Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

Supporting information

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

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Table of Contents

Text S1. Example for sodium dodecyl sulfate (SDS)

The anionic surfactant sodium dodecyl sulfate ("SDS", CAS number: 151-21-3) is reported to have a fish BCF of 1-7.4 L kg^{-1}, as reported in various studies submitted for dossier 2126 of the REACH database of the European Chemical Agency (ECHA)[2] (https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/2126/5/4/2).

K_{OW} values for SDS

A recent study on obtaining experimental logP values for different types of surfactants showed poor correlation amongst test methods, within homologs of the same surfactant type, and relative to common computational approaches, particularly for ionic surfactants[1]. Table S1 shows an example of the wide range of logP values derived experimentally for SDS, as submitted for dossier 2126 of the REACH database of the European Chemical Agency (ECHA)[2] (https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/2126/4/8). Table S1 also lists the variable results from common computational approaches to derive the logP (i.e. the K_{ow} for the neutral form) or logD (pH-dependent octanol-water distribution coefficient determined at a specific pH, e.g. at pH 5.5, denoted as $logD_{5.5}$, where the dissociated form of SDS accounts for >99.99%). Two reported phospholipid-water distribution coefficients ($logD_{MLW}$) are listed for comparison.

Table S1. Example of the variability in logP values for sodium dodecyl sulfate and difference between logP values and $logD_{MLW}$ values

Experimental findings listed in the EU REACH dossier 2126 for SDS *	**"logP"**	**Computational approach**	**"logP"**	**$logD_{5.5}$**	**$logD_{7.5}$**
• experimental solubility ratio, based on maximum solubility in water (CMC) and octanol	<-2.03	• EPISuite KowWIN (via chemspider), NB. It is unclear if this relates to the neutral or ionic form	2.42		
• experimental slow-stirring method, OECD 123[3]	0.83	• ACD/Labs (via chemspider)	5.39	0.62	0.62
• experimental Leo & Hansch, likely via shake-flask method, OECD 107[4]	1.6	• ChemAxxon (via chemspider)	4.42	2.04	2.04
			(pH<0)		
		• SPARC - ionic strength=0	~5.30	2.68	2.68
		SPARC - ionic strength=1	~5.30	1.04	0.49
		SPARC - ionic strength=10	~5.30	0.96	0.044
		SPARC - ionic strength=100	~5.30	0.95	0.034

Experimental phospholipid binding studies with SDS	**Reference**	**$logD_{MLW}$ pH 7-7.4**
Dialysis system with unilamellar liposomes, with measurements made at dissolved SDS concentrations close to the CMC	Kalmanzon et al., 1992	3.52
Adapted SSLM assay, using series of measurements for a wide range of SDS concentrations well below the CMC	Droge, 2019	4.6

* https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/2126/4/8

The listed logP values obtained with experimental methods span 3.6 log units, and although the applied pH is often not reported, the range is indicative of the problems deriving a reliable logP/logD value for surfactants experimentally. Computational approaches lack transparency on how the ionic component is derived. Only SPARC allows for adjustment of ionic strength, which is known to influence K_{OW} for organic ions almost proportionally with a higher salinity due to facilitated "ion-pairing"[5-8]. Moreover, whereas octanol is a neutral dipolar solvent, various types of natural organic matter contain high densities of ionogenic moieties besides hydrophobic pockets. Soil organic matter is abundant in carboxylic and phenolic acid groups, phospholipids contain zwitterionic headgroups, and all proteins are abundant in both positively and negatively charged amino acid side chains. For cationic surfactants, octanol has been shown to strongly underestimate the sorption affinity to soil organic matter[9] and phospholipids[10-12], because octanol does not account for the favorable contribution of electrostatic interactions between sorbent and sorbate[11].

The logP recommended value in the REACH dossier, is the one based on the maximum solubility in octanol divided by the maximum solubility in water, as it can be obtained quite unambiguously experimentally. It can be questioned if the critical micelle concentration (CMC) is acceptable as maximum aqueous solubility of dissolved monomers. With a logP value of -2 (using a maximum octanol solubility of 1.2 g/L and CMC >130 g/L), it is the lowest value reported.

The relevance of unreliable K_{OW} values for surfactants has recently been discussed in terms of toxicity predictions.[13] A key finding of that study is illustrative to repeat here for the realism that the current paper also aims to address. For neutral chemicals, the K_{OW} is commonly used as a proxy to the lipid water partition coefficient (K_{lipw}), and for most neutral chemicals (except strong H-bond donors) the relationship is almost 1:1.[14] As baseline toxicity is related to a constant critical membrane concentration of 50-300 mmol/kg lipid for any species[15-18] (average derived at 140 mmol/kg[17]), K_{OW} is strongly correlated to baseline toxicity[19-21]. For the anionic SDS, average fish and daphnid toxic concentrations are reported in the range of 9.1 ± 8.2 mg/L (lowest value of 0.59 mg/L) and 11.0 ± 5.5 mg/L, respectively.[13] The recommended log*D* in the SDS-REACH dossier of -2 would predict a narcotic LC_{50} at 14000 mmol/L (4000 g/L, i.e. impossible), while the highest log*D* of 1.7 would predict a narcotic LC_{50} at 2.8 mmol/L (805 mg/L, i.e. possible). With observed acute LC_{50} values >80 times lower than this highest log*D* value, SDS could be considered to be acting by specific toxic mode(s) of action.

