{MLW}	ref
Cationic surfactants		
alkylamines		
$C_8NH_3^+$	3.10	55
$C_{10}NH_3^+$	4.30	55
$C_{12}NH_3^+$	5.58	55
N-methylalkylamines		
$C_8N(CH_3)H_2^+$	2.76	55
$C_{10}N(CH_3)H_2^+$	3.98	55
$C_{12}N(CH_3)H_2^+$	5.39	55
dialkylamines		
$C_6N(C_6)H_2^+$ [a]	3.15	55
$C_8N(C_8)H_2^+$	4.65	55
N,N-dimethylalkylamines		
$C_8N(CH_3)_2H^+$	2.35	55
$C_{10}N(CH_3)_2H^+$	3.65	55
$C_{12}N(CH_3)_2H^+$	5.30	55
N,N,N-trimethylalkylammonium		
$C_8N(CH_3)_3^+$	2.18	55
$C_{10}N(CH_3)_3^+$	3.34	55
$C_{12}N(CH_3)_3^+$	4.35	55
$C_{14}N(CH_3)_3^+$	5.46	55
benzalkonium cations		
C_6-BAC$^+$ [b]	2.12	55
C_8-BAC$^+$	3.11	55
C_{10}-BAC$^+$	4.01	55
alkylpyridinium cations		
C_8-PYR$^+$ [b]	2.28	58
C_{12}-PYR$^+$	4.89	55
ionic liquid salts		
IM1$^+$-6 [c]	<1.5	58
IM1$^+$-8	2.06	58
IM1$^+$-10	3.15	58
IM1$^+$-12	3.76	58
IM1$^+$-14	4.09	58
IM1$^+$-16	4.48	58
IM7$^+$-7 [c]	4.03	58
IM1$^+$-8OH [d]	2.06	58
IM1$^+$-12-IM1$^+$ [e]	2.29	58
Pyr$^+$8 [f]	2.18	58
P$^+$2228 [g]	2.24	58
bis(2-ethylhexyl)-amine		
BEHNH$_2^+$	3.59	*new*
ester-based C_{12}-amine		
$C_{12}AcNH_3^+$ (2-aminoethyl laurate)	3.76	*new*

[a] $C_6N(C_6)H_2^+$ = dihexylamine; [b] BAC = benzalkonium chloride, and PYR = pyridinium chloride; [c] IM1$^+$-6 = 1-hexyl-3-methylimidazolium chloride, and IM7$^+$-7 = 1,3-diheptylimidazolium chloride; [d] IM1$^+$-8OH = 1-(8-hydroxyoctyl)-3- methylimidazolium bromide; [e] IM1$^+$-12-IM1$^+$ 2Br$^-$ = 1,1’-(1,12-dodecanediyl)bis[3-methylimidazolium] dibromide; [f] Pyr8$^+$ = 1-octylpyrrolidinium chloride; [g] P2228 = triethyloctyl-phosphonium; [h] SLES = sodium laurylether sulfonate – pure C_{12}-EO_4-SO_4^-.Na$^+$ was used; n.a. not available

RESULTS AND DISCUSSION

Fish oil-water distribution ratios for non-ionic and ionic surfactants

Measured aqueous concentrations in twin-vial systems with surfactant spiked in a 3 mm fish oil layer show that equilibration is reached within 1 day, for $C_{12}EO_4$, C_{11}-2-LAS$^-$, or $C_{12}SO_4^-$ (Figure S3). It is thus assumed that $C_{12}N(CH_3)_3^+$ and $C_{12}SO_3^-$ are also sufficiently equilibrated between the fish oil layer and the stirred water in this system within the 2 days before sampling.

Non-ionic surfactant. For the alcohol ethoxylate surfactant $C_{12}EO_4$, only oil-spiked systems resulted in measurable aqueous concentration. As listed in Table 1, initial oil concentrations >1 g/L still resulted in equilibrated aqueous concentrations below the critical micelle concentration (CMC) of 18 mg/L.[71] Using different concentrations spiked in fish oil and analysis of both phases, a log linear sorption isotherm was fitted to the data (Figure S4). The resulting fitted fish oil-water distribution ratio of 4000 L/L is considered representative for the storage lipid-water distribution ratio (logD_{SLW} of 3.6, Table 1 and Table S3). The D_{SLW} is only a factor of 3 (0.5 log units) lower than the membrane lipid-water distribution coefficient D_{MLW} (logD_{MLW} of 4.13, extrapolated from $C_{12}EO_5$ (see Table 1), with the EO-increment of -0.12 log units,[56] see also eq.1). Although this single non-ionic surfactant may not be fully representative of other non-ionic surfactants, it can be deduced from a large set of other neutral chemicals[64] that the logD_{MLW} is a conservative indicator of the sorption affinity to the total lipid pool in tissue for non-ionic surfactants.

Ionic surfactants. For all three tested anionic surfactants ($C_{12}SO_4^-$, C_{11}-2-LAS$^-$, and $C_{12}SO_3^-$), and the cationic quaternary ammonium surfactant $C_{12}N(CH_3)_3^+$, spiked aqueous concentrations remained constant despite addition of 10% v/v of fish oil (Table S3). However, all these ionic surfactants were clearly detected in oil at a fraction of <0.1-1% of the total amount in the system. When using oil-spiked systems, oil concentrations of surfactants dropped, e.g., from 104 mg/L to 2.9-5.3 mg/L for $C_{12}N(CH_3)_3^+$, while water concentrations reached even higher concentration, e.g., 9.5-11.3 mg/L for $C_{12}N(CH_3)_3^+$. The mass balance remained within 100-120%. Although the logD_{SLW} derived from water-spiked systems was somewhat lower than that derived from oil-spiked systems (-1.0 vs. -0.4 for $C_{12}N(CH_3)_3^+$), the logD_{SLW} for ionic surfactants were in all cases < 0 (-1.1 ± 0.16 for $C_{12}SO_3$, -0.8/-1.1 for C_{11}-2-LAS, -1.45 ± 0.14 for $C_{12}SO_4^-$). Ionic surfactants have a higher affinity for water than for fish-oil. Comparing logD_{SLW} values for these ionic surfactants to logD_{MLW} values (e.g., 4.35 for $C_{12}N(CH_3)_3^+$), as listed in Table 1, it is evident that ionic surfactants sorb at least five orders of magnitude stronger to phospholipids than to neutral lipids.

