

Use of OECD TG 249 Aquatic Toxicity Test for Assessing Hazardous Waste for Complex Mixtures

Krista Enos,^a Gilly Stoddart,^b Christopher Fassbender,^b Stephan Fischer,^c Kristin Schirmer^d

^aHP Inc., 1070 NE Circle Blvd, Mailstop 524D, Corvallis, OR 97330, United States; ^bPETA Science Consortium International e.V., Frieolzheimer Str. 3, 70499 Stuttgart, Germany
^caQuaTox-Solutions GmbH, Else-Züblin-Str. 11, 8404 Winterthur, Switzerland ^dEawag, Department of Environmental Toxicology, Überlandstr. 133, 8600 Dübendorf, Switzerland

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 Samples¹ of the California Department of Toxic Substances Control² (DOHS 22³).
- OECD Test Guideline (TG) 249⁴ 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 items

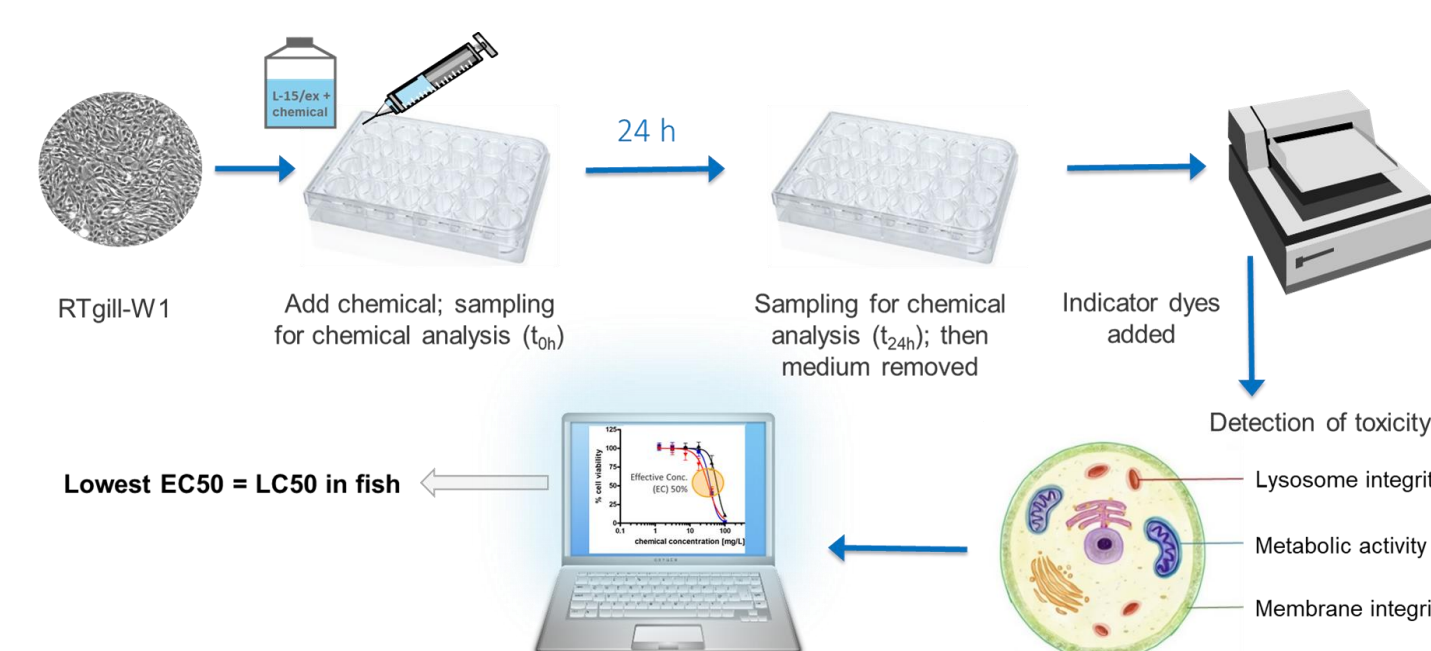
- HP 3DM650 Binding Agent: a water-based 3D binding agent including a latex polymer, cosolvent, biocide, cyan colorant, and other additives
- HP 3D800 FA: a water-based 3D fusing agent including cosolvent, biocide, black colorant, and other additives
- HP 3D800 DA: a water-based 3D detailing agent including cosolvent, biocide, and other additives.