D_{MLW} values for SDS

The first reported liposome-water partition coefficient is made for SDS in a 1992 study using ^{14}C radiolabeled SDS in dialysis systems with dissolved unilamellar phospholipid bilayers[22]. The logD_{MLW} was reported to be 3.5, although test concentrations were near the CMC. In 2019, a sorbent dilution assay with solid-supported lipid membranes (SSLM), based on bilayers non-covalently coating microporous silica beads, showed that the sorption affinity of SDS for phospholipids was constant over a broad concentration range well below the CMC, with a logD_{MLW} of 4.6. Although this is a log unit higher than that determined in the ^{14}C-SDS study, the value for SDS proved consistent with a

series of surfactant analogues and is considered to be more accurate.[13] Importantly, both D_{MLW} are orders of magnitude higher than the logK_{OW} values ("logP or logD") reported in the EU REACH registration dossier for this chemical, and the outcomes of different commonly used computational tools (Table S1). The 2009 measured D_{MLW} for SDS is more than 6.6 log units higher than the recommended logP value (-2.03) for SDS in the EU REACH dossier. This is likely not a unique case nor an extreme case in risk assessment dossiers for ionic surfactants.

The relevance of phospholipid-water distribution coefficient in understanding the mode of action for surfactants is evident from a calculation presented in the 2009 study, where the logD_{MLW} was determined; based on the logD_{MLW} of 4.6 (Table S1), and the 140 mmol/kg critical membrane burden, a narcotic LC_{50} of ~1 mg/L is predicted, i.e. close to the average fish and daphnid toxic concentrations that are reported in the range of 9.1 ± 8.2 mg/L (lowest value of 0.59 mg/L) and 11.0 ± 5.5 mg/L, respectively. As a result, SDS is considered a baseline narcotic ionic chemical, as expected.

Thus far, four studies have determined the sorption affinity to artificial phospholipid bilayers (D_{MLW}) for a systematic series of 60 pure surfactant structures in total[23-26]. D_{MLW} values were published using dissolved unilamellar liposomes for a series of eight pure homologue alcohol ethoxylates, varying in both alkyl chain length and number of ethoxylate units, using both dialysis and ultracentrifugation[23, 24]. Two recent studies focused on cationic surfactant compounds, including permanently charged quaternary ammonium compounds (QACs) such as benzalkonium chloride[25] and ionic liquid cations[26]. In 2019, D_{MLW} values for a series of anionic surfactants, including alkylsulfonates, alkylsulfonates, as well as perfluorinated carboxylates and perfluorinated sulfonates was published using a sorbent dilution series with bilayers that are non-covalently coating microporous silica beads[13]. These D_{MLW} values for the perfluorinated anions were confirmed by a different research group using a dialysis system with fully dissolved liposomes[27]. One additional D_{MLW} value for a short-alkyl chain (C_8) alkylbenzene sulfonate was determined by dialysis with dissolved liposomes as part of a wider variety of anionic compounds[28]. All values are listed in Table 2 of the current study.

Text S2. Methodological details.

Fish-oil/water distribution assay

The nonionic surfactant $C_{12}EO_4$ was tested in oil spiked concentrations (1.3 g/L in 6 vials, 4 g/L in 2 vials, and 13 g/L in 2 vials). Because of visible mixing in some vials, these samples were not used. The cationic surfactant $C_{12}N(CH_3)_3^+$ and anionic surfactants C_{11}-2-LAS, $C_{12}SO_3$, and $C_{12}SO_4$ were tested both with spiked oil (all ~100 mg/L) and spiked water (all ~10 mg/L, except ~100 mg/L for $C_{12}SO_4^-$) in a separate series of four replicates each, ensuring that aqueous concentrations remained below the CMC and above detection limits. After 2 days equilibration, both phases were sampled, with oil samples diluted at least 20x with methanol, and water samples diluted at least 20x with 50% methanol/water, before analyses by LC-MS/MS.

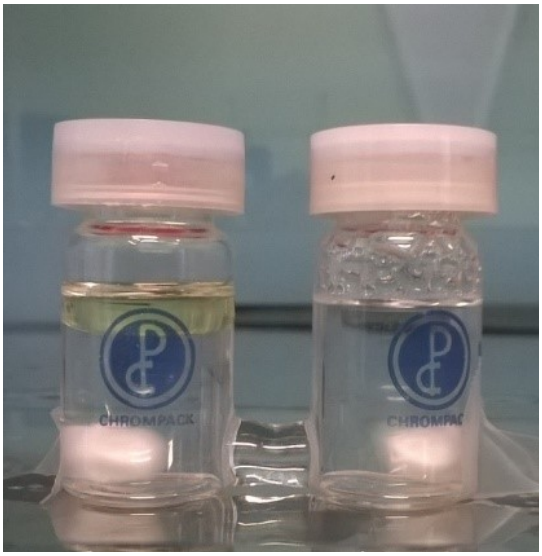


Figure S1. Twin vial with PTFE spinbar used in oil/water tests, with 0.5 mL oil in the left vial

Due to a high background signal for $C_{12}SO_4$ (sodium dodecyl sulfate; SDS), relatively high water concentrations had to be tested. Water spiked samples decreased to aqueous concentrations of ~80 mg/L (CMC 1000-3000 mg/L), and ~3 mg/L in the oil (Table S3).

Figure S2. A 6 vial sorbent-dilution series strip from the TRANSIL^{XL} 96-well plate system transferred to glass inserts.

Increasing volumes of the white TRANSIL bead suspension can be seen from left to right. The outer two plastic 96 well vials have only a white plastic bottom, and are used as control solutions without beads. All sorbent beads are transferred from the plastic wells into glass vials (picture on the right), replacing the aqueous buffer from PBS to 0.1 M ammonium acetate with 1mg/L sodium azide (with glass inserts 0.25 mL, without glass inserts 1.6 mL, but also 10mL glass vials can be used). In the picture on the right, glass inserts are used, and vials are centrifuged.

