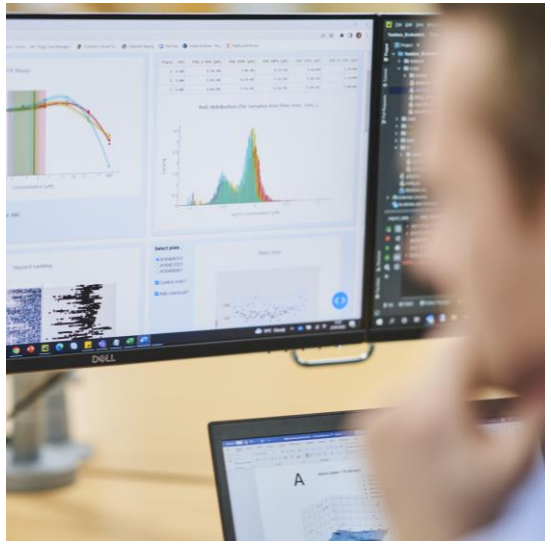
Using An *In Silico* NAMs Approach To Predict Bioaccumulation In Fish:

A Case Study For Anionic Surfactants Within A Regulatory Context



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Introduction

- Bioaccumulation endpoint required for registered substances exceeding the 100 t/y threshold (Annex IX)

- *Hyalella azteca* bioconcentration test (HYBIT)
- Intrinsic hepatic/S9 clearance in vitro assays (OECD 319/IVIVE methods)
- QSAR models e.g. Episuite, T.E.S.T, VEGA

Specific consideration for surfactants

- No longer possible to waive BCF based on $logK_{ow}$
- Experimental methods technically challenging for surfactants
- Computational methods also have limited reliability
- Weight of Evidence (WoE) approach using $logK_{mw}$ in conjunction with toxicokinetic models

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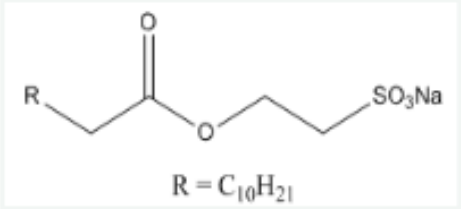
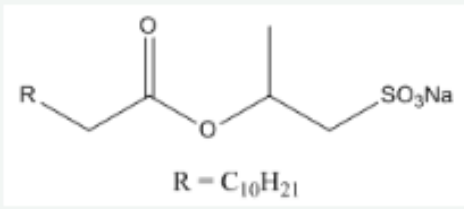
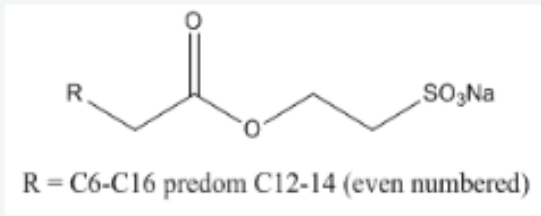
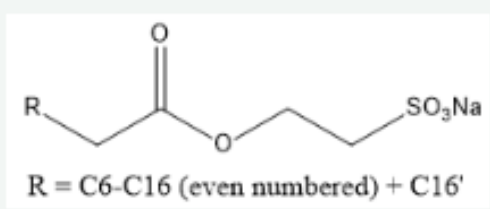
Case study – alkyl isethionates

Substance	SLI	SLMI	SCI	DEFI
EC Identifier	EC: 230-949-8	EC: 700-150-3	EC: 263-052-5	EC: 287-024-7
Structure	 $R = C_{10}H_{21}$	 $R = C_{10}H_{21}$	 R = C6-C16 predom C12-14 (even numbered)	 R = C6-C16 (even numbered) + C16'

- SLI = Sodium Lauryl Isethionate C12 chain
- SLMI = Sodium Lauryl Methyl Isethionate C12 chain + methyl branch
- SCI = Sodium Cocoyl Isethionate C8-C18 (predominately C12-C14)
- DEFI = De-Esterified Fatty Isethionate C8-C18 (predominately, C12,16,18)
- pKa = 1.08 (will exist in the ionised form under environmental conditions)