DOHS 22

- Screening test 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 h post-exposure; lethal concentration 50% (LC₅₀) calculated at 96 h using a binomial test

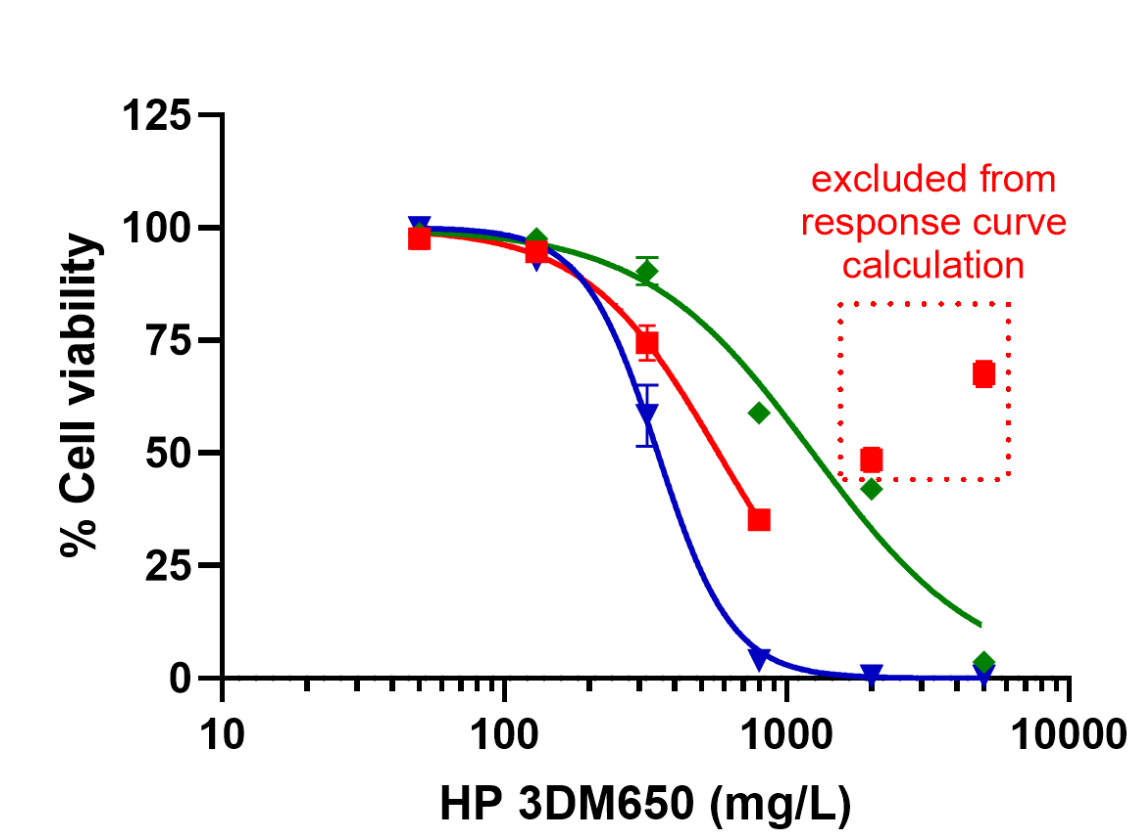
OECD TG 249

- aQuaTox Solutions conducted range-finder test and definitive test in RTgill-W1 gill cells
- Three cellular endpoints evaluated (which can provide information on mechanism of action)
- Effective concentration 50% (EC₅₀) were determined (the lowest being the predicted fish LC₅₀ value)

