# EVALUATION OF THE RTgill-W1 CELL LINE ASSAY (OECD TG 249) FOR PREDICTING ACUTE FISH TOXICITY OF SURFACTANTS

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## BACKGROUND

- The RTgill-W1 Fish Cell Line Acute Toxicity assay (OECD TG 249) demonstrates good correlation with existing *in vivo* data across multiple substance types. However, data is limited for surfactants.
- Surfactants can exist as mono- or multi-constituent and/or UVCBs, and their surface-active nature, solubility, lipophilicity and/or presence of ionizable functional groups can make the application of standard and alternative methodologies challenging.
- An Environment and Health – Risk Assessment & Management (ERASM) Task Force has gathered existing high-quality in vitro RTgill-W1 data and generated new experimental data to evaluate the applicability of OECD TG 249 for surfactants.
- *In vivo* data selection prioritized high-quality available data (e.g., compositional data, measured concentrations).

## PRELIMINARY DATA

- Existing OECD TG 249 and historical OECD TG 203 data align within one order of magnitude with higher cellular sensitivity observed for anionic surfactants.
- Cationic surfactants appear to show slight deviation from unity; likely due to uncertainties within the available *in-vivo* data such as exposure concentration due to their absorptive properties and test duration.

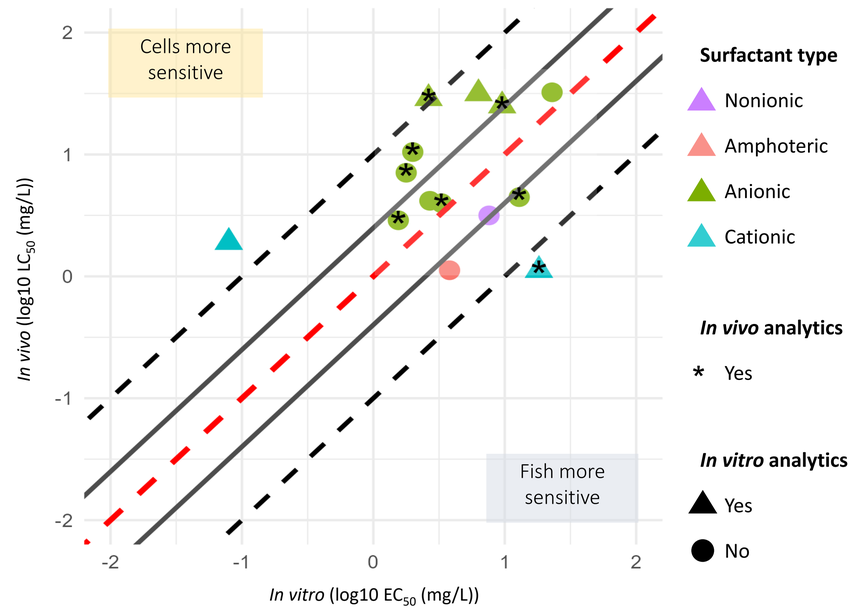
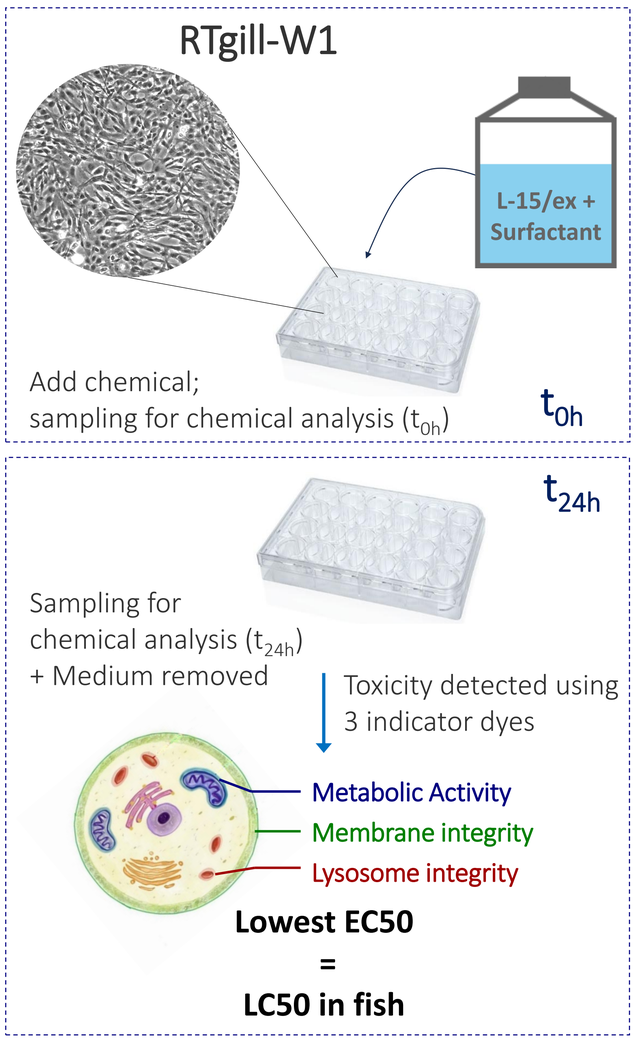