Phospholipid-water SSLM assay

The phospholipid material used in three surfactant D_{MLW} studies is applied as bilayers non-covalently coating macroporous silica particles, called solid-supported lipid membranes (SSLM), or Transil® beads. It is a commercially available pre-dosed sorbent dilution assay. Once the SSLM beads are in suspension, sorption equilibrium is rapidly attained (less than an hour) due to the minimal distance between bulk medium and the bilayer sorbent sites. The low dose of applied surfactant does not solubilize phospholipids from the suspended solid lipid membrane (SSLM). Due to the high density of the silica, the SSLM particles are readily centrifuged into a pellet, allowing for easy sampling of freely dissolved concentrations in the supernatant, and calculation of sorbed concentrations through a mass balance approach. Up to a D_{MLW} of $10^{5.5}$ can be readily obtained with high confidence, covering a large range of homologues for all types of surfactants.

The SSLM assay comes packaged in frozen 96-well plates, which can be separated in 12 strips of 8 wells (see Figure S2) that form a dilution series (factor 1.8 dilution steps), of which two do not contain lipids. As outlined by Timmer and Droge,[25] there are certain features and limitations to the SSLM assay that should be regarded when deriving D_{MLW} values for surfactants in this assay, particularly for strongly binding surfactants and when using LC/MS/MS analysis:

(i) the TRANSIL beads may leak a small (~1%) fraction of lipids into the medium (probably upon thawing). This lipid fraction will remain in the supernatant. Changing the medium ≥3 times by 80% with fresh medium should render negligible dissolved lipids, but care should be taken to ensure this. In the original 96 well plate set up, typically the leaching is highest for the vials with the highest lipid content, so these may overestimate the aqueous concentration even more for strongly-binding chemicals Timmer and Droge reported that 1% of lipid leaching noticeably affects the testing of compounds with D_{MLW} >4.5.[25]

(ii) surfactants may accumulate at the air-water interface, but certainly also at the glass-water interface or at the glass-water film above the suspension. We therefore recommend to use larger water volumes in glass surfaces than the plastic well plates with silicone caps, preferably filled up to remain only a small headspace air bubble.

(iii) many surfactants are best, or only, detected by LC-MS/MS. In our experience, the high concentrations of involatile salts in the PBS medium that is provided (as a standard) in the commercial TRANSIL assay can strongly influence the ionization even minutes after the injection, depending on column type and eluent composition. We therefore switched to 0.1M ammonium acetate as test medium, which is easy to make, should give a pH of 7 when working with clean glassware and surfactants added as salts, and which is often added as 10mM eluent anyway.

In order to reduce effects of ~1% phospholipids leaking in the medium after thawing[25], and to renew the test medium from PBS to 0.1M ammonium acetate[13], at least three cycles of 'centrifugation-decantation-medium renewal' were used to transfer the total amount of lipid from each well into glass vials, using 1.5 mL autosampler vials for most surfactants studied.

For each sorbent dilution series, only data was collected if the sorbed fraction was larger than 30%, i.e. if the dissolved concentration had decreased to less than 70% compared to the reference vials. The reason for this is that only the aqueous concentration is measured and the sorbed concentration is derived with a mass balance approach, and an overall analytical and experimental uncertainty of ±10% needs to be taken into account. This should limit the uncertainty in the sorbed concentration to <33%, and should account for a robust mass balance calculation to derive D_{MLW}. Moreover, by taking multiple samples from a sorbent dilution series, higher fractions of sorbed compound are included which reduces the uncertainty via the mass balance approach, and fitting a sorption isotherm should provide a weighted average of the D_{MLW}. Depending on the sorption affinity, either expected according to extrapolation from analogue compounds or based on pilot experiments, the lipid:solution ratio was adjusted by using either a higher density TRANSIL series (HD, 0.9-16.7 µL lipid) or a lower density TRANSIL series (LD, 0.047-0.9 µL lipid), and performing the sorption studies in either 250 mL suspension in 300mL glass inserts, 1.6 mL in glass autosampler vials, or in 10 mL glass vials. On average, this can be approached by the equation:

$$\text{fraction dissolved} = \frac{1}{1 + D_{MLW} \cdot \frac{V_{ML}}{V_W}}$$

with V_{ML} and V_W both in the same volumetric units. Accordingly, at a 70% fraction dissolved, the ratio

$D_{MLW} \cdot \frac{V_{ML}}{V_W}$ must be > (1-0.7)/0.7 = 0.43.

In the application of the HD series, the lowest V_{ML} is 0.9 µL, so when a V_W of 250 µL is used (e.g. glass inserts), the lowest D_{MLW} that can be tested to have all 6 vials with SSLM material with >30% sorbed is 119 L/kg. At a lower D_{MLW} value such as 10, only the vial of the series with the highest sorbent amount will reduce the freely dissolved concentration by more than 30%.

For the lowest sorbent amount in the LD series, the maximum water volume of 12 mL (that can be used to fill a standard 10 mL glass vial with a small remaining headspace bubble) is estimated to result in a sorbed fraction of 30% for a logD_{MLW} of ~5. For a compound with a logD_{MLW} of 6, using the second lowest sorbent amount (0,0857uL lipid) of the LD series in 12 mL medium will already decrease the aqueous concentration by >90%, which may give problems with the analytical LOD, because given the maximum allowable sorbed concentration is ~6 g/L lipid, the maximum aqueous concentration with a logD_{MLW} of 6, is 6 µg/L. The highest measured logD_{MLW} values were for C_{12}-2-LAS.