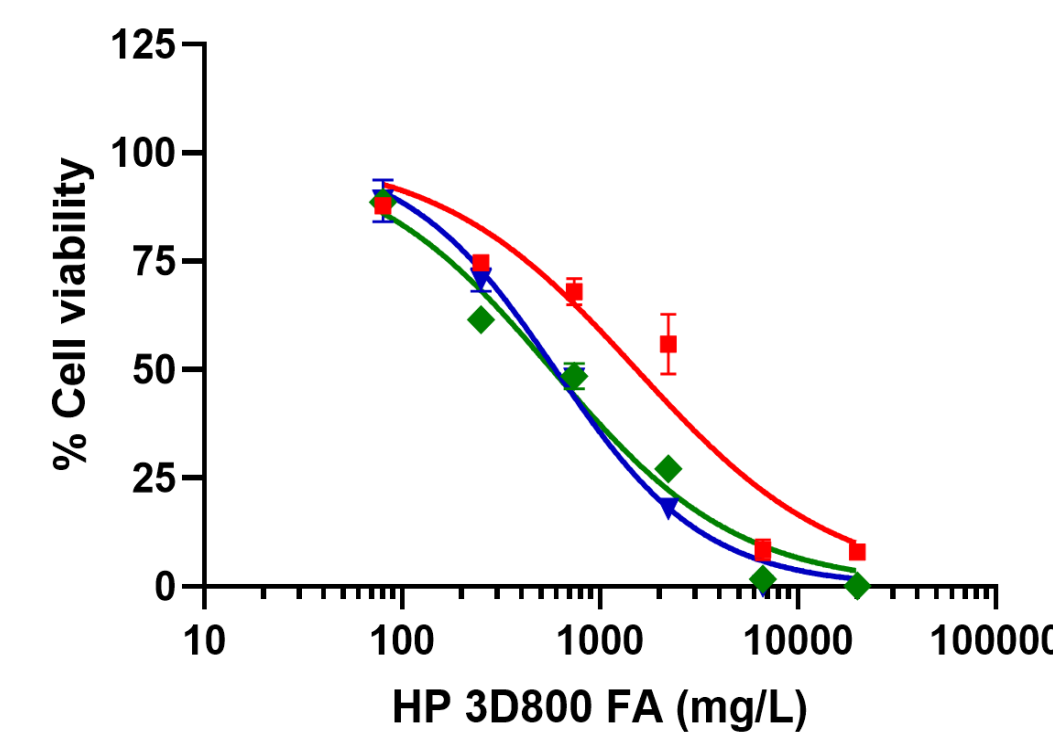


Results

Test items tested using OECD TG 249

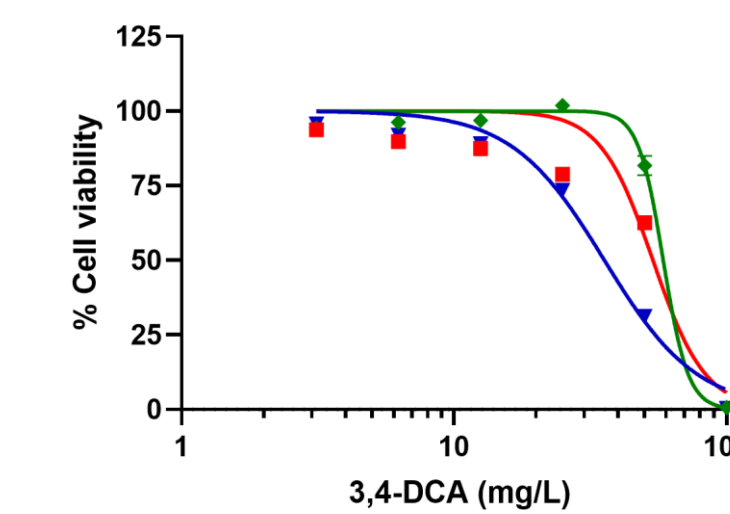


[Data are from one biological replicate with error bars representing standard deviation of technical triplicates.]



