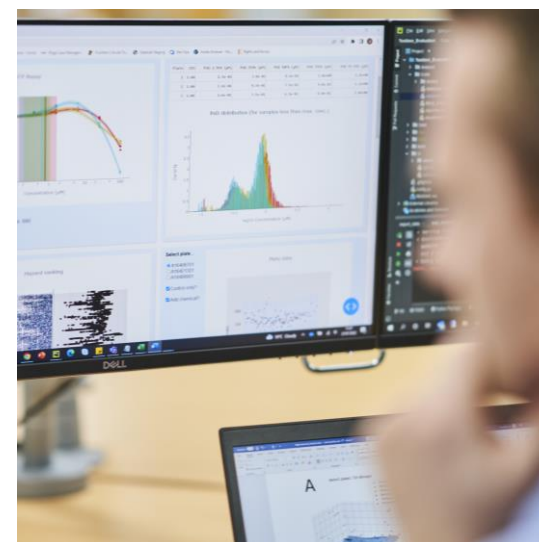
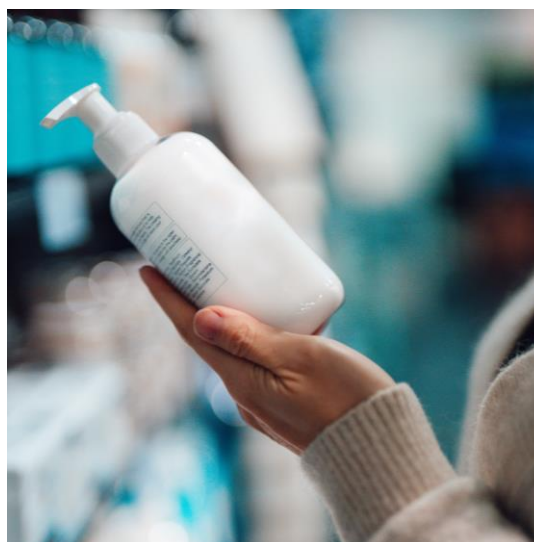


# Using An *In Silico* NAMs Approach To Predict Bioaccumulation In Fish: A Case Study For Anionic Surfactants Within A Regulatory Context



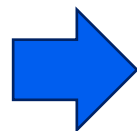
## Introduction

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



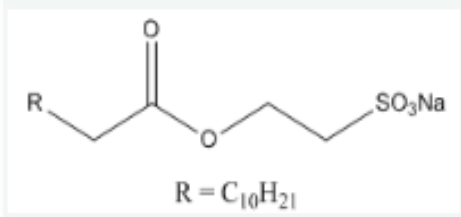
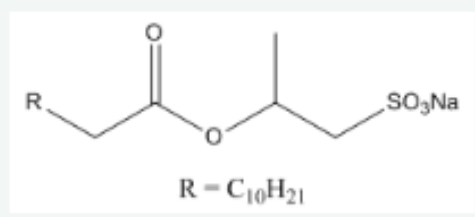
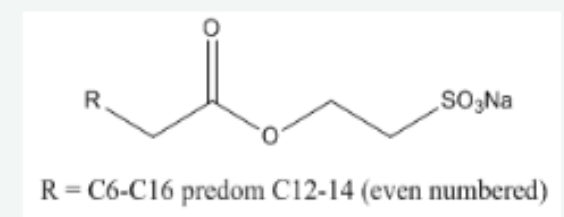
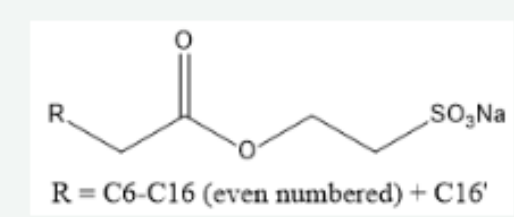
- *Hyalella azteca* bioconcentration test (HYBIT)
- Intrinsic hepatic/S9 clearance in vitro assays (OECD 319/IVIVE methods )
- QSAR models e.g. Episuite, T.E.S.T, VEGA

