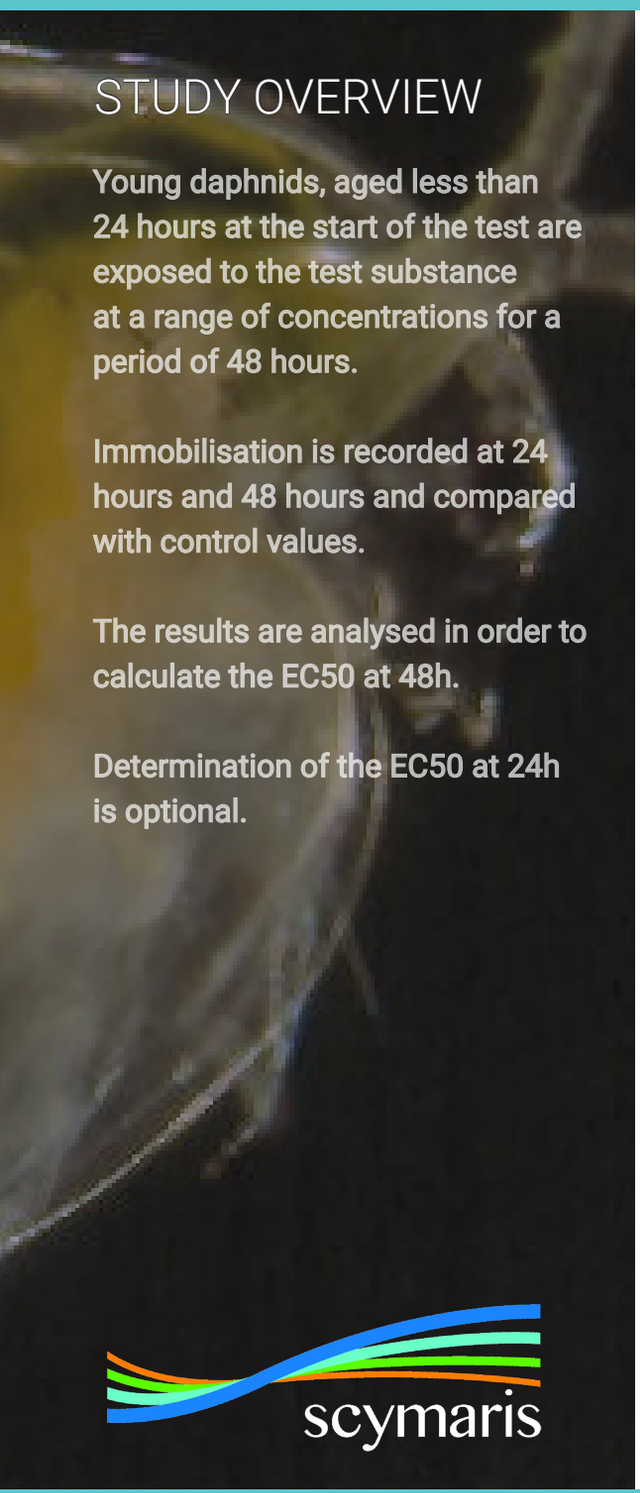
Technical Approach

OECD 202: *Daphnia sp*. Acute Immobilisation Test



STUDY OVERVIEW

Young daphnids, aged less than 24 hours at the start of the test are exposed to the test substance at a range of concentrations for a period of 48 hours.

Immobilisation is recorded at 24 hours and 48 hours and compared with control values.

The results are analysed in order to calculate the EC50 at 48h.

Determination of the EC50 at 24h is optional.

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OUR CAPABILITIES

Our team comprises proven experts in testing routine and problematic (hydrolytically unstable, highly sorptive, volatile, low solubility) materials and regularly develop novel methodologies, exposure systems and analytical techniques to support bespoke testing strategies.

We operate in modern GLP-compliant facilities striving to provide the highest quality service possible.

We offer a comprehensive range of acute, chronic and bespoke aquatic ecotoxicology studies with a variety of test species and we can conduct testing to a variety of test guideline requirements.

