

# Case Study: OECD TG 249 Aquatic Toxicity Test for Assessing Hazardous Waste for Complex Mixtures

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## Background

- HP invents and manufactures complex chemical mixtures (inks, toners, 3D-printing agents, and powders).
- During new product development, aquatic toxicity testing is required to assess hazardous waste, e.g., according to the Static Acute Bioassay Procedures for Hazardous Waste Samples<sup>1</sup> of the California Department of Toxic Substances Control<sup>2</sup> (DOHS 22<sup>3</sup>).
- OECD Test Guideline (TG) 249<sup>4</sup> predicts acute fish toxicity and could be used to fulfil the requirement to assess wastewater.

**Aim: Investigate whether OECD TG 249 can predict acute fish toxicity for the assessment of wastewater for complex mixtures**

## Methods

### Test Item

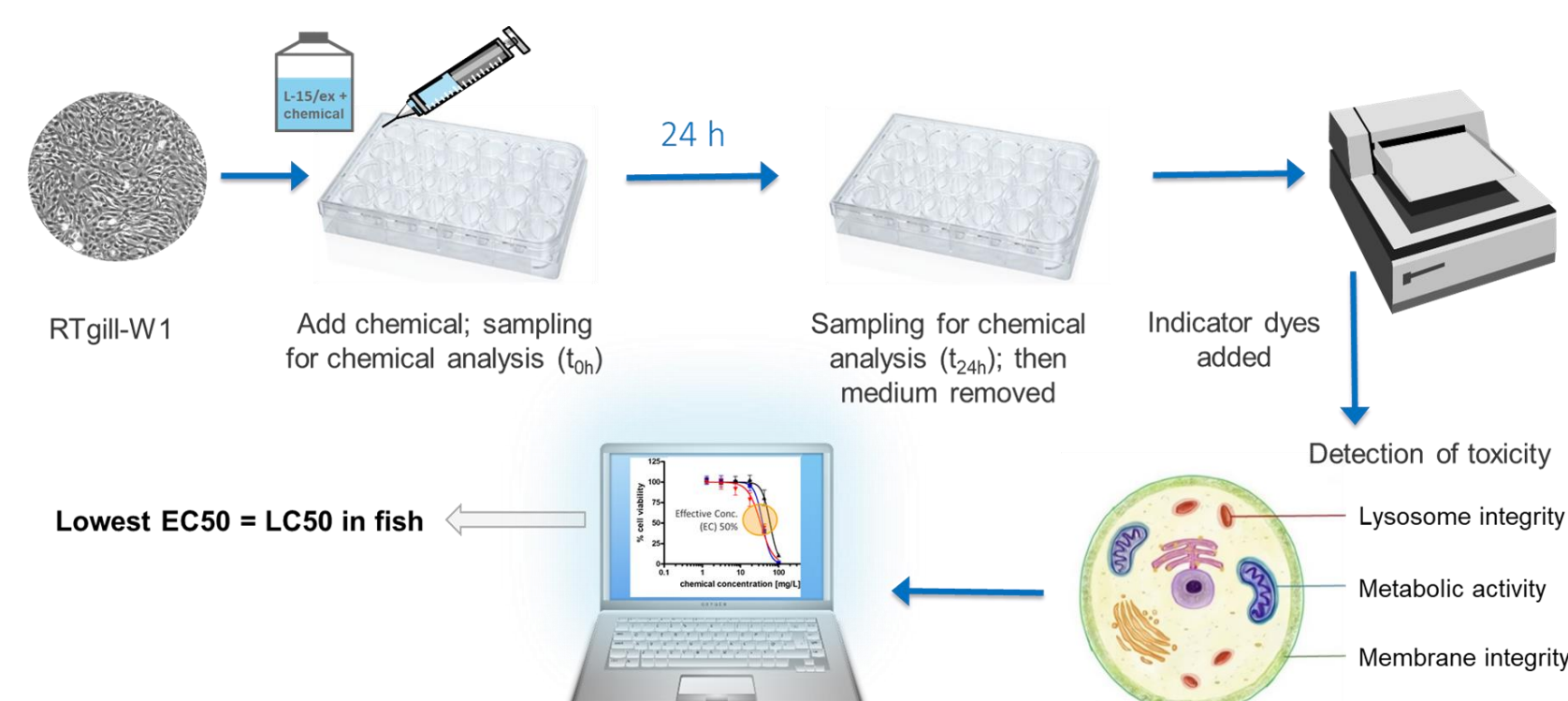
- HP 3DM650 Binding Agent: a water-based three-dimensional binding agent including a latex polymer, cosolvent, biocide, cyan colorant, and other additives