**Figure 1. Correlation between *in vivo* (OECD TG 203) and historical *in vitro* (OECD TG 249) log10 LC50/EC50 data for four surfactant classes (nonionic, amphoteric, anionic and cationic).** Unity line is dashed red line. 10-fold deviation from line of unity is dashed black lines. Grey line represents 2.5-fold deviation from line of unity.

## EXPERIMENTAL SET UP




## EXPERIMENTAL PHASE - METHODS

- Exposure concentrations were analytically determined.
- Measured concentrations ranged from 31% to 83% of nominal concentrations.
- $EC_{50}$ values are based on geometric mean of measured concentrations with the lowest value being used for comparison with *in vivo* values as instructed by OECD TG 249.
- Testing of cationic test items was expected to be challenging due to their adsorptive nature: a Water Accommodation Fraction (WAF) and solvent dosing (using an organic solvent: 0.5% v/v MeOH) were compared.

## EXPERIMENTAL PHASE - RESULTS

- A good correlation between $LC_{50}$ obtained in different fish species and new *in vitro* $EC_{50}$ data was found (Figure 2; Table 1).
- Results of WAF vs solvent dosing are equivalent based on geometric mean of measured concentrations (Figure 3). Both approaches provide consistent exposure for cationic substances despite their adsorptive behaviour.

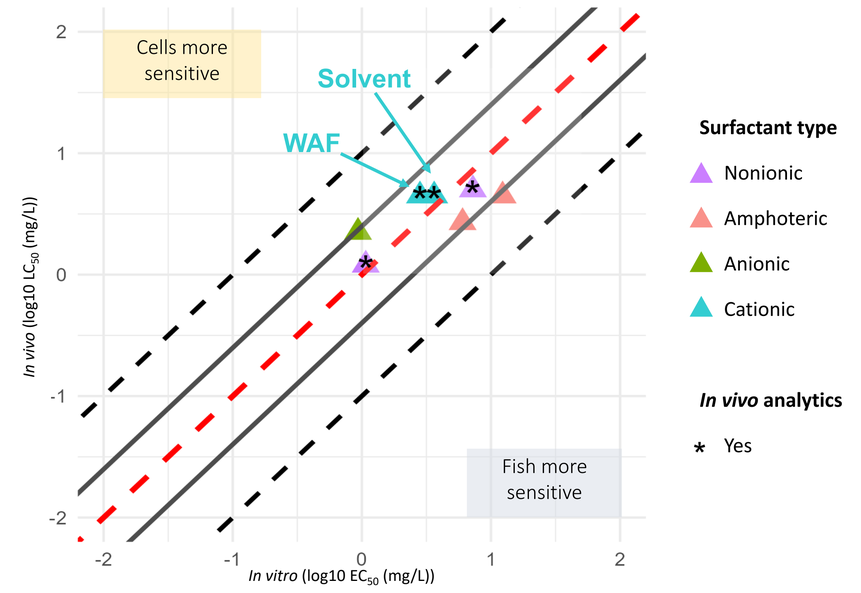



**Figure 2. Correlation between *in vivo* (OECD TG 203) and *in vitro* (OECD TG 249) log10 LC50/EC50 data for four surfactant classes (nonionic, amphoteric, anionic and cationic).** Unity line is dashed red line. 10-fold deviation from line of unity is dashed black lines. Grey line represents 2.5-fold deviation from line of unity.

