Suitability of the RTgill-W1 Cell Line Assay (OECD TG 249) to Predict Acute Fish Toxicity for Surfactants

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Background and Key Objective

- The RTgill-W1 Fish Cell Line Acute Toxicity assay (OECD TG 249) offers a viable alternative to *in vivo* testing, showing good correlation with existing fish data. However, there are limited data to support its application to surfactants.
- The Environment and Health – Risk Assessment & Management (ERASM) Task Force is working to gather new and existing high-quality *in vitro* (OECD 249) data on surfactants, to compare with existing *in vivo* (OECD TG 203) and fish embryo toxicity data (OECD TG 236) to inform regulatory practices and the wider surfactant industry.

Data collation

- Existing *in vivo* and *in vitro* experimental data (OECD TG 203, OECD TG 249) were collated from external sources (e.g. ECHA dossiers, scientific literature) and supplemented with additional proprietary data from ERASM TaskForce members, for a range of nonionic, anionic, cationic and amphoteric surfactants.
- Surfactants frequently exist as multi-constituents, UVCBs or polymers, thus detailed compositional data was gathered to ensure adequate interpretation of the toxicity data.
- *In vivo* studies with analytically confirmed exposure concentrations were prioritised over nominal concentrations.
- High quality *in vivo* fish data were available for all surfactant classes, with the majority of data being for nonionic surfactants. RTgill-W1 data was extensively available for anionic surfactants.
- Only a limited number of fish embryo toxicity (OECD TG 236) data have been identified thus far (primarily for amphoteric surfactants) and have therefore not been included in this analysis.

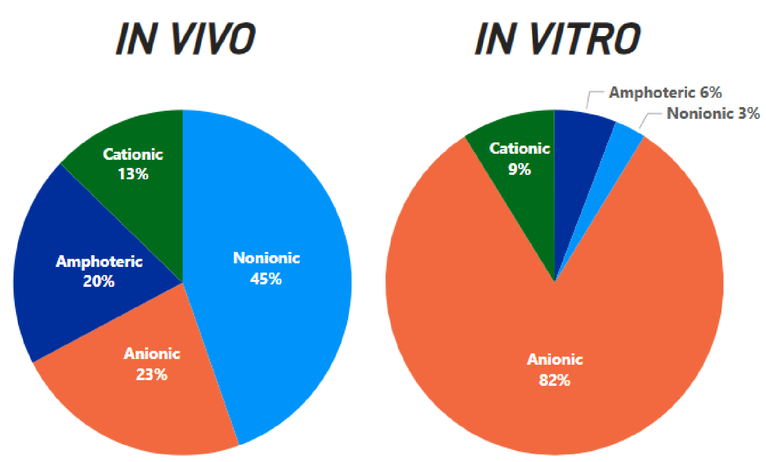


Figure 1. *Literature based In vivo* and *in vitro* data for cationic, anionic, amphoteric, and nonionic surfactants. *92 in vivo entries and 35 in vitro entries were collected. 14 entries overlap the two datasets.*

N.B. OECD 236 data has not been included

Correlation of existing *in vivo/in vitro* data across all surfactant classes

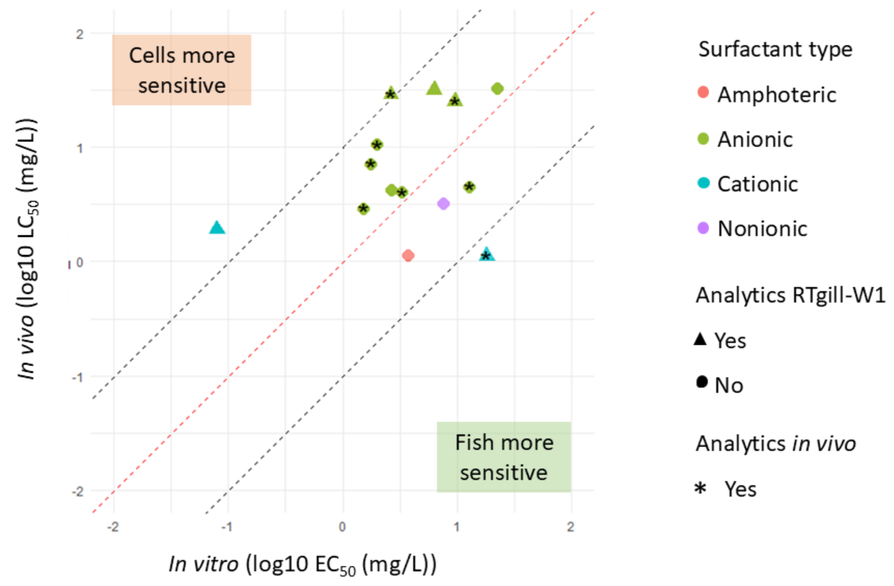


Figure 2. Correlation between *in vivo* and *in vitro* log10 LC50/EC50 data for four surfactant classes (amphoteric, anionic, cationic, and nonionic).

Unity line is dashed red line. 10-fold uncertainty is dashed black lines.