Phospholipid-water distribution ratios for new surfactant types

For 18 surfactants, new D_{MLW} values were measured using the SSLM sorbent dilution series. Table 2 provides details for each assay on consistency between control duplicates, deviation of control

concentrations from nominal values, sample size and 95% confidence margins of D_{MLW}. The measured aqueous concentrations and mass-balance calculated phospholipid concentrations are presented for each tested surfactant in Figure 1. At least 6 valid data points were available from one or two different SSLM sorbent dilution series for each surfactant, except for the zwitterionic amine oxide $C_{10}A^{+}O^{-}$, for which only three valid points were obtained (See Figure 1E). As shown in Figure 1, the plotted data points for each surfactant indicate that sorbed concentrations increase linearly with dissolved concentrations, with 95% confidence margins within 0.2 log units for the isotherms fitted with a slope of 1 on the double logarithmic plots. Only for $C_{10}A^{+}O^{-}$, the confidence margin was larger (0.42 log units for D_{MLW}), but there is good consistency with its homologue $C_{12}A^{+}O^{-}$, based on the 0.55 increment per CH_2 unit (see section *Influence of surfactant specific molecular fragments* below). The logD_{MLW} values derived from the isotherms for 17 monoprotic surfactants, and four pH-dependent logD_{MLW} values for the diprotic diamine, are summarized in Table 3 (labeled '*new*'), together with sixty D_{MLW} values published previously for surfactants in peer reviewed literature. Text S3 describes in further detail how molecular fragments impact the D_{MLW} values for the newly measured surfactants, grouped as Linear Alkylbenzene Sulfonates (LAS), strongly branched AlkylBenzene Sulfonates (ABS), dialkyl-based surfactants, and surfactants with various polar moieties between alkyl chain and ionic head group. However, the main goal of measuring these additional surfactants in this study was to establish and evaluate a more complete data matrix on D_{MLW} for a wide range of surfactant types.

Influence of surfactant specific molecular fragments on D_{MLW}

Anionic surfactants. Table S3 presents a matrix of the molecular fragments shared by subsets of the 31 anionic surfactants and D_{MLW} values (including 10 perfluorinated surfactants). Presence or absence of most fragments is denoted by a 1 or a 0, respectively, except for 'alkyl chain length' and 'branching factor'. With inclusion of the newly derived D_{MLW} values for LAS and ABS surfactants and bis(2-ethylhexyl) structures in this study, the effect of alkyl chain branching can be distinguished to various degrees in comparison to linear alkyl chains. For simplification, the 'branching factor' is pragmatically assigned to fixed values: a value of 1 is used for C_x-2-LAS^{-}, a value of 2 for the more inner positioned C_x-6-LAS^{-} and "bis(2-ethylhexyl)" structures, and a value of 3 for the branched ABS^{-} compounds. Anionic units are -SO_3^{-} , -SO_4^{-} and -CO_2^{-}, with -CO_2^{-} deduced entirely from perfluorinated carboxylic acids. Hydrophobic tail fragments are separated into number of carbon atoms in the chain '#CH_x', effect of a 'dialkyl' structure (for the two bis(2-ethylhexyl) anions), 'perfluorination' of the chain or not. Specific other moieties (distinct from the anionic functional group) include the number of repetitive ethoxylate ("EO") units (4 for the SLES^{-} compound tested), the acetate ester unit in $C_{12}AcS^{-}$, and the phenyl ring in LAS and ABS. Using multiple linear regression on the full set of 31 structures (using the LINEST function in Excel software), the following coefficients (with standard errors in parentheses) were derived for 9 specific fragments to predict logD_{MLW} for anionic surfactants, using the -SO_3^{-} functional group as the reference point:

$$\log D_{MLW\ (SO_3=0)} = -2.77\ (0.17) + 0.56\ (0.02)\cdot[\#CH_x] + 0.77\ (0.09)\cdot[SO_4] - 0.12\ (0.05)\cdot[\#EO] + 2.24\ (0.13)\cdot[\text{benzyl}] + 0.62\ (0.11)\cdot[\text{acetate}] - 2.00\ (0.21)\cdot[\text{dialkyl}] - 0.49\ (0.09)\cdot[\text{branching factor}] + 3.20\ (0.16)\cdot[\text{perfluor}] - 0.91\ (0.00)\cdot[CO_2] \quad \text{eq.1}$$

with df = 21, $r^2 = 0.985$, sy.x (standard deviation of the residuals) = 0.17, F=155, ss_{reg} (regression sum of squares) =41, $ss_{resid.}$ (sum of squared residuals) = 0.61. A plot of the measured logD_{MLW} vs. predicted logD_{MLW} is presented in Figure 2.

