Australian Government
Department of the Environment and Energy
Supervising Scientist

internal report 645

Updated procedure for the 72 hour algal growth inhibition toxicity test using *Chlorella* sp.

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September 2016

Release status – unrestricted

The Department acknowledges the traditional owners of country throughout Australia and their continuing connection to land, sea and community. We pay our respects to them and their cultures and to their elders both past and present.

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How to cite this report:

Pease C, Mooney T, Trenfield M, Costello C & Harford A 2016. Updated procedure for the 72 hour algal growth inhibition toxicity test using *Chlorella* sp. Internal Report 645, September, Supervising Scientist, Darwin.

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The Supervising Scientist is a branch of the Australian Government Department of the Environment and Energy.

Supervising Scientist

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Executive summary

Chlorella sp. is one of five species routinely used in the Supervising Scientist ecotoxicology laboratory to derive site-specific water quality guideline values that are implemented in the creeks surrounding Ranger uranium mine in northern tropical Australia. Data for this species, which is native to the region, have contributed to the derivation of toxicity estimates for a number of contaminants of potential concern. The procurement of a new flow cytometer, which is able to quantify lower algal cell densities compared to previous methods, has allowed for the refinement of the test method to be more ecologically relevant.

Previous studies have demonstrated an increase in sensitivity of algae to metals associated with lower algal cell inoculation density (Moreno-Garrido et al 2000, Franklin et al 2002). Reducing the algal cell density within the test system also allows for a reduction in the concentration of nutrients added to the test medium. Dissolved metals and nutrients are known to interact, and this has the potential to interfere with the assessment of metal toxicity. Consequently, studies were undertaken to assess algal cell inoculation density and nutrient concentrations in order to optimise the sensitivity of the 72 h *Chlorella* sp. growth inhibition toxicity test. The outcome of these studies was an updated test method that uses an algal inoculation density of 3×10^3 cells ml^{-1} (one order of magnitude lower than the original test method) and nitrate and phosphate concentrations of 3.63 mg L^{-1} nitrate and 34.5 μg L^{-1} phosphate (one quarter of the concentrations in the original test method). These refinements have resulted in a test system that is more representative of natural systems, which is an important requirement for developing toxicity tests. This report describes the revised test method and the studies used to define the test parameters.

1 Introduction

The tropical microalga, *Chlorella* sp. has been cultured continuously in the Supervising Scientist ecotoxicology laboratory for 25 years. This is one of five species routinely used to derive site-specific water quality guideline values, which are implemented in the creeks surrounding Ranger Uranium Mine in the Alligator Rivers Region in tropical northern Australia. The protocol, detailed in (Riethmuller et al 2003) uses an algal density of 3 x 10^4 cells ml^{-1}, and is measured by performing electronic cell enumeration using electro-zoning methods (i.e. Coulter Multisizer II Particle Analyser, Beckman-Coulter). This protocol has been effective for assessing the toxicity of contaminants to *Chlorella* sp.. The reproducibility of the protocol (as evident from results of the reference toxicity program) means a high level of reliability can be associated with the results of these studies. While the protocol is currently effective, it is important to continue to refine and improve protocols to make them as reproducible and sensitive as possible.

Studies have shown increased sensitivity to metals in toxicity tests when lower initial densities of algae are used (Moreno-Garrido et al 2000, Franklin et al 2002). This reduced sensitivity is likely to be due to increased metal accumulation rates at lower densities (Franklin et al 2002). Lower algae densities also enable nutrients, which are required within the test medium, to be reduced to concentrations that better reflect those which occur in the environment. Nutrients, such as phosphate, readily bind with some contaminants and can reduce their bioavailability and toxicity (Mkandawire et al 2006, Mkandawire et al 2007). Therefore, reducing the levels of nutrients within the test medium often increases the sensitivity of the test. Therefore, it is ideal to minimise both initial algal cell densities and concentrations of nutrients to levels as close as possible to environmental concentrations.

The use of flow cytometry instead of electro-zonation or manual count methods for counting algal cells has made it possible to accurately measure much lower densities of microalgal cells per millilitre of test medium. The acquisition of a flow cytometer by the Supervising Scientist ecotoxicology laboratory has, thus, enabled the ability to optimise the 72 h *Chlorella* sp. growth inhibition toxicity test for both initial algal cell density and, consequently, nutrient concentrations. This report describes the revised method for the *Chlorella* sp. toxicity test, and also provides details of the studies undertaken to enable the method to be updated.

2 Objective

The objective of a test series (i.e. 3-4 definitive tests) is to determine the concentrations of a specified chemical or whole effluent that shows the median effect concentration (e.g. EC50). This is the concentration of a chemical in solution that is estimated to cause a 50% effect concentration of a sublethal response of test organisms. This is measured as the 50% effect population growth of the tropical alga *Chlorella* sp. over 72 h (Riethmuller et al 2003).

3 Principle of the test

Exponentially growing cells of *Chlorella* sp. are exposed to a concentration range of a toxicant or water of interest over a 72 h period. The cell counts are performed at 48 h, 72 h, and occasionally 24 h, to calculate cell division rates for the algal population over time. The concentration-response relationship is modelled using non-linear regression and toxicity estimates are derived from this model. This test is based on international standards, specifically:

- OECD (2011) test number 201: Freshwater alga and cyanobacteria, growth inhibition test; and
- USEPA (2002) Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, 5th edition.

4 Test organism

The unicellular green alga, *Chlorella* sp., has been continuously cultured in the ecotoxicology laboratory for the past 25 years (see Riethmuller et al (2003) for details). *Chlorella* sp. was isolated from surface waters of Magela Creek in Kakadu National Park (Georgetown Billabong) by Armando Padovan in 1991.

5 Dilution water

Depending on the aim of the test, either synthetic soft water (SSW) or uncontaminated natural water (often Magela Creek water (MCW) for Ranger related toxicity tests) is used as the test diluent.

Magela Creek water is collected from one of two locations along the creek. When there is flow in the creek during the wet season, water is collected by boat near Georgetown Billabong (Map Grid of Australia (MGA) Zone 53, 275320.954 East, 8597972.198 North). Throughout the rest of the year water is collected from Bowerbird Billabong closer to the source of the creek (MGA Zone 53, 287190 East, 8587265 North) where there is a persistent water body during the drier months.

Synthetic soft water was created to simulate the inorganic composition of MCW during the wet season. As a result, it lacks any form of organic carbon. Magela Creek water is very soft and slightly acidic with low alkalinity and low electrical conductivity. The SSW is made using the method in Appendix D. SSW is prepared as close to the start of an experiment as possible and is stored in the refrigerator in sealed polyethylene containers for up to two weeks.

6 Chemical solutions

All reagents used are analytical grade and stock solutions are made up in ultra-pure water (18 MΩ, Milli-Q, Millipore). The date of stock preparation, source and the person who made the solution are marked on each bottle. A label displaying the chemical name, formula and any required hazard symbols and first aid information must also be included on the bottle. These labels must adhere to the Supervising Scientist Branch (SSB) chemical labelling protocol (See SSB document WHS-029).

