

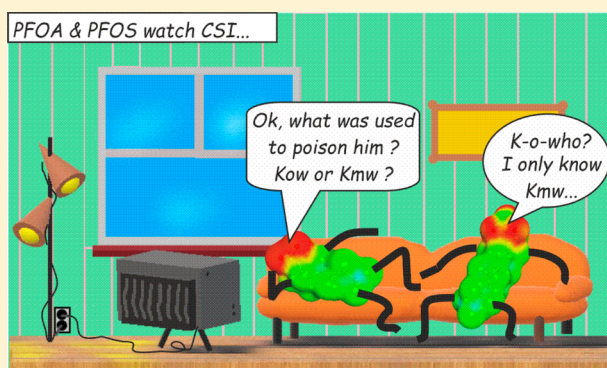
Membrane–Water Partition Coefficients to Aid Risk Assessment of Perfluoroalkyl Anions and Alkyl Sulfates

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Supporting Information

ABSTRACT: This study determined the sorption affinity to artificial phospholipid membranes (K_{MW}) for series of perfluorinated carboxylates (PFCAs), perfluorinated sulfonates (PFSAs), alkyl sulfates (C_xSO_4), and 1-alkanesulfonates (C_xSO_3). A sorbent dilution assay with solid supported lipid membranes (SSLM) showed consistent CF_2 unit increments of 0.59, and CH_2 unit increments of 0.53, for the log K_{MW} of perfluorinated and hydrogenated anions, respectively. PFSAs sorbed 0.90 log units stronger than analogue PFCAs; C_xSO_4 sorbed 0.75 log units stronger than analogue C_xSO_3 anions. The log K_{MW} values for the octyl analogues increase in the order $H(CH_2)_8SO_3^-$ (1.74) < $H(CH_2)_8SO_4^-$ (2.58) < $F(CF_2)_8CO_2^-$ (PFNA, 4.04) < $F(CF_2)_8SO_3^-$ (PFOS, 4.88). Intrinsic partition ratios determined on a phospholipid coated HPLC column (IAM-HPLC) closely aligned with SSLM K_{MW} values. COSMO-RS based molecular calculations of K_{MW} aligned with SSLM K_{MW} values for hydrogenated anions with C_8 – C_{14} alkyl chains but strongly underestimated CF_2 and CH_2 unit increments for C_4 – C_8 based anions. Dividing the critical narcotic membrane burden of 100 mmol/kg by the experimental K_{MW} predicts lethal baseline toxicity concentrations ($LC_{50,narc}$). The $LC_{50,narc}$ coincides with the lowest reported acute LC_{50} values for several anionic surfactants but were on average about an order of magnitude lower.



INTRODUCTION

Perfluoroalkyl substances (PFAS) are highly persistent chemicals and abundantly used for many purposes and, therefore, reach the environment via all kinds of waste streams. Widely varying structures of PFAS have been detected ubiquitously in humans and the environment.^{1–6} Many PFAS are ionogenic in the common pH range of field conditions and biotic tissues and act as surfactants.⁷ Due to the limited data available on basic physical–chemical properties of the majority of these PFAS, such as dissociation constants and partition coefficients, exposure assessment models such as global fate models and toxicokinetic models may provide uncertain outcomes.^{8,9} The dissociation constant (pK_a) of PFAS has proven to be difficult to determine experimentally and is simply unknown for most “emerging” PFAS.^{6,10,11} As a result of their surfactant behavior, octanol–water partition coefficients (K_{OW} , for neutral species) or octanol–water distribution coefficients (D_{OW} , at a certain pH) cannot be determined experimentally, or only with relatively high uncertainty.^{12–14} Often only C_{18} based chromatographic retention measurements are available to estimate PFAS hydrophobicity, but such data do not adequately reflect the influence of ionic interactions between PFAS and environmentally relevant sorbents.^{15–18} Due to the lack of experimental data, QSARs to predict K_{OW} based on molecular

fragments are not properly calibrated for most ionogenic PFAS.^{11,19,20} In most risk assessment models, however, a chemical’s K_{OW} value is used as a key descriptor to relate the chemical “hydrophobicity” to the environmental distribution, bioavailability, bioaccumulation, and toxicity.^{21–23} Knowing the uncertainty on the fraction of ionized and neutral species at a certain environmental pH of emerging PFAS, and the unknown link between molecular structure and a PFAS’s “hydrophobicity”, requires adequate partition measurements on relevant sorbents such as individual soil and tissue components.

A wealth of PFAS measurements in different tissues of a wide variety of biota has become available. For the two most abundant PFAS worldwide, the C_8 -sulfonate PFOS and the C_8 -carboxylate PFOA, at least 100 unique studies measured levels in tissues of an organism (125 hits on Scopus search term “PFOS AND bioaccumulation AND blood”, and excluding “review”, June 2018). Based on these developing data sets, it rapidly became clear that PFOA and PFOS are strongly bound to serum proteins such as albumin, and accumulated poorly in

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storage lipid blubber. This sparked a wave of studies measuring and modeling (serum) protein binding.^{24–34} The observed tissue distribution, relative differences in half-life, and isomer specific (branching) accumulation profiles could often be related to protein binding differences. However, for humans and several other species, and even for gender differences, enzymatic renal absorption processes may even enhance the apparent tissue retention (decrease the elimination rate) of specific PFAS more strongly.^{35,36}

Despite observations on the low storage lipid accumulation of PFAS, some studies also suggest that phospholipid binding could play a significant role in tissue distribution of PFAS.^{19,20,37,38} However, phospholipids binding measurements with PFAS are to my best knowledge limited to several calorimetric PFOS binding studies with liposomes by one research group,^{39–41} one neutron reflectometry study to investigate lipid bilayer interactions at the molecular level,⁴² a column retention study using immobilized phosphatidylcholine phospholipid coating,⁴³ and a quantum-chemical modeling approach for a series of perfluorinated carboxylates (PFCAs) and perfluorinated sulfonates (PFSA) (Figure S7 in ref 33). The derived phospholipid membrane–water partition coefficient (K_{MW}), ranging between 4.4×10^4 to 8.8×10^4 , indicates indeed that PFOS is phospholipophilic.