Equation 1 indicates several key structural features that are shared for many of the anionic surfactants: (i) the contribution of the anionic headgroup to D_{MLW} increases with additional oxygen atoms: CO_2^- < SO_3^- by 0.9 log units and SO_4^- > SO_3^- by 0.8 log units. Note that the CO_2^- increment was derived with perfluorinated carboxylate acids only, but that a separate increment accounts for the effect of fluorination; (ii) the carbon chain length has a constant increment of 0.56 log units (s.e. 0.02), including perfluorinated chains; (iii) two alkyl chains instead of a single chain decrease the D_{MLW} with 2 log units, which can be interpreted as the first two carbon atoms in each of the two chains alongside the anionic group not contributing to the D_{MLW}; (iv) branching reduces the D_{MLW} by a maximum of 1.5 log units (-0.49 times 3 for highly branched ABS); (v) a benzyl unit as part of the hydrophobic chain increases D_{MLW} by 2.2 log units; (vi) ethoxylate chains preceding the anionic SO_4^- unit decrease the D_{MLW} with 0.1 log units per EO unit, which compares well to the EO increment for neutral alcohol ethoxylates (see Eq.3 below); (vii) an acetate group between the anionic SO_3^- unit and alkyl chain decreases the D_{MLW} with 0.6 log units, and (viii) perfluorination of the alkyl chain appears to result in a consistent increase of 3.2 log units compared to its analogue hydrogenated alkylcarboxylate and alkylsulfate structures.

Cationic surfactants. The same fragment approach for anions was followed for the cationic surfactants, listed in Table S4. This group does not include perfluorinated chemicals, but is a mixture of ionizable amines and quaternary ammonium compounds, including a set of imidazolium-based ionic liquids. The cationic headgroup fragments are divided according to the number of hydrogen atoms (NAi) on the protonated/cationic form of the amine/ammonium, following previous studies[72,73]; NAi = 3 for primary amines, 2 for secondary amine, 1 for tertiary amine, and 0 for quaternary ammonium compounds. Pyridinium units and 3-methyl-imidazolium units are treated as additional fragments (besides the NAi of 0). The tails are again represented by number of carbons in the chain '#CH_x', a 'branching factor' based on the new bis(2-ethylhexyl) structure $BEHN^+$, and 'dialkyl factor'. Specific other moieties include the benzyl unit as part of benzalkonium structures, and the acetate ester of $C_{12}AcN^+$.

The D_{MLW} study with cationic surfactants[55] indicated that ~1% lipids leaked from the SSLM beads upon thawing and resulted in underestimation of the actual D_{MLW} for those compounds. In the D_{MLW} study with the imidazolium cations this was not accounted for,[58] and D_{MLW} values for the longest chain analogues (C_{12} and C_{14}) are therefore not included in the equation.

Based on a multiple linear regression for 8 fragments in 25 different structures, the following surfactant fragment-D_{MLW} equation was derived for cationic surfactants (with standard errors for each coefficient in parentheses):

$$\log D_{MLW} = -2.22\ (0.33) + 0.35\ (0.06)\cdot[\text{NAi}] + 0.55\ (0.02)\cdot[\#CH_x] - 2.34\ (0.23)\cdot[\text{dialkyl}] - 0.71\ (0.16)\cdot[\text{branching factor}] - 0.34\ (0.18)\cdot[\text{3-methyl-imidazolium}] + 0.26\ (0.21)\cdot[\text{pyridinium}] + 0.68\ (0.20)\cdot[\text{benzyl}] - 1.73\ (0.29)\cdot[\text{acetate}] \quad \text{eq.2}$$

with df = 16, $r^2 = 0.96$, sy.x = 0.25, F=55, $ss_{reg} = 28.0$, $ss_{resid.} = 1.03$. A plot of the measured logD_{MLW} vs. predicted logD_{MLW} is presented in Figure 2.

The D_{MLW} for cationic surfactants increases with alkyl chain length by 0.55 log units (s.e. 0.03) per carbon unit, similar to the anionic surfactants. Also, the dialkyl fragment is comparable to that for anionic surfactants. The cause for the reduced affinity for phospholipid membranes for dialkyl compounds is that parts of the alkyl chains close to the charged moiety don't protrude in the hydrophobic core of the bilayer, but reside in the partially hydrated outer domain with the zwitterionic phospholipid headgroups.[55] The acetate fragment on the primary amine cation (-1.73) is much more negative than for the sulfonate anion (-0.6), for reasons currently unknown. The benzyl moiety in benzalkonium cations only increases D_{MLW} by 0.86 log units, while this increase is 2.2 log units in LAS anions. The reason for this may be due to the position of the benzyl unit relative to the chain; opposite of the hydrophobic tail in benzalkonium, while it is part of the 'tail' in LAS. The pyridinium ring only increases D_{MLW} by 0.26 log units (with relatively large standard error), while the 3-methylimidazonium unit even decreases D_{MLW}, by 0.34 log units.

The data matrix in Tables S3 and S4 can be readily extended if new D_{MLW} data becomes available for additional surfactant types. This may result in slight changes for some of the currently derived fragment coefficients, as several fragments are represented by only one or a few chemicals. The goal of the fragment-activity equations is not to serve as a separate calculation tool from which D_{MLW} values can be derived for any new surfactant type. It is recommended that for each new surfactant type, the D_{MWL} is measured for experimentally feasible pure/isomeric surfactant components. The fragment models clarify the contribution of surfactant specific units to the overall D_{MLW} parameter, which helps to derive some rules of thumb. It is valuable to confirm that branched structures have a lower affinity than fully linear structures, that there appears to be a consistent offset for dialkyl structures compared to single chain analogues, and that additional polar groups may reduce the D_{MLW}. The coefficients could also be used to align different *in vitro* or *in silico* tools to predict D_{MLW}. However, the most critical outcome of the fragment models is that we established the consistency of the chain length contribution. This allows for the extrapolation of the measured D_{MLW} for a single surfactant component to analogues with a different chain length, and select those structures for which a refined BCF assessment is relevant, as discussed further below.