7 Test solutions

All test waters, regardless of diluent, need to be supplemented with nutrients in order to promote algal growth that is sufficient to measure a toxicological response. A buffer (1 mM HEPES; *N*-2hydroxypiperazine-*N'*-2ethanesulfonic acid) is required to minimise pH drift throughout the 72 h test period (Appendix B). The following steps must be followed when preparing test solutions:

- 5 L of nutrient-supplemented test water is made (using either MCW or SSW) to final nitrate and phosphate concentrations of 3.63 mg L^{-1} nitrate (NO_3^-) and 34.5 µg L^{-1} phosphate (PO_4^3), respectively, and a final HEPES concentration of 1 mM.
- 500 ml of the above test water is allocated for each treatment.
- For each treatment, 50 ml aliquots are dispensed into three Erlenmeyer flasks. The remaining test solution for each treatment is used for the validation of toxicant and nutrient concentrations and water quality measurements.

The nutrient concentrations in this protocol differ from the original algal testing protocol (3.63 mg L^{-1} NO_3^- and 34.5 µg L^{-1} PO_4^{3-} compared to 14.51 mg L^{-1} NO_3^- and 138 µg L^{-1} PO_4^{3-} previously). The tests and method used to determine the new nutrient concentrations are shown in Appendix A.

8 Test conditions and quality analysis

At the start of each toxicity test, sub-samples (40 ml) of the control, a procedural blank and an ultra-pure water blank are collected in plastic sample bottles and acidified with 1% v/v nitric acid (HNO_3, Chem-supply). Samples are analysed by an analytical chemistry laboratory for Al, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Na, Ni, Pb, Se, SO_4, U and Zn using ICP-MS or ICP-OES. Samples are also taken from all other treatments and analysed for toxicants and modifying elements relevant to the test in question. Verification of nutrient concentrations (NO_3^- and PO_4^{3-}) are performed by taking a 50 ml sub-sample from the control treatment and an ultra-pure water blank. Tests are conducted at 29 ± 1° C using a constant temperature growth cabinet with a 12 h light: 12 h dark photoperiod. Light intensity ranges between 100-150 μmol m^{-2} s^{-1} and is checked quarterly using a light meter.

Physico-chemical water quality parameters – i.e. electrical conductivity (EC), pH (WTW Multiline P4 Meter) and dissolved oxygen (DO, WTW inoLab Multiline Level 1) - are measured at the start and conclusion of the test using a pooled sample of each treatment. Temperature is monitored with a probe placed in a 50 ml vial containing 30 ml of ultrapure water at 5 min intervals and recorded using a remote logging system (Testo Saveris Professional, Lenzkirch, Germany). During the testing period, test containers are removed from the temperature controlled chamber for the minimum amount of time to maintain as constant temperature as possible throughout the test.

Results of the chemical analyses are considered acceptable if the control, procedural blank and ultra-pure water blank are free of contamination and the measured spiked toxicant concentrations are within 20% of nominal concentrations. Water quality data were considered acceptable if: the recorded temperature of the incubator remained at 29 ± 1°C; the recorded pH of the control group was within ± 1 unit of Day 1 values; the EC for the control solution was within 10% of the values obtained on Day 1; and the DO concentration was greater than 70% saturation throughout the test.

9 Apparatus and test equipment

9.1 Container preparation

All equipment that comes into contact with test organisms, control water or test solutions should be made of a material which is suitable to the toxicant in question. For metals, a plastic (e.g. polyethylene) should be used, where possible, whereas for organics, glass is a more suitable material. All plastic and glassware is washed by soaking in 5 % HNO_3 for 24 h before undergoing a detergent wash (Neodisher Laboclean, phosphate free, Miele, Gütersloh, Germany) and two rinses in a laboratory dishwasher (Miele, Gütersloh, Germany) with deionised reverse osmosis water (Elix, Millipore, Massachusetts, USA). If metals are being tested, glassware used for the incubation of algae in toxicity tests must be silanised with 2% dimethyldichlorosilane in 1,1,1-trichloroethane (Coatasil, Thermofisher Scientific, Massachusetts, USA) to decrease metal adsorption to the glass.

9.2 Equipment

- Flow cytometer (e.g. Accuri C6, BD)
- 15 ml glass tissue homogeniser with Teflon pestle
- Disposable 5 ml polystyrene round-bottomed tubes
- Light-tight constant temperature growth chamber (set at 29 ºC)
- Merck ultra-pure water purification system or similar
- Refrigerator (set at 4 ºC)
- pH, electrical conductivity and dissolved oxygen meters
- A-grade volumetric flasks (500 ml and 5 L)
- Chemicals and reagents
- Analytical balance and weigh boats
- Centrifuge – 4 x 500 ml capacity with swing out buckets
- Plastic centrifuge tubes (50 ml)
- Vortex mixer
- Borosilicate glass 200 ml Erlenmeyer flasks with aluminium caps
- Automatic adjustable pipettes (100 µL, 1 ml and 5 ml)
- Disposable microlitre pipette tips
- Light meter
- Magnetic stirrer and stirrer bars
- Polyethylene storage containers (1 L)
- 120 ml polystyrene water parameter vials
- Cell scraper
- Testo Saveris™ temperature monitoring system
- Random number sheet

- 40 ml and 15 ml sterile plastic sample tubes
- 50 ml plastic bottles for nutrient samples

10 Area for test preparation

The preparation of test solutions should be carried out in an area with ample room and free of contamination from harmful vapours, dust or disturbance. Throughout the test workers should take care not to introduce any contaminants during daily observations and water exchanges by washing hands and arms and wearing disposable gloves. When working with uranium additional precautions must be taken such as using designated benches within the laboratory and disposing of uranium waste down specific sinks within the laboratory (See eriss ecotoxicology lab uranium ASA for the full requirements).

11 Recording data

The number of algal cells in each test flask is counted at 48 h, 72 h and, occasionally, 24 h after test commencement (see Section 12 and Appendix E for details). Two additional flasks are inoculated at the beginning of the test (i.e. Day 0) and these are counted to measure the starting algal cell density. These data are then used to calculate algal growth rate (Appendix G). The pH, dissolved oxygen and electrical conductivity are measured on a subsample of test waters at the start and end of the test. Continuous temperature data is collected throughout the test by the Testo Saveris™ temperature probe inside the incubator.

12 Test procedure

Day 0

Chlorella sp. cells are harvested from a 4-5 d old stock culture as these are in the exponential phase of growth. The algal cells should be centrifuged in a 50 ml plastic centrifuge tube at 1310 *g* for 7 minutes. The supernatant is then decanted and the cell-pellet resuspended in approximately 30 ml of ultra-pure water by vortexing. The washing process is repeated three times to ensure that the nutrient-enriched culture medium is removed.

After three rinses the pellet is resuspended in approximately 15 ml of ultra-pure water. Two dilutions of this cell suspension are prepared; a 1:10 dilution (e.g. 2 ml cells into 20 ml ultra-pure water) and a 1:100 dilution (0.2 ml of 1:10 dilution into 20 ml ultra-pure water). The 1:100 dilution is used to determine the volume of the 1:10 dilution inoculum required to have the correct final cell density.

