Testing Poorly Water-Soluble Test Chemicals According to the Updated OECD Guidance Document 23 on Aqueous-Phase Aquatic Toxicity Testing of Difficult Test Chemicals

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Introduction

The Organisation for Economic Co-operation and Development (OECD) Guidance Document on Aqueous-Phase Aquatic Toxicity Testing of Difficult Test Chemicals (GD 23)[1] supplements OECD test guidelines (TGs) for regulatory studies. For example, GD 23 provides essential guidance on maintaining consistent exposure to the dissolved test chemical throughout aquatic tests while minimising conditions that may lead to experimental artefacts (e.g. physical effects).

First published in 2000, GD 23 was updated in 2019 in an effort led by the European Commission, ICAPO and the USA to provide state-of-the-art approaches to aquatic toxicity testing involving difficult-to-test chemicals.

Particular attention was paid to updating methods available for testing poorly water-soluble test chemicals while avoiding the use of solvents. Thus, the need for a solvent control group is eliminated, reducing the number of test animals used.

Content of GD 23

Preparing aqueous solutions and selecting exposure systems for chemicals that are:	
• **Poorly/sparingly water soluble**	• Coloured
• Volatile	•Hydrophobic
• Easily degraded	• Ionisable
• Highly adsorptive	• Multi-component substances
• Complex forming	• Alloys

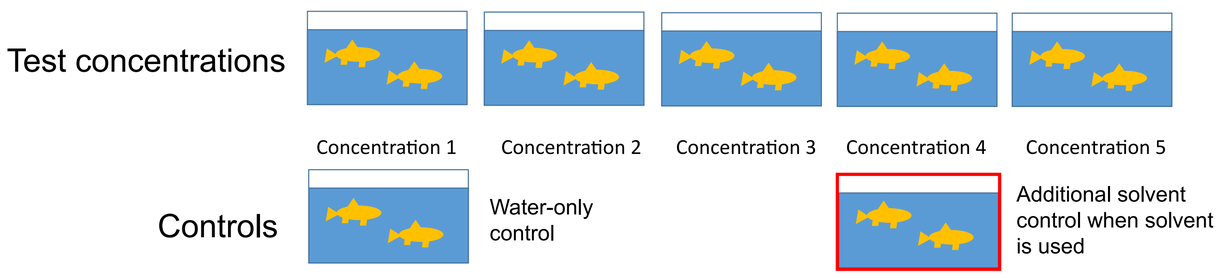
Additional topics:	
• Preliminary assessment of stability	• Testing at low chemical concentrations
• Exposure system considerations	• Sampling schedules
• Testing at water saturation	• Calculation and expression of test results

This poster focuses on the expansion of the guidance on testing of poorly water-soluble test chemicals.

Solvent Controls

- Poorly/sparingly water-soluble test chemicals present unique challenges.
- Solvents are sometimes used to facilitate dissolution but can affect study results.
- Solvent use increases the number of animals used by up to 50% per test in a limit design and up to 29% in a full design, depending on the TG, because an additional solvent control group is included.

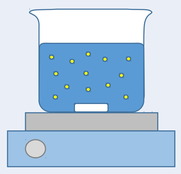
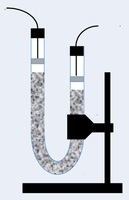
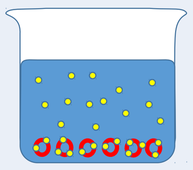
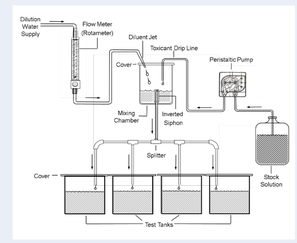
Example of an aquatic toxicity test design:



Number of Fish Used in Selected OECD TGs						
OECD TG	**# fish per control**	**# test concentrations**	**# fish per test concentration replicate**	**# of replicates**	**# fish per test if solvent used (total)**	**# fish saved if no solvent used (%)**
TG 203: Fish, Acute Toxicity	7	5	7	1	49	7 (17)
TG 210: Fish, Early-Life Stage Toxicity (FELS)	20	5	20	4	560	80 (17)
TG 229: Fish Short Term Reproduction Assay (FSTRA)	6	3	6	4	120	24 (25)
TG 234: Fish Sexual Development Test (FSDT)	30	5	30	4	840	120 (17)
TG 240: Medaka Extended One Generation Reproduction Test (MEOGRT)	504 (total)	5	42 (total)	6–24	2,268	504 (29)

Exposure Without Solvents

Substantial revisions were introduced to, *inter alia*, reduce occasions when solvents are used. The following techniques described in GD 23 may not require solvents in the exposure systems.

Direct addition	Generator systems	Passive dosing	Flow-through exposure systems
 Stirring, shaking, blending, homogenisation, sonication, pH adjustment	 Liquid/liquid saturator  Saturator column	 Loaded polymer used as partitioning donor to maintain freely dissolved concentrations	 Maintain stable water quality and test concentrations (*Diagram courtesy of EAG*)
• Guidance on methods for dissolution of the test chemical is provided. • Undissolved test chemical must be removed prior to test initiation.	• Saturator column preparation techniques, temporal limitations of use, and analytics are described. • Test medium must not be recirculated through columns after being used in exposure.	• Guidance on loading principles and techniques is provided. • References to publications describing different geometries of dosing polymers are provided. • Limitations of the method are described.	• Recommendations are provided for the number of tank volume turnovers per 24 hours, flow-rate calibration, stock solution preparation, and the equilibrium pre-test period.

Saturators With Various Chemicals and Media

Saturation method	Test chemical	Measured water solubility, mg/L (OECD TG 105)	Dilution water type	Temp °C	Measured saturation concentration, % solubility
Saturation column	Substance A	3	tap water	13	59%
			tap water	23	56%
			Elendt M4	20	65%
	Substance B	0.0281	tap water	13	62%
			tap water	25	104%
			Elendt M4	20	82%
	Substance C	0.3	AAP	23	41%
			tap water	25	52%
			Elendt M4	20	73%
Solid-liquid saturator	Substance D	0.27	AAP	23	97%
Liquid-liquid saturator (static)	Substance E	3.1	Elendt M4	20	44%
Liquid-liquid saturator (recirc.)			Elendt M4	20	84%

Water solubility is determined under highly controlled conditions in ultrapure water at 20°C and the saturation concentration in environmentally relevant water and test conditions can vary. Saturator methods allow the best opportunity to achieve high concentrations near solubility while avoiding the presence of solvents and undissolved chemicals.

Conclusions

- The updated GD 23 represents a collaborative effort among numerous experts from industry, OECD member countries, non-governmental organisations, and academia.
- The updated GD 23 will help government agencies, industry, and contract research organisations conduct valid and reliable aquatic toxicity studies on difficult-to-test chemicals while minimising the number of animals used and the need to repeat studies.

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Reference

[1]OECD. Guidance Document on Aqueous-Phase Aquatic Toxicity Testing of Difficult Test Chemicals. 8 February 2019. http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=ENV/JM/MONO(2000)6/REV1&docLanguage=En.

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