Executive summary

The CEFIC-LRI project ECO-37 "D-BASS: **D**eveloping a **B**ioaccumulation **A**ssessment **S**trategy for **S**urfactants" had the general objective to conduct hypothesis-driven experimentation to develop a scientifically defensible, mechanistically-based method for bioaccumulation assessment of surfactants. The current final ECO37 Deliverable (D16) presents an overview of all data collected during ECO37, and the key insights gained during analysis of the data. More details are provided in the Deliverables D1-D15.

Many types of surfactants are used in households and industrial processes, and although adequate waste water treatment is able to remove large fractions, there is a risk of environmental contamination and accumulation of these chemicals in food webs. The bioconcentration factor (BCF) is the ratio between the chemical concentration accumulated in the tissue of an organism exposed to contaminated water (C_{org}), and the aqueous concentration (C_{aq}) at steady-state (i.e., no significant change in concentrations over time). For chemicals with a hydro*phobic*/lipophilic character, the BCF (C_{org}/C_{aq}) can be above a certain criterion which would be a cause of regulatory concern. In the PBT assessment used in REACH, the criterion to classify a chemical as bioaccumulative is 2000.

Although all surfactant molecules have a hydro*philic* headgroup unit, they also all have a hydro*phobic* chain of hydrocarbon units (alkyl chain) that together give these chemicals their surface active properties. The hydrophobic part of a surfactant molecule may result in it having a BCF above regulatory criteria. The BCF for surfactants such as nonionic alcohol ethoxylates (AEO) and anionic LAS are relatively well studied,[1-4] and mostly indicate the BCF to be well below regulatory criteria. However, there are scarce measured BCF data for other surfactant types.[5-7] As a result, chemical risk assessment for most types of surfactants depends on estimates of BCF, not measurements. In addition to BCFs the octanol-water partition coefficient (K_{OW}) is often used to screen chemicals for their bioaccumulative properties. For example, under REACH, chemicals with log K_{OW} > 4.5 are identified for "B" assessment.

The amphiphilic character of all surfactants and the charged unit in ionogenic surfactants, render these chemicals challenging for regulatory environmental assessment. One problem encountered in regulatory assessment is that K_{OW} values are highly uncertain for all surfactant types, both when derived experimentally and when predicted.[8] More importantly for BCF assessment, though, multiple studies have shown that octanol-water distribution ratios do not reflect the comparatively high affinity of ionic organic compounds for phospholipid cell membranes.[9-11] BCF predictions for ionic surfactants are therefore likely to be much higher than current estimations based on reported logP values.[12,13] To address uncertainty in quantifying the fish-water partitioning behaviour of surfactants in aqueous systems, the ECO37 project measured sorption affinities of surfactants to artificial cell membranes (D_{MLW}) *in vitro,* and BCFs *in vivo*, for a wide range of surfactants.

The ECO37 starting hypothesis was that measured sorption affinities of surfactants to artificial cell membranes would provide a major advancement in a more realistic and scientifically defensible assessment of the BCF for surfactants. Since fish BCF values for several surfactants (AEO and LAS) have been shown to be lower than expected from equilibrium partitioning due to significant elimination of biotransformation metabolites, realistic BCF assessment of other types of surfactants also needs to

account for active elimination via biotransformation. Information on the biotransformation potential of surfactants is also mostly lacking in regulatory assessment. The ECO37 project aimed to address this second issue by creating a data matrix for intrinsic *in vitro* hepatic clearance rates. These parameters should be incorporated into a suitable BCF model package, and tested (evaluated) by *in vivo* BCF data.

The project was divided into three work packages (WP) that would work in synergy to achieve the objective of an improved scientific basis for the BCF assessment of surfactants. WP1 aimed to create a sound data matrix of measured *in vitro* parameters on tissue-water distribution and intrinsic hepatic clearance rates. WP2 aimed to derive a systematic series of newly measured bioconcentration factors of surfactants in fish, with a particular focus on critical knowledge gaps for cationic surfactants. WP3 aimed to further refine an existing bioaccumulation model for ionogenic compounds ("BIONIC") and evaluate the model performance with the *in vitro* parameters from WP1, against the *in vivo* BCF values from WP2.