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Tiered approach

Droge et al (2021) *Environ. Sci.: Processes Impacts*, 2021,23, 1930-1948


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PAPER

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

***In silico* profiling**

Tier 1 – BCF screening equation using membrane-water partition/distribution co-efficient (log K/D_{mlw})

Realistic sorption affinity to fish tissue
NO BIOTRANSFORMATION INCLUDED

Tier 2 – Higher tier model refinement (BIONIC v3)

INCLUDES BIOTRANSFORMATION

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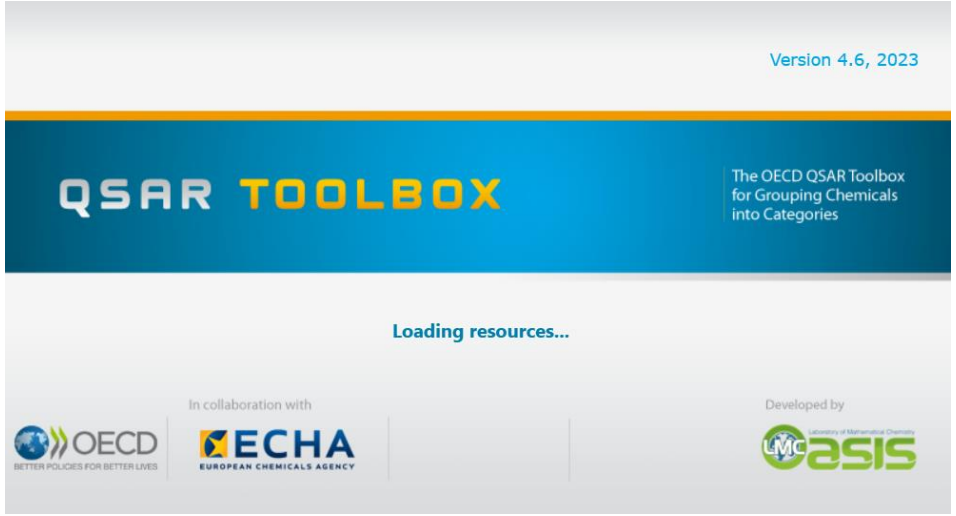
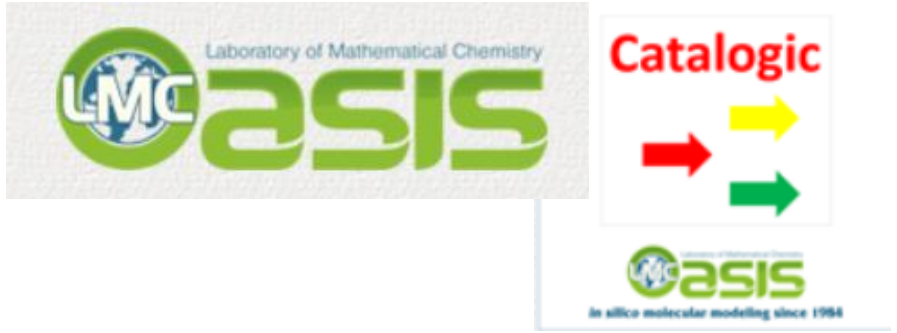
In silico screening

- Bioaccumulation profiling conducted for all components in each substance



Bioaccumulation – Metabolism Alerts
Bioaccumulation – Metabolism half-lives

'Fast' or 'very fast' biotransformation/ metabolism half-lives for all components



LogBCF generated using the BCF baseline model DP v.02.08 from CATALOGIC v5.16.1

Positive correlation with chain length, all components < 2000 L/kg but this is logK_{ow} based therefore of limited applicability to surfactants