Besides providing explanations on tissue distributions in exposed biota, insight into K_{MW} is of high relevance for understanding PFAS hazard levels since baseline, nonspecific toxicity (i.e., narcosis) is considered to be driven by critical chemical accumulation levels in phospholipid cell membranes. As defined for a wealth of toxicity data across species and chemical classes, including ionogenic compounds, the baseline critical target lipid burden (C_L^*) is in the range of 50–300 mmol/kg phospholipid.^{44–48} For many inert neutral chemicals, the K_{OW} is a relatively accurate and readily available proxy for phospholipid membrane–water partitioning (K_{MW}), and K_{MW} a simplified proxy related to the bioconcentration factor of a chemical from water into membrane phospholipids. Accordingly, the freely dissolved concentrations in aquatic environments that possibly induce narcosis ($LC_{50,narc}$) are then approached by

$$LC_{50,narc} \text{ (mmol/L)} \\ = C_L^* \text{ (as } \sim 100 \text{ mmol/kg)} \cdot K_{MW}^{-1} \text{ (L/kg)} \quad (1)$$

which, for a broad set of neutral chemicals up to $\log K_{OW}$ 5.3,²¹ has been redefined with the $\log K_{OW}$ as

$$\log(LC_{50,narc}) = 0.945 \log(K_{OW}) + \log C_L^* \quad (2)$$

in which $\log C_L^*$ could be refined per species, certain types of chemicals, and acute/chronic effect levels. Using this powerful K_{OW} -based concept, observed toxic effect concentrations can be compared to predicted narcotic concentrations, which is informative on the extent to which specific toxic modes of action influence a chemical's toxicity. However, in the absence of adequate experimentally derived or QSAR-based K_{OW} values for ionogenic surfactants, measurements of K_{MW} for surfactants could simply be applied to eq 1. More importantly, K_{MW} measurements for surfactants are relatively simple and consistent, but unfortunately standardized protocols such as OECD test guidelines are still not available. Once a substantial K_{MW} data set has been generated for any surfactant class, this could allow for the creation of K_{MW} -QSARs or the validation

of software calculations based on simulations of the phospholipid binding affinity.^{49–51} For example, a recent study applied two assays to determine phospholipid sorption for 19 cationic surfactants.⁵² With this data set it was demonstrated that quantum-chemistry based predictions with COSMOmic software were accurate for these types of surfactants.⁵² These could in turn be used for predictions of toxic effect concentrations in *in vitro* studies.⁵³

The main aim of the current study was to derive a starting set of K_{MW} values for PFAS structures, including both PFOS and PFOA and different chain length analogues and to compare several available tools to do so. A second aim of this study was to compare K_{MW} values for PFASs with non-fluorinated alkyl sulfonates and alkyl sulfates, for which also hardly any consistent K_{MW} values have been determined. The results of two experimental tools to measure sorption of a set of common PFAS structures to artificial phospholipids were evaluated: batch sorption tests with solid supported lipid membranes (SSLM) and retention on a chromatographic column coated with immobilized artificial membrane phospholipids (IAM-HPLC). The SSLM assay is considered to provide reference sorption data for bilayer phospholipid membranes. The IAM-HPLC assay applies monolayer phospholipid coated silica particles and is a commonly used tool in, e.g., drug development and allows for high throughput measurements. However, IAM-HPLC can be problematic for ionogenic compounds due to pH- and salinity-dependent confounding electrostatic surface attraction to the silica-based particles, as discussed in detail in a study focused on organic cations⁵⁴ and briefly outlined in the [Supporting Information section S3](#) (SI–S3). The series of PFAS and alkylsulf(on)ate measurements allowed for a systematic examination of the applicability domain of the IAM-HPLC assay to organic anions. In relation to these experimental data, it was evaluated to what extent the quantum-chemistry based software COSMOtherm (COSMOmic module) is able to predict K_{MW} particularly for the studied anionic PFAS. The final aim was to evaluate how K_{MW} values could aid risk assessment of PFAS and alkylsulf(on)ates by comparing the $LC_{50,narc}$ values predicted with eq 1 to observed acute LC_{50} values for the selected compounds.

MATERIALS AND METHODS

Chemicals. Suppliers and purity of the selected nine linear perfluoroalkyl carboxylic acids, three linear perfluoroalkyl sulfonates, nine linear 1-alkanesulfonates, and nine linear *N*-alkyl sulfate are provided in Supporting Information Table S1. Abbreviated names will be used according to those listed in SI Table S1.

K_{MW} Based on Solid-Supported Phospholipid Bilayers. For SSLM experiments, sorbent-dilution based TRANSIL^{XL} Intestinal Absorption kits was purchased from Sovicell GmbH (Leipzig, Germany). The sorbent consists of phospholipid bilayers noncovalently linked to macroporous silica (TRANSIL beads). The assay is prepared on a 96 well plate system, in which one row of eight wells includes two reference wells with only phosphate-buffered saline (PBS) medium, and a series of six wells with increasing amounts of TRANSIL beads in PBS. Following the adapted SSLM protocol of Timmer and Droge,⁵² the aim was to (i) increase the volume/surface ratio of the testing system by using series of glass vials instead of the well plate, and thus reduce surface accumulation effects of surfactants, (ii) change from PBS to