Inoculum volume is determined from a 25 µL sub-sample using the flow cytometer (for complete instructions on measuring algal density and setting up the Accuri C6 flow cytometer, see Electronic cell enumeration section below and Appendix E).

The cell count from the flow cytometer is multiplied by the dilution factors (40 × for the 25 µL subsample taken by the flow cytometer and 100 × for the initial 1:100 dilution) to give the density of the washed cell suspension in cells ml^{-1}. This value is then divided by the desired final cell density in each 50 ml aliquot of test solution to give the volume of the 1:10 dilution to add to each test flask (see example below). Two additional flasks are also inoculated and sampled on day 0 to confirm that the cell inoculation density is 3×10^3 cells ml^{-1}.

Example:

Algal counts (P1 gate):	= 738, 728, 714, 657
$\bar{x}$	= 709.25
x100	= 70925
x40	= 2.84 x 10^6

$$\frac{3 \text{ x } 10^3 \times 50\ mL}{2.84 \text{ x } 10^6}$$

= 0.0528

= 53 µL added to each test flask of 50 ml test solution

Test solutions are prepared as described in section 6 and are equilibrated for 2 h.

Tests are performed with three replicates per treatment at an algal cell density of 3 x 10^3 cells ml^{-1}. Given the variability between replicates within a treatment is usually low ($\bar{x} = 2.31$ % CV in controls of tests to date), this design may be changed to two replicates per treatment if required. This also allows for more toxicant concentrations to be tested.

One flask with test solution but without algae is used as a background count correction for the flow cytometer for each treatment.

NOTE: If test solutions are similar in composition, one blank flask can use used for all treatments.

The test flasks are then placed randomly on the shelves in a light-tight constant temperature growth chamber at 29 ± 1°C on a 12:12 h light, dark cycle at 100-150 photons PAR m^{-2} s^{-1}.

Days 1-3

Each flask is gently agitated by hand twice daily throughout the test to avoid gas limitation; this is done by swirling the solution approximately six times in the clockwise and anti-clockwise direction.

The cell density in each flask is determined at both 48 and 72 h by using the flow cytometer (as per Step 3 and Appendix E).

Electronic cell enumeration

Each flask should be gently agitated using a cell scraper to resuspend the algal cells. A 2 ml sub-sample is then taken and placed in a glass tissue grinder and homogenised using a Teflon pestle to break down any clumps of algal cells. 500 μL of this sample is then placed in a clean 2 ml test tube and placed on the flow cytometer sampling stage. Three 25 μL sub-samples are analysed by the flow cytometer and an average taken of the number of events that occur within the gated region that represents algae auto-fluorescing in the red colour. This process is then repeated for each replicate per treatment with the blank flask containing no algae measured first to determine background levels of fluorescence.

The flow cytometer is back-flushed between each treatment. The homogeniser and pestle are also rinsed with ultra-pure water and two new test tubes are used for each treatment.

13 Randomisation

Each day, a new set of random numbers must be used to assign the position of each flask within the incubator. Each flask has a number written on the top of the tin foil lid and is placed into the incubator according to the order of the numbers on the random number sheet. The flasks need to be positioned on the edges of each shelf as close to the light source as possible to ensure that light exposure is maximised. Randomisation of flask position in the incubators is an important part of the experimental design. Random numbers are obtained from a random number table or generator for each day of the test. A set of random numbers is unique for each test and is not to be reused.

14 Reference toxicants

The use of reference toxicants enables the response of the test organism to be assessed over time to ensure the response is reproducible (see Appendix C for the current reference toxicity program). This process also checks the proficiency of operators and laboratory standards. Uranium (U, added as uranyl sulphate) is used in a concentration range from 5-80 μg L^{-1}. Synthetic soft water is used as the diluent. The EC50 value, calculated from the concentration-response curve should fall within 3 standard deviations (SDs) of the mean on the quality control chart for the test species. If the value falls outside 2 SDs of the mean, it is a warning that there may be something wrong with the test or the sensitivity of the organism has changed. It is important to note that a control chart cannot be produced or considered reliable with less than 5 values.

15 Acceptability of test data

The test data are considered acceptable if:

1. The recorded temperature of the incubator remains within the prescribed limits (29 ± 1ºC),
2. The growth rate of the control algae is within the range 1.9 ± 0.15 (mean ± standard deviation) doublings per day,
3. There is <20% co-efficient of variability (CV) in the control growth rate,
4. The recorded pH is within the prescribed limits and,
5. The results of reference toxicity testing are within the set limits.

16 Analyses of test data

The growth rate of the algae in each flask is calculated using linear regression analyses (Appendix F). A regression is plotted for $\log_{10}$ cell density vs. time (h) to determine the slope of the line for each flask, which is equivalent to the cell division rate per h (μ) for each treatment. Doublings per day are calculated by multiplying the cell division rate by 24 (the hours in a day) then by the constant of 3.32. The algal population growth constant is based on calculations done by CSIRO and are shown in Appendix F. The growth rates of each treatment are presented as a function of the control response and these are plotted against measured toxicant concentrations. The endpoint of the algal growth test is measured as the 72 h EC50. Linear interpolation is used to calculate EC values when performing reference toxicity tests using uranium (U) to allow for comparison with historical reference toxicity data but in all other toxicant tests non-linear regressions (e.g. generally three parameter log-logistic models) are used as they provide a more accurate fit to the data. Toxicant concentration is log transformed in all analyses.

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Appendix A
Nutrient reduction trials

1 Aims

Tests were performed to establish the minimum concentration of nitrate (NO_3^-) and phosphate (PO_4^{3-}) that can be added and produce acceptable algal growth over 72 h without nutrients being a limiting factor within the test system.

2 Method

Five tests were conducted with reduced cell density (3 x 10^3 cells ml^{-1}) and reduced nutrients. Nutrient levels ranging from 1/64th of the original nutrient concentration (2.16 µg L^{-1} PO_4^{3-} and 227 µg L^{-1} NO_3^{-1}) to the full ration (14.51 mg L^{-1} NO_3^- and 138 µg L^{-1} PO_4^{3-}) were tested to determine the minimum concentration of nutrients required to maintain acceptable control growth (Table A1). The complete list of tests is shown in Appendix G. All tests were performed in SSW amended with different volumes of PO_4^{3-} and NO_3^- added using the method described above.