Non-ionic surfactants. A structure-activity relationship was derived for the set of non-ionic alcohol ethoxylates in an earlier publication.[56] Starting from the logD_{MLW} of 4.01 for $C_{12}EO_5$, the relationship is based on carbon chain length [#CHx] and number of additional ethoxylate units:

$$\log D_{MLW} = 4.01 + 0.45\,[\#CHx\text{-}12] - 0.12\cdot(\#EO\text{-}5) \qquad \text{eq.3}$$

The coefficients are derived in the original study as average for the logD_{MLW} differences between 5 pairs of analogue structures. The 95% confidence interval for #CHx is 0.05, and for #EO also 0.05.

For $C_{14}EO_8$, for example, this results in a predicted logD_{MLW} of 4.55, which compares closely to the experimental result of 4.45. There are a wide variety of much more complex ethoxylated surfactants in use, sometimes with ethoxylate chains well beyond 20 units. It is not advisable to extrapolate eq.3 to such structures, as the thickness of the phospholipid layer may not fully absorb such long polar chains. Ethoxylated surfactants are almost always technical mixtures, for which an average logD_{MLW} may be derived based on individual components using an average chain length and ethoxylate number. For more simple non-ionic surfactant structures that lack ethoxylated chains or similar extensive polar moieties, such as fatty acid alkanolamides, the D_{MLW} can be approximated by correlation with measured or predicted K_{OW} values according to Endo *et al.*[64]:

$$\log D_{MLW} = 1.01\cdot \log K_{OW} + 0.12 \qquad \text{eq.4}$$

Zwitterionic surfactants. The two alkyldimethylamine-oxides and two alkylbetaine structures for which the D_{MLW} was measured are insufficient to derive a separate fragment approach for zwitterionic surfactants. It is not yet clear if these zwitterions could be included either in the fragment approach for cationics or that for anionics. Alkyldimethylamine-oxides are zwitterionic compounds where the oppositely charged moieties are in very close proximity, with the pK_a of the acidic oxide moiety not clearly defined. The D_{MLW} difference between the C_{10} and C_{12} analogue is 1.12 log units, displaying a similar linear CH_2 unit increment as for other surfactant types[55,59] (Figure 1E). The acetate group of the alkylbetaine $C_{12}A^+Ac^-$ has a pK_a of 3.6 (https://echa.europa.eu/registration-dossier/-/registered-dossier/14910/4/22), i.e., low enough to render the compound to be >99.99% zwitterionic at pH 7. The logD_{MLW} of 3.0 for $C_{12}A^+Ac^-$ is 1.35 log units less than the cationic analogue $C_{12}N(CH_3)_3^+$ which only lacks the dissociated carboxylic acid unit in comparison. The carboxylate group of the zwitterionic $C_{12}A^+Bu^-$ has a pK_a of 4.5 (chemicalize.org), and $C_{12}A^+Bu^-$ is therefore largely (>99.5%) zwitterionic at pH 7. Surprisingly, the logD_{MLW} of $C_{12}A^+Bu^-$ was 0.32 log units lower than that of $C_{12}A^+Ac^-$ (Figure 1E), despite having two additional CH_2 units between the two charged moieties. The position of these spacer CH_2 units are most likely located in the polar headgroup domain of the phospholipid bilayers when sorbed. As was shown for example for the methylation units on amine analogues or dialkyl

structures,[55] CH_2 units that reside in the polar headgroup domain hardly increase, or even decrease, the logD_{MLW}.

Polyprotic surfactants. The polyprotic surfactant *N*-dodecylpropane-1,3-diamine was tested in four buffers with various pH (Figure 1F). The pK_a of the two amine units are poorly defined. A pK_a of 10.5 was predicted for the outer primary amine, and a pK_a of 8.15 for the inner secondary amine (http://archemcalc.com/sparc.html). The main reason for selecting this surfactant was to deduce whether the diprotic form had a substantially higher or lower D_{MLW} than the monoprotic form. As the D_{MLW} increases with pH, apparently the diprotic form reduces the D_{MLW}, by about 1.5 log units. However, the difference in D_{MLW} of 0.36 log units between pH4 and pH5 suggests that the second pK_a is lower than 8.15, more in the range of 6, but there are too few data points to fit the speciation profile.

A $BCF_{baseline}$ screening approach to derive critical chain lengths for different surfactant types that potentially surpass B thresholds

A baseline screening approach for BCF could assume that equilibrium partitioning is reached between dissolved concentrations in the water and only the target lipid fraction in the total wet tissue, thereby neglecting (i) biotransformation, which, where it occurs, will lower BCF,[74] (ii) the possibility of equilibrium not being reached due to slow membrane permeation,[29,75] and (iii) other tissue phases contributing to the sorption of surfactants (e.g., plasma and structural proteins).[76,77] The phospholipid fraction of total fat in the whole body of fish is estimated to be approximately 25%.[75,78] For a fish with 5% fat the phospholipid fraction would thus be 1.25%. Using the 1.25% phospholipid fraction (f_{ML}) as the target lipid fraction for ionic surfactants, as partitioning to storage lipid is negligible for these chemicals as shown above in the fish oil distribution experiments, and 5% total lipid content for non-ionic surfactants, the wet weight fish $BCF_{baseline}$ screening value is estimated as:

$BCF_{baseline}$ (ionic surfactants) = $0.0125 \cdot D_{MLW}$, or: $\log BCF_{baseline} = \log D_{MLW} - 1.9$ eq.5

$BCF_{baseline}$ (non-ionic surfactants) = $0.05 \cdot D_{MLW}$, or: $\log BCF_{baseline} = \log D_{MLW} - 1.3$ eq.6

Using the baseline screening BCF approaches above, ionic surfactants could be screened as potentially 'B' for the EU REACH threshold if $\log D_{MLW} \geq 5.2$ (See Figure 3), and non-ionic surfactants if $\log D_{MLW} \geq 4.6$. Similarly, ionic surfactants could be screened as potentially 'vB' for the EU REACH threshold if $\log D_{MLW} \geq 5.6$ (See Figure 3), and non-ionic surfactants if $\log D_{MLW} \geq 5.0$. Ideally, the $BCF_{baseline}$ is considered a conservative Tier 1 screening metric and should not underestimate the actual BCF.