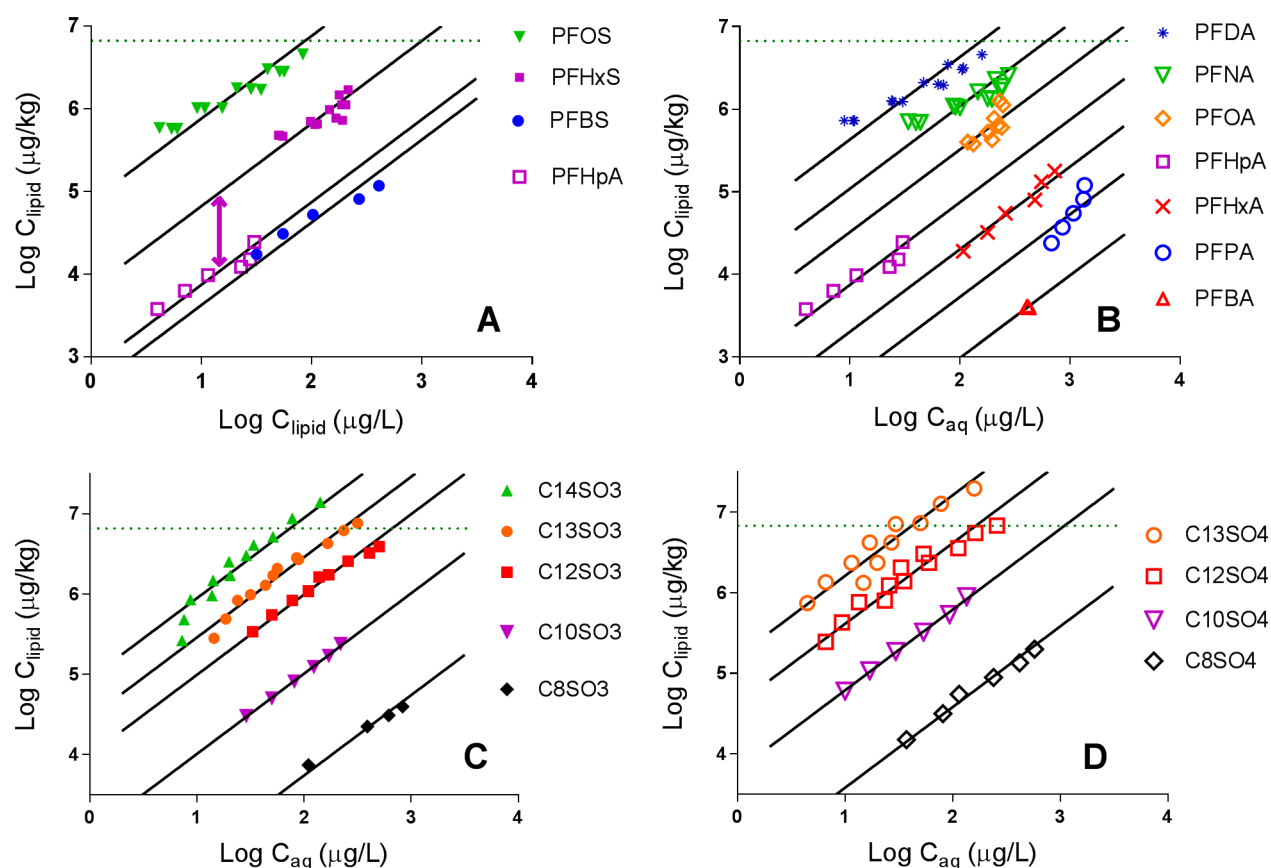


Figure 1. Sorption isotherms of perfluorinated (A, B) and hydrogenated (C, D) anionic surfactants using the solid-supported lipid membrane assay. All fitted log-linear lines have a forced slope of 1. Horizontal broken lines indicate a sorbed fraction of ~ 1 mol/60 mol phospholipid.

the desired buffer (pH 7, 100 mM ammonium acetate in pure water with 1 mmol/L of the biocide NaN_3 in all cases), (iii) strongly reduce the amount of nonvolatile salts in the medium to facilitate direct injection of water samples in LC-MS/MS, and (iv) remove phospholipid material possibly detached from the beads to negligible amounts. The standard TRANSIL^{XL} kit ranges from 0.048 to 0.9 μL phospholipid per vial (so also in our HPLC vials), whereas a high density TRANSIL^{XL} kit (for low affinity compounds) has a complementary higher range of lipids from 0.884 to 16.7 μL . Both series apply 1.8 dilution factors (supplier specification sheet). Further testing details on spiking, glass vial types, replicates, and testing concentrations are provided in SI section S1.

Only for samples where the measured concentrations in supernatant were at least 30% reduced compared to controls without sorbent, lipid-sorbed concentrations were determined from the decreased dissolved concentration relative to sorbent free controls and amount of applied lipid. The sorbed fraction of (mixtures of) organic anions remained less than 2 mol % per mol phospholipid, as recommended to avoid confounding charge effects.

All valid data for each compound were plotted on a double logarithmic scale and the K_{MW} was derived by fitting a linear curve with a slope of 1 according to

$$\log C_{\text{lipid}} = \log C_{\text{aq}} + \log K_{\text{MW}} \quad (3)$$

Concentrations were measured using a HPLC system (Prominence UFLC-XR, Shimadzu) coupled to a tandem mass spectrometer (QTRAP 4000, Applied Biosystems), using external calibration standards prepared in the same medium.

Details on chromatographic separation (SI Table S2) and detection of each compound (SI Table S3) are provided in SI section S1.

K_{MW} Based on Retention Capacity Factors on IAM-HPLC Column. A 100 \times 4.6 mm IAM.PC.DD2 column (Regis Technologies, Inc., Morton Grove, IL USA) was used with an IAM.PC.DD2 10/300 guard cartridge in front. A flow rate of 1.0 mL/min was applied with the column maintained at a controlled temperature of 25 $^{\circ}\text{C}$. Methanol stock solutions of the test chemicals were diluted >100 times in aqueous buffer of similar composition as the applied eluent, and 20 μL was injected.

Based on series of retention experiments with organic cations,⁵⁴ Droge et al. showed that the surface charge of silica appeared to be net positive at pH < 5 (reducing the apparent retention time of organic cations), and net negative at pH > 5 (enhancing the apparent retention time of organic cations). At a lower eluent salinity, these effects are enhanced further (for more details see ref 54). This confounding effect on retention time is independent of the sorption to the zwitterionic (net neutral) phosphatidylcholine lipid coating and needs to be accounted for (partly or permanently) ionized solutes. The confounding retention effects relate to attraction/repulsion of ions from bulk eluent to the diffusive water layer (or electrical double layer) surrounding the IAM particle surfaces. This process acts equally on all likewise charged compounds (and does not occur for neutral compounds) and is fully separated from the intrinsic molecular interaction with the phospholipid coating. The confounding electrostatic attraction (Boltzmann factor $B_{\text{pH}/1}$) is simply an addition to the intrinsic interaction

with the phospholipid monolayer ($\log K_{\text{IAM,intr}}$) and accounted for as ref ⁵⁴

$$\log K_{\text{IAM,apparent}} = \log K_{\text{IAM,intr}} + \log B_{\text{pH}/1} \quad (4)$$

As a result, it was advised to either apply a corrective Boltzmann factor $B_{\text{pH}/1}$ for IAM-HPLC measurements at pH 7.4, or to perform retention studies at pH 5.0, at which the IAM surface charge appeared to be net neutral.^{55,56} Therefore, besides comparing IAM-HPLC retention data to SSLM K_{MW} values, the IAM-HPLC tests were also used to evaluate the influence of pH and ionization on the retention on IAM-HPLC of a series of organic anions, to confirm net neutral eluent pH conditions, and to define Boltzmann factors $B_{\text{pH}/1}$ for several important eluent conditions.

For IAM-HPLC experiments, a mixture of PFAS compounds as well as single solute PFCAs, PFSA, alkylsulfonates, and alkylsulfates were injected onto the chromatographic column connected to the same LC-MS/MS as for the SSLM assay. Eluents with different methanol/buffer ratios, at pH 3.0 (formic acid/ammonium formate buffer) 4.0, 5.0, 5.5, 6.0 (acetic acid/ammonium acetate buffers), 7.0, 7.4, and 7.8 (formic acid/ammonia/ammonium bicarbonate buffers), at high salinity (100 mM of the buffer salt, close to physiological saline buffers) and lower salinity (10 mM, recommended for LC-MS/MS) were used and kept running at 1 mL/min for >1 h before the first injection. Although extensive series of various solvent/buffer measurements (10–50% methanol mixed with pH 5 acetate buffer) of the retention capacity factor were made, final values were obtained with fully aqueous buffer as eluent. Citric acid or C_4SO_3 were used to determine the net void retention volume, depending on the tested pH (see [Results and Discussion](#)).

COSMOmic Predictions of K_{MW} . The COSMOmic module of COSMOtherm software V17 was used to calculate the weighed partition ratio between water and a hydrated zwitterionic 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) phospholipid bilayer ($K_{\text{DMPC-W}}$) developed by Jakobtorweihen et al.⁵⁰ (time-average atomic distribution from a MD run). The same positive membrane dipole potential settings were used as recommended by Bittermann et al. for that DMPC bilayer.⁵¹ The most relevant ionized conformers were created with COSMOconf V4.0 at the “TZVP” (triple- ζ for valence electrons plus polarization function) parametrization. Conformers were included automatically in the weighed $K_{\text{DMPC-W}}$ calculations.