Table A1 Summary of reduced nutrient trials showing growth rate range and nutrient concentrations tested

Test Code	Growth rate range (doublings d^{-1})		Concentrations tested (µg L^{-1})[a]
1366G	0.62 – 1.70	N	453, 907, 1209, 1814, 3628, 7255, 14510
		P	4.31, 8.63, 11.5, 17.3, 34.5, 69, 138
1373G	1.35 – 1.89	N	907, 1209, 1814, 2418, 3628
		P	8.63, 11.5, 17.3, 23, 34.5
1376G	0.71 – 1.43	N	227, 453, 605, 907, 1209, 1814
		P	2.16, 4.31, 5.75, 8.63, 11.5, 17.3
1378G	0.91 – 1.70	N	907, 1209, 1451, 1814, 2418
		P	8.63, 11.5, 13.8, 17.3, 23
1380G	1.08 – 1.66	N	1209, 1451, 1814, 2418, 2902
		P	11.5, 13.8, 17.3, 23, 27.6

[a] The original concentration of PO_4^{-3} and NO_3^- added to test medium was 14.51 mg L^{-1} NO_3^- and 138 µg L^{-1} PO_4^{-3}

3 Results

Algal population growth rates consistently above the original test acceptability criterion of 1.4 ± 0.3 doublings d^{-1} (Figure A1.) were observed in all concentrations of nutrients above 1/8 of the original nutrient provision (i.e. 1.815 mg L^{-1} NO_3^- and 17.3 µg L^{-1} PO_4^{3-}). Based on this information, a new nutrient concentration of ¼ of the original nutrient dose was selected (i.e. 3.63 mg L^{-1} NO_3^- and 34.5 µg L^{-1} PO_4^{3-}). This value was selected due to the consistently high growth rates and small variance observed. A lower concentration was not selected to ensure that nutrients would not become a limiting resource in the testing environment. All measured nutrient

concentrations and quality control checks associated with these data are shown in Appendices I and J.

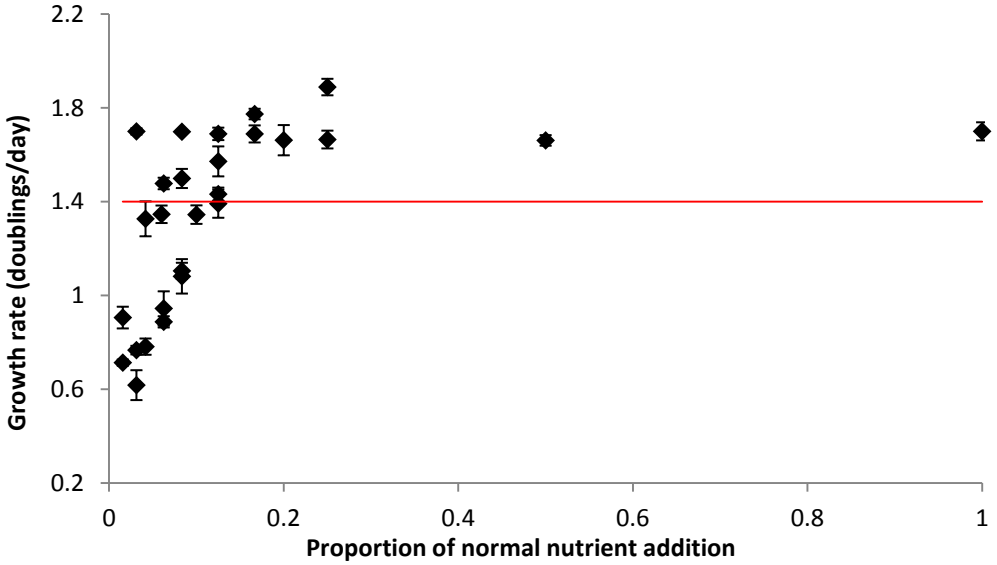


Figure A1 Pooled population growth rates for the five reduced nutrient trials performed (± S.E.). Red line represents the original population growth acceptability criteria of 1.4 doublings d^{-1}.

4 Discussion

We established that a reduction of nutrients to a quarter of the original ration provided sufficient nutrients to *Chlorella* sp. at a reduced cell density of 3×10^3 cells ml^{-1}. A nutrient reduction to 3.63 mg L^{-1} NO_3^- and 34.5 µg L^{-1} PO_4^{3-} produced an average control growth rate over the nine successful reference toxicity tests of 1.9 doublings d^{-1}. This growth rate was higher than the original test protocol acceptability criterion for population growth rate (1.4 doublings d^{-1}) and seeing as this is a new methodology a new acceptability criterion was created. A higher growth rate would be expected with a reduced algal cell density due to there being a lower number of cells per ml of medium and, as such, more capacity for growth before nutrients become a limiting factor. It may have been possible to reduce the nutrient levels further, as there was little reduction in population growth rate observed at nutrient concentrations as low as 1/8 of the initial ration. However, it was decided to select slightly higher concentrations to ensure that nutrients did not become limiting. Statistical reports associated with this data can be found in Appendix K.

Appendix B
Use of HEPES buffer in algal toxicity tests

1 Aims

Tests were performed to establish whether HEPES (*N*-2hydroxypiperazine-*N*'-2ethanesulfonic acid) was required in the new test system. Due to the reduction in nutrients and *Chlorella* sp. population starting density within the new system it was possible that pH would remain within the acceptability criterion of 1 pH unit in the absence of a buffer as fewer waste products would be produced by the algae present.

2 Methods

Three tests were conducted with an additional control treatment without HEPES included. Tests were performed in SSW with a *Chlorella* sp. starting cell density of 3 x 10^3 cells ml^{-1}. Nutrients were added in the reduced concentrations of 3.63 mg L^{-1} of NO_3^- and 34.5 µg L^{-1} of PO_4^{3-}. Algal population change was measured with flow cytometry using the method outlined in Section 12 of the main report. The complete list of tests is shown in Appendix H and associated quality control checks are in Appendices I and J. Nominal nutrient concentrations were used for analyses as the measured concentrations we received from an external analysis company were inaccurate (see Appendix J, Table J2). This has been an ongoing problem in the *Supervising Scientist* ecotoxicology lab for some time, a solution for measuring nitrate and phosphate accurately is needed.

Table B1 pH variation and percent coefficient of variation (% CV) in control treatments with and without 1mM of the buffer HEPES added to the test system after 72h (n=3). pH samples were measured from a pooled sample taken from the three test flasks.

	pH variation		% CV	
Test	1mM HEPES	No HEPES	1mM HEPES	No HEPES
1455G	0.31	0.48	0.72	13.9
1466G	0.07	0.59	0.19	1.63
1468G	0.09	0.34	0.93	21.5

