Exploring the Applicability of the OECD TG 249 Fish Cell Line Acute Toxicity Assay in Environmental Safety Assessment of Cosmetic Product Ingredients

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Background:

Global efforts to advance animal-free safety assessments continue, yet acute fish toxicity (AFT) data remain a commonly required component of environmental hazard and risk evaluations under international chemical regulations. To address this, alternative methods such as the OECD TG 249 RTgill-W1 assay (a rainbow trout gill cell-based cytotoxicity test) are being evaluated for their ability to reliably predict toxicity to fish while reducing animal use, with current research, led by ICCS, focused on expanding the assay's domain of applicability across diverse, cosmetic-relevant chemistries and use cases.

- OECD TG 249 RTgill-W1 shows strong concordance with traditional *in vivo* tests
- Ongoing work evaluates performance across diverse chemistries and properties
- Focus includes difficult-to-test substances and functional ingredient relevance

Objectives:

1. Identify available data (and gaps) for chemicals tested against the OECD TG 249 RTgill-W1 assay.
2. Generate high-quality OECD TG 249 RTgill-W1 assay data for cosmetic ingredients to evaluate their capacity to predict acute fish toxicity.
3. Identify potential chemical-specific limitations of OECD TG 249 to ensure appropriate use in contextualized hazard assessments.
4. Drive NAM regulatory acceptance and confidence.

Ingredient Selection

A diverse set of cosmetic ingredients was chosen to understand the applicability domain of the RTgill-W1 assay, with attention given to substances that might be difficult to test using standard aquatic toxicity methods (Table 1).

Important factors included:

- Representation of multiple functional classes of cosmetic ingredients
- Broad spectrum of physicochemical properties, such as Log Kow, water solubility, and ionization status
- Ensuring the dataset mirrors real-world ingredient variety
- Existing *in vivo* fish acute toxicity data allowing the comparison with RTgill estimated toxicities and the exploration of potential limitations

*The acute toxicity value was greater than the maximum water solubility of the *substance* **AM: Antimicrobial*

Material Name	Material Class	Log Kow	Water solubility (~20 °C)	Acute Fish Toxicity (LC_{50}; mg/L) *(in vivo)*	RTgill solubilization method	RTgill max. solubility (mg/L; nominal)	RTgill final test range (mg/L)
Octocrylene	UV filter	6.1	40 µg/L	> 0.5*	DMSO	2	0.06 - 2
Octisalate	UV filter	5.94	74 µg/L	> 82*	DMSO	4	0.13 - 4
Methylparaben	Preservative	1.98	1.9 g/L	59.5	cell medium	1500	9.38 - 300
Propylparaben	Preservative	2.8	500 mg/L	6.4	cell medium	250	1.56 - 50
Phenoxyethanol	Preservative	1.2	25 g/L	344	cell medium	5000	156.3 - 5000
Benzyl alcohol	Preservative	1.05	40 g/L	460	cell medium	2500	78.13 – 2500
Sodium benzoate	Preservative	1.88	556 g/L	484	cell medium	250000	512 – 50000
Sodium salicylate	Preservative	-1.49	576 g/L	> 100	cell medium	2500	25.60 – 2500
Lauryl alcohol	Emollient	5.3	1 mg/L	1	Ethanol	4	TBD
1-Hexanol	Solvent	1.8	1.3 g/L	97	cell medium	1000	31.25 - 1000
Cetrimonium bromide	AM**/Conditioning	3.18	55 g/L	0.2	cell medium	1000	0.08 - 8

Table 1. List of cosmetic ingredients selected for testing using OECD 249 RTgill-W1 with physico-chemical properties. Acute Fish Toxicity LC_{50} are fish *in vivo* toxicity data from acute tests available in data summaries on the ECHA CHEM database .

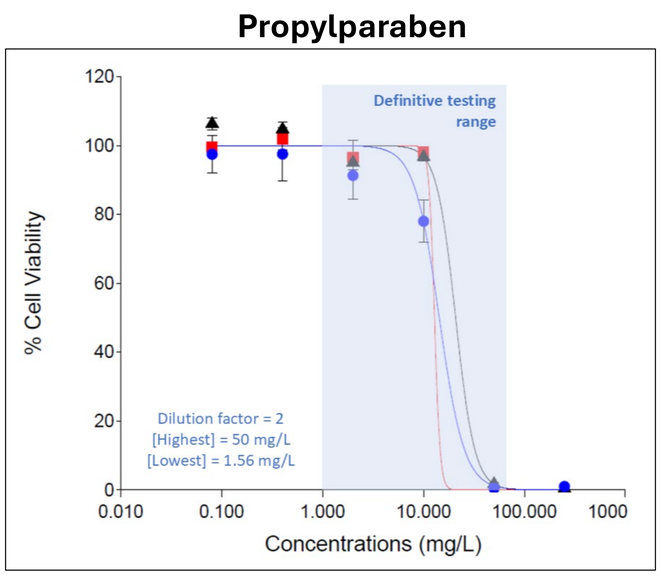

Figure 1. Range finder results from Propylparaben with the definitive testing range defined (1.56 – 50 mg/L)

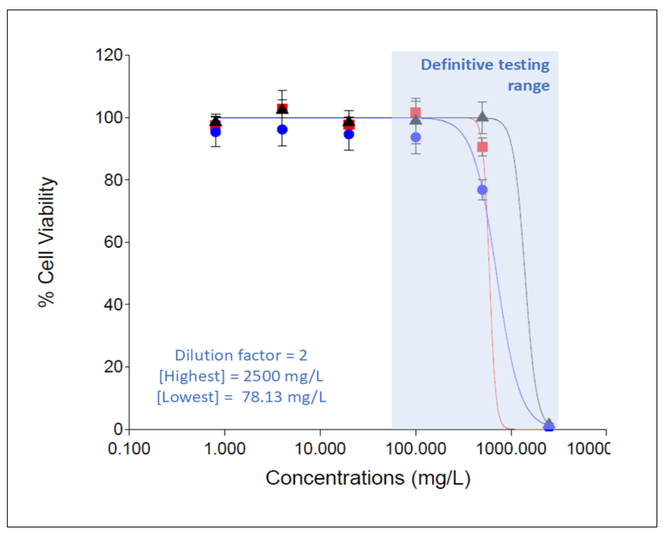

Figure 2. Range finder results for Benzyl alcohol with the definitive testing range defined (78.13 – 2500 mg/L)

Early Learnings

- **Solubility is not limiting** when reasonable measures (e.g., solvent, sonication, vortex, etc.) are implemented.
- **Range finding tests are reasonably predictive** of acute fish toxicity values: cetrimonium bromide appears to be the most toxic compound of the series, in vivo and according to RTgill range finders. Cosmetic ingredients that are less toxic to fish show generally low effects in RT gill preliminary tests.
- **Analytical verification will be leveraged** to assess relevance of effects from materials with limited solubility.
- **Preliminary results support use of OECD TG 249** to assess the acute toxicity of cosmetic ingredients to fish.

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C1 - Internal use