RESULTS AND DISCUSSION

K_{MW} Based on SSLM and Relationships with Surfactant Structure. Although phospholipid membranes are anisotropically structured, membranes provide for a uniform bulk absorptive phase for each solute, and competition to specific sorption sites is not expected.^{52,57} Particularly when a dissolved concentration range of more than a factor of 10 was covered, the SSLM sorption isotherm data ([Figure 1](#)) indicate a concentration independent sorption coefficient for all tested anions. Due to too high membrane loadings with charged chemicals, repulsion effects may influence sorption linearity. In order to avoid creating significant confounding charge effects, the maximum advised level to sorb chemicals into liposome of 1.7 mol % (1 mol/60 mol phospholipids) corresponds to $\sim 10^{6.8}$ $\mu\text{g/kg}$ (log 6.8 on the Y-axis in [Figure 1](#)). Overall, the fitted isotherms show consistent increments in $\log K_{\text{MW}}$ for

series of analogue anions so the various applied beads/solution ratios did not matter and it was assume all fitted values are valid representations of the sorption process.

The SSLM assays provided between 5 and 13 valid measurements from which K_{MW} values could be determined for a series of PFCAs, from PFPA up to PFDA ($\text{F}(\text{CF}_2)_4\text{CO}_2^-$ – $\text{F}(\text{CF}_2)_9\text{CO}_2^-$), and the PFSA PFBS, PFHxS, and PFOS ($\text{F}(\text{CF}_2)_4\text{SO}_3^-$ – $\text{F}(\text{CF}_2)_8\text{SO}_3^-$). Only a single sample for PFBA from a testing series where the sorbed fraction was >30% and considered valid. The observed total difference over 6 (CF_2) units for the C_3 – C_9 PFCAs series is 3.63 log units ([Table 1](#)).

Table 1. Phospholipid Bilayer Sorption Coefficients and Predicted Acute Toxicities for Selected Surfactants

name	$\log K_{\text{MW}}$ (SSLM)	95% C.I.	N^a	$\text{LC}_{50,\text{narc}}$ via eq 1 (mg/L)
PFBA	1.0		1	2130.0
PFPA	1.73	1.54–1.92	5	489.7
PFHxA	2.31	2.23–2.38	6	153.3
PFHpA	2.87	2.76–2.99	6	49.0
PFOA	3.51	3.44–3.59	11	12.8
PFNA	4.04	3.95–4.13	13	4.2
PFDA	4.63	4.54–4.73	13	1.2
PFBS	2.63	2.45–2.81	5	70.1
PFHxS	3.82	3.75–3.90	13	6.0
PFOS	4.88	4.79–4.97	13	0.7
C_8SO_3	1.74	1.64–1.85	4	380.3
C_{10}SO_3	3.01	2.99–3.03	6	23.2
C_{12}SO_3	3.99	3.95–4.04	10	2.7
C_{13}SO_3	4.46	4.41–4.51	12	1.0
C_{14}SO_3	4.95	4.85–5.04	12	0.3
C_8SO_4	2.58	2.52–2.65	6	59.2
C_{10}SO_4	3.79	3.77–3.81	6	4.1
C_{12}SO_4	4.61	4.54–4.69	12	0.7
C_{13}SO_4	5.21	5.12–5.30	11	0.2

^aNumber of data points used to fit K_{MW} which all had a $f_{\text{sorbed}} > 30\%$.

For alkylsulfates and alkylsulfonates, alkyl chains between C_8 and C_{14} were tested. For shorter chain lengths the lower $\log K_{\text{MW}}$ would lead to too small reductions in the water dissolved concentrations to render accurate mass balance calculations. For larger chain lengths with higher $\log K_{\text{MW}}$ the strong reduction of the spiked concentration through sorption to lipid would result in concentrations below our limit of detection (LOD). The tested range of nonfluorinated surfactants span six or five hydrocarbon units.

This unique set of consistent K_{MW} values for series of fluorinated and nonfluorinated analogues allows for deriving the, currently poorly understood, relative contributions of different structural moieties of surfactants to membrane–water partition coefficients: the hydrophobic tails, the anionic headgroups, and overall effects of perfluorination compared to hydrogenated analogues.

Contributions of Hydrophobic Surfactant Chains to K_{MW} . As expected, sorption increases with longer alkyl chain lengths of any surfactant. For the set of seven cojoining nonfluorinated analogues, the average $\log K_{\text{MW}}$ increment is 0.53 (± 0.08 stdev). The total difference over six (CH_2) units for the C_8 – C_{14} alkylsulfonate series is 3.21 log units. Cationic surfactants displayed a comparable average $\log K_{\text{MW}}$ increment of CH_2 (0.59 ± 0.03 stdev).⁵² The alkyl chains of both types of

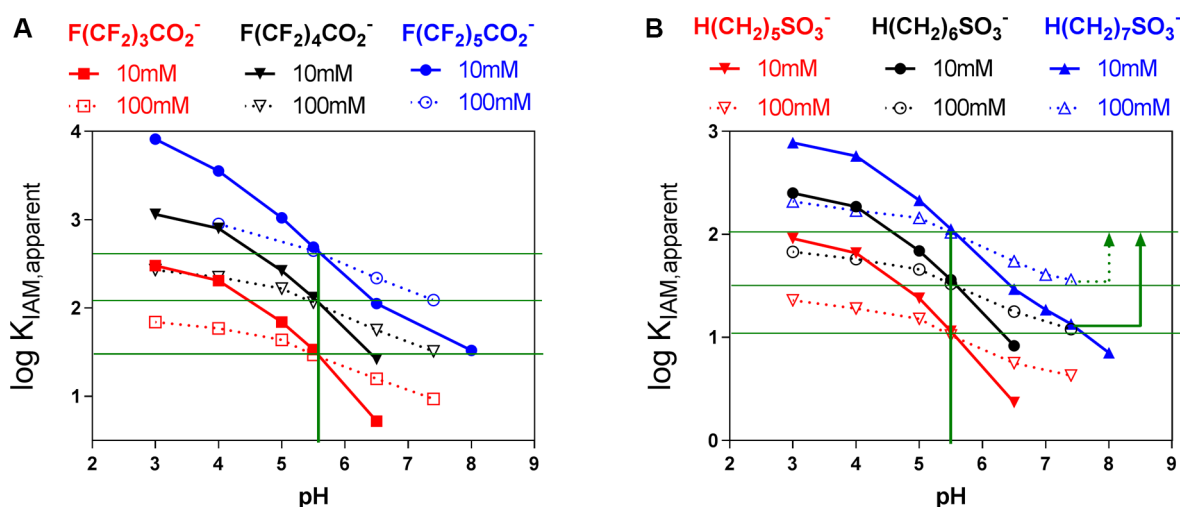


Figure 2. Apparent partition ratios between aqueous buffer eluent and the IAM column material ($K_{IAM,apparent}$) for (A) perfluorinated carboxylates; (B) alkylsulfonates. Each compound was tested at two series of fully aqueous buffers adjusted at either 0.010 or 0.1 M to a certain pH. Blue, red, and black lines are only connecting sequential pH data for visual aid. The green vertical line indicates the pH where ionic strength did not influence the $K_{IAM,apparent}$, indicating a net neutral IAM surface charge. The horizontal green lines indicate the intrinsic sorption affinity to the phospholipid monolayer ($K_{IAM,intr}$) for each compound at the pH with net neutral IAM. The green arrows indicate the difference between $K_{IAM,apparent}$ and $K_{IAM,intr}$ at pH 7.4 at 10 and 100 mM ionic strength, which for all tested compounds were 0.9 and 0.3 log units, respectively.