Specific consideration  
for surfactants



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

## 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	 <p>R = C<sub>10</sub>H<sub>21</sub></p>	 <p>R = C<sub>10</sub>H<sub>21</sub></p>	 <p>R = C6-C16 predom C12-14 (even numbered)</p>	 <p>R = C6-C16 (even numbered) + C16'</p>

- 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)

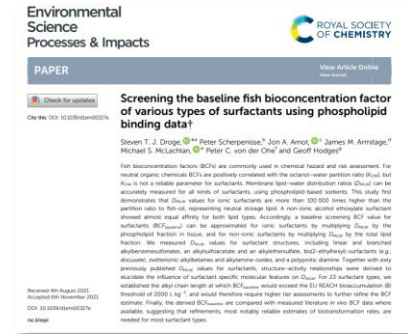
# Tiered approach

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

*In silico* profiling

**Tier 1 – BCF screening equation using membrane-water partition/distribution coefficient ( $\log K/D_{mlw}$ )**

**Tier 2 – Higher tier model refinement (BIONIC v3)**



Realistic sorption affinity to fish tissue  
NO BIOTRANSFORMATION INCLUDED

INCLUDES BIOTRANSFORMATION

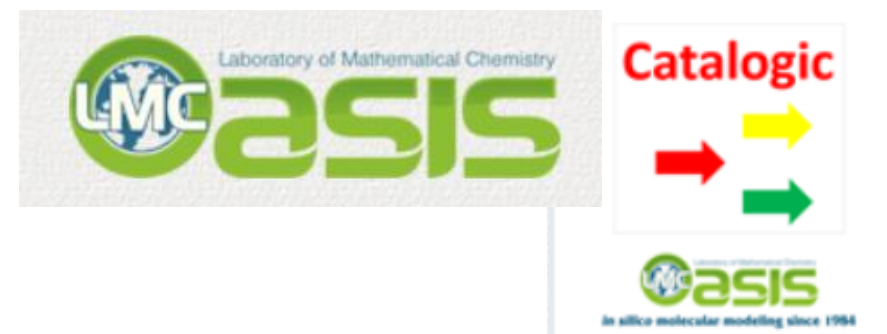
## *In silico* screening

- 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<sub>ow</sub> based therefore of limited applicability to surfactants**

## Tier 1 – Log $D_{mlw}$ baseline screening

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

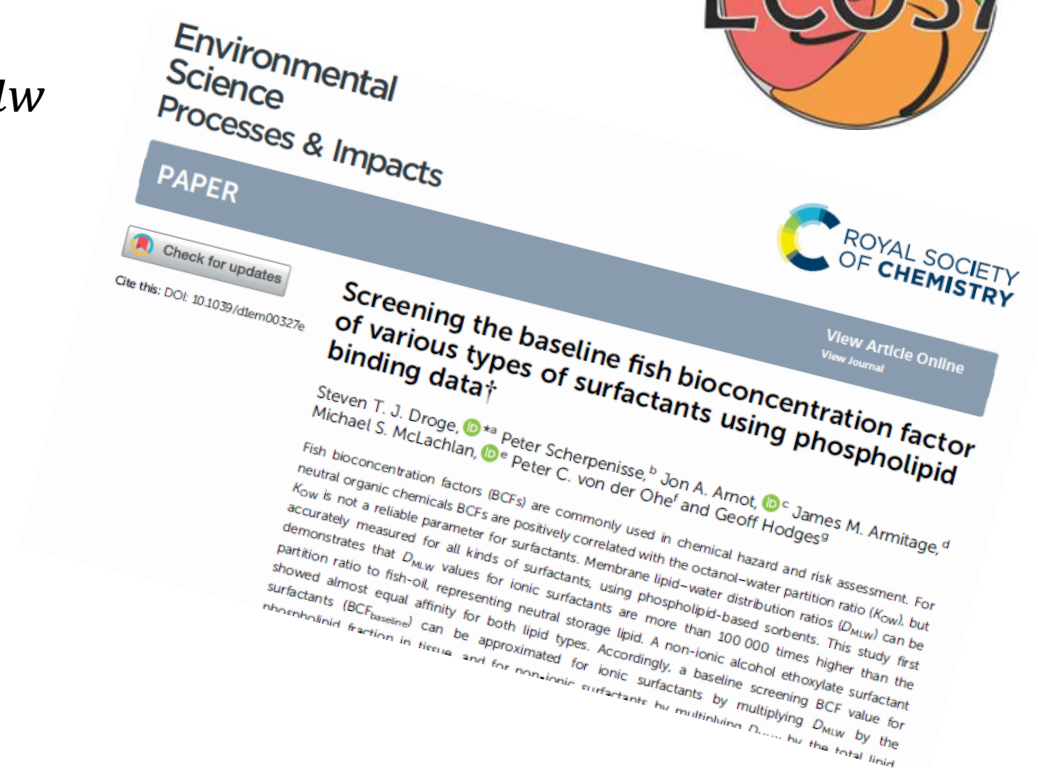
where 0.0125 = phospholipid fraction of fish