- Values for majority of chemicals fall within one order of magnitude from unity (Figure 2).
- Due to the lack of data across surfactant types, a best fit line for individual surfactant types could not be generated.
- For anionic surfactants, the *in vivo* and *in vitro* data suggest a tendency for cells to be slightly more sensitive than the fish with none of the values falling below the 10-fold uncertainty line indicating that the OECD TG 249 is likely protective for anionic surfactants.
- Cationic surfactants tended to fall outside of this range, though some uncertainty exists with *in vivo* data for one chemical since exposure duration was only 24hrs.

Experimental Phase – Preliminary Results

- Further OECD TG 249 data is being generated to expand the dataset and strengthen comparisons.
- Experimental work is still on-going however preliminary results for anionic and amphoteric surfactants are shown in Table 1.
- Exposure concentrations have been analytically determined. EC50 values are based on geometric mean of measured concentrations.
- *In vivo* data selection based on best available for data quality (e.g. compositional data, measured concentrations etc).

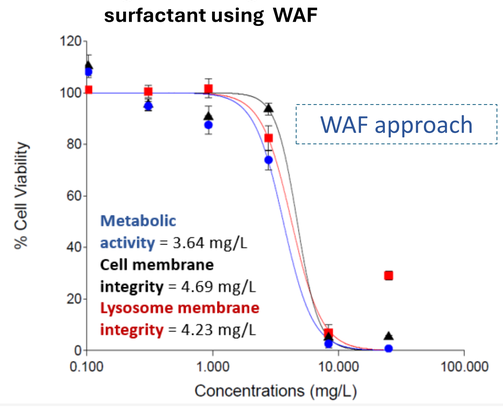
Table 1. Preliminary data for completed *in vitro* studies

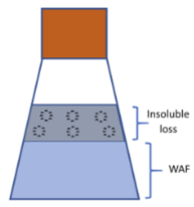
Surfactant Type	Surfactant class	CAS/EC		*In vitro 24 hr* EC50 (mg/L)*			*In vivo* 96 hr LC50 (mg/L)**
				Metabolic activity	Cell membrane integrity	Lysosome membrane integrity	
Nonionic	C12-13, branched & linear, alcohol ethoxylate (EO 1-2.5)	160901-19-9/ 500-457-0	EC50	1.17	1.76	**1.08**	1.19 (m) (*P. promelas*)
			95% C.I.	0.88 – 1.46	1.47 – 2.04	**0.8-1.37**	0.89-1.45
	C10 alcohol ethoxylate (1-2.5 EO)	26183-52-8/ 500-046-6	EC50	**7.29**	12.01	11.05	4.97 (m) (*P. promelas*)
			95% C.I.	**4.04 – 10.53**	6.14 – 17.88	8.58 – 13.53	3.81-6.49
Amphoteric	C12-C14 Alkyl Dimethyl Betaine (ADB)	66455-29-6/ 931-700-2	EC50	**12.29**	23.69	15.28	4.44 (n) (*D. rerio*)
			95% C.I.	**10.98 – 13.60**	9.83 – 37.55	13.35 – 17.20	
	C12-C14 Alkyl Dimethyl Amine Oxide (ADAO)	308062-28-4/ 931-292-6	EC50	**6.04**	8.9	7.96	2.7 (n) (*P. promelas*)
			95% C.I.	**5.15 – 6.92**	3.80 – 14.00	5.56 – 10.36	

m = mean measured concentration, n = nominal concentration 95% C.I . = 95% confidence intervals
* Geometric mean of analytically determined exposure concentrations ** existing literature data

- As testing of cationic surfactants is expected to be challenging due to their adsorptive nature, both a Water Accommodated Fraction (WAF) approach and solvent dosing (using organic solvent) are compared.
- Initial rangefinder results of WAF vs solvent dosing approaches for cationic surfactants suggest results are comparable based on nominal loading rate (Figure 3a & b).

Figure 3a. Rangefinder results for cationic surfactant using WAF





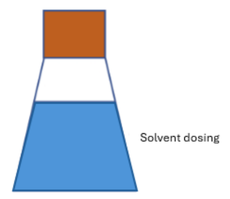
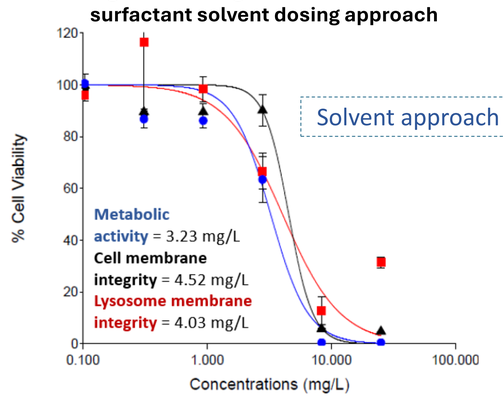


Figure 3b. Rangefinder results for cationic surfactant solvent dosing approach



Preliminary conclusions & Next Steps

- Initial results indicate that *in vitro* data for nonionic and amphoteric surfactants is indicative of *in vivo* acute toxicity. Additional testing is ongoing for a single anionic (C12-C13 alkyl benzene sulphonate) and two cationic (primary fatty amine ethoxylate) surfactants.
- Perform thorough comparisons between new experimental data from the RTgill-W1 cell line and existing fish acute toxicity data (OECD TG 203) as well as fish embryo toxicity data (OECD TG 236) to ensure robust conclusions.
- Offer guidance on the applicability of OECD TG 249 as a suitable alternative to using vertebrates in surfactant testing.

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