

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.

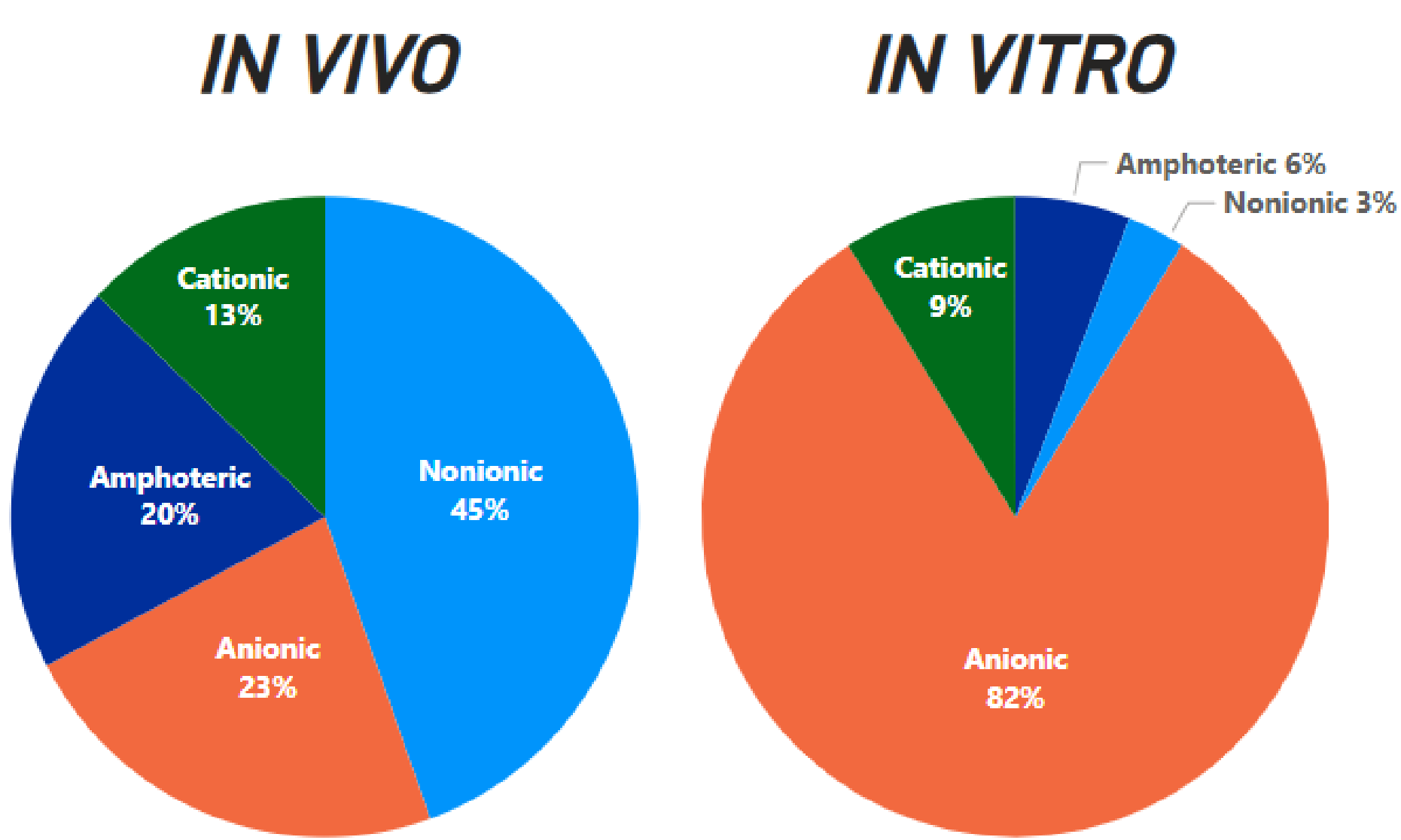
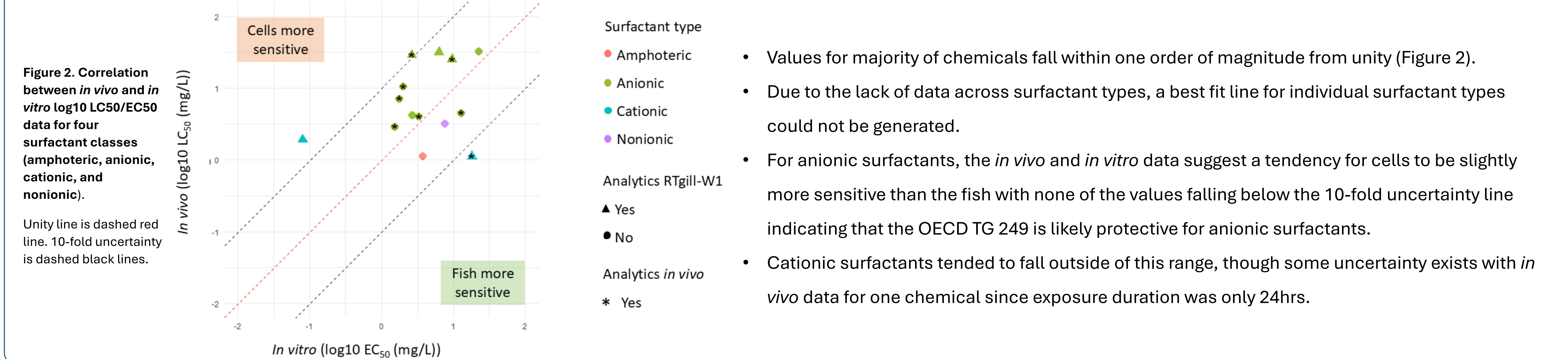


Figure 1. Literature based *in vivo* and *in vitro* data for cationic, anionic, amphoteric, and nonionic surfactants. 92 *in vivo* entries and 35 *in vitro* entries were collected. 14 entries overlap the two datasets.