or

$$\log BCF = \log D_{mlw} - 1.9$$

Screening cut-offs:

$$\begin{aligned} \geq 5.2 \text{ (ionic)} &= BCF \geq 2000 \quad \text{potentially B} \\ \geq 5.6 \text{ (ionic)} &= BCF \geq 5000 \quad \text{potentially vB} \end{aligned}$$



## Tier 1 – Log $D_{mlw}$ baseline screening

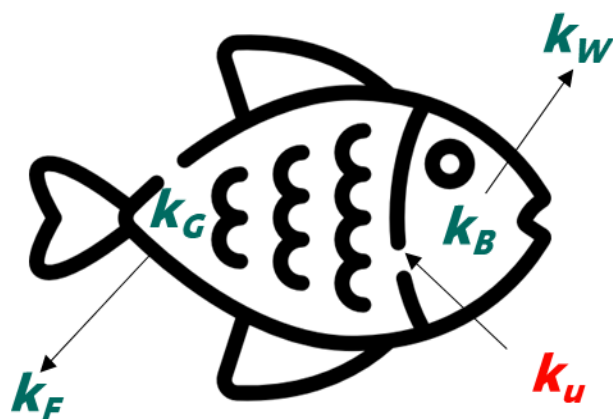
Name	Chain length (CL)	log $D_{MLW}$	logBCF	BCF (L/kg)
<b>Sodium caproyl isethionate</b>	8	3.50	1.60	39.81
<b>Sodium decanoyl isothionate</b>	10	3.63	1.73	53.70
<b>Sodium lauroyl isethionate</b>	12	4.42	2.52	331.13
<b>Sodium myristoyl isethionate</b>	14	4.95	3.05	1122.02
<b>Sodium palmitoyl isethionate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

## Tier 2 BIONIC model

### Base of the BIONIC model

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

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



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

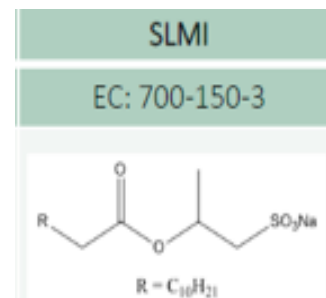
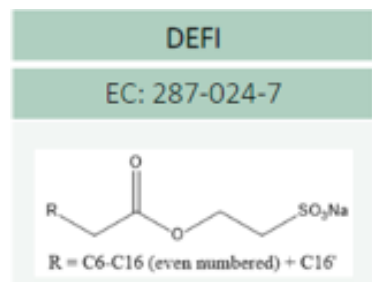