In case a single sorbent dilution series allowed only for 1-3 valid datapoints, a second or third dilution series was tested, using at a different spiked concentration (e.g a factor 3-10 different spiking concentration). This created sufficient independent measurements for the few valid lipid:medium ratios that together were used to plot a sorption isotherm.

In addition, only data was collected where the sorbed concentration was <3mol% of the phospholipid molecules, because at higher sorbed concentrations of ionic sorbates, electrostatic repulsion may affect the sorption affinity.[29, 30] The total suspension volume for each test vial was obtained by weighing the full vials and subtracting the pre-recorded weight of each empty vial. A Rainin Pipet-Lite XLS electronic multichannel pipet with adjustable spacer (Mettler Toledo BV, Tiel, The Netherlands) was used to transfer a constant volume of a surfactant stock solution in 50% methanol to the respective autosampler vials of a single sorbent dilution series, thereby ensuring that the test solution contained <1.5% methanol.

The spiked bead suspensions in the glass vials were then placed on a Stuart SRT9 roller mixer (Boom BV, Meppel, The Netherlands) for at least 15 min at 33 rpm, after which the vials were centrifuged at 750 g (20 °C) for 10 min. From each vial, 100 µL of the supernatant was mixed together with 100 µL of acetonitrile in a 0.3 mL glass insert in an autosampler vial, turning vials upside down repeatedly to ensure mixing, after which they were stored at 4°C until LC-MS/MS analysis.

LC-MS/MS analysis

Fish-oil/water distribution samples

For all tested compounds, oil samples (20 µL) were diluted 50x in methanol (incl. internal standard), water samples (20 µL) were diluted 50x in a 50% methanol/water solution (incl. internal standard). A GraceSmart 150 x 2.1 mm column + guard C18 (5µm) was used for anionic and nonionic surfactants, and a Phenomenex Kinetex XB-C18 100 x 3 mm (2.6µm) column for the cationic surfactants.

For $C_{12}SO_3$ and C_{11}-2-LAS, and isocratic eluent was used with 60% A/40%B (A: 90/10 methanol/water + 5 mM ammonium acetate; B: 50/50 methanol/water + 5 mM ammonium acetate). For $C_{12}SO_4$ isocratic eluent was used with premixed 70% methanol/30% water + 5 mM ammonium acetate.

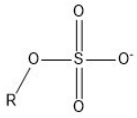
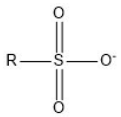
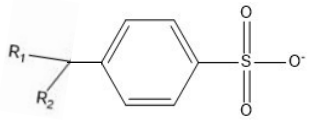
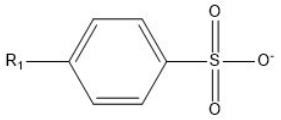
For $C_{12}EO_4$, an isocratic eluent was used with 80% methanol/20% water +5 mM ammonium acetate. For C_{12}TMAC, a gradient elution was used from 70/30% to 20/80% (water + 0.1% formic acid)/(acetonitril + 0.1% formic acid). LC-MS/MS m/z signals are listed in Table S2.

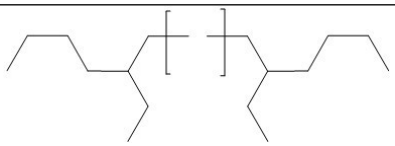
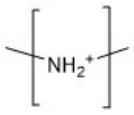
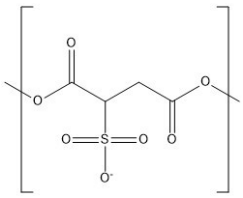
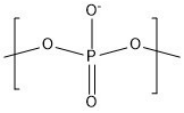
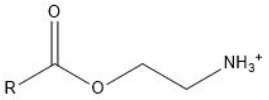
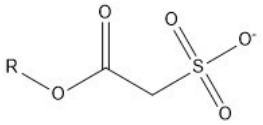
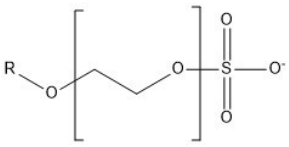
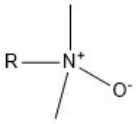
SSLM assay samples

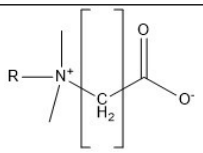
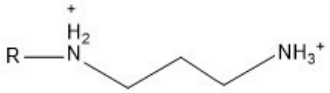
SSLM samples and external calibration standards consisted of 50/50% test medium/methanol. For all tested compounds, a Supelco Ascentis Express 100 x 2.1mm column was used (40°C), with gradient elution from 60/40% to 10/90% (water +10 mM ammonium acetate and 0.1% formic acid)/(methanol + 0.1% formic acid). LC-MS/MS m/z signals are listed in Table S2.

Tables and Figures

Table S2. Overview of suppliers, pKa estimates, LC-MS/MS signals and surfactant structures of the chemicals tested.