### DOHS 22

- Screening test commissioned by HP Inc. and conducted at third-party laboratory
- Test species: Fathead minnow (average length: 37 mm; average weight: 0.59 g)
- Test concentrations: 0 (control), 400 mg/L, and 750 mg/L
- Two replicates per concentration; one control replicate, each with 10 fish (i.e., 50 fish in total)
- Endpoint: survival at 0, 24, 48, 72, and 96 hours post-exposure; lethal concentration 50% (LC<sub>50</sub>) calculated at 96 hours using a binomial test

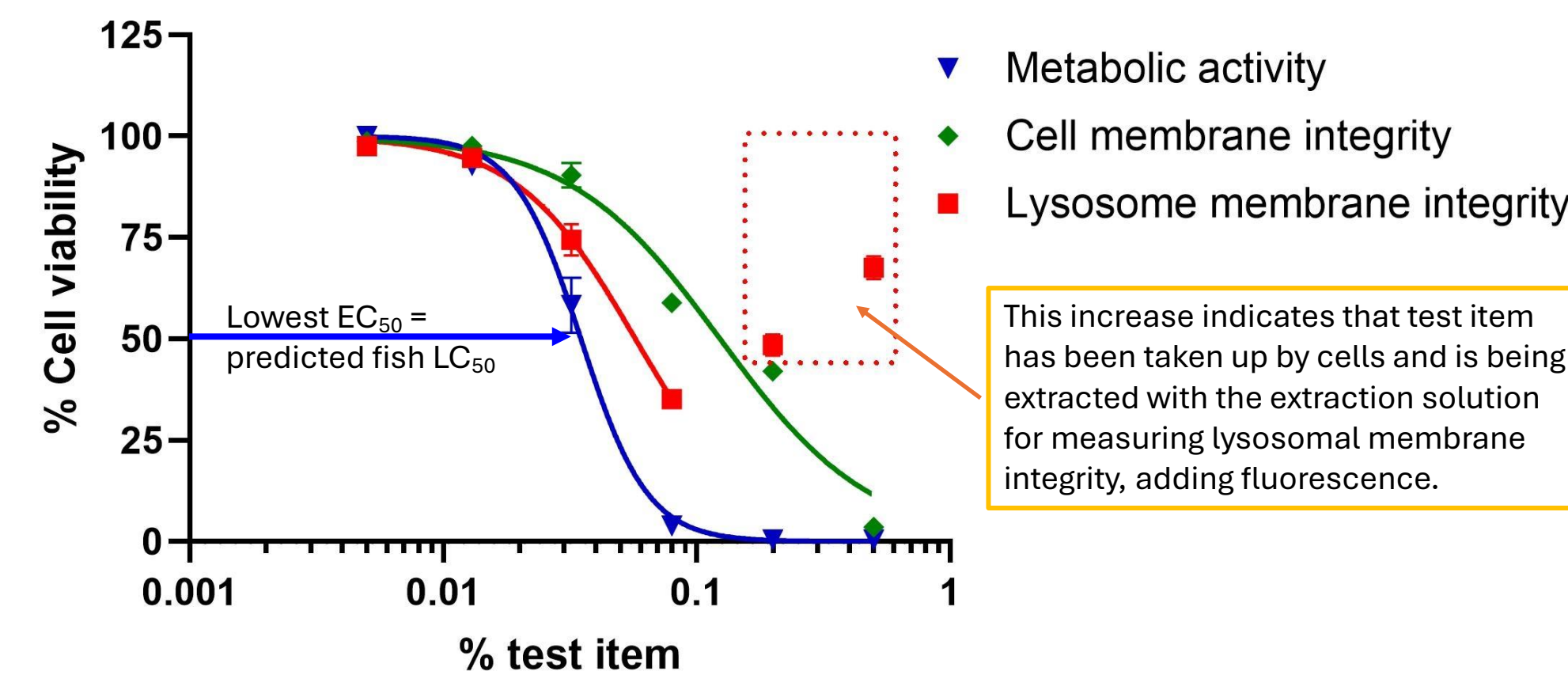
### OECD TG 249

- Solubility, range-finding, and a definitive test with RTgill-W1 cells and a positive control conducted by aQuaTox-Solutions
- Three cellular endpoints evaluated (which can provide information on the mechanism of cellular action)
- Effective concentration 50% (EC<sub>50</sub>) determined