Certain perfluorinated surfactants could function as benchmark chemicals for this simplified $BCF_{baseline}$ approach based on D_{MLW} and f_{ML}, because these anionic surfactants are not expected to be

biotransformed in fish and are well known to bind to proteins. However, only perfluorinated surfactants like PFOS that accumulate mainly due to uptake/elimination via the gills and sorption affinity to the tissue are suitable for this purpose.[48] As shown in Table 3, the logD_{MLW} for PFOS is 4.9, so the screening log$BCF_{baseline}$ is 3.0, or 1000 L kg^{-1} according to eq.5. The BCF determined on carcass of rainbow trout (liver and gut removed)[42] was 1100 L kg^{-1}, although for the whole fish a somewhat higher BCF may be derived as the liver indicated a BCF of 5400 L kg^{-1}, while sampled blood indicated 4300 L kg^{-1}. Another BCF study determined a whole body BCF of 720-1300 L kg^{-1} for PFOS in carp, which is again consistent with the screening $BCF_{baseline}$.[43]

Higher experimental fish BCF than the $BCF_{baseline}$ may be the result of contribution of tissue components other than phospholipids. The relatively higher BCF of PFOS in the fish blood sample than the carcass is due to PFOS having a relatively high sorption affinity to blood proteins (Table 3) and fatty-acid binding proteins in liver, but these make up a small proportion of the overall protein pool, and total tissue weight. Neglecting the contribution of sorption to proteins to the overall BCF could result in this $BCF_{baseline}$ approach underestimating the worst case BCF, which would be undesirable. An option could be to extend equations 5 and 6 in the screening phase if protein binding data are available with a wet fish weight fraction of 0.3% serum albumin multiplied by blood protein-water distribution ratios (D_{BPW}) and 15% muscle protein multiplied by muscle protein-water distribution ratios (D_{MPW}). The research field of surfactant-protein interactions is extensive, but highly diverse. Often the surfactant binding is studied at concentrations well above the critical micelle concentration (CMC) in terms of detergency performance or protein separations, but sorption coefficients for monomeric levels on purified surfactants are scarce.[79-81] Although only 4 surfactants are included in Table 3, a more targeted search could result in more binding coefficients. For PFOA and PFOS, including D_{BPW} would result in an increase of $BCF_{baseline}$ by a factor of 2.2 and 1.1, respectively. Sorption affinities of PFOS to structural proteins are not available, to our knowledge. Data on binding to protein material are retrieved from literature for some surfactants.[68-70,82] Fish specific D_{MPW} (as L kg^{-1} in Table 3) are reported for two surfactants (C_8-1-LAS, and C_8-benzalkonium).[70] The albumin binding coefficient for the anionic C_8-1-LAS is more than tenfold higher than D_{MPW},[69] and therefore binding of anionic surfactants to albumin is likely not indicative of binding to fish muscle protein. The available D_{MPW} are also tenfold or more below D_{MLW}, but most fish have about tenfold higher structural protein content compared to phospholipid. Although protein binding may be relevant for the overall fish BCF of surfactants, the current data set is insufficient to include D_{MPW} in estimating a $BCF_{baseline}$. Protein binding may best be included as a higher tier refinement option. In the absence of D_{BPW}, a conservative option could be to set a factor of 2 lower trigger value for the $BCF_{baseline}$ compared to existing regulatory triggers below which the substance is concluded to be "not bioaccumulative" (nB), and above which higher tier refinements of BCF assessment would be required (including sorption to protein). For example, the $BCF_{baseline}$ threshold of 1000 L kg^{-1} could be used in relation to the EU REACH threshold of 2000 L kg^{-}

[1]. However, setting a threshold depends on regulatory acceptance, regulatory requirements, and further evaluation of relevant BCF studies.

The D_{MLW} measurements summarized in Table 3 and as discussed above, demonstrate that for nearly all linear chain surfactants, both ionic and non-ionic, the CH_2 increment of the chain length contributes about 0.5-0.55 log units (1.0-1.1 log unit per 2 CH_2 units). Lower CH_2 unit increments apply to branched structures. Figure 3 shows all C_{12}-chain based surfactant structures for which logD_{MLW} have been determined, or for which shorter chain homologues can be used to extrapolate to C_{12} with high confidence, including perfluorinated anions. This C_{12}-chain surfactant set covers 26 structures, including non-ionic, anionic, cationic and zwitterionic structures. The "critical" chain length that would surpass the EU REACH B threshold of 2000 L kg^{-1} in this $BCF_{baseline}$ screening approach is presented in Figure 3 in the bottom row, derived for each surfactant structure using the log 0.55 CH_2 increment derived from equations 1 and 2. Note again that this does not yet include the uncertainty factor due to possible contribution of binding to protein in tissue. Overall, this $BCF_{baseline}$ screening of the "critical" chain length of surfactants based on D_{MLW} is a major improvement over current K_{OW} based estimates, because it is based on consistent experimental data on sorption affinities relevant for tissue. In the absence of *in vivo* data for most types of surfactants, or long chain homologues, the $BCF_{baseline}$ value serves as a conservative screening estimate of the BCF in fish, which can be further refined with standardized *in vitro* assays and *in silico* modeling when this is considered relevant. Refinement may be based first on additional *in vitro*-parameters such as intrinsic clearance rates of liver fractions (e.g. OECD guidelines 319A for hepatocyte cells[83] and 319B for S9 fractions,[84] liver microsome fractions, or liver cell spheroids), as well as Quantitative Structure-Activity Relationships for predicting biotransformation half-lives in fish,[85-88] and subsequent BCF modeling.[75] The extent to which the screening $BCF_{baseline}$ is overestimating the actual fish BCF is further examined below using non-fluorinated surfactants for which *in vivo* BCF studies are available.