Chemical	Supplier	pKa	m/z Detection LC-MS/MS (mode)	Structure
				Alcohol ethoxylate
$C_{12}EO_4$	Sigma-Aldrich	-	380.5/89 (pos)	Nonionic, listed name e.g. Tetraethylene glycol monododecyl ether
				Quaternary ammonium
$C_{12}N(CH_3)_3^+$	Sigma-Aldrich	-	228.3/60 (pos)	permanently cationic: *N,N,N*-Trimethyldodecyl-ammonium chloride
				Alkylsulfate
$C_{12}SO_4^-$	Sigma-Aldrich	<-1	265.2/97 (neg)	
				Alkylsulfonate
$C_{12}SO_3^-$	Sigma-Aldrich	<-1	249.3/80.1 (neg)	
				linear alkylbenzene sulfonates 
C_{10}-1-LAS$^-$	From the synthesized batches produced by Tolls et al. [31]	<-1	297.0/170.0 (neg)	anionic (R_1 = 10, R_2 = 0)
C_{10}-2-LAS$^-$		<-1	297.0/183.0 (neg)	anionic (R_1 = 9, R_2 = 1)
C_{11}-2-LAS$^-$		<-1	311.0/183.0 (neg)	anionic (R_1 = 10, R_2 = 1)
C_{12}-2-LAS$^-$		<-1	325.0/183.0 (neg)	anionic (R_1 = 11 R_2 = 1)
C_{12}-6-LAS$^-$		<-1	325.0/183.0 (neg)	anionic (R_1 = 6, R_2 = 6)
				branched alkylbenzene sulfonates 
C_{11}-ABS$^-$	AkzoNobel (components of Witconate P-1059)	<-1	311.0/197.0 (neg)	anionic (R_1 = 11)
C_{12}-ABS$^-$		<-1	325.0/197.0 (neg)	anionic (R_1 = 12)
				bis(ethylhexyl) compounds

Chemical	Supplier	pKa	m/z Detection LC-MS/MS (mode)	Structure
				
Bis(2-ethylhexyl) amine^{+} (BEHN^{+})	Sigma-Aldrich	>10	242.1/130.3 (pos)	cationic 
Bis(2-ethylhexyl) sulfosuccinate (Docusate sodium salt, DOSS^{-})	Sigma-Aldrich	<-1	421.3/81.3 (neg)	anionic 
Bis(2-ethylhexyl) phosphate (BEHP^{-})	Sigma-Aldrich	-0.3 ([c]) 1.5 ([d,e]) 1.94([b]) 3.1([a])	323.0/99.0 (neg)	anionic 
			surfactants with other polar moieties	
2-aminoethyl laurate (LEN^{+})	Sigma-Aldrich	>10	244.0/227.2 (pos)	cationic 
Sodium lauryl sulfoacetate (SLAcS^{-})	Sigma-Aldrich	<-1 ([a])	325.0/197.0 (neg)	anionic 
Sodium laureth sulfoate Na.C_{12}-EO_4-SO_4 (SLES^{-})	Na-salt; >95% supplied by Procter and Gamble	1.24 ([a])	441.3/97 (neg)	anionic 
			alkyldimethylammonium oxides 	
C_{10}-$N(CH_3)_2O$ ($C_{10}A^{+}O^{-}$)	Sigma-Aldrich	~4-5 ([a],[32])	202.4/58 (pos)	zwitterionic
C_{12}-$N(CH_3)_2O$ ($C_{12}A^{+}O^{-}$)	Sigma-Aldrich	~4-5 ([a],[32])	230.4/58 (pos)	zwitterionic
				Alkylbetaines

Chemical	Supplier	pKa	m/z Detection LC-MS/MS (mode)	Structure
				
C_{12}-N$(CH_3)_2(CH_2)CO_2$	Sigma-	3.6	272.3/104.2	Zwitterionic
($C_{12}A^+Ac^-$)	Aldrich	([a])	+ 272.3/58 (pos)	[CH_2]
C_{12}-N$(CH_3)_2(C_3H_6)CO_2$	Sigma-	~4.5	LC-MS only:	zwitterionic
($C_{12}A^+Bu^-$)	Aldrich	([b])	300.3 (pos)	[C_3H_6]
				diamine cation
C_{12}-N(C_3H_6)N	AK Scientific	7.6/	244.2/227.3	
($C_{12}N^+PN^+$)		>10 ([a])	(pos)	

[a] Information on pKa from REACH dossiers:
SLES: https://echa.europa.eu/registration-dossier/-/registered-dossier/5371/4/22
SLSA: no data in https://echa.europa.eu/substance-information/-/substanceinfo/100.015.847
Amine oxides: https://echa.europa.eu/registration-dossier/-/registered-dossier/10062/4/22
Betaines: https://echa.europa.eu/registration-dossier/-/registered-dossier/14910/4/22
Alkyl-propanediamine: https://echa.europa.eu/registration-dossier/-/registered-dossier/1782/4/22
BEHP: https://echa.europa.eu/registrationdossier/-/registered-dossier/13106/4/22

[b] Chemicalize.org

[c] COSMO*therm* V18.2, COSMO*logic* GmbH & CoKG (3ds Dassault Systèmes)

[d] ACD/Labs

[e] Guthrie, J.P. Hydrolysis of esters of oxy acids: pKa values for strong acids; Brønsted relationship for attack of water at methyl; free energies of hydrolysis of esters of oxy acids; and a linear relationship between free energy of hydrolysis and pKa holding over a range of 20 pK units. Canadian Journal of Chemistry 1978, 56, 2342-2354.