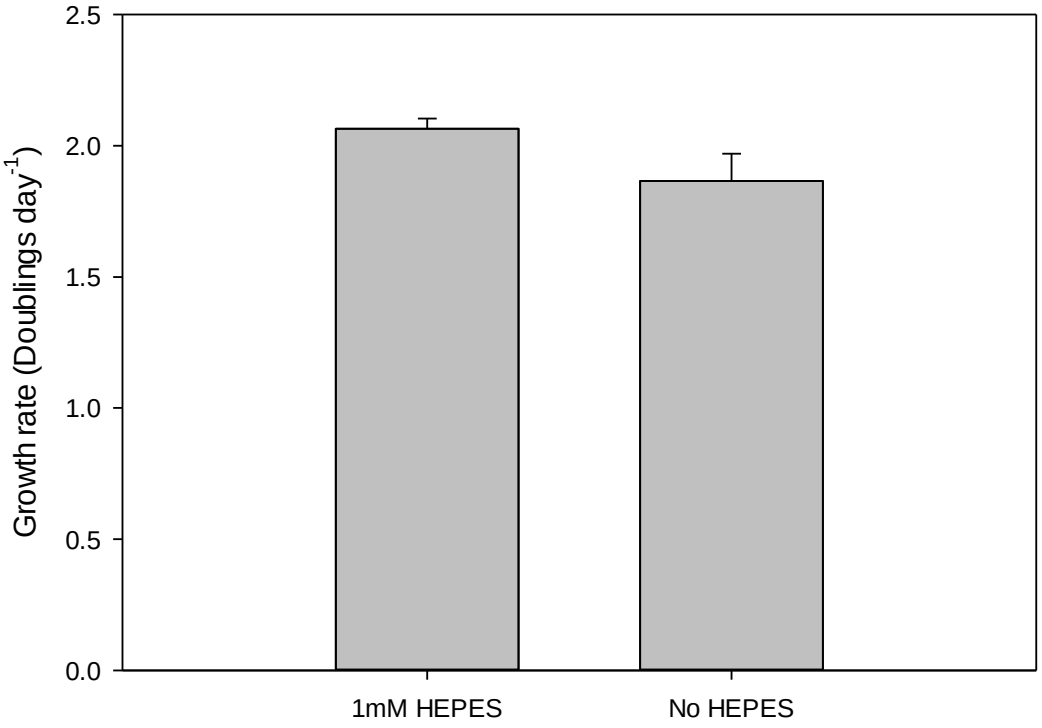


Figure B1 *Chlorella* sp. population growth rate in SSW with and without the inclusion of 1mM HEPES (n = 3).

3 Results and discussion

Reducing the initial starting density notably improved pH control with, at most, a 0.2 unit drift in pH in the nutrient trials and 0.3 of a unit in reference toxicity testing. Even in the absence of the HEPES buffer, which is used to control pH in algal tests, a change of up to 0.59 units was observed. While pH control was within the 'acceptability criterion of 1 pH unit' in the absence of HEPES (Table B1) the population growth rates were lower (Figure B1) and more variable between replicates (%CV HEPES $\bar{x}$ = 0.61, No HEPES $\bar{x}$ = 12.35). Moreover, pH changes of around 0.5 of a unit can still result in marked changes in metal speciation and bioavailability. Consequently, it was considered appropriate to retain HEPES in the test medium. In two of the tests performed abnormally high concentrations of iron, aluminium and manganese were detected; this indicates a source of contamination. Results for these tests fit within our expectations and as such these tests were included in analyses.

Appendix C
Reference toxicity testing with uranium in Synthetic Soft Water

1 Aims

On finalisation of the new methodology reference toxicity tests were performed to determine how the new method would affect the sensitivity of *Chlorella* sp. to uranium.

2 Method

Uranium (U) toxicity tests were performed using the reduced nutrient concentrations and starting cell density of 3 x 10^3 cells ml^{-1} in SSW. Initially, 7 U concentrations were tested ranging between 5 and 160 μg L^{-1} to establish definitive concentration–response curves. After 5 repeatable tests, this was reduced to 5 concentrations ranging between 20 and 160 μg L^{-1}**.**

Control growth rates for each test were higher than with the past protocol of 1.4 ± 0.3 doublings d^{-1} (Reithmuller *et al.*, 2003, Table C1) and a new acceptability criterion for population growth rate was calculated. After six tests, a growth rate acceptability criterion was derived by calculating the average and standard deviation of the growth rate of the control treatment for each test. The average was made the acceptability criterion and the standard deviation was used as the acceptable variance surrounding the criterion. Based on the control treatment growth rates in the reference toxicity tests a new acceptability criterion of 1.90 ± 0.15 doublings day^{-1} was calculated. All measured U concentrations and quality control checks associated with this data are shown in Appendices I, J and K.

3 Results

Nine valid U toxicity tests were performed in synthetic soft water (Appendix D) using the nutrient concentrations (¼ of the original nutrients of Reithmuller et al (2003)) of 3.63 mg L^{-1} NO_3^- and 34.5 μg L^{-1} PO_4^{3-}. The 50% Effect Concentrations (EC50) for the nine tests ranged between 9 and 53 μg L^{-1}. The current running mean (± 2 Standard Deviations, SD) for the new method is 35 (6, 63) μg L^{-1} compared to 42 (7, 76) μg L^{-1} of U in the original method (Figure C1).

Table C1 Control population growth rates and toxicity estimates for reference toxicity tests using a starting algal density of 3 x 10^3 cells ml^{-1}. Values in parentheses in the 'Control population growth rate' column represent % CV and in the 'EC50' column represent 95% confidence limits.

Test Code	Date	Control population growth rate (Doublings d^{-1})	EC50 (µg L^{-1} U)
1383G	03/02/14	1.84 (8.36)	40 (35, 45)
1389G	18/02/14	1.88 (2.39)	41 (37, 45)
1404G	13/05/14	1.86 (0.42)	39 (37, 40)
1415G	05/08/14	1.74 (3.44)	49 (46, 55)
1431G	09/12/14	1.78 (1.58)	53 (41, 86)
1455G	17/03/15	2.11 (0.72)	37 (28, 48)
1466G	09/06/15	2.17 (0.19)	9 (8, 10)
1468G	22/06/15	1.91 (0.93)	19 (18,19)
1479G	07/09/15	2.05 (2.78)	25 (23, 27)

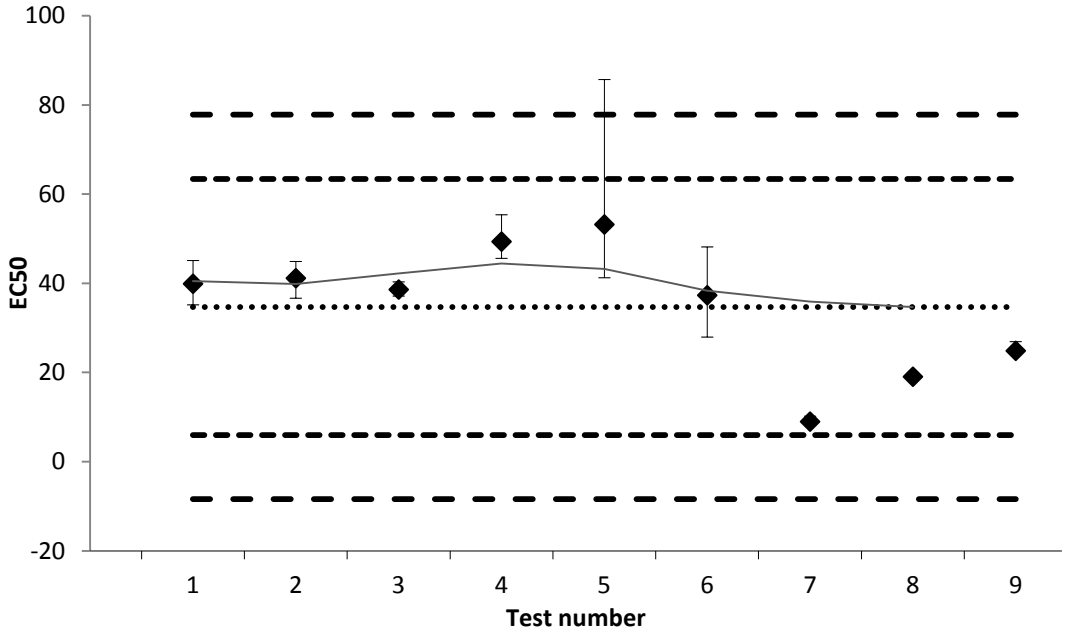


Figure C1 Reference toxicant control chart for *Chlorella* sp. using population growth rate as an endpoint. Data points represent EC50 µg L^{-1} U toxicity estimates and their 95% confidence limits (CLs). Reference lines represent the following: broken lines – upper and lower 99% confidence limits (± 3 standard deviations) of the whole data set; dotted lines – upper and lower warning limits (± 2 standard deviations); unbroken line – running mean