Comparison of $BCF_{baseline}$ with available *in vivo* BCF data

Non-ionic surfactants. For non-ionic surfactants that are (expected to be) within the applicability domain of a K_{OW} assessment, measured or predicted K_{OW} values and commonly used B assessment tools (e.g., the BCFBAF module in EPI Suite ver.4.11) are sufficient for a $BCF_{baseline}$ screening approach. For more complex non-ionic surfactant structures, such as those with ethoxylated chains, D_{MLW} values for experimentally feasible structures, or analogues, should be used. For alcohol ethoxylates, logD_{MLW} increases slightly for analogues with shorter EO units (eq.3). Based on the $BCF_{baseline}$ approach in equation 6, Figure 3 shows that even the shortest C_{12}-alcohol ethoxylates will not surpass the B limit. However, Figure 3 indicates that the $BCF_{baseline}$ for C_{14}-alcohol ethoxylates with 8 EO units and less will surpass the B limit, and longer alkyl chain homologues will mostly approach or surpass B limits in this screening phase. *In vivo* BCF studies with fathead minnows, however, have shown that

biotransformation strongly reduces the actual bioaccumulation potential of this type of surfactants in fish[37,38]. For example, based on total ^{14}C-labeled signals, the homologue $C_{13}EO_8$ indicated a BCF of 224 L kg^{-1} (which compares favourably with a $BCF_{baseline}$ of ~400 L kg^{-1}), whereas measured steady state parent compound concentrations in fish and water indicate a BCF of 31 L kg^{-1}.[37] For the same fish species, the *in vivo* BCF of the less ethoxylated $C_{13}EO_4$ and the longer chain $C_{16}EO_8$ were 232 and 387 L kg^{-1}, respectively,[38] while the respective $BCF_{baseline}$ values were ~1,000 and 14,000 L kg^{-1}, i.e. a factor of 4-36 higher. Biotransformation of $C_{13}EO_8$ and $C_{16}EO_8$ has been confirmed in hepatic microsomes and liver hepatocytes from two fish species,[89,90] but *in vitro* clearance data for a systematic series of alcohol ethoxylates may be required to confirm whether further extrapolation to this entire group is appropriate.

Anionic surfactants. Figure 3 demonstrates that the $BCF_{baseline}$ screening would not classify alkylsulfonates, alkylsulfates, or alkylether sulfates (with ~4 EO units) with chain lengths of 12 carbon atoms as bioaccumulative under EU REACH. For SDS ($C_{12}SO_4^-$), multiple fish BCF studies are available that report BCF values between 1-7.2 L kg^{-1} (listed in [41]), while the proposed screening BCF approach predicts a $BCF_{baseline}$ of 500 L kg^{-1}. The substantial difference suggests a strong impact of biotransformation. Rapid RT-S9 clearance of SDS has also been reported.[91] With sufficient evidence on the influence of additional CH_2 units on D_{MLW}, analogue alkylsulfonate, alkylsulfate, and alkylether sulfate surfactants with chain lengths of 15, 13, and 14, respectively, have screening $BCF_{baseline}$ values above the B threshold. For these substances, we would recommend proceeding to the next tier in a BCF assessment framework and evaluating biotransformation. Unpublished fish BCF values for the technical mixture $C_{14/15}SO_4$ are in the range of 180-972 L kg^{-1} for catfish and fathead minnows (listed in [41]), with 6.5 times higher BCF for $C_{15}SO_4$ compared to $C_{14}SO_4$. These values are higher than a BCF of 73 L kg^{-1} reported for the longer chain homologue $C_{16}SO_4$;[92] all of the *in vivo* values are actually much smaller than the respective screening $BCF_{baseline}$.

For the whole range of different inner isomers of the C_{12}-chain linear alkylbenzenesulfonates (LAS), the $BCF_{baseline}$ screening values slightly surpass the B-limit, but shorter chain LAS would not classify as B. BCF studies with fathead minnows demonstrated that rapid biotransformation reduces the actual BCF in these fish by >40%. The pure homologue C_{12}-2-LAS and further inner isomers had a more than tenfold lower BCF (100-200 L kg^{-1})[39,93] than predicted by $BCF_{baseline}$ based on logD_{MLW}. Longer chain LAS structures, such as C_{13}-2-LAS, however, do show higher BCF values in the same fish species (370-1000 L kg^{-1})[39,93] in both lab studies and caged field exposures; still below, but closer to the EU REACH 'B' thresholds. The isomer mixture of more strongly branched C_{12}-alkylbenzenesulfonates (C_{12}-ABS) are predicted to have a tenfold lower B potential than the LAS isomers, and the lower contribution of branched CH_2 units to logD_{MLW} (0.3 in comparison to C_{11}-ABS) suggest that even C_{14}-ABS may still be below the B threshold in the screening assessment. However, considering the historical issues with poor biodegradation properties associated with branched surfactants[4], such structures are expected to also

have a much lower biotransformation potential than their linear analogues. Actual fish BCF values for ABS may thus be closer to the $BCF_{baseline}$ compared to LAS.

The logD_{MLW} of 4.24 for the SLES structure $C_{12}EO_4S^-$ suggests that a C_{14}-analogue would surpass the threshold B limit (Figure 3). The logD_{MLW} for analogue $C_{14}EO_2S^-$ is predicted to be ~5.4, rendering a screening $BCF_{baseline}$ of 3162 L kg^{-1}. A previous study derived a BCF estimate of 12 L kg^{-1} for the SLES structure $C_{14}EO_2S^-$ in fish, based on an assumed logK_{OW} of 2.1, accumulation into 10% fat content, and included the hepatic clearance based on measured *in vitro* clearance rates.[90] Clearly, this approach differs from our proposed $BCF_{baseline}$ approach with optional further refinement using measured *in vitro* clearance rates, but also note the orders of magnitude difference in predicted logK_{OW} and experiment-based logD_{MLW}. $C_{14}EO_2S^-$ has been demonstrated to be biotransformed *in vitro*,[90] which will lower the actual fish BCF. Unfortunately, there are no publicly available BCF data for pure or technical alkylether sulfates, or validated modeling approaches for anionic surfactants, to verify the predictions that the BCF is below or still above the B limit.