Table S3. Fragment matrix for anionic surfactants for multiple linear regression with logD_{MLW}.

	ref	SSLM log D_{MLW}	chain fragments				headgroup fragm.			other fragments			predicted log D_{MLW}
			#C	branching	di-alkyl	per-fluor	CO_2	SO_3	SO_4	benzyl	acetate	#EO	
$C_8SO_3^-$	13	1.74	8	0	0	0	0	1	0	0	0	0	1.70
$C_{10}SO_3^-$	13	3.01	10	0	0	0	0	1	0	0	0	0	2.82
$C_{12}SO_3^-$	13	3.99	12	0	0	0	0	1	0	0	0	0	3.94
$C_{13}SO_3^-$	13	4.46	13	0	0	0	0	1	0	0	0	0	4.50
$C_{14}SO_3^-$	13	4.95	14	0	0	0	0	1	0	0	0	0	5.06
C_8-1-LAS$^-$	28	3.61	8	0	0	0	0	1	0	1	0	0	3.95
C_{10}-1-LAS$^-$	([a])	5.1	10	0	0	0	0	1	0	1	0	0	5.07
C_{10}-2-LAS$^-$	([a])	4.79	10	1	0	0	0	1	0	1	0	0	4.57
C_{11}-2-LAS$^-$	([a])	5.33	11	1	0	0	0	1	0	1	0	0	5.13
C_{12}-2-LAS$^-$	([a])	5.62	12	1	0	0	0	1	0	1	0	0	5.69
C_{12}-6-LAS$^-$	([a])	5.36	12	2	0	0	0	1	0	1	0	0	5.19
C_{11}-ABS$^-$	([a])	4.16	11	3	0	0	0	1	0	1	0	0	4.14
C_{12}-ABS$^-$	([a])	4.44	12	3	0	0	0	1	0	1	0	0	4.70
SLAcS$^-$	([a])	4.26	12	0	0	0	0	1	0	0	1	0	4.56
DOSS$^-$	([a])	4.58	16	2	1	0	0	1	0	0	2	0	4.43
BEHP$^-$	([a])	3.81	16	2	1	0	0	0	1	0	0	0	3.96
PFBS$^-$	13	2.63	4	0	0	1	0	1	0	0	0	0	2.66
PFHxS$^-$	13	3.82	6	0	0	1	0	1	0	0	0	0	3.78
PFOS$^-$	13	4.88	8	0	0	1	0	1	0	0	0	0	4.90
$C_8SO_4^-$	13	2.58	8	0	0	0	0	0	1	0	0	0	2.47
$C_{10}SO_4^-$	13	3.79	10	0	0	0	0	0	1	0	0	0	3.59
$C_{12}SO_4^-$	13	4.61	12	0	0	0	0	0	1	0	0	0	4.71
$C_{13}SO_4^-$	13	5.21	13	0	0	0	0	0	1	0	0	0	5.27
$C_{12}EO_4SO_4^-$	([a])	4.24	12	0	0	0	0	0	1	0	0	4	4.24
PFBA$^-$	13	1.0	3	0	0	1	1	0	0	0	0	0	1.19
PFPA$^-$	13	1.73	4	0	0	1	1	0	0	0	0	0	1.75
PFHxA$^-$	13	2.31	5	0	0	1	1	0	0	0	0	0	2.31
PFHpA$^-$	13	2.87	6	0	0	1	1	0	0	0	0	0	2.87
PFOA$^-$	13	3.51	7	0	0	1	1	0	0	0	0	0	3.43
PFNA$^-$	13	4.04	8	0	0	1	1	0	0	0	0	0	3.99
PFDA$^-$	13	4.63	9	0	0	1	1	0	0	0	0	0	4.55

#C is the number of carbon atoms as part of the hydrophobic chain. Branching factors set to '1' for the more external isomers Cx-1-LAS and Cx-1-LAS and each 2-ethylhexyl unit, '2' for the more internal LAS isomer Cx-6-LAS, and '3' for the branched clusters of Cx-ABS.

([a]) determined in this study

Table S4. Fragment matrix for cationic surfactants for multiple linear regression with logD_{MLW}.

	ref	SSLM log D_{MLW}	chain fragments			headgroup			other		predicted log D_{MLW}
			#C	di-alkyl	branching	NAi	PYR	3-methyl imidazolium	acetate	benzyl	
$C_8NH_3^+$	25	3.1	8	0	0	3	0	3	0	0	3.26
$C_{10}NH_3^+$	25	4.3	10	0	0	3	0	3	0	0	4.37
$C_{12}NH_3^+$	25	5.58	12	0	0	3	0	3	0	0	5.48
LEN^+	([a])	3.75	12	0	0	3	0	3	1	0	3.75
$C_8N(CH_3)H_2^+$	25	2.76	8	0	0	2	0	2	0	0	2.91
$C_{10}N(CH_3)H_2^+$	25	3.98	10	0	0	2	0	2	0	0	4.02
$C_{12}N(CH_3)H_2^+$	25	5.39	12	0	0	2	0	2	0	0	5.13
$C_6N(C_6)H_2^+$	25	3.15	12	1	0	2	0	2	0	0	2.79
$C_8N(C_8)H_2^+$	25	4.65	16	1	0	2	0	2	0	0	5.01
$BEHN^+$	([a])	3.59	18	1	2	2	0	2	0	0	3.59
$C_8N(CH_3)_2H^+$	25	2.35	8	0	0	1	0	1	0	0	2.57
$C_{10}N(CH_3)_2H^+$	25	3.65	10	0	0	1	0	1	0	0	3.68
$C_{12}N(CH_3)_2H^+$	25	5.30	12	0	0	1	0	1	0	0	4.79
$C_8N(CH_3)_3^+$	25	2.18	8	0	0	0	0	0	0	0	2.22
$C_{10}N(CH_3)_3^+$	25	3.34	10	0	0	0	0	0	0	0	3.33
$C_{12}N(CH_3)_3^+$	25	4.35	12	0	0	0	0	0	0	0	4.44
$C_{14}N(CH_3)_3^+$	25	5.46	14	0	0	0	0	0	0	0	5.55
C_8-PYR^+	25	2.28	8	0	0	0	1	0	0	0	2.48
C_{12}-PYR^+	25	4.89	12	0	0	0	1	0	0	0	4.69
C_6-BAC^+	25	2.12	6	0	0	0	0	0	0	1	1.97
C_8-BAC^+	25	3.11	8	0	0	0	0	0	0	1	3.08
C_{10}-BAC^+	25	4.01	10	0	0	0	0	0	0	1	4.19
$IM1\text{-}8^+$	26	2.06	8	0	0	0	0	0	0	0	1.88
$IM1\text{-}10^+$	26	3.15	10	0	0	0	0	0	0	0	2.99
$IM1\text{-}12^+$	26	3.76	12	0	0	0	0	0	0	0	4.10

#C is the number of carbon atoms as part of the hydrophobic chain. Branching factor is set to '1' for each 2-ethylhexyl unit.