\* steady-state,  $dC/dt = 0$

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>

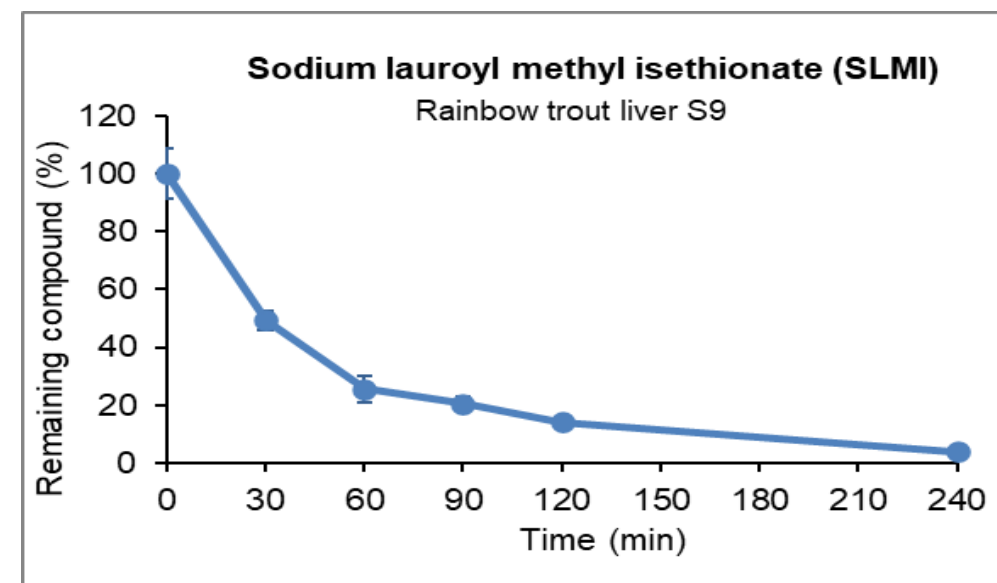
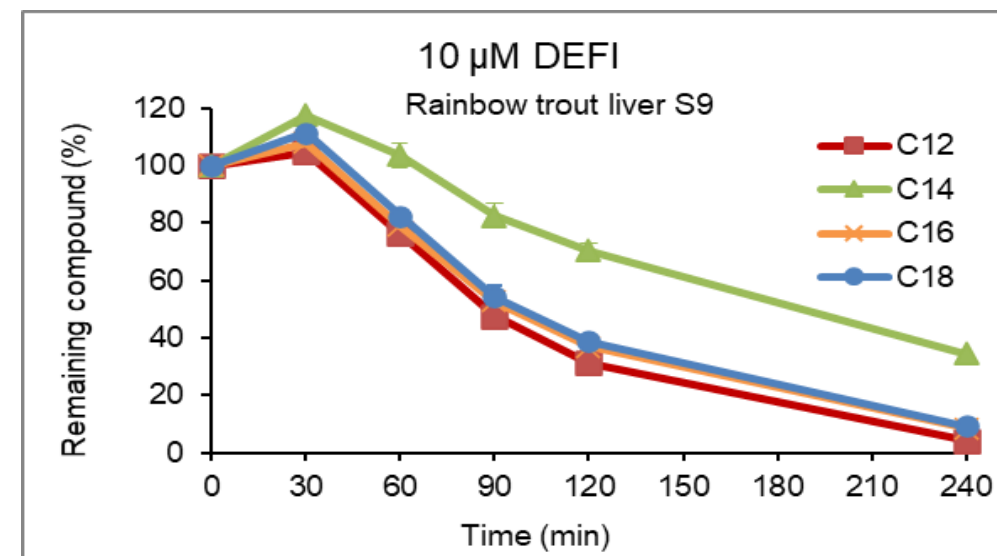


## *In vitro* hepatic clearance S9 assay (OECD 319B)



### S9 IVIVE Biotransformation

	$k_e$ (min <sup>-1</sup> )	$t_{1/2}$ (min)
<b>C12</b>	<b>0.03</b>	<b>21.71</b>
<b>C14</b>	<b>0.01</b>	<b>89.10</b>
<b>C16</b>	<b>0.01</b>	<b>57.05</b>
<b>C18</b>	<b>0.01</b>	<b>58.04</b>
<b>C12 branched</b>	<b>0.01</b>	<b>54.89</b>