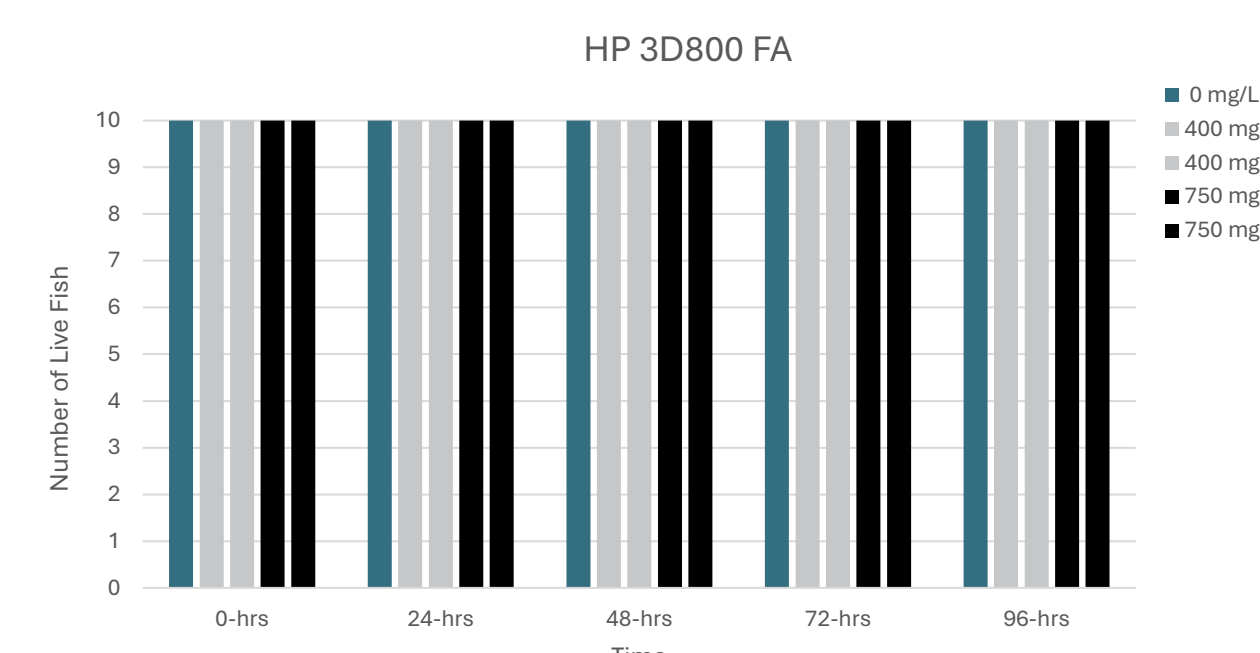
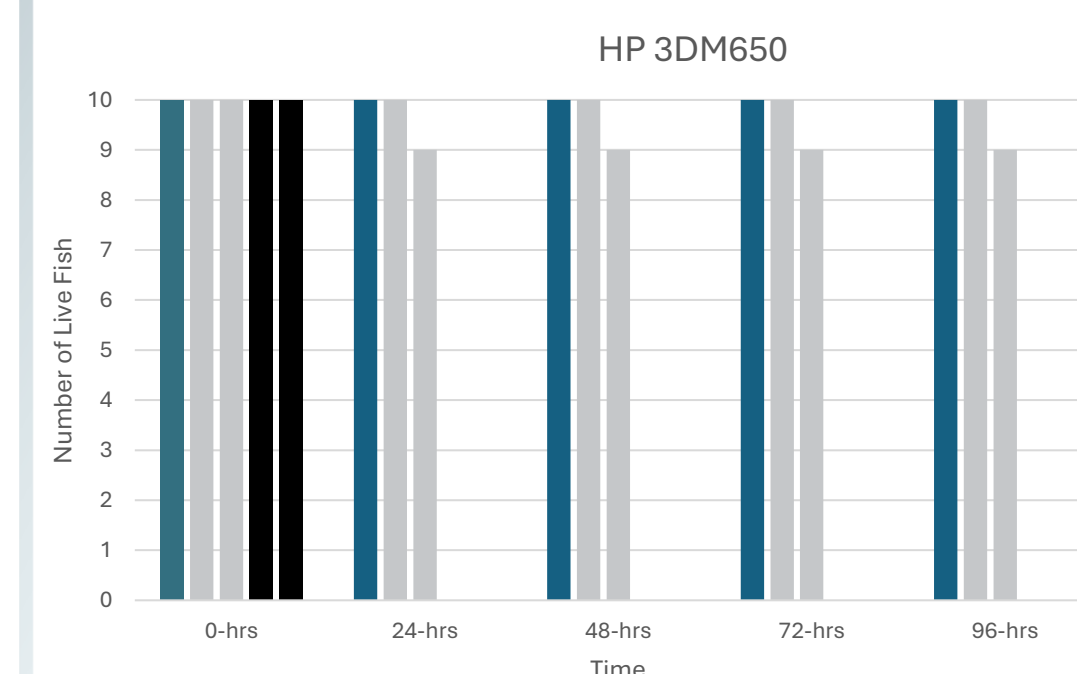
[Date are from two biological replicates with error bars representing the deviation of the two means, derived from technical triplicates.]