The bis(2-ethylhexyl)-based anionic surfactants included in this assessment are well below B thresholds in the $BCF_{baseline}$ screening approach, but longer alkyl C-chain analogues of these forms are also marketed (e.g., 1,4-diisodecyl and 1,4-diisotridecyl sulphosuccinate analogues (CAS: 29857-13-4 and 55184-72-0)). Bis(2-ethylhexyl)sulfosuccinate (or "di-octylsulfosuccinate", "DOSS", or "docusate") is a significant component of the oil dispersants Corexit 9500 and Corexit 9527A. Both dispersants were used (estimated 6.8 million liters[94]) in efforts to remediate the Deepwater Horizon oil spill in the Gulf of Mexico. $DOSS^-$ is also a food additive in beverages as a wetting agent or solubilizer for flavor emulsion stabilizers. A BCF range of 3-8 L kg^{-1} has been reported for $DOSS^-$ in common carp (ref [95], and listed in [75]). The log $BCF_{baseline}$ is almost 2 orders of magnitude higher (500 L kg^{-1}). This may be related to rapid biotransformation, as $DOSS^-$ was observed to be cleared relatively fast in S9 from trout liver.[91] For $BEHP^-$ the available *in vivo* BCF of 3 L kg^{-1} in common carp,[75] is also less than $BCF_{baseline}$ (79 L kg^{-1}).

Cationic surfactants. As indicated in Figure 3, $BCF_{baseline}$ for ionizable linear alkylamines with chain lengths of C_{12} and longer exceed the B threshold for EU REACH. A BCF study with rainbow trout exposed to systematic mixtures of cationic surfactants at pH 7.6 indeed reported that the BCF exceeded 2000 L kg^{-1} for 4 amines with chains ≥C_{13}.[45] This is a relevant example demonstrating that the $BCF_{baseline}$ approach is able to conservatively select surfactants for which refined BCF assessments are required. Due to the relatively lower D_{MLW} for dialkylamines (fragment value of -2.34 log units, eq.2), only dialkylamines with two nonyl or decyl chains have $BCF_{baseline}$ values >2000 L kg^{-1}. No *in vivo* BCF data are available for dialkylamines to confirm this yet.

The $BCF_{baseline}$ values for quaternary ammonium compounds suggest that a higher tier BCF assessment is needed using *in silico* and *in vitro* approaches for trimethylalkylammonium cations with chain lengths

of $\geq C_{14}$, as well as benzalkonium and pyridinium-based quaternary ammonium compounds (QAC) with $\geq C_{13}$. Several of such compounds are in use as (part of technical mixtures of) plant protection products and biocides, for example $C_{12\text{-}16}$ benzalkonium (e.g., wood preservative, https://echa.europa.eu/documents/10162/b9030b10-c8af-211b-456a-4f4b11d509b7) and cetylpyridinium (e.g., used as antiseptic drug in some toothpastes, https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/17221/3/1/6). The EU REACH dossier for $C_{12\text{-}16}$ benzalkonium reports a steady-state BCF (whole fish) of 79 L/kg for bluegill (https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/22044/5/4/2) based on measured ^{14}C residues, indicating much lower BCF values than expected based on the $BCF_{baseline}$. In BCF studies with rainbow trout exposed to systematic mixtures of cationic surfactants,[45] $C_{14}N(CH_3)_3^+$ had a steady state BCF based on parent compound analysis of 50 L kg^{-1}. Tissue distribution analysis of rainbow trout indicated that $C_{14}N(CH_3)_3^+$ was poorly taken up systemically, with only low levels detected in blood but not in other tissues, while the chemical accumulated at external surfaces such as gill and skin (mucus).[44] The permanently charged form of quaternary ammonium compounds seems to strongly limit permeation through gill cells, while the minor neutral fraction for analogue ionizable amines with pKa > 10 drives systemic uptake of these chemicals from water.

Zwitterionic surfactants. Alkyldimethylamine-oxide (amine oxides) mixtures may contain C_{16} and C_{18} components.[96] Whereas a substantial toxicity dataset is available for amine oxide zwitterionics,[97] bioconcentration studies to evaluate the logD_{MLW}-based $BCF_{baseline}$ approach are as yet lacking for all zwitterionic surfactants. Amine oxides are suggested to deprotonate from net cationic to zwitterionics, going from low to high pH,[98] but the pK_a determined to be 4.95 indicates that at pH 7.0 applied in the current study, and in most environmental and physiological pH, the cationic form contributed <1%. Since these compounds are predominantly net neutral, it may be of interest to study partitioning in storage lipids in future efforts. If only equilibration to the phospholipids of cell membranes is considered, the $BCF_{baseline}$ screening suggests that even C_{16} based zwitterions may not surpass screening B thresholds. The same applies to betaine-based zwitterionics and analogues.

Alternative ways to derive $BCF_{baseline}$ and options for further refinement

D_{MLW} may be difficult to derive experimentally for strongly sorbing surfactants (e.g., chain lengths $>C_{14}$).[55] For most surfactants shorter pure homologues are available, or can be synthesized, which will allow for extrapolation of the D_{MLW} to longer chain lengths. D_{MLW} values for technical mixtures could be assessed based on those for pure compounds and mixture compositions. As shown for the branched anionic surfactant formulation Witconate P-1059 (Figure S5), specific signals in a mass spectrometer can be used to derive the D_{MLW} for specific isomeric clusters within the experimentally feasible range. Just as commonly done for alcohol ethoxylate mixtures,[99] an average D_{MLW} may be derived for the whole substance based on mixture composition.