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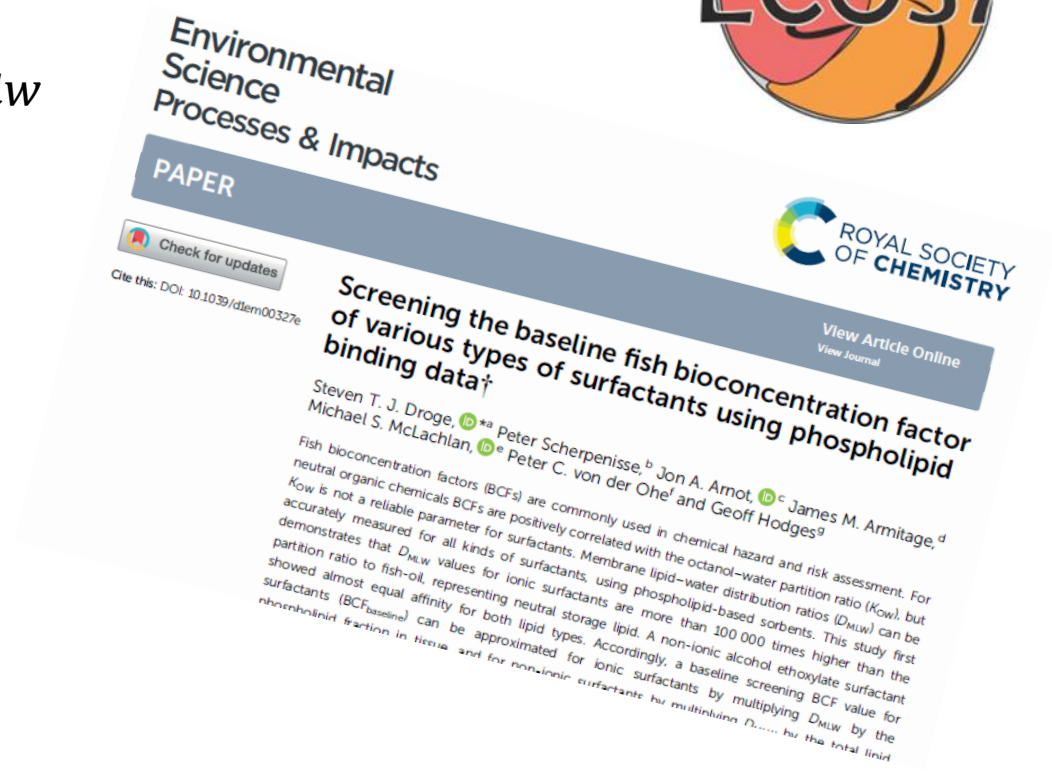
Tier 1 – LogD_{mlw} baseline screening

$$BCF_{baseline}\ (ionic\ surfactants) = 0.0125\ * D_{mlw}$$

where 0.0125 = phospholipid fraction of fish

or

$$logBCF = logD_{mlw} - 1.9$$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Cite this: DOI: 10.1039/d1em00327e

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ROYAL SOCIETY OF CHEMISTRY

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

Steven T. J. Droge, *[a] Peter Scherpenisse,[b] Jon A. Arnot,[c] James M. Armitage,[d] Michael S. McLachlan,[e] Peter C. von der Ohe[f] and Geoff Hodges[g]

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

Screening cut-offs:

≥ 5.2 (ionic) = BCF ≥ 2000 potentially B

≥ 5.6 (ionic) = BCF ≥ 5000 potentially vB

Droge et al (2021) *Environ. Sci.: Processes Impacts*, 2021,23, 1930-1948

Tier 1 – Log D_{mlw} baseline screening

Name	Chain length (CL)	$logD_{MLW}$	logBCF	BCF (L/kg)
Sodium caproyl isethionate	8	3.50	1.60	39.81
Sodium decanoyl isothionate	10	3.63	1.73	53.70
Sodium lauroyl isethionate	12	4.42	2.52	331.13
Sodium myristoyl isethionate	14	4.95	3.05	1122.02
Sodium palmitoyl isethionate	16	5.41	3.51	3235.94
Sodium stearoyl isethionate	18	5.92	4.02	10568.18
Sodium lauroyl methyl Isethionate	12(1)	4.79	2.89	776.25