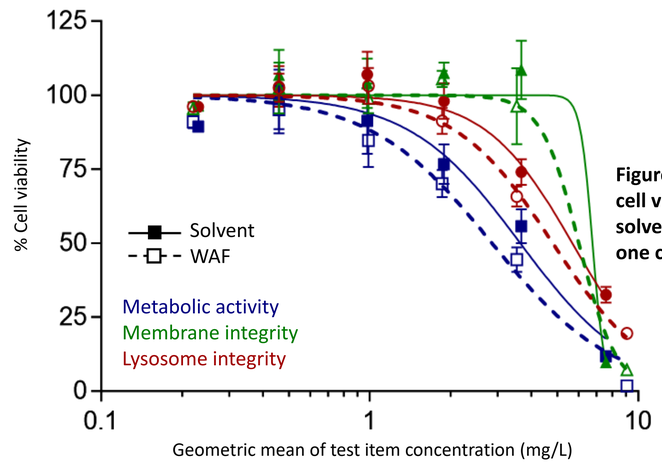



**Figure 3. Comparison of cell viability results using solvent dosing VS WAF for one cationic test item**

## TAKE-HOME MESSAGES

- The OECD TG 249 was successfully evaluated across multiple surfactant classes (2 nonionic, 2 amphoteric, 2 cationic, 1 anionic) ; one cationic surfactant test item is pending final analysis.
- Surfactant specific considerations (e.g. critical micelle concentration, solubility, stability) were addressed using preliminary solubility and rangefinder data without requiring protocol modifications during definitive testing.
- Different methods of preparation (WAF, solvent) showed no differences in toxicity outcomes.
- Based on currently available data, OECD TG 249 appears to adequately predict acute fish toxicity for different surfactant classes; newly generated *in vitro* data falls within 2.5-fold of the line of unity.

**Table 1. Data for new RTgill-W1 OECD TG 249 studies vs LC50**

| Surfactant class | CAS /EC | *In vitro* 24 h EC50 (mg/L)* Metabolic activity | Cell membrane integrity | Lysosome integrity | In vivo 96h LC50** | Ratio *In vivo/In vitro* |
|---|---|---|---|---|---|---|
| C12-13, branched & linear, alcohol ethoxylate (EO 1-2.5) | 160901-19-9/ 500-457-0 | 1.17 (0.88 – 1.46) | 1.76 (1.47 – 2.04) | **1.08** (0.8 – 1.37) | 1.19 (m) (*P. promelas*) | 1.10 |
| C10 alcohol ethoxylate (1-2.5 EO) | 26183-52-8/ 500-046-6 | **7.29** (4.04 – 10.53) | 12.01 (6.14 – 17.88) | 11.05 (8.58 – 13.53) | 4.97 (m) (*P. promelas*) | 0.68 |
| C12-C14 Alkyl Dimethyl Betaine | 66455-29-6/ 931-700-2 | **12.29** (10.98 – 13.60) | 23.69 (9.83 – 37.55) | 15.28 (13.35 – 17.20) | 4.44 (n) (*D. rerio*) | 0.36 |
| C12-C14 Alkyl Dimethyl Amine Oxide | 308062-28-4/ 931-292-6 | **6.04** (5.15 – 6.92) | 8.9 (3.80 – 14.00) | 7.96 (5.56 – 10.36) | 2.7 (n) (*P. promelas*) | 0.45 |
| Benzenesulfonic acid, C10-13-alkyl derivs., sodium salts | 68411-30-3/ 270-115-0 | **0.94** (0.75 – 1.13) | 1.45 (-8.51 – 11.42) | 1.14 (1.10 – 1.17) | 2.22 (*P. promelas*) | 2.36 |
| Ethanol, 2,2'-iminobis-, N-C12-18-alkyl derivs (Solvent method) | 71786-60-2/ 276-014-8 | **3.64** (2.64 – 4.64) | 7.04 (-51.03 – 65.12) | 5.68 (4.63 – 6.72) | 4.44 (m) (D. rerio) | 1.22 |
| Ethanol, 2,2'-iminobis-, N-C12-18-alkyl derivs (WAF method) | 71786-60-2/ 276-014-8 | **2.85** (2.08 – 3.63) | 6.01 (4.45 – 7.56) | 4.80 (4.01 – 5.60) | 4.44 (m) (D. rerio) | 1.56 |

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

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