## 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 =  $0.15 \text{ h}^{-1}$
- For surfactants without significant clearance but for which homologues did show clearance, estimated reaction rate of:

**LL-S9**  
**3**

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

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

**In Vitro In Vivo Extrapolation (IVIVE) S9 & BCF Calculation**  
Complete Phys-Chem, Special Considerations, Test System and Biota data inputs first

Assay Composition (2 mg S9/ml)

AutoFill Assay DEFAULTS

REQUIRED INPUT FROM USER

Blood Composition				Donor Fish & In Vitro Data			Inputs for IVIVE		
f <sub>SL</sub>	f <sub>PL</sub>	f <sub>Serum Alb</sub>	f <sub>struct Protein</sub>	Mass (g)	Reaction Rate (k, h <sup>-1</sup> )	[S9] mg / ml assay	Liver S9 mg S9/g liver	Liver fraction of BW	Cardiac output fraction to liver
0.007	0.007	0.0225	0.1235	375	0.50	2	163	0.015	0.259
0.007	0.007	0.0225	0.1235	375	0.50	2	163	0.015	0.259
0.007	0.007	0.0225	0.1235	375	0.50	2	163	0.015	0.259
0.007	0.007	0.0225	0.1235	375	0.50	2	163	0.015	0.259

AutoFill Blood DEFAULTS

AutoFill IVIVE DEFAULTS

IVIVE & CALCULATE BCFs

ID	Name
1	Neutral
2	Acidic1
3	Acidic2
4	Acidic3

AutoFill ALL DEFAULTS

DELETE ALL INPUTS

## 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	<b>39.81</b>
C10	1.46	0.00193	0.04	0.0008	0.0016	16.78	36.03	<b>53.70</b>
C12	1.46	0.00087	0.24	0.0007	0.0016	2.84	6.76	<b>331.13</b>
C14	21.96	0.00229	0.07	0.0008	0.0016	9.92	315.25	<b>1122.02</b>
C16	49.38	0.00214	0.10	0.0007	0.0016	6.54	467.04	<b>3235.94</b>
C18	106.91	0.00160	0.10	0.0004	0.0016	6.51	1005.14	<b>10568.18</b>
C12 branched	11.05	0.00227	0.11	0.0008	0.0016	6.00	96.47	<b>776.25</b>

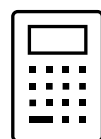
$k_U$  = gill uptake,  $k_W$  = gill elimination,  $k_B$  = biotransformation,  $k_F$  = faecal elimination,  $k_G$  - growth dilution

## 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



$$\log BCF = \log D_{mlw} - 1.9$$

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**

# Future recommendations

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

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

### Coarse-Grained Molecular Dynamics Simulations of Passive Partitioning of Ionic Surfactants into Cell Membranes

Eoin Kearney<sup>1</sup>, Mark A. Miller<sup>1</sup>, Elin Barrett<sup>2</sup>, Adriana Bejarano<sup>3</sup>, Jens Bietz<sup>4</sup>, Kristin Connors<sup>5</sup>, James Dawick<sup>6</sup>, Steven Droge<sup>7</sup>, Marc Geurts<sup>8</sup>, Geoff Hodges<sup>9</sup>, Diederik Schowaneck<sup>9</sup> and Sabrina Wilhelm<sup>9</sup>

(1) Department of Chemistry, Durham University, UK; (2) Unilever - Safety and Environmental Assurance Centre (SEAC), UK; (3) Shell Global Solutions, US; (4) Clariant Produkte (Deutschland) GmbH; (5) Procter & Gamble Brussels Innovations Centre, Belgium; (6) Innospec Limited, UK; (7) Environmental Risk Assessment, Wageningen Environmental Research, Netherlands; (8) Nouryon Chemicals B.V., Netherlands; (9) BASF Personal Care and Nutrition GmbH, Germany