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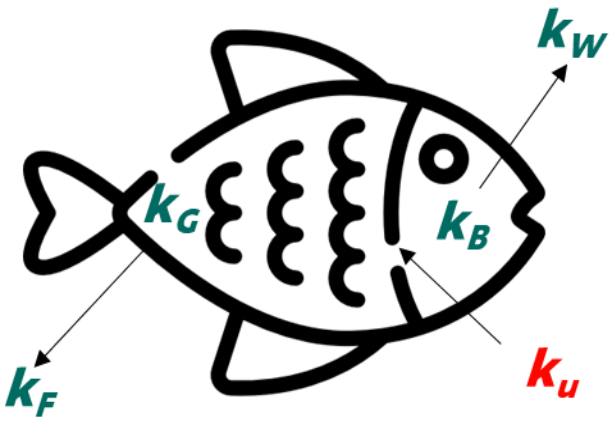
Tier 2 BIONIC model

Base of the BIONIC model

BCF_{ss*} = concentration in fish (C_B)/concentration in water (C_W) = k_U/k_E



$$\frac{\text{uptake}}{\text{elimination}} = \frac{\text{gill uptake } (k_U)}{\text{gill elimination } (k_W) + \text{Biotransformation } (k_B) + \text{faecal elimination } (k_F) + \text{growth dilution } (k_G)}$$

$$\frac{dC_B}{dt} = k_U C_W - k_E C_B$$

* steady-state, dC/dt = 0

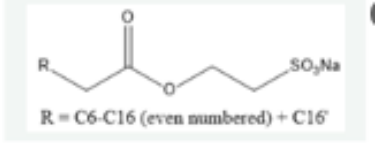
Armitage, J. M., Erickson, R. J., Luckenbach, T., Ng, C. A., Prosser, R. S., Arnot, J. A., Schirmer, K., & Nichols, J. W. (2017). Assessing the bioaccumulation potential of ionizable organic compounds: Current knowledge and research priorities. Environmental Toxicology and Chemistry, 36(4), 882–897. https://doi.org/10.1002/etc.3680

https://sites.google.com/view/bionic-v3/home

In vitro hepatic clearance S9 assay (OECD 319B)