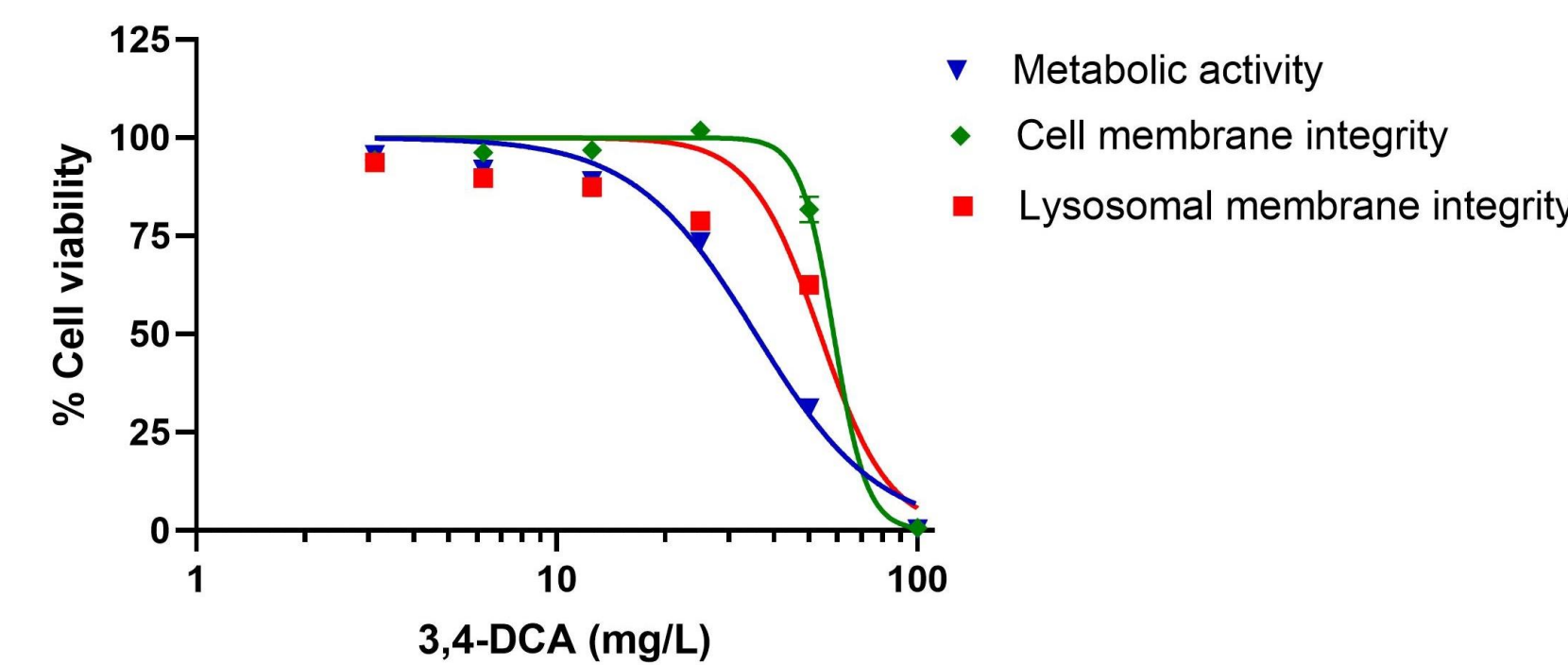


## Results

### HP 3DM650 tested using OECD TG 249

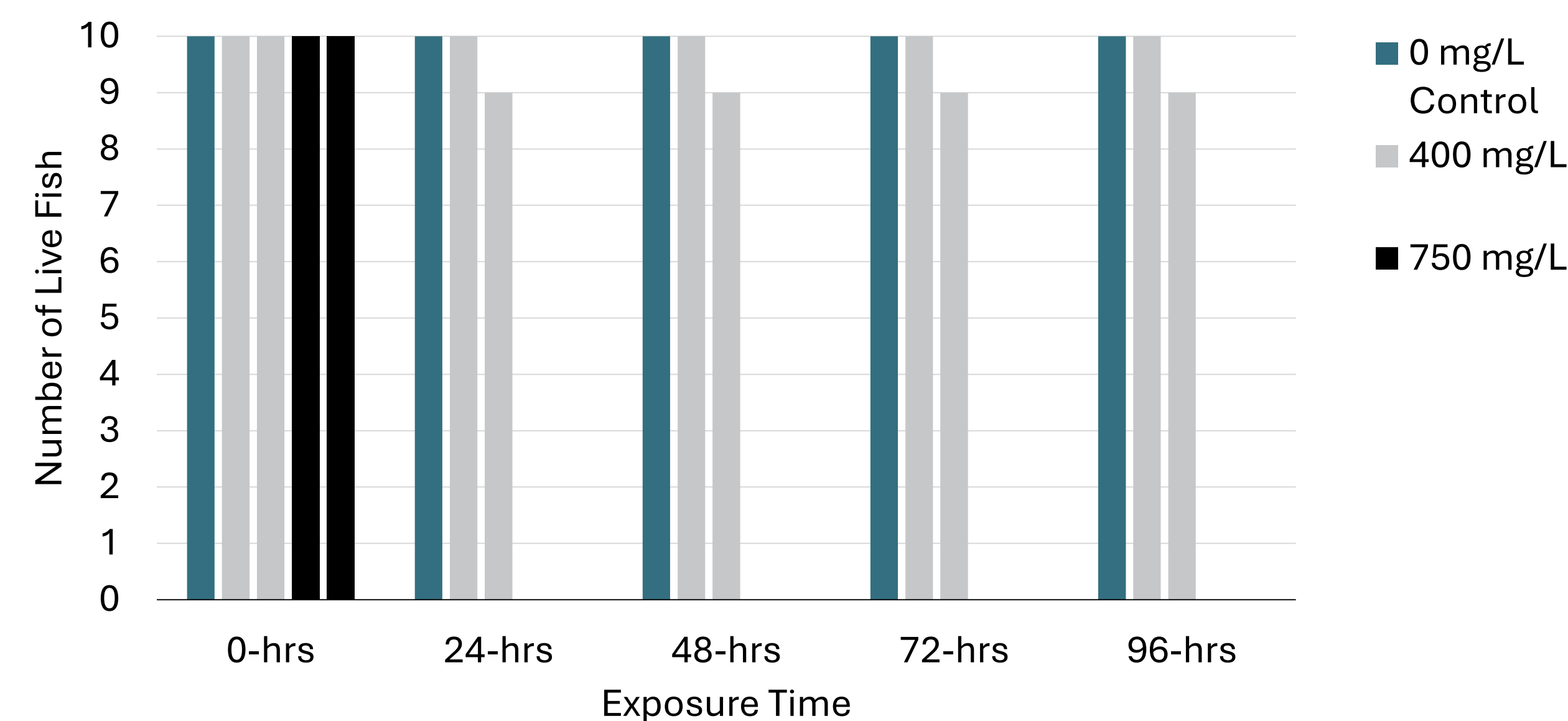


### Positive control in OECD TG 249: 3,4-dichloroaniline (DCA) – passing all validity criteria



HP 3DM650	Metabolic Activity	Cell Membrane Integrity	Lysosome Membrane Integrity
EC50 (% v/v of test item)	0.035	0.125	0.057
95% CI (% v/v of test item)	0.03 to 0.04	0.08 to 0.17	0.05 to 0.06

### HP 3DM650 tested using DOHS 22



### EC<sub>50</sub> and LC<sub>50</sub> values for HP 3DM650

OECD TG 249	DOHS 22
24-h EC <sub>50</sub> = 0.035% v/v (350 mg/L)	96-h LC <sub>50</sub> = 565.8 mg/L

## Discussion

- DOHS 22 and OECD TG 249 results were similar.
- OECD TG 249 results are derived from a full concentration-response curve and are therefore likely to be more accurate than DOHS 22 results, which are based on only two concentrations.
- The results in the red box for the neutral red uptake curve were excluded from this study due to interference from the HP 3DM650 cyan colorant, which impacted these results (see orange box).