surfactants likely take up the same position in the hydrophobic bilayer core. The CH_2 unit K_{MW} -increment for neutral alkylbenzenes is in a comparable range.⁴⁹

For the set of eight cojoining perfluorinated analogues, the average $\log K_{MW}$ increment is $0.59 (\pm 0.07 \text{ stdev})$ per CF_2 , and the single value for PFBA fits in this trend. These CF_2 increments to K_{MW} values for these anionic PFASs were clearly lower than liposome–water sorption coefficients for neutral fluorotelomer alcohols (FTOHs), which increased with $0.74 \log$ units per CF_2 unit.⁵⁸

The anionic surfactants do sorb somewhat weaker than analogue primary amine or quaternary ammonium surfactants though, depending on the headgroup type. For example, $C_{10}NH_3^+$ and $C_{10}N(CH_3)_3^+$ showed $\log K_{MW}$ of 4.3 and 3.3, respectively, on the same SSLM assay, while $C_{10}SO_3^-$ and $C_{10}SO_4^-$ showed respective $\log K_{MW}$ of 3.0 and 3.8. This may seem counterintuitive if one considers that the membrane dipole potential is net positive (+), but it likely relates to a slightly different average position of the oppositely charged phosphatidylcholine moieties in the headgroup region of phospholipid bilayers: phosphate anions are on average closer to the hydrophobic core than the choline trimethylammonium cations (Figure S4 shows the atomic distribution of the DMPC bilayer applied in COSMOmic). As a result, carbon chains of cationic surfactants may be protruding deeper in the hydrophobic core than those of analogue anionic surfactants (Figure S4 shows the center of C_{12} -based cationic surfactants positioned deeper in COSMOmic's DMPC bilayer than C_{12} -based anionic surfactants).^{55,56}

Contributions of Hydrophilic Head Groups of Surfactants to K_{MW} . Unexpectedly, the sulfate anion sorbed stronger for each nonfluorinated sulfate–sulfonate analogue pair, on average for four pairs by $0.75 \log$ units ($\pm 0.09 \text{ stdev}$). It was expected that sulfate ions would sorb weaker to phospholipids compared to analogue sulfonate ions because the additional oxygen atom would render the molecule overall more polar (EPISuite KOWWIN predicts a $\log P$ for neutral form 1-dodecylsulfonic acid 2.42 and 3.02 for dodecyl hydrogen sulfate). Contrastingly, for this one extra oxygen

between $R-O-SO_3^-$ and $R-SO_3^-$, $\log K_{MW}$ increased even more than by a CH_2 unit. This may be partially due to a larger hydration of the $R-SO_3^-$ headgroup (see the next section on perfluorination).¹⁸ However, just like the above notion that cationic surfactants may extend deeper into the hydrophobic core than anionic surfactants, the extra oxygen of sulfate allows for a deeper position of the alkyl chains of alkylsulfates into the hydrophobic bilayer core.

The carboxylate PFCA anions showed a lower $\log K_{MW}$ compared to analogue sulfonate PFSA anions with the same amount of perfluorinated carbons (e.g., PFHpA vs PFHxS, Figure 1). This contrasts most K_{OW} predictions. For the three PFCA–PFSA pairs the average difference is 0.90 log units. In this case, the sulfonate group is larger than the carboxylate anion headgroup, but it is less clear than for the sulfate/sulfonate and cation/anion examples above if this results in a deeper position into the membrane. This lower K_{MW} for PFCAs shows that the contribution of the fraction neutral carboxylate species at the tested pH of 7.0 is negligible: both types of perfluorinated compounds have very low pK_a values (<1).

Perfluorinated Surfactant Tails Compared to Hydrogenated Surfactants Tails. It becomes immediately apparent from the tested range of chain lengths that perfluorinated compounds sorb stronger to membranes lipids than nonfluorinated analogues in the order $C_xSO_3^- < C_xSO_4^- < F(CF_2)_xCO_2^- < F(CF_2)_xSO_3^-$. This is exemplified by the $\log K_{MW}$ values for the octyl analogues: $H(CH_2)_8SO_3^-$ (1.74) $< H(CH_2)_8SO_4^-$ (2.58) $< F(CF_2)_8CO_2^-$ (PFNA, 4.04) $< F(CF_2)_8SO_3^-$ (PFOS, 4.88). The larger molecular volume of the fluorine atom compared to the hydrogen atom may render a higher energy gain by leaving the water phase. However, the CF_2 unit contribution to K_{MW} of PFAS is not significantly higher than the CH_2 unit increment for alkyl sulf(on)ates. The large differences between these four series of surfactants appears to be mainly on the interactions of the surfactant headgroup with the phospholipid headgroups. The effect of fluorine molecules on the headgroup contribute strongly to that, as exemplified by the difference of a factor 1000 between

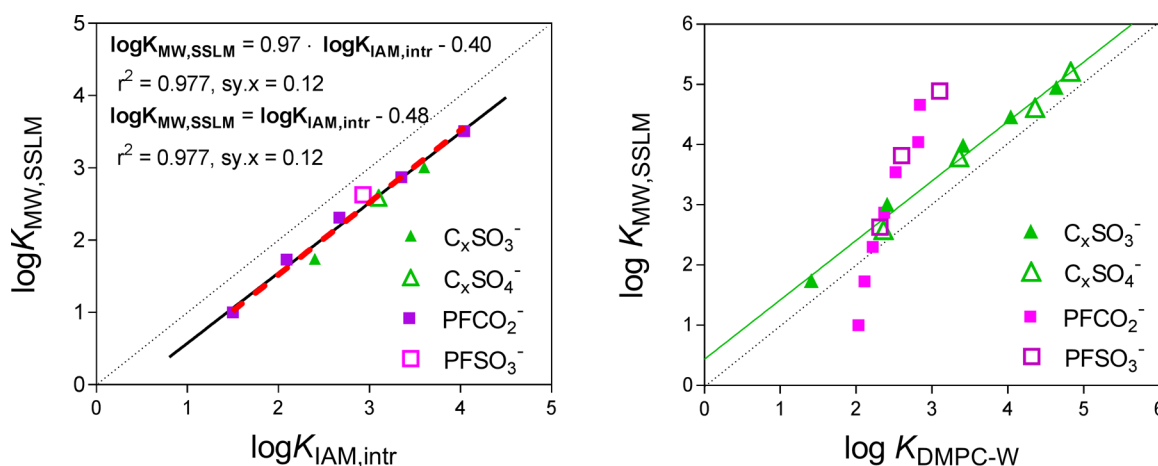


Figure 3. (A) Experimental phospholipid bilayer data obtained with the SSLM assay ($\log K_{MW,SSLM}$) for four types of organic anions plotted against intrinsic IAM-HPLC partition ratios to phospholipid monolayer (IAM.PC.DD2). The dotted line in A indicates 1:1 overlap, the broken line in A is the fit with a slope of 1, the drawn line in A is the linear fit through all nine data points. (B) COSMOmic predicted partition coefficients to a hydrated DMPC phospholipid bilayer ($\log K_{DMPC-W}$) for four types of organic anions plotted against experimental phospholipid bilayer data obtained with the SSLM assay ($\log K_{MW,SSLM}$). The dotted line indicates 1:1 predictions, the drawn line is the linear fit through all alkylsulfonate and alkylsulfate data together.