N.B. OECD 236 data has not been included

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



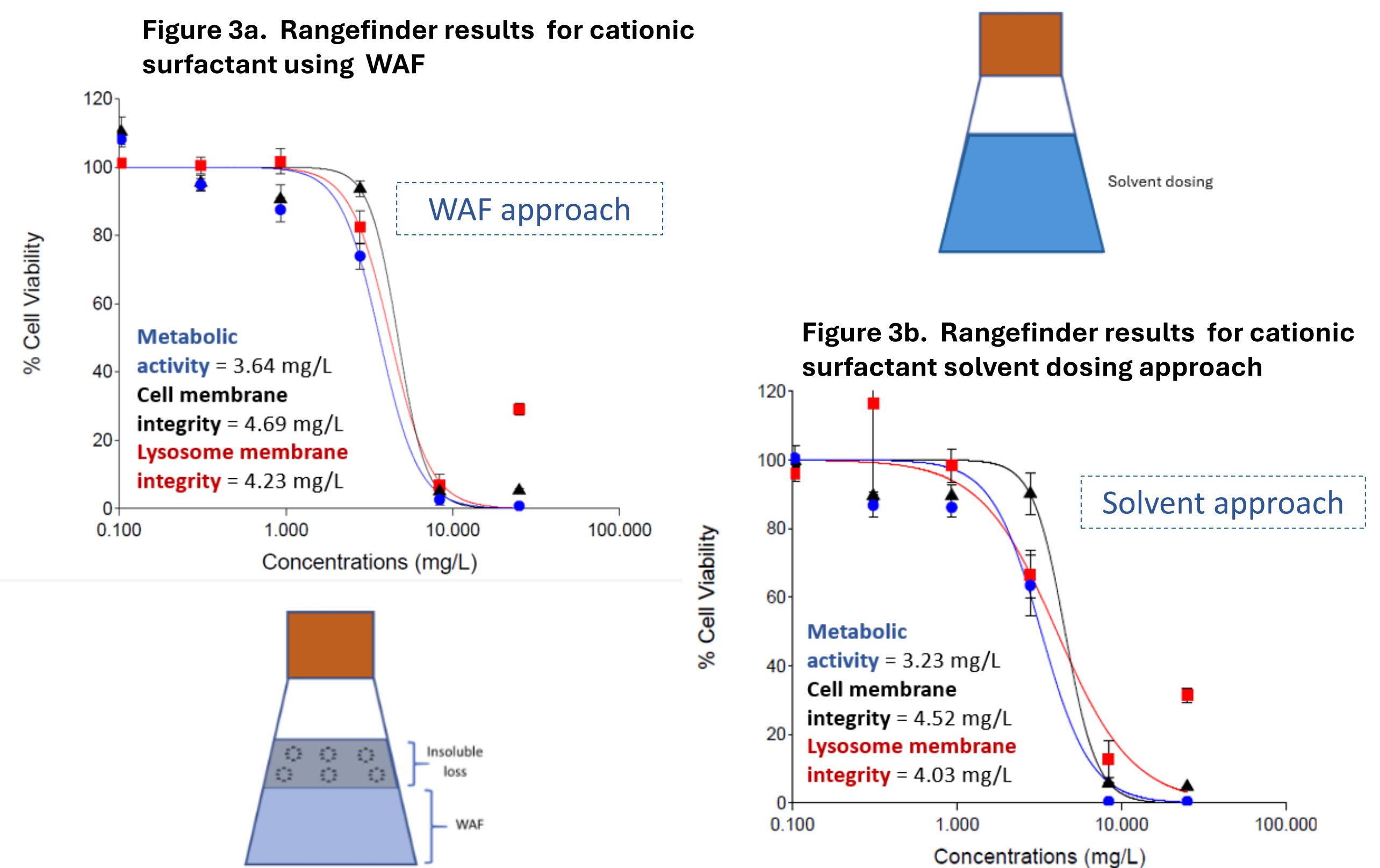
- Values for majority of chemicals fall within one order of magnitude from unity (Figure 2).
- Due to the lack of data across surfactant types, a best fit line for individual surfactant types could not be generated.
- For anionic surfactants, the *in vivo* and *in vitro* data suggest a tendency for cells 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).
- 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).

Table 1. Preliminary data for completed <i>in vitro</i> studies							
Surfactant Type	Surfactant class	CAS/EC	<i>In vitro</i> 24 hr EC50 (mg/L)*			<i>In vivo</i> 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) (<i>P. promelas</i>)
			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) (<i>P. promelas</i>)
			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) (<i>D. rerio</i>)
			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) (<i>P. promelas</i>)
			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



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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