- OECD TG 249 provides information on the products' cellular mechanism of action: Metabolic activity was most affected.
- Products tested according to DOHS 22 with an LC<sub>50</sub> of 500 mg/L are designated as "hazardous". Therefore, in this case study, the OECD TG 249 results, falling below the threshold, are more protective than the DOHS 22 test results.

## Next Steps

- Test if significantly more or less toxic products can be equally well evaluated using OECD TG 249
- Depending on the outcome, the *in vitro* fish cell assay should be considered an acceptable alternative for new product hazardous waste assessments.

## References

- <sup>1</sup>California Department of Fish and Game Water Pollution Control Laboratory. Static acute bioassay procedures for hazardous waste samples. Updated November 1988. Accessed August 22, 2024. [https://dtsc.ca.gov/wp-content/uploads/sites/31/2021/03/HWMP\\_bioassay\\_report\\_ADA2.pdf](https://dtsc.ca.gov/wp-content/uploads/sites/31/2021/03/HWMP_bioassay_report_ADA2.pdf)
- <sup>2</sup>California Department of Toxic Substances Control. Acute Aquatic Toxicity. [DTSC's Hazardous Waste Classification training course.] Accessed August 22, 2024. <https://dtsc.ca.gov/acute-aquatic-toxicity>
- <sup>3</sup>California Department of Health Services (DOHS). Title 22 California Code of Regulations 66261.24(1)(6). Last updated August 9, 2024. Accessed August 22, 2024. [https://govt.westlaw.com/calregs/Document/I8430AAA95B6111EC9451000D3A7C4BC3?viewType=FullText&originationContext=documenttoc&transitionType=CategoryPageItem&contextData=\(sc.Default\)](https://govt.westlaw.com/calregs/Document/I8430AAA95B6111EC9451000D3A7C4BC3?viewType=FullText&originationContext=documenttoc&transitionType=CategoryPageItem&contextData=(sc.Default))
- <sup>4</sup>OECD. Test no. 249: Fish cell line acute toxicity—the RTgill-W1 cell line assay. *OECD Guidelines for the Testing of Chemicals*. Section 2. OECD Publishing; 2021. <https://doi.org/10.1787/c66d5190-en>
- <sup>5</sup>aQuaTox-Solutions Services. Accessed September 11, 2024. <https://aquatox-solutions.ch/de/services-2>