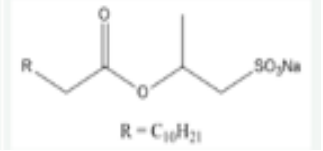
DEFI

EC: 287-024-7

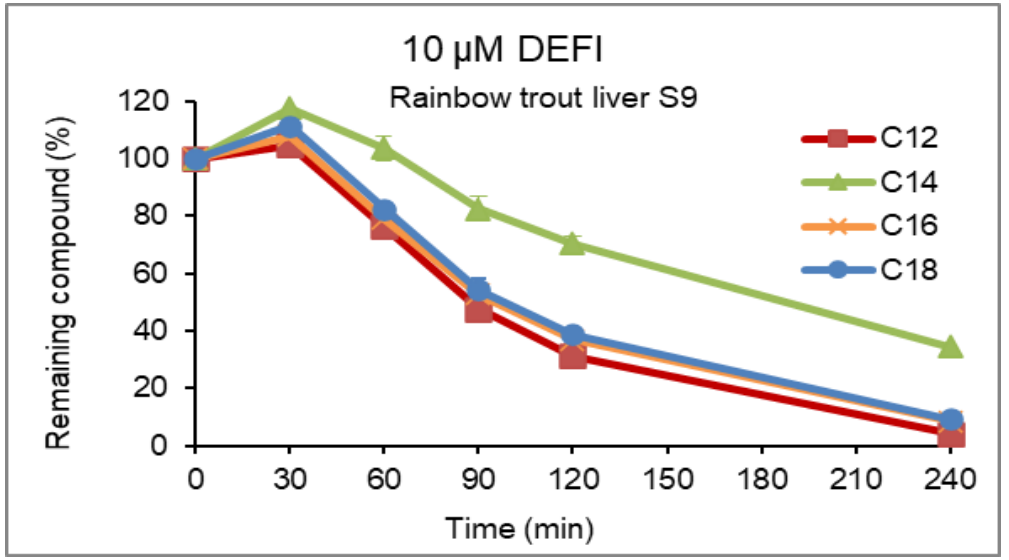


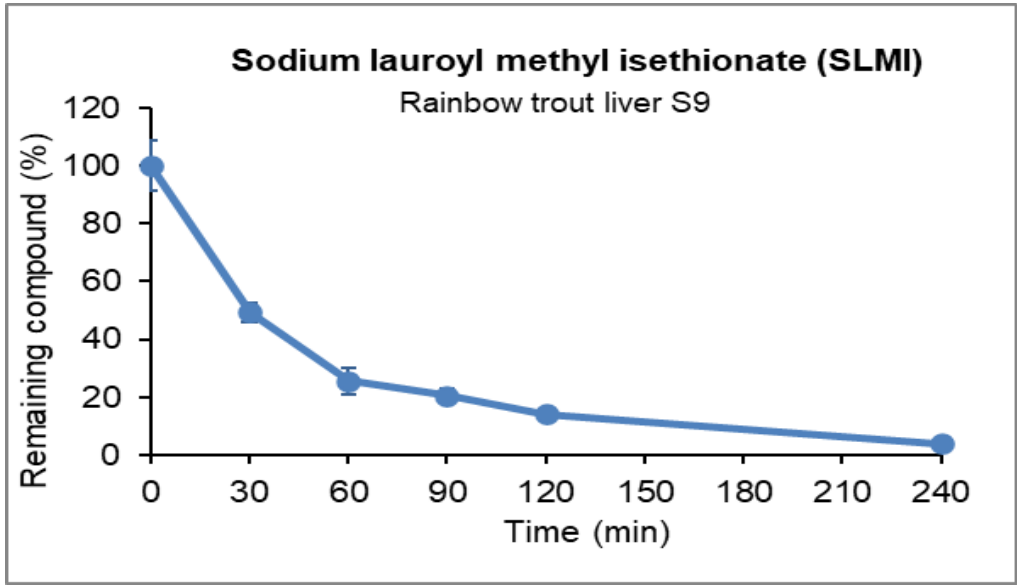
SLMI

EC: 700-150-3



	S9 IVIVE Biotransformation	
	k_e (min $^{-1}$)	$t_{1/2}$ (min)
C12	0.03	21.71
C14	0.01	89.10
C16	0.01	57.05
C18	0.01	58.04
C12 branched	0.01	54.89





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BIONIC - *In vitro in vivo* extrapolation (IVIVE)