([a]) determine in this study

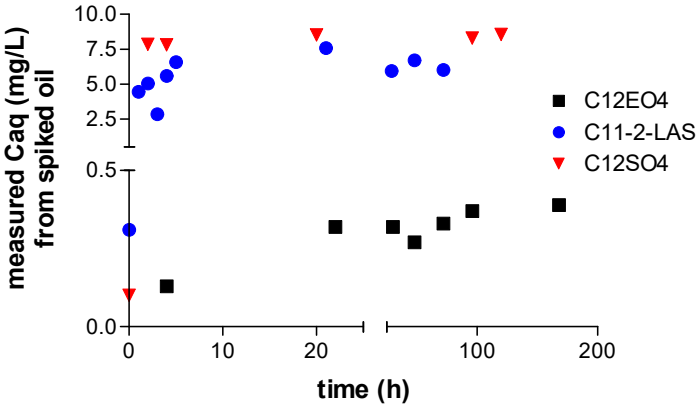


Figure S3. Time series of aqueous concentrations in the fish-oil/water assay for three surfactants.

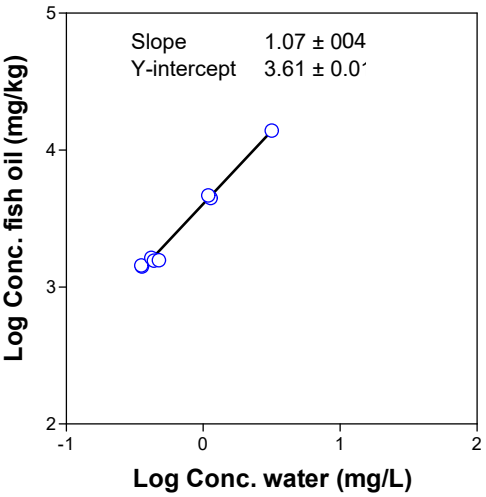


Figure S4. Fish-oil/water sorption isotherm for $C_{12}EO_4$ (B).

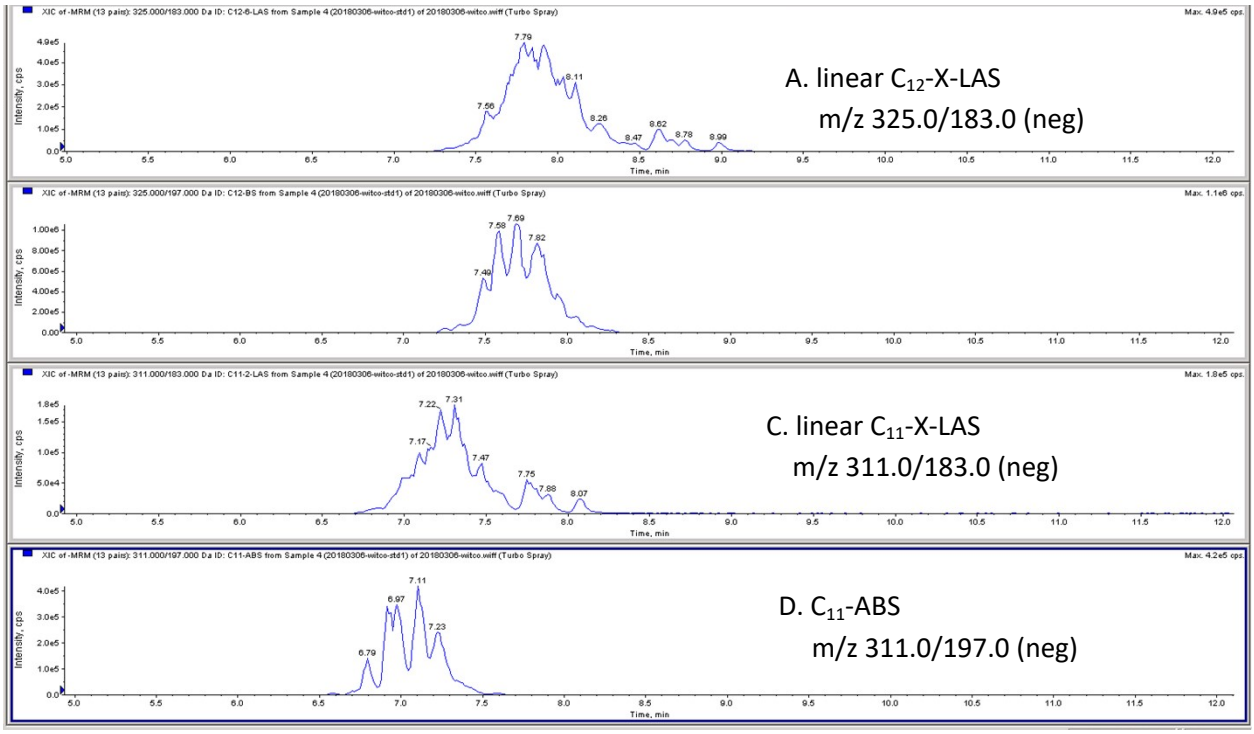


Figure S5. Chromatograms of the specific fragment signals separating branched ABS from linear LAS.