Although Table 3 presents a rich set of D_{MLW} values derived with SSLM assays and dialysis sampling with liposomes, D_{MLW} values may be further explored by using alternative methods to determine phospholipid binding. Chromatographic retention on immobilized phospholipid coated silica[100] has been validated for anionic and cationic surfactants.[55,59,101] Correlations with other chromatographic descriptors may allow for further extrapolations.[102] Quantum-chemical predictions, such as from the COSMOmic module of the COSMOtherm software, can make use of the three-dimensional structure of ionic chemicals and how these align most favorably in a model hydrated phospholipid.[65,103] However, a misalignment between for example COSMOmic calculations and SSLM measurements of D_{MLW} has been reported for perfluorinated anions.[59,104] This indicates that each alternative method needs to be carefully validated as part of the applicability domain of D_{MLW} data obtained with phospholipid bilayers when considering new specific surfactant types.

The pH-dependent D_{MLW} for the multiprotic *N*-dodecylpropane-1,3-diamine presented in this study indicates that deriving D_{MLW} at neutral pH may be insufficient for regulatory assessment. Most of the ionizable surfactants (alkylamines, sulfonates, sulfates, phosphate) have such small fractions of neutral species in the environmentally relevant pH range of 6-9 that Eq. 5 is valid. In tissue, the pH is often tightly controlled at pH 7-7.8, but can be exceptionally low in some organelles (e.g., lysosomes). Also, the pH near the gill surface may be specific to certain conditions.[105] In such more extreme pH environments deviating from pH at which D_{MLW} is derived, but also for surfactants with a more intermediate pK_a, the neutral species may influence the overall D_{MLW}. In such cases, for example for alkyldiethanolamines, the neutral species may be included based on a K_{OW} approach (eq.1) and Henderson-Hasselbalch speciation calculations.

In case the $BCF_{baseline}$ prediction for a surfactant exceeds a regulatory threshold, further refinement should be explored using information from *in vitro* and *in silico* approaches. Mass balance models like BIONIC have been developed and evaluated to simulate the toxicokinetics and BCFs of IOCs in fish[75] and BIONIC can incorporate chemical distribution information (e.g., D_{MLW} but also protein binding) and biotransformation rate data to obtain higher tiered BCF estimates. The Bioaccumulation Assessment Tool (BAT),[106] and subsequent BCF modeling provides a weight-of-evidence framework to incorporate multiple lines of evidence (i.e., *in vivo*, *in silico* and *in vitro* data), including measured and predicted field metrics of bioaccumulation (i.e., bioaccumulation factors, biomagnification factors), to inform B assessment decision-making. Technically and ethically less demanding and more cost-effective than full *in vivo* studies, such a tiered approach would allow for an enhanced realism in predicting the bioconcentration potential of surfactants for assessments as compared to estimates based on K_{OW} and existing BCF models used for neutral chemicals. A substantial role of biotransformation resulting in reduced actual BCF may be demonstrated *in vitro* by substrate depletion data with fish liver material, for which OECD guidelines have become available (OECD 319A[83] and 319B[84,107,108]). Fish liver S9 substrate depletion rates have been determined already for limited series of surfactants.[89-91,109] This does,

721 however, require further optimization of various *in vitro-in vivo* extrapolation factors that are currently
722 developed using K_{OW}.[29,75] If the surfactant is within the applicability domain of QSARs for predicting
723 the *in vivo* whole body level biotransformation rate (half-life),[85-88] these data can also be used to refine
724 B predictions, e.g., using BIONIC or BAT. It is noted that the biotransformation rate QSAR in BCFBAF
725 includes K_{OW} in the descriptor set and predictions from this QSAR may therefore be more uncertain than
726 other biotransformation rate QSAR predictions that do not include this parameter in the QSAR
727 descriptors. There is sufficient evidence that the nearly fully ionized sulfate-base and sulfonate-based
728 surfactants,[39] as well as ionizable alkylamines,[44] are taken up systemically in fish. Fish BCF for ionizable
729 alkylamines is found to be pH-dependent with increased BCF at higher pH, likely due to the increased
730 fraction of neutral species facilitating gill permeation. Quaternary ammonium cations, however, poorly
731 permeate through the gills and mostly sorb on outer tissues only, resulting in relatively low BCF
732 values[44,45] compared to ionizable analogues. And although the uptake mechanisms and toxicokinetics of
733 per/polyfluorinated surfactants are widely studied in many species, a move to a fish BCF model focused
734 on D_{MLW} provides significant improvement in preliminary screening for BCF of aquatic organism
735 breathing via gills compared to models based on K_{OW}. More studies on surfactants, but also ionic
736 chemicals in general, are needed to derive relevant *in chemico* data for other factors relevant in a higher
737 tier bioaccumulation assessment, particularly addressing the role of specific tissue derived components
738 such as structural and blood proteins, lysosomes, and the presence of anionic phospholipids in cell
739 membranes.[29]

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ASSOCIATED CONTENT

Supporting Information

The following files are available free of charge.

Additional details of experimental methods and supportive figures (.pdf)

Corresponding Author

* Steven T.J. Droge (steven.droge@gmail.com)

Author Contributions

Experimental work was performed by SD and PS. The manuscript was written by SD with contributions of all authors. All authors have given approval to the final version of the manuscript. The conclusions expressed in the paper represent the expert judgement of the authors, but not necessarily the opinion of their affiliation.

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Conflict of interest

There are no conflicts of interest to declare. The contribution by SD is based on his work during employment at the University of Amsterdam, which ended December 31st, 2019.

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