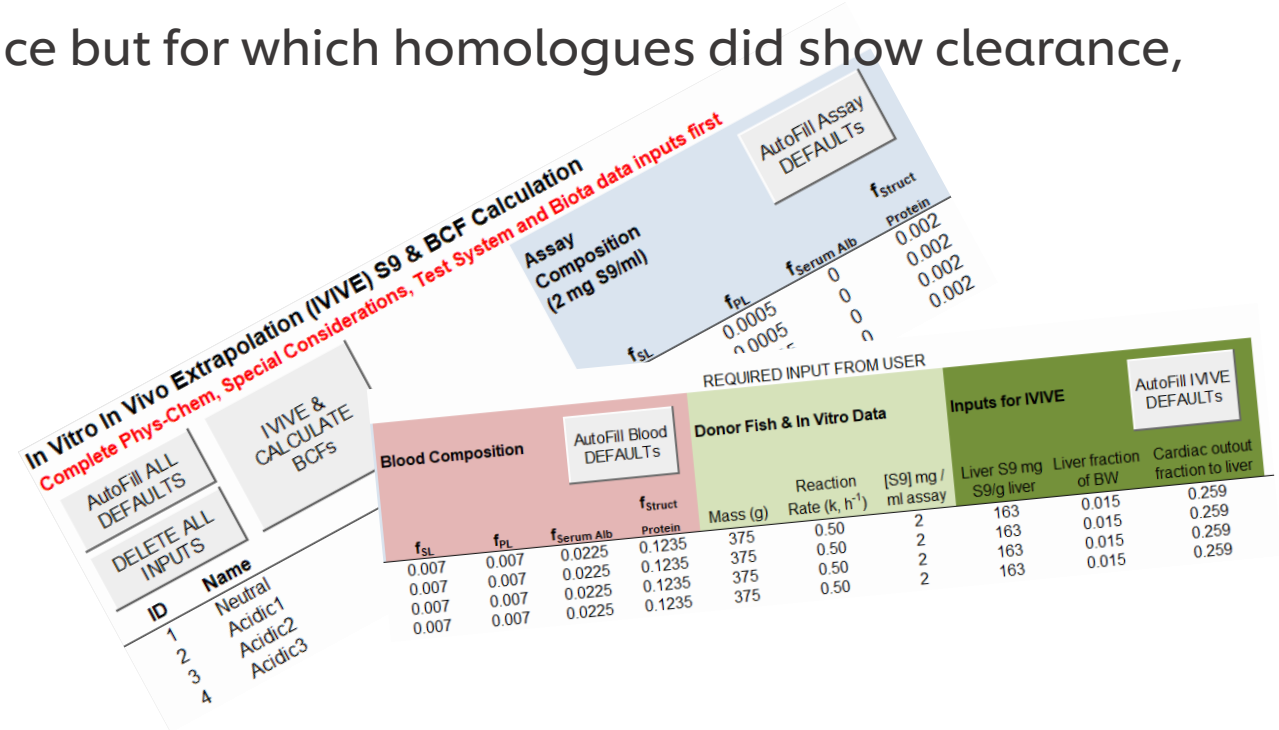
Special consideration for C8 & C10 isethionate constituents

- Ribbenstedt et al reports lowest detectable clearance rate (LL-S9) in OECD 319B for surfactants = 0.15 h^{-1}
- For surfactants without significant clearance but for which homologues did show clearance, estimated reaction rate of :

LL-S9
3

C12 clearance rate = 1.916 h^{-1} (measured)

C8 & C10 clearance rate = 0.05 h^{-1} (estimated)



Ribbenstedt et al. *Environ Sci & Tech* **2022** *56* (10), 6305-6314
DOI: 10.1021/acs.est.1c05543

BIONIC outputs

Chainlength	Uptake (L/kg/d) & Elimination rate constants (1/d)*					Total Elimination half-life (d)	Tier 2 BCF (L/kg)	Tier 1 BCF (L/kg)
	k_U	k_W	k_B	k_F	k_G			
C8	0.96	0.00185	0.04	0.0008	0.0016	15.28	21.90	**39.81**
C10	1.46	0.00193	0.04	0.0008	0.0016	16.78	36.03	**53.70**
C12	1.46	0.00087	0.24	0.0007	0.0016	2.84	6.76	**331.13**
C14	21.96	0.00229	0.07	0.0008	0.0016	9.92	315.25	**1122.02**
C16	49.38	0.00214	0.10	0.0007	0.0016	6.54	467.04	**3235.94**
C18	106.91	0.00160	0.10	0.0004	0.0016	6.51	1005.14	**10568.18**
C12 branched	11.05	0.00227	0.11	0.0008	0.0016	6.00	96.47	**776.25**

k_U = gill uptake, k_w = gill elimination, k_B = biotransformation, k_F = faecal elimination, k_G - growth dilution