**Objectives**

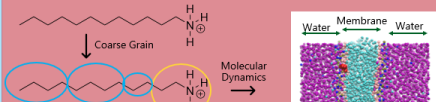
- Environment and Health – Risk Assessment & Management (ERASM) is a joint research platform of the European Detergents and Surfactants Industries. The ERASM 'Membrane Water Partitioning of Surfactants' project aims to evaluate the alignment between 3 experimental and 3 computational methods to measure the phospholipid membrane-water partition ratio ( $K_{MW}$ ) for 12 surfactant structures, covering 4 surfactant types. This poster focuses on computational methods.
- For the underpinning experimental work see poster 3.01P-Th136 (Droge *et al.*).
- Previously our group developed an automatic coarse-graining script to allow rapid setup of membrane-water partitioning simulations using molecular dynamics.
- We aim to derive best practice for use of the Martini coarse-grained force field for simulation of charged surfactants, and ultimately to benchmark it and other computational methods.

**Background**




- Membrane-water partitioning ( $K_{MW}$  for  $D_{50}$ ) is a key metric for baseline toxicity (narcosis)

**Computational Methods: Molecular Dynamics Simulation**

- cg\_param is a python script to convert a SMILES code into a coarse-grained structure, ready for simulation in a coarse-grained membrane.
- The script uses two main parts:
  - A graph-based spectral mapping algorithm to break large molecules up into roughly four-atom beads while preserving symmetry.
  - ALOGPS [2] a web-based neural network to generate  $\log K_{MW}$  values for the fragments, allowing parameterisation into the Martini force field [3].



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

### Assessment of methods for measuring the membrane-water partition ratio ( $K_{MW}$ ) for surfactants

Steven Droge<sup>1\*</sup>, Nina Jensen<sup>1</sup>, Elin Barrett<sup>2</sup>, Adriana Bejarano<sup>3</sup>, Jens Bietz<sup>4</sup>, Kristin Connors<sup>5</sup>, James Dawick<sup>6</sup>, Marc Geurts<sup>7</sup>, Geoff Hodges<sup>9</sup>, Eoin Kearney<sup>8</sup>, Mark A. Miller<sup>8</sup>, Diederik Schowaneck<sup>9</sup>, Sabrina Wilhelm<sup>9</sup>

\*Environmental Risk Assessment, Wageningen Environmental Research; Netherlands; <sup>2</sup>Safety and Environmental Assurance Centre, Unilever, UK; <sup>3</sup>Shell Global Solutions, US; <sup>4</sup>Clariant Produkte (Deutschland) GmbH; <sup>5</sup>Procter & Gamble Brussels Innovations Centre; <sup>6</sup>Innospec Limited; <sup>7</sup>Nouryon; <sup>8</sup>Department of Chemistry, Durham University, UK; <sup>9</sup>BASF Personal Care & Nutrition GmbH

**Objective**

The Environment and Health – Risk Assessment & Management (ERASM) is a joint research platform of the European Detergents and Surfactants Industries. The ERASM 'Membrane Water Partitioning of Surfactants' project aims to evaluate the alignment between 3 experimental and 3 computational methods to derive the **phospholipid membrane-water partition ratio ( $K_{MW}$ )** for 12 surfactant structures, covering 4 surfactant types. Here we discuss results from the experimental methods (for computational methods, see Poster Corner TUE 3.12 Kearney *et al.*)

**Results**

- Even for non-surfactant reference chemicals, the range  $\sim 1$  log units for the 3

**Figure 2:** Average membrane-water partition ratios (SGLH and SGLH) plotted against the most reliable  $K_{MW}$  values using the low

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

# Thank You

**Andrea Gredelj (Unilever)**

**Geoff Hodges (Unilever)**

**Steve Gutsell (Unilever)**

**Nicola Haywood (Unilever)**

**James Dawick (Innospec)**

**Lauren McAnally (Innospec)**

**Marc Geurts (Nouryon)**

**James Armitage (for answering our questions on BIONIC!)**