We provide both standard and bespoke studies with *Daphnia Magna* and *Ceriodaphnia*

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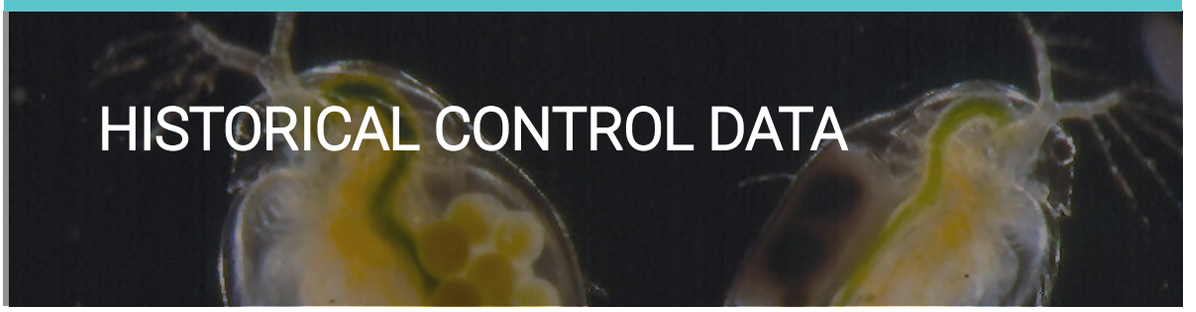

HISTORICAL CONTROL DATA

The OECD 202 Test Guideline details the following performance criteria for the test validity:
In the control, no more than 10% of the daphnids should have been immobilised or show other signs of stress.

	Control Type	Number immobilised across all studies (48 hr)
2018	DWC	0%
2019	DWC	0%
2020	DWC Solvent	0.008%
2021	DWC Solvent	0.002%
2022	DWC Solvent	0%

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METHOD DEVELOPMENT APPROACHES TO UVCB'S AND DIFFICULT SUBSTANCES TO SUPPORT ECOTOXICOLOGY STUDIES

For chromatographically resolvable compounds (surfactants, petrochemicals etc.)	**For non-chromatographically resolvable compounds (polymers, resins etc.)**
• Review compound information and characterisation data/ methods supplied by the sponsor and from other literature sources • Determine the most appropriate technique for proposed studies (LC-UV/MS/radio, GC-FID/MS, IC) this may include test injections on each type of system • Whether MS is used as the final detector or not, LC-Accurate Mass or GC-EI-MS will be used to characterise the substance and identify the key components to monitor using the chosen analytical method. • Select components to monitor during the proposed studies, and agree with the Sponsor prior to method validation. We attempt to quantify each component of the test substance >10% and to account for >85% of total components. This is not always possible with certain UVCB's. • Determine homogeneity and reproducibility of the test item and working solutions. •Determine sample preparation procedures to extract the components from the test medium.	• Review compound information and characterisation data/methods supplied by the Sponsor and from other literature sources. • Determine the most appropriate technique for proposed studies (TOC, UV-Vis, LSC, GPC/SEC or other chromatographic methods*) this may include tests on each type of system. • Determine homogeneity and reproducibility of the test item and working solutions. • Determine sample preparation procedures to extract the components from the test medium. *Although GPC/SEC or other chromatographic methods may show resolvable peaks in the chromatography these are not likely to be individual components and therefore these are not considered specific methods.
Presentation of results	**Presentation of results**
Unless individual potencies / reference standards of each component are available results are presented in mg total substance /L (or equivalent). Each component is used to estimate the concentration of the total substance, and these estimates can be widely different dependant on the physical properties of each component (e.g water solubility). We have observed this may be preferred by regulators over summation methods which attempt to generate a single value for the measured concentration of the substance in the test solutions. Data used for dose verification only, ecotox studies use WAF loading rates or nominal concentrations to determine the endpoints (NOEC / LOEC etc.)	Total substance is reported in mg/L (or equivalent). For GPC/SEC or other chromatographic methods this may include summation of multiple peak areas. Data used for dose verification only, ecotox studies will use WAF loading rates or nominal concentrations to determine the endpoints (NOEC / LOEC etc.). Optional - to support regulatory submissions a non-GLP report can be included to demonstrate that chromatographic resolution of individual components was not possible/practical.

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