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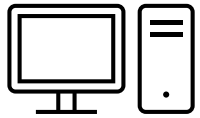
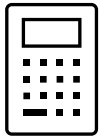
Final Weight of Evidence approach (submitted under Annex XI, section 1.2 "weight of evidence")



In silico profiling → Predicted fast/very fast metabolism for all components



$logBCF = logD_{mlw} - 1.9$

Tier 1 → Low potential for bioaccumulation for all components except C16 & C18 (without accounting for biotransformation)

Tier 2 → Rapid biotransformation results in low potential for bioaccumulation for all components (BCF < 2000)

Exposure data → Alkyl isethionates are readily biodegradable with high removal (>99%) in sewage treatment plants and half-life of 0.015 days) in treated effluent-surface water mixing zones

Alkyl isethionates have a low potential for bioaccumulation – further testing is scientifically unjustified and contrary to Article 25 of REACH

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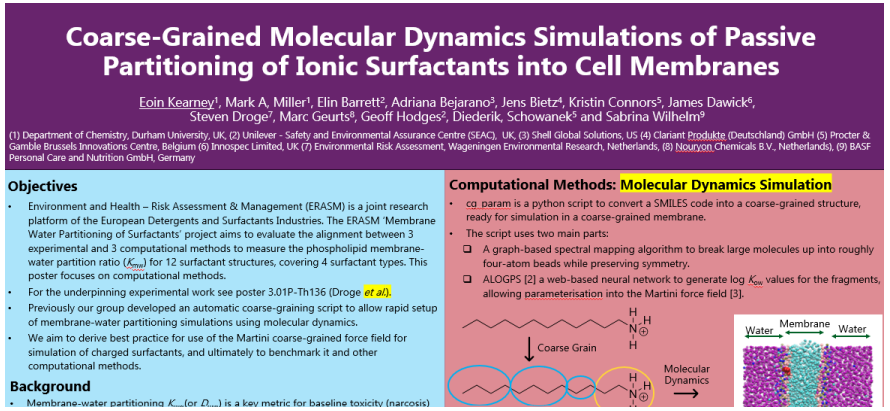
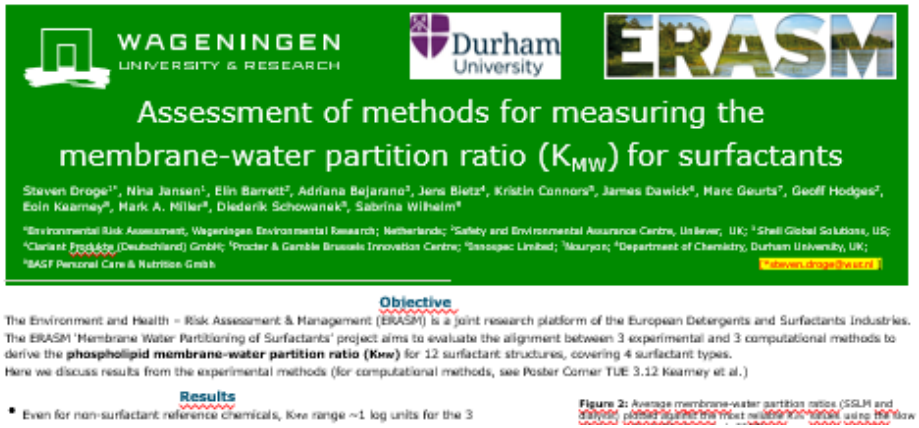
Future recommendations

- Standardised, robust and reliable empirical/computational methods for logK_{MW} e.g. OECD Guideline?

3.12.P-Tu247 Coarse-Grained Simulations of Passive Partitioning of Ionic Surfactants into Cell Membranes



3.01.P-Th136 Assessment of Methods for Determining The Membrane-Water Partition Ratio for Surfactants



- Investigate use of BIONIC v3 model across a wider range of surfactants types/classes to understand its applicability and limitations

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Thank You

Andrea Gredelj (Unilever)

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Unilever