C_8 analogues $H(CH_2)_8SO_3^-$ ($\log K_{MW}$ 1.74) and $F(CF_2)_8SO_3^-$ (PFOS, $\log K_{MW}$ 4.88), but still a factor 240 for C_4 analogues, as in IAM retention based K_{MW} (Table S5, discussed below) for $H(CH_2)_4SO_3^-$ ($\log K_{MW,IAM}$ 0.5) and $F(CF_2)_4SO_3^-$ (PFBS, $\log K_{MW,IAM}$ 2.9). Similarly, Jing et al. determined $\log K_{OW}$ differences of ~ 2 orders of magnitude for a series of perfluorinated and hydrogenated anions.⁵⁹ Clearly, perfluorination also strongly affects the pK_a of the terminal carboxylic acid,¹⁰ and this is likely the result of the influence of the arrangement of water molecules around the surfactant as a hydration sphere. The perfluoroalkyl group has a strong electron-withdrawing effect on oxoanion groups, while hydrocarbon tails have an electron-donating effect.¹⁸ The oxoanion group of perfluorinated compounds is therefore poorly hydrated (chaotropic) and may more readily release these water molecules than hydrocarbon tail anions. This most likely also explains the stronger sorption affinity of perfluorinated anions to phospholipid membranes, but also the higher affinity of surfactants with a larger headgroup.

SI section S2 presents correlations between $\log K_{MW}$ and several single molecular parameters (SI Table S4 and Figure S1). Molecular weight does not align perfluorinated anions with hydrogenated anions, but molecular volume did, either approached by McGowan's V_x or COSMOtherm's conformer average volume. Both fit linearly with r^2 0.93 and 0.33 standard deviation of the residuals.

K_{MW} Based on IAM-HPLC and Effects of Eluent Conditions. From Apparent to Intrinsic IAM Partition Ratios. As shown for three perfluorinated and three non-fluorinated test compounds in Figure 2, the retention of all tested anions on IAM-HPLC column strongly depended on pH and salinity, in nearly the exact opposite trends as for organic cations.⁵⁴ At the low pH range (pH_{IAM+}), the lower salinity buffer results in a stronger retention of all anions. At the high pH range (pH_{IAM-}), this effect of buffer salinity is reversed, anions elute faster at lower salinity. For the series with 10 mM buffers, the apparent partition ratio to the IAM surfaces differs by a factor of 100 between pH 3.0 and pH 7.4 for these fully ionized anions only due to changes of the eluent pH on the IAM surface chemistry. All retention capacity

factors (k_{IAM}) for compounds in this study are already converted to apparent IAM partition ratios ($K_{IAM,apparent}$) by multiplying k_{IAM} with the eluent:sorbent phase ratio of 18.9 for IAM.PC.DD2 type columns (SI Table S5, section S3).^{54,60} The confounding attraction at low pH agrees with observations for inorganic anions (Br^- , NO_3^-) in the study with organic cations.⁵⁴ The pH where no effect of buffer salinity is observed (pH_{IAM-0}) appeared to be 5.4–5.6, as indicated by the green vertical lines in Figure 2. This is slightly higher than the pH_{IAM-0} of 5.0 determined for cations⁵⁴ on a IAM-HPLC column from an older batch (Regis Technologies SER no. 48056, currently SER no. 59666). The tested organic anions become gradually more repulsed from the IAM surface at pH further above the net neutral surface charge and much stronger at lower salinity.

The green arrows at Figure 2B give an example of the required Boltzmann correction factor for a certain pH and ionic strength ($B_{pH/I}$) to account for electrostatic IAM effects to derive an “intrinsic phospholipid partition ratio” ($K_{IAM,intr}$) from $K_{IAM,apparent}$. An eluent pH of 7.0 and 7.4 is commonly used as this has direct relevance for physiological purposes, but the derived $K_{IAM,apparent}$ clearly needs to be corrected to $K_{IAM,intr}$. Averaging the difference in $K_{IAM,apparent}$ at pH_{IAM-0} (pH 5.5) and pH 7.0 or 7.4 for all anions for which these experimental data were available, the corrective increments for electrostatic IAM repulsion in 10 mM buffer ($\log B_{pH7.0/0.01M}$ and $\log B_{pH7.4/0.01M}$) are +0.74 and +0.92 log units. $\log B_{pH7.0/0.1M}$ and $\log B_{pH7.4/0.1M}$ are +0.29 and +0.48 log units. These electrostatic corrective increments apply to all (monoprotic) anions on the current column, IAM-HPLC columns from other batches may give slightly different $B_{pH/I}$ values. $B_{pH/I}$ values apply also to partially ionized weaker acids, as discussed in SI section S3. Using the repulsion of anions at physiological pH and applying cosolvent gives the potential benefit of IAM-HPLC over the SSLM method of reducing the retention of organic anions with strongly phospholipid affinities such as PFOS and $C_{11}SO_3^-$. However, these could already be determined with SSLM (up to $C_{14}SO_3^-$, Table 1), and also the LC-MS compatible bicarbonate buffer for pH 7.4 is rather unstable.