Example of a positive control in OECD TG 249: 3,4-dichloro-aniline (DCA)



- ▼ Metabolic activity
- ◆ Cell membrane integrity
- Lysosome membrane integrity

Test items tested using DOHS 22



EC₅₀ and LC₅₀ values for Test items

	OECD TG 249			DOHS 22 96-LC ₅₀ (mg/L)
	Metabolic Activity (mg/L)	Cell Membrane Integrity (mg/L)	Lysosome Membrane Integrity (mg/L)	
HP 3DM650	349.5 (95% CI: 328.7 to 372.7)	958.0 (95% CI: 903.0 to 1030)	573.5 (95% CI: 542.4 to 607.3)	565.8
HP 3D800 FA	594.9 (95% CI: 508.0 to 695.1)	573.0 (95% CI: 441.5 to 739.9)	1533 (95% CI: 963.1 to 2379)	>750
HP 3D800 DA	167.5 (95% CI: 146.0 to 192.1)	321.8 (95% CI: 261.7 to 395.7)	162.6 (95% CI: 132.7 to 199.2)	575

Discussion

- OECD TG 249 results were consistently more sensitive than the DOHS 22 bioassay; the lowest obtained value is taken as proxy for fish acute toxicity.
- 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 for HP 3DM650 were excluded for concentration-response calculations due to the interference from the HP 3DM650 cyan colorant impacting these results.
- Most sensitive mechanism of action has varied between test items, which can improve understanding of how mixtures or components within mixtures are contributing to aquatic toxicity.
- Products tested according to DOHS 22 with an LC₅₀ of ≤500 mg/L are designated as “hazardous”. Two of the products fell below this threshold with the OECD TG 249 results, whereas one did not, in agreement with the DOHS 22. Overall, OECD TG 249 results are more protective than the DOHS 22 test results.

Next Steps

- Test if other types of complex mixtures 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

- ¹California Department of Fish and Game Water Pollution Control Laboratory. Static acute bioassay procedures for hazardous waste samples. Updated November 1988. Accessed Aug 22, 2024. https://dtsc.ca.gov/wp-content/uploads/sites/31/2021/03/HWMP_bioassay_report_ADA2.pdf
- ²California Department of Toxic Substances Control. Acute Aquatic Toxicity. DTSC's Hazardous Waste Classification training course. Accessed Aug 22, 2024. <https://dtsc.ca.gov/acute-aquatic-toxicity/>
- ³California Department of Health Services (DOHS). Title 22 California Code of Regulations 66261.24(1)(6). Last updated August 9, 2024. Accessed Aug 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))
- ⁴OECD. 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>
- ⁵aQuaTox-Solutions Services. Accessed March 01, 2025. <https://aquatox-solutions.ch/de/services-2/>