4 Discussion

At a reduced cell density and nutrient level, *Chlorella* sp. sensitivity to U increased slightly (17% reduction – 42 to 35 μg L^{-1}), albeit non-significantly. This result was driven by the last three tests, which were more sensitive than the previous six tests (Figure C1). An increase in sensitivity would be expected, as ligands such as phosphate cause speciation changes when complexation occurs with U in solution (Markich 2002). This complexation is known to reduce U toxicity to freshwater organisms (Markich 2002, Trenfield et al 2011).The lower starting algal density would also contribute to the reduction in sensitivity as less metals have been found to bind to algal cells at higher algae densities (Franklin et al 2002). Seeing as only nine tests have been performed so far, it is difficult to determine whether the more sensitive results in the last three tests were due to a change in the *Chlorella* sp. culture or due to natural variation in U sensitivity. When a comparison is made between the variation in sensitivity of the current and previous method we see that it is similar, if not a little more variable in the old method (current test method EC50 range = 8.96 – 53.17 μg L^{-1}, old test method EC50 range = 10.12 – 74.91 μg L^{-1}). Further reference toxicity testing will allow for this to be better characterised. In one of the reference toxicity tests abnormally high concentrations zinc were detected; this indicates a source of contamination (Table J3). The growth rate and toxicity estimate for this test fit within our expectations and as such this test was included in analysis. Statistical analyses associated with this data are found in Appendix K.

Appendix D
Synthetic Soft water preparation

1 Safety

Check the SDSs for any chemicals you are about to use prior to making up solutions to ensure you are aware of any WH&S issues and the appropriate PPE. There are no risks associated with SSW due to the low concentrations of all chemicals added. Gloves, eye protection and a lab coat must be worn while preparing Synthetic Soft Water.

2 Preparation of solution

- Fill a 5 L volumetric flask with ultra-pure water and pour this into a clean 25 L plastic barrel designated for synthetic water preparation.
- Partially refill the 5L flask with ultra-pure water and add the appropriate amount of the 7 stock solutions (see Table 1) to the flask. Make the flask up to volume with ultra-pure water and pour into the barrel.
- Fill the 5 L flask twice more to make the volume in the barrel equal 20 L.
- Aerate overnight to allow mixing and gaseous exchange.
- Check pH after a minimum of 12 h aeration and adjust to 6.0 ± 0.15 using 0.05 M H_2SO_4 or 0.05 M NaOH.
- The water can be stored at 4°C for up to two weeks if necessary. The pH needs to be checked before use to ensure it remains within range.

3 Making stocks

Stock solutions are made up every 18 - 24 months.

- Add the appropriate amount of chemical in column 2 of Table D1.
- Make up 1 L of each stock solution at a time.

Table D1 Stock solutions used to prepare synthetic soft water.

	Ingredient	Stock Solution (g L^{-1})	Volume of stock per 20 L	Nominal conc. of element in SSW
1	$NaHCO_3$	72.34	1 ml	0.99 mg L^{-1}
2	$Al_2(SO_4)_3.18H_2O$*	17.26	1 ml	0.075 mg L^{-1}
3	$MgSO_4.7H_2O$	121.52	1 ml	0.599 mg L^{-1}
4	$CaCl_2.2H_2O$	32.96	1 ml	0.449 mg L^{-1}
5	KCl	14.09	1 ml	0.3107 mg L^{-1}
6	$FeCl_3.6H_2O$	10	1 ml	0.1285 mg L^{-1}
7	Trace Element Solution	In 1 L add:	0.5 ml	
	$CuSO_4.5H_2O$	0.11		0.975 µg L^{-1}
	$ZnSO_4.7H_2O$	0.123		0.699 µg L^{-1}
	$Pb(NO_3)_2$ (from EnRad)	0.008		0.125 µg L^{-1}
	$MnSO_4.H_2O$	1.188		9.654 µg L^{-1}
	$UO_2SO_4.3H_2O$ (use 5gL^{-1} U stock in fridge 2)	0.007		0.1125 µg L^{-1}

*Requires heating to dissolve

Appendix E
Analysis of algal densities using the Accuri C6 flow cytometer

Prior to commencing algal density measurements the Accuri C6 must be run through a start-up process and performance check. The methods are detailed below.

1 Start-up clean

- Open the 'H_2O Start-up' file in the CFlow workspace.
- Select the back flush button in the workspace and wait for this process to run.
- Ensure there is a tube of at least 2 ml of ultra-pure water on the SIP.
- Select the next free data cell, label it with the date and run the ultra-pure water for 10 minutes.
- Save and close file.
- Place a tube of Extended Flow Cell Clean solution on the SIP.
- Open the 'Clean' file in the Maintenance folder.
- Select the next free data cell and run for 2 minutes.
- Save and close file. Reopen 'H_2O start-up' file.
- Select the back flush button in the workspace and wait for this process to run.
- Replace the tube of ultra-pure water on the SIP.
- Select the next free data cell and run the ultra-pure water again for another 10 minutes.
- Save and close file.

2 Validation beads

2.1 The need for validation

Prior to analysis of experimental samples, the performance of the Accuri C6 should be checked using validation beads. The validation should verify whether the lasers are correctly positioned.

The Accuri C6 should be able to distinguish a defined number of peaks within each fluorescence channel i.e. the 8-peak beads should show 8 peaks when a sample is run and the 6-peak beads should show 6 peaks. If these peaks are not discernible, there may be a problem with either the beads or the instrument. If it is a case of the latter, any data collected may be incorrect. Thus, each data collection event should be accompanied by a file that shows the validation bead data. It is important not to proceed with the analysis of any experimental samples until the validation process has been completed successfully.

2.2 Setup

A template for bead validation has been created which has pre-defined settings. The file name is 8 and 6 peak template.c6t. A workspace has been created for routine bead validations. Each workspace file has the capacity to hold 48 x 8-peak validation data points and 48 x 6-peak data points. The workspace should be saved with the date that corresponds with the first entry (e.g., 8 and 6 peak 200313). Once all 96 wells are filled, then a new workspace can be created, using the

pre-saved template. If for some reason, a new template needs to be created, ensure the following settings are entered:

Type *50000* in the *events* edit box and select *Ungated Sample* from the associated drop-down list (Figure E1). Set the fluidics to **Slow** rate (Under Fluidics Control in the Control Panel).

Figure E1 Run Limit: 50000 Events.