Using IAM-HPLC to Predict Sorption of Organic Anions to Cell Membranes. As summarized in SI Table S5, there are nine organic anions for which now both $K_{IAM,intr}$ and $K_{MW,SSLM}$ are available, and these are plotted in Figure 3. The $\log K_{IAM,intr}$ for the coated phospholipid monolayer are consistently higher than the SSLM-based K_{MW} phospholipid bilayer values. The values for the two types of perfluors follow exactly the same trend as the alkylsulfonates and alkylsulfate compounds. The SSLM based K_{MW} values should be considered reference values, as they are derived for bilayer phospholipids without electrostatic confounding factors. It should be kept in mind that also SSLM bilayers are not similar to actual cell membranes (see last two paragraphs in the section on Perspectives for Risk Assessment). Still, assuming a constant offset for all anions (forcing a slope of 1) results in an IAM-SSLM corrective increment ($\Delta_{IAM-SSLM,anions}$) of -0.47 log units, about a factor of 3. There may be several factors influencing $\Delta_{IAM-SSLM,anions}$. First, if the tested anionic compounds only sorb to the outer phospholipid half of the bilayer (in the applied time frame), they logically already deviate by a factor of 2 lower from the IAM monolayer partition ratios. Another feature specific to IAM surfaces is that the residual protonated propylamine groups may provide for additional protonated sorption sites embedded in the rather hydrophobic core, rendering stronger interactions than the headgroup choline groups. For now it is suggested to simply use the empirical $\Delta_{IAM-SSLM,anions}$ of -0.47 log units for strongly dissociated acids, combined with $\log B_{pH/I}$ values if necessary, in order to use IAM-HPLC to experimentally derive $\log K_{MW}$ values. The observed consistency between $\log K_{IAM,intr}$ and SSLM for diverse anions in this study shows that this may be a viable route compared to the more labor intensive SSLM assay or alternative (dialysis) experiments when a (large set of) screening value(s) is required for lower tier risk assessment.

K_{MW} Based on COSMOmic Calculations, Alignment, and Shortcomings. Experimental measurements are not always possible for (perfluorinated) anionic surfactants, for example when standards are not available, too expensive to obtain, or sorption coefficients are too strong to accurately detect dissolved (eluent) concentrations (e.g., $C_{14}SO_4^-$ sorbs critically strong for experiments). COSMOtherm can use 3-dimensional conformers of both solute and partition phase molecules to calculate partitioning constants to one-half of a phospholipid bilayer. Figure 3 plots COSMOmic predicted $\log K_{DMPC-w}$ values against the SSLM-based K_{MW} values for the tested organic anions (SI Table S4). For the anionic perfluorinated compounds, COSMOmic seems to strongly misinterpret the contribution of the perfluorinated alkyl chain length. The overall predicted CF_2 increment by COSMOmic is 0.17 log units (purple squares, Figure 3), which results in overestimated $\log K_{MW}$ for the smallest perfluor anions and underestimated $\log K_{MW}$ for the largest perfluor anions, differing over orders of magnitude for the tested series. Interestingly, as noted before,³³ the CF_2 increment for anionic perfluor carboxylates with chain lengths longer than $(CF_2)_{10}$ increases to 0.4 – 0.6 log units. Peculiarly, the neutral forms of the two series of PFAS do show a consistent CF_2 increment of 0.6 for $\log K_{DMPC}$ (SI Figure S3). This could allow for application of the same Δ_{MW} scaling factor [$\log K_{MW,N} - \log K_{MW,ion}$] of between -2 to -3 for anions as proposed by Armitage et al.²⁰ but now based on COSMOmic input for the neutral species. It is noteworthy that the review on K_{MW} values for ionogenic compounds and subsequent optimization of

COSMOmic for ionogenic compounds did include a single value for PFOS,⁵¹ seemingly covering perfluorinated compounds. However, as it turns out, a single representative compound is not sufficient to adequately confirm that a “model” is predictive for an entire series or class of compounds.

In contrast to the PFAS anions, and in line with cationic surfactants and most other ionogenic compounds,^{51,52,55,56} COSMOmic provides accurate predictions for the SSLM data for nine alkylsulfonates and alkylsulfates (green triangles, Figure 3) in a shared regression:

$$\log K_{MW} = 0.987 \log K_{DMPC-w} + 0.435$$

$$(df = 7, r^2 = 0.987, sy.x = 0.141, F = 535) \quad (5)$$

The slope of nearly 1 indicates that COSMOmic accurately predicts the influence of the hydrocarbon chain. However, the shortest alkylchain of this set is C_8SO_3 . When considering the IAM-HPLC data for smaller chain C_4 – C_8 alkylsulf(on)ates (Table S5), each CH_2 units for $K_{PLIPW-IAM}$ is still close to 0.5 log units, but COSMOmic calculations increase on average only by 0.09 log units per CH_2 moiety, a similar misinterpretation as for equal sized PFAS anions (Figure S3B). This was further confirmed by a series of phenylalkylcarboxylic acids. Apparently, COSMOmic tends to strongly overestimate the K_{MW} for small organic anions. For small organic cations, however, COSMOmic calculates constant CH_2 unit contribution of 0.5 log units, while methyl groups close to the charged nitrogen indeed have little contribution to the K_{MW} .⁵⁶ The alignment between alkylsulfonates and alkylsulfates and very low $sy.x$ indicates that COSMOmic adequately captures the influence of the increased sorption affinity due to the extra oxygen in the SO_4 structures. This is a clear improvement over any (calculated) octanol–water based prediction which always results in lower predictions for alkylsulfate analogues. The offset of $+0.435$ indicates a constant underestimation of $\log K_{MW,SSLM}$ by COSMOmic's $\log K_{DMPC-w}$. In contrast, the COSMOmic alignment study with ionogenic compounds found COSMOmic to overestimate K_{MW} for anions with 0.37 log units, despite applying the same DMPC input structure.⁵¹ This difference may relate to using a consistent SSLM assay, while the reviewed data were mostly from a variety of dissolved liposome vesicles, but also the near lack of sulfonic acids and sulfates in the alignment study.

Perspectives of Using K_{MW} for Risk Assessment. Phospholipid–water partition coefficients for perfluorinated compounds can be measured with both SSLM and IAM-HPLC if chemical standards are available and adequate analytical detection is possible. These K_{MW} values could be applied as one of the key input parameters in several steps/tiers of chemical risk assessment. Examples are bioconcentration model predictions for uptake/elimination of organic anions from the aqueous phase in whole organisms (e.g., using the BIONIC model for ionogenic compounds²⁰). The higher $\log K_{MW}$ value for PFOS than PFOA (4.9 vs 3.5 , respectively) corresponds with fish BCF values (~ 1000 vs 7 , respectively, in Carp⁶¹), while (predicted) K_{OW} based predictions would have predicted this the other way round. More specific toxicokinetic models for chemical distribution within the body (e.g., as described for PFAS^{33,36,38}) could aid the assessment of which tissues of impacted biota pose the highest risk for consumption.