The technical substance of "Witconate P-1059" was found to contain clearly separated signals of linear $C_{10\text{-}13}$ alkylbenzenesulfonates (Graphs S5A and C), as well as $C_{11\text{-}13}$ branched alkylbenzenesulfonates (Graphs S5B and D). Lacking a reference standard for the branched C_{11} analogue, the total weight equivalent concentrations were used. The signal defined to be C_{11}-ABS was thus only related to the total weight of the chemical added to each vial. The C_{11}-ABS MS/MS specific signal comprised a cluster of isomers (Graph S5D), clearly isolated from the MS/MS fragment for linear C_{11}-LAS isomers (Graph S5C)[33], and which was not visible in linear LAS standards. The total peak area of this cluster was used to quantify the area.

Text S3. Detailed evaluation of new D_{MLW} data.

Linear alkylbenzene sulfonates (LAS)

Anionic LAS surfactants are composed of mixtures with alkyl chain lengths between C_{10}-C_{14}, and only inner-isomers of the benzosulfonate group are included, i.e., homologues with benzosulfonate in the terminal position are not in use. The current study is the first to report D_{MLW} values for multiple components of this typical anionic detergent. C_{10}-1-LAS (logD_{MLW} of 5.1) is thus mainly of interest for structure-activity comparisons, and its D_{MLW} is 1.6 log units higher than for the shorter C_8-1-LAS, measured with dissolved POPC liposomes in dialysis systems[28]. For the tested series of pure LAS isomers (Figure 1A), we make the following key observations: (i) the terminal position of the benzosulfonate group results in a ~0.3 log units higher D_{MLW} value in comparison to the benzosulfonate group on the second carbon (C_{10}-1-LAS *vs.* C_{10}-2-LAS); (ii) isomers with an even more inner position of the benzosulfonate group may have a slightly lower D_{MLW} value up to another 0.3 log units (C_{12}-6-LAS *vs.* C_{12}-2-LAS); (iii) the isomer C_{12}-2-LAS (logD_{MLW} 5.6), more or less the representative average structure of environmental LAS mixtures[34], has a 1.6 log unit higher sorption affinity than the analogue lacking the benzyl unit $C_{12}SO_3$; (iv) the alkyl chain effect of LAS is comparable to the alkylsulfonates and neutral chemicals (i.e., CH_2 increment of ~0.5 log units)[13].

Branched alkylbenzene sulfonates (ABS)

The logD_{MLW} of 4.16 for the cluster of branched isomers identified as 'C_{11}-ABS' (see text with Figure S5 for further details) is 1.2 log units lower than that observed for the linear isomer C_{11}-2-LAS. C_{12}-ABS had a D_{MLW} 0.28 log units higher than C_{11}-ABS. The branched isomer mixtures contain a range of isomers that may have different D_{MLW}'s, so the value for the mixture may reflect an average of individual D_{MLW}'s ranging over ± 0.2 log units. However, we did not observe a clear shift in the m/z fingerprint of the mixture in the SSLM buffer, compared to the standards added. We found no background signal of the ABS compounds in blanks, whereas background LAS signals were relatively high for C_{11} and C_{12} isomers.

Dialkyl-based surfactants

The anionic surfactant $DOSS^-$ (also named 'docusate') has two specifically branched 2-ethylhexyl tails, connected via esters to a sulfosuccinate unit. The logD_{MLW} of $DOSS^-$ is 0.77 log units higher than the more simplified bis(2-ethylhexyl) anion analogue $BEHP^-$ (only phosphate between the branches). This was not expected, because the two ester moieties in $DOSS^-$ increase the molecules' polarity. However, the phospholipophilicity of $DOSS^-$ may be increased compared to $BEHP^-$ because the branched hydrocarbons of $DOSS^-$ can extend to deeper positions in the phospholipid bilayer, further

way from the anionic group. On the other hand, with a total of 16 hydrocarbon units in the two branched chains surrounding the ionic moiety, $BEHP^-$ has a D_{MLW} 2.2 log units lower than would be expected for a single linear C_{16}-chain sulfonate analogue (predicted logD_{MLW} ~6 based on $C_{14}SO_3$ + 1 log unit, as for LAS above and other surfactants[25,13]). Similar large differences have been observed for dialkylamine cations, where dihexylamine has a D_{MLW} 2.25 log units lower than single chain analogue *N*-methyldodecylamine[25]. Again, this most likely results from the deeper intercalation depth of the single linear alkyl chain in the phospholipid bilayer[25].

The cationic $BEHN^+$ has a logD_{MLW} that is comparable (0.2 log units lower) with its anionic analogue $BEHP^-$. Notably, the two branched chains of $BEHA^+$ result in a 1 log unit lower D_{MLW} than the linear dialkyl chain analogue dioctylamine (Table 1, 3.6 vs. 4.6).

Surfactants with polar moieties between alkyl chain and ionic head group

For neutral chemicals, an additional acetate ester reduces the K_{OW} by about a log unit, e.g., 1-hexyl acetate (2.8) compared to hexane (3.9). The cationic LEN^+ has a D_{MLW} 1.8 log units lower than an analogue dodecylamine without the acetate group. Surprisingly, however, the sulfoacetate anion $SLAcS^-$ had a D_{MLW} 0.26 log units higher than the analogue alkylsulfonate without the acetate group (Figure 1D). The ethoxylated sulfate $SLES^-$ (Figure 1C) showed a D_{MLW} 0.4 log units lower than an analogue alkylsulfate, which corresponds well to the influence of EO-units for non-ionic surfactants[23, 24].

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