WP1 measured two key *in vitro* parameters for BCF assessment for >40 surfactants – the membrane lipid/water distribution coefficient (D_{MLW}), and the intrinsic *in vivo* clearance rate ($CL_{int,in\ vivo}$, in mL h^{-1} g $liver^{-1}$) determined with rainbow trout liver S9 (RT-S9). D_{MLW} is used to describe sorption affinity to the phospholipid fraction as the major sorptive tissue component for surfactants (Droge, 2019, ES&T 53, 760-770), and $CL_{int,in\ vivo}$ is further converted to a whole body biotransformation rate for a 10 g fish ($k_{B,N}$, in d^{-1}) using *in vitro-in vivo* extrapolation (IVIVE) models. Although batch sorption studies with artificial liposome bilayers provide high confidence D_{MLW} values, there is no OECD guideline available yet. Chromatographic retention capacity factors, using HPLC columns with immobilized artificial membrane (IAM, with phospholipid monolayers on silica) allow for efficient, readily standardized, high throughput measurements that are demonstrated to align with liposomal D_{MLW} values. For surfactants with D_{MLW} values beyond log 6, experimental measurements are not feasible though for any of the assays, and it is shown that one can rely on measured series of shorter homologue surfactants instead, or apply a surfactant specific D_{MLW}-QSARs. *In vitro* hepatic clearance rates were obtained according to OECD 319B, and can be determined for any chemical that dissolves. These $CL_{int,in\ vivo}$ values provide for experimental support for a weigh of evidence approach on fish biotransformation rates alongside QSAR-based estimates of $k_{B,N}$. Including previously published data, the data matrix for these two *in vitro* parameters spans 23 surfactant classes, and provides a sound basis to perform a Baseline Screening BCF that circumvents the logP uncertainty. Preliminary "critical chain lengths" have been derived for all evaluated surfactant types, below which no further refinements of BCF would be required. Biotransformation rate estimates and effect of ionization on gill membrane permeation are refinements that would be taken into account with the surfactant-adapted BIONIC-BCF model in WP3.

WP2 measured *in vivo* fish BCF values for 11 anionic surfactant structures, and 12 cationic surfactant structures using uptake/elimination studies. The cationic surfactants included 10 alkylamines (4 primary, 2 secondary, and 4 tertiary amines) and 2 quaternary alkyltrimethylammonium compounds (ATMACs). A positive correlation between BCF and the alkyl chain length of both types of surfactants was observed. Four of the tested chemicals (protonated alkylamines with chain length of C_{13}, C_{14}, and C_{16}) had BCFs in excess of 2000 L kg^{-1}, the threshold for bioaccumulative substances in the REACH regulation. This indicates that bioaccumulation of surfactants can be relevant in a regulatory context and underscores the need for better understanding of the underlying processes. For all cationic surfactants, additional fish

tissue distribution experiments were performed. Much lower BCFs for QAC cations relative to analogue alkylamines was observed, which relates to lower uptake of the permanently charged QAC across the gills. BCF measurements for six cationic surfactants at a lower pH indicated lower BCF values for alkylamines, but not for QAC, demonstrating the influence of the minor neutral amine fraction on gill uptake rates. Cationic surfactants are analytically and technically challenging chemicals due to the surface active property, which results in increased potential losses, that are more difficult to prevent or control, for surfactants with carbon chain lengths $\geq C_{16}$. For tested cationic surfactants with chain lengths up to C_{16}, aqueous concentrations in the *in vivo* BCF study (adapted OECD 305) could be kept stable for 2 weeks in the flow through system, although chemicals with C_{16} chain lengths had concentrations less than 50% of nominal. For anionic surfactants (also up to C_{16}), more rapid equilibration with fish and more rapid elimination from fish in comparison to the cationic surfactants, allowed for shorter uptake and elimination phases. Testing with longer chain surfactants may pose additional technical and analytical challenges with fish BCF testing.

WP3 refined the BIONIC V2 model, a mechanistically-based bioconcentration model for aquatic organisms established after CEFIC-LRI ECO21, to additionally include bioavailability, permanently charged surfactant chemicals, ion trapping in low pH lysosome, membrane specific properties, and intrinsic *in vitro* hepatic clearance rates using current IVIVE models. The BIONIC model refines the "Baseline Screening BCF" values which are based only on D_{MLW}. BIONIC adequately captures the influence of biotransformation for nonionic AEOs and most anionic surfactants, and the limited gill permeation of QACs. For alkylamine surfactants, the current parameterization appears to be insufficient because *in vivo* BCF values are consistently higher than model calculations that include biotransformation on the basis of the *in vitro* RT-S9 substrate depletion rates ($CL_{int,in\ vivo}$). To deepen our understanding of bioconcentration of surfactants, we need to address data gaps in biotransformation rates, sorption affinities to blood proteins and structural proteins, measurements of volume of distribution (blood:tissue distribution), more detailed physiology based uptake and assimilation efficiency for ionogenic chemicals, and surfactant bioavailability in the water column.