Technical Approach

OECD 201: Freshwater Alga & Cyanobacteria, Growth Inhibition Test

STUDY OVERVIEW

The purpose of this test is to determine the effects of a substance on the growth of freshwater microalgae and/or cyanobacteria.

Exponentially growing test organisms are exposed to the test substance in batch cultures over a period of normally 72 hours.

In spite of the relatively brief test duration, effects over several generations can be assessed.

scymaris

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 culture 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.

OVERVIEW

- 106 studies across 5 species including ***Pseudokirchneriella subcapitata, Synechococcus leopoliensis, Anabaena flos-aquae, Desmodesmus subspicatus,*** and ***Navicula pelliculosa***
- Control types include DWC, DWC (WAF), Solvent Control (THF), and Solvent Control (DMF)

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

Scymaris separates in to two categories. Currently we do not work with Biologics.

Chromatographically resolvable (surfactants, petrochemicals etc.)	**Non-chromatographically resolvable (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, agreed with the Sponsor prior to method validation. We attempt to follow everything individually >10% and to account for >85% of total components. This is not always possible. • 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 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 to be preferred by ECHA 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 ECHA submissions, at an additional charge, a non-GLP report can be included to demonstrate that chromatographic resolution of individual components was not possible/practical.

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OECD 201 – APPROACH TO DIFFICULT SUBSTANCES

Poorly soluble	Highly adsorptive	Unstable (e.g hydrolysis, photolysis, volatility etc)
Pre-study dosing trials: • WAF • Direct addition saturation + filtration • Solvent addition • Saturator column • Coulter counts to determine particulate interference Optional – To support submission, a non-GLP report can be produced and referenced in the GLP final report to demonstrate that the maximum achievable concentration was used in the test. Additional charge.	• Pre-conditioning of test vessels and centrifuge tubes, effectiveness checked in dosing trials or range finding. • Analysis of blank vessels to determine the proportion of test substance adsorbed to algae Optional - If pre-conditioning has failed to stabilise the test concentration there is an option to treat as "Unstable" see right-hand column.	Due to the test design of OECD 201 reduction of instability is often impossible (e.g flow though and semi-static testing not practical) • Sacrificial analytical test vessels, prepared and analysed at 24-hour intervals to monitor concentration throughout the test. Gives better accuracy on the geometric mean. • Sacrificial sealed biology test vessels analysed at each sampling occasion for volatile substances.

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HISTORICAL CONTROL DATA

	Species	Control Type	Average Control Mean Yield (10^4 cells/ mL)	Average Control Mean 0-72h Growth Rate/ day	Average Validity Criteria - Exp.Increase in Biomass	Average Validity Criteria - CoV Sectional Growth Rates (%)	Average Validity Criteria - CoV 0-72h Growth Rates (%)
2017	Pseudokirchneriella subcapitata	DWC Solvent Control (THF) Solvent Control (DMF)	34.5	1.39	65.10	14.33	1.99
2018	Pseudokirchneriella subcapitata Anabaena flos-aquae	DWC Solvent Control (THF) Solvent Control (DMF)	61.95	1.40	53.74	13.37	1.49
2019	Pseudokirchneriella subcapitata	DWC DWC (WAF) Solvent Control (THF) Solvent Control (DMF)	51.83	1.46	82.25	7.59	1.46
2020	Pseudokirchneriella subcapitata Desmodesmus subspicatus Anabaena flos-aquae	DWC DWC (WAF) Solvent Control (DMF)	84.3	1.47	86.68	17.61	1.87
2021	Pseudokirchneriella subcapitata Anabaena flos-aquae Navicula pelliculosa	DWC Solvent Control (DMF)	90	1.41	83.24	27.45	2.84
2022	Pseudokirchneriella subcapitata	DWC	103.68	1.38	119	20.6	1.78
2023	Pseudokirchneriella subcapitata Anabaena flos-aquae	DWC Solvent Control (THF)	73.1	1.44	71.69	15.6	1.61

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