Run 8-Peak Validation Beads

1. Open the current 8- and 6- peak validation workspace.
2. Prepare a sample of suspended 8-peak validation beads by adding 4 drops of the beads to 1 ml Ultra-pure water. A new set of validation beads is made up at the beginning of each test.
3. Vortex and place the tube on the Sample Introduction Platform (SIP).
4. Select the next empty data cell following the previous 8- peak validation. If this is the first run in a new template, select A1. Make sure the data cell in the CFlow template is empty before starting the run. If the button displays ADD TO, the cell already contains data.
5. Check that the fluidics is set to Slow
6. Click on the RUN button to start acquisition. Acquisition automatically stops after 50,000 total events are acquired.
7. Name the sample by typing a name in the text box just above the Sample Grid. Include the date in the sample name to differentiate it from samples collected on other dates (Figure E2). Samples can be named before, during or after collection.
8. When the collection is finished, remove the sample tube
9. Back flush the SIP. This can be achieved by either selecting the back flush icon or Instrument > Run Back flush. Ensure a blotter or empty sample tube is under the SIP to catch the dripping fluid. Wipe excess fluid from the SIP with a lint-free Kimwipe.

NOTE: The R1 region may not encompass the main population of bead events on the FSC-H vs. SSC-H plot. This is common and acceptable at this stage.

Figure E2 Sample Name: 8-Peak Beads.

Run 6-Peak Validation Beads

1. Prepare a sample of suspended 6-peak validation beads by adding 3 drops each of Peak 1 and Peak 2 beads to 1 ml of Ultra-pure water.
2. Vortex and place the tube on the SIP.
3. Select the next empty data cell following the previous 6- peak validation and name the sample. If this is the first run in a new template, select E1 (Figure E3).
4. Again, verify that *Events* is still enabled and set at 50,000 in *Ungated Sample* and the fluidics is set to *Slow*.

5. Click on the *RUN* button.
6. Name the sample including the date in which it was processed.

NOTE: The R2 region may not encompass the main population of bead events on the FSC-H vs. SSC-H plot. This is common and acceptable at this stage.

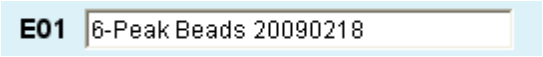


Figure E3 Sample Name: 6-Peak Beads.

Saving validation bead data

By default, CFlow automatically saves validation bead data at the end of each run.

Sample and prompts you to save when moving between different workspaces. You can also save data manually at any time by selecting *File* > *Save*.

Analysing and recording validation bead data

After you collect the bead data, analyse the data using the *Collect* tab of CFlow to ensure that the C6 is functioning properly. The template has already predefined appropriate gate settings for the 8-peak and 6-peak validations.

To analyse the bead data:

1. Click on the well that contains the most recent 8-peak bead data.
2. On the first FSC-H vs. SSC-H plot (scatter plot) in the bead file, adjust the pre-drawn region (R1) to encompass the main population by dragging the border of the region (Figure E4). R1 should contain more than 75% of all events.

NOTE: There is usually a "shadow" population (called *bead doublets* or *clumps*) that is slightly higher in FSC-H than the main cluster of beads; this is normal for these beads. Do not include the shadow group in R1.

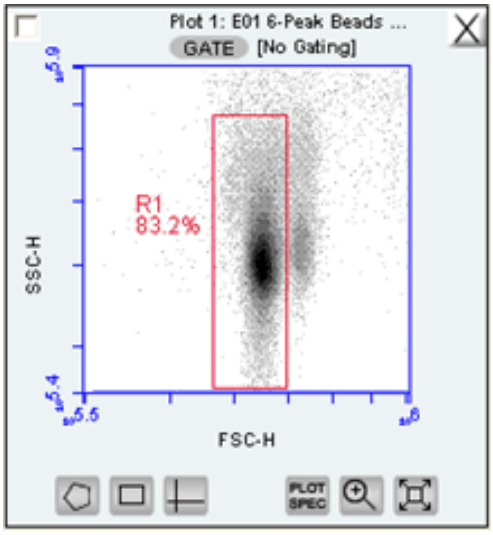


Figure E4 Plot with Bead Doublets.

Verify that the next three plots (FL1-H, FL2-H, and FL3-H) are gated on scatter region R1 and that the plots display the message *R1 in all* next to the *GATE* button (Figure E5). If it is not displayed, click on the *GATE* button and select *R1in all events* from the pop-up dialog box.

NOTE: Gates may need to be moved slightly when a new packet of beads are opened as different batches of beads have slightly different characteristics. Move the gate to encompass the main population of beads but not the "shadow" population.

Figure E5 Example of data for 8-peak beads. The number of expected peaks is: FL1-H (8), FL2-H (8) and FL3-H (6). The peaks in FL4-H are not relevant.

Monitoring the Validation Bead data

A convenient way to monitor the Validation Bead data, and thus the C6 performance, is to store the 8- and 6- peak validation bead data in a single CFlow file, saving the data from each day in its own cell. Then, using the Statistics tab, a table can be created of the Mean channel numbers and Coefficient of Variation (CVs) for the top peaks, and for the forward scatter of the beads to make it easy to determine if the C6 performance is stable.

However, on a daily basis, it is sufficient to record the R1 and R2 percentages along with the CV for each of the M1, M2 and M4 peaks in the lab record book. This allows the user to quickly compare the results with previous entries. The R1 and R2 percentages should be over 75% and the FSC-H, FL1-H and FL2-H CVs should be less than 6%.

3 Final clean

1. When the collection is finished, remove the sample tube from the SIP and wipe off the end of the SIP with a lint-free tissue.
2. Back flush the SIP.
3. Open the workspace "H_2O start-up" in the maintenance folder and advance to the next empty data cell.

4. Check that the *Time* check box (*Min Sec*) in the Instrument Control Panel is set to ten minutes and that the fluidics are set to *Fast*

5. Click on the *RUN* button.

6. When the run is finished, leave the tube on the SIP.

The C6 is ready for experimental work after successful completion of the validation beads analyses.

4 Analysis of test samples

A template specifically for *Chlorella* sp., has been created and the file is named *Chlorella* TEMPLATE. Templates will have file name ending with .c6t (e.g. *Chlorella* TEMPLATE.c6t). The *Chlorella* TEMPLATE has predefined settings in place specifically tailored for *Chlorella* sp., algal cells. Figure E6 shows healthy algal counts at 48h in the template workspace.

To open a file template select *File* > *Open CFlow File or Template* >*Templates*> *Chlorella* TEMPLATE.c6t. Automatically, a blank workspace will open with all required parameters set. The C6 will prompt you to save this file before the first analysis can proceed.