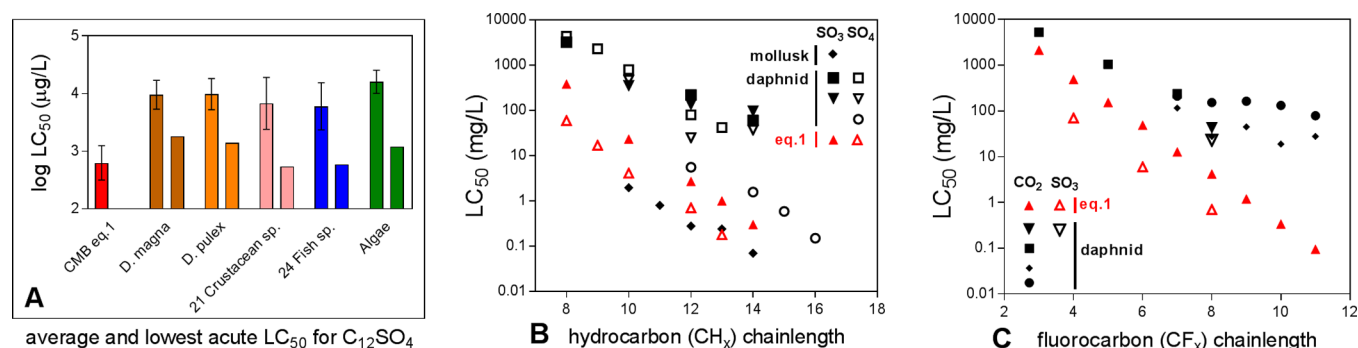


Figure 4. Predicted acute narcotic log $LC_{50,narc}$ (red bar in A, red upward triangles in B and C, based on the critical membrane burden range divided by K_{MW}) plotted together with observed acute narcotic log LC_{50} data from (A) ECOTOX database extracts on $C_{12}SO_4$ with group average (first bar) and lowest (second bar) reported acute log LC_{50} values for several species and species classes; (B) studies that tested series of alkyl sulfates (open symbols) and alkylsulfonates (closed symbols) for toxicity on *D. magna* and mollusk *Crassostrea gigas* (squares ref 76, circles ref 72, downward triangles ref 77, diamonds [Cardwell et al. 1979 in ECOTOX database]); (C) studies that tested series of perfluorocarboxylates (close symbols) and perfluorosulfonates (open symbols) on daphnids (*D. magna* data for squares ref 68, dots ref 7, downward triangles ref 69, and *Chydorus spheridae* for diamond ref 7).

The current limited focus is on more direct predictions of acutely narcotic concentrations $LC_{50,narc}$ as in the simplified way of eq 1. For selected organic anions listed in Table 1, toxicity data was collected from literature and compared to the calculated $LC_{50,narc}$ using the experimental log K_{MW} and the critical narcotic membrane burden (CMB_{narc}) of 100 mmol/kg⁶² (Figure 4). This way, expected $LC_{50,narc}$ values for analogue C_8 anions PFOS, PFNA, C_8SO_4 , and C_8SO_3 are 0.7, 4, 60, and 380 mg/L, respectively (Table 1). Typically, the CMB_{narc} is related to acutely toxic effect concentrations with sufficient equilibration time for chemicals into the test organism, e.g. 3d algal growth, 2d daphnids immobility and 4d fish mortality.^{44,62–65} The EPA-ECOTOX database (<https://cfpub.epa.gov/ecotox>) was checked for relevant acute (2–7d) LC_{50} data for crustaceans and fish as common and other relevant test organisms (SI Table S6A–D). Only for the common soap ingredient $C_{12}SO_4$ a wealth of toxicity data are available, also because this compound has been recommended and applied as (nonvolatile) toxic reference compound.

For $C_{12}SO_4$, the predicted $LC_{50,narc}$ is 0.7 mg/L (0.34–1.4 mg/L for the CMB range 50–200 mmol/kg). Figure 4A shows the average 2d LC_{50} values reported for the crustacean species *Daphnia magna* and *Daphnia pulex* to be about an order of magnitude higher: 11.0 ± 5.5 mg/L (s.d., $N = 47$) and 11.7 ± 8 mg/L ($N = 35$), respectively (SI Table S6C). The lowest reported LC_{50} values for both species (second bar for each color in Figure 4A), however, are close to the $LC_{50,narc}$ with 1.8 and 1.4 mg/L. The average acute LC_{50} for 21 other crustacean species is comparable (average 10.7 ± 10.2 mg/L with a lowest value of 0.55 mg/L), just as the average acute LC_{50} for 23 fish species (9.1 ± 8.2 mg/L with a lowest value of 0.59 mg/L). Algae are on average less sensitive, with an average 2–4d 50% inhibition at 37 mg/L, although the marine diatom *Skeletonema costatum* showed an EC_{50} at 1.2 mg/L.

Similarly as for $C_{12}SO_4$, but with less toxicity data on acute effects and from studies that often did not measure actual test concentrations, the CMB_{narc} approach appeared to predict narcotic toxic concentrations an order of magnitude lower than the average reported toxicity for most aquatic test species for other alkylsulfonates (Figure 4B, SI Table S6E)⁶⁶ and the perfluorinated anions (Figure 4C, SI Tables S6A/B). Details are discussed further in SI section S6. Noteworthy exceptions

were the acute LC_{50} values of mollusks for series of alkylsulfonates (closed diamonds in Figure 4B), which were up to an order of magnitude lower than expected by the CMB_{narc} , while daphnid toxicity strongly leveled off of LC_{50} values going from PFOA–PFDoDA (closed dots in Figure 4C)⁷ compared to eq 1 predictions, even though PFCA concentrations had been measured. A higher toxicity than $LC_{50,narc}$ may relate to specific modes of toxic action, while leveling off may relate to dissolution limitations.⁶⁷

This general finding of mostly lower observed acute toxicity than predicted baseline effect levels based on log K_{MW} can have different causes. First, the SSLM reference phosphatidylcholine phospholipid may overestimate the affinity of organic anions to actual cell membranes, which are often negatively charged due to enrichment with anionic lipids. This could indeed cause a 4 times lower sorption affinity at high membrane loadings required to induce narcosis.^{57,70} In addition, the CMB for anions may be higher than the 50–200 mmol/kg for neutral compounds. Rapid biotransformation⁷¹ may be a cause for $C_{12}SO_4$, but this would not apply as a cause to the findings for PFOA and PFOS. Organic anions partition slower into cells than neutral compounds,⁷² but it is unlikely that less than 10% equilibrium is reached within the 2–8d acute tests, as fish uptake studies with the strongly anionic surfactant C_{12} -2-LAS indicate 90% SS within 2 days.^{73,74}

From this K_{MW} based analysis, eq 1 seems to be overly protective for lower tier predictions of the baseline toxicity level for organic anions, based on the CMB defined for neutral narcotics and standardization to phosphatidylcholine phospholipids. However, this simplified K_{MW} approach is based on consistent experimentally derived values and coincides with acute empirical LC_{50} values for the most sensitive species and thus provides a conservative indication of acute narcotic effect concentrations that is useful for risk assessment of surfactants. On average the $LC_{50,narc}$ is within an order of magnitude for many of the organic anions evaluated. This K_{MW} approach could also allow for evaluation of empirical effect concentrations that appeared to induce specific modes of action, or the lowest concentrations that significantly induced a molecular initiating event (MIE)⁷⁵ of more specific adverse outcome pathways for (perfluorinated) anions in relation to these baseline effect levels.

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b05052.

Details on adapted protocol for the SSLM assay, LC and MS details, dependency of K_{MW} of linear alkyl anions on molecular descriptors, detailed IAM-HPLC data, details for COSMOmic predictions, ECOTOX database extracts, and more detailed comparisons between predicted and reported acute aquatic toxicity data (DOCX)

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