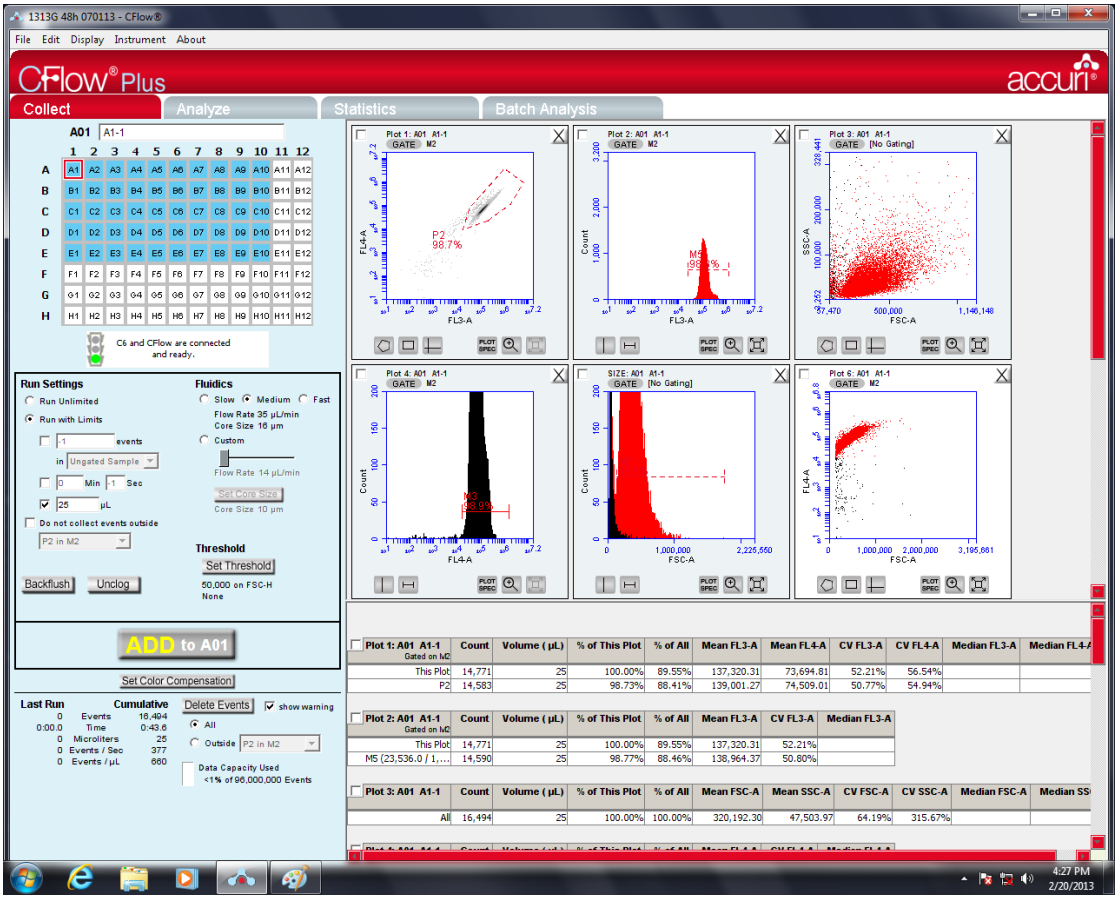


Figure E6 Typical control *Chlorella* sp., count setting at 48 h.

4.1 Preparing a sample

1. Thoroughly mix the sample so that a representative sub-sample can be taken. Scrape the bottom of the flask using a Teflon scraper before sub-sampling.

2. Pipette a sub-sample (min. 500µL) into a tissue grinder and homogenise the sample. Pour the sample into sample tube. Suitable tubes for algal samples are polystyrene falcon tubes with round bottoms. Algal cells can stick to the surface of the container so it is recommended that

samples are prepared just before being analysed. I.e. do not allow the sample to sit for longer than a few minutes.

4.2 Analysing a sample

1. Shake or agitate the sample in the tube before placing it onto the SIP.
2. If you are working from a predefined template, all data cells should have been pre-labelled with the sample ID. If you are not working from a template, type your sample ID in the text box. Click on the data cell.
3. Check that run limits and fluidics settings are correct. If you are working from a predefined template, these parameters should already be set. *Chlorella* sp., fluidics should be set to *Medium* speed and *25 µL sample volume*.
4. Ensure the traffic light is green.
5. If green, then click the Run button.
6. Repeat steps 2-5 twice more for triplicate readings. Several readings can be done on the same sample to achieve higher replication.
7. Back flush the fluidics line before moving on to a new treatment.
8. Repeat the above steps for the remainder of the test.
9. For the *Chlorella* sp., template, the region being counted is gated to the P2 region (Figure E7).

Plot 1: A01 A1-1 Gated on M2	Count	Volume (µL)
This Plot	14,771	25
P2	14,583	25

Figure E7 *Chlorella* sp. gated region counts vs. full plot counts

5 Shutdown procedure

When you finish collecting samples, rinse out the SIP to ensure cells or other particles are not left in the SIP.

1. Open the 'H_2O shutdown' file in the Maintenance folder.
2. Place a tube with 2 ml of filtered, de-ionized water on the SIP and advance to any empty data cell.
3. It should be set to run for 10 minutes.
4. Click on the RUN button.
5. Save this file and open the 'Clean' file in the Maintenance folder. Place a tube with 2 ml of extended clean solution on the SIP.
6. Select an empty data cell.
7. The time should be set to two minutes and the fluidics speed to *fast*.
8. Click on the *RUN* button.
9. Once the run is finished, remove the tube of cleaning solution from the SIP.
10. Select the back flush button in the workspace and wait for this process to run.
11. Re-open the 'H_2O shutdown' file.
12. Place a tube with 2 ml of ultra-pure water on the SIP.

13. Select an empty data cell.
14. Click on the *RUN* button.
15. Once this is complete turn the C6 off.

Appendix F
Calculation of algal growth constant

At any time (t), the number of cells (N) is expressed as:

$$N = N_0 e^{\mu t} \quad (1)$$

Where μ is the specific rate constant.

The actual rate is $\frac{dN}{dt}$

The specific growth rate - $\mu = \frac{1}{t}\frac{\ln N}{N_0}$ (Take logarithm of eq. 1.) (2)

Convert to natural log

$$\mu = \frac{1}{t}\log\frac{N}{N_0} \text{ x } 2.303 \quad (3)$$

Meanwhile, using eq. 3 to take the time for the number of cells to double yield.

$$\mu = \frac{1}{t_{2x}}\log\frac{2N_0}{N_0}\ (2.303) = \frac{1}{t_{2x}}\ln 2 \quad (4)$$

Rate of doubling

$$\frac{1}{t_{2x}} = \frac{\mu}{\ln 2} \quad (5)$$

Rate of doubling per day

$$\frac{\mu}{\ln 2} \text{ x } 24\text{h/day} \quad (6)$$

Since $\mu = \frac{1}{t}\log\frac{N}{N_0}2.303$

Then substituting into eq. 6.

Rate of doubling per day

$$\frac{1}{t}\log\frac{N}{N_0}\ (2.303\ \div \ln 2) \text{ x } 24$$

$$\therefore\ \frac{1}{t}\log\frac{N}{N_0}\ 3.32 \text{ x } 24$$

Where μ = slope of log N x t in hrs

Specific growth rate (μ)

$$\mu = \frac{1}{t}\ln\frac{N}{N_0} \quad (1)$$

Convert to logs

$$\mu = \frac{1}{t}\log\frac{N}{N_0}\ 2.303 \quad (2)$$

Meanwhile, using eq. 2, calculate the doublings, i.e. time for the number of cells to double yield

$$\mu = \frac{1}{t_{2x}}\log\frac{2N_0}{N_0}\ 2.303 \quad (3)$$

Convert to natural log

$$\mu = \frac{1}{t_{2x}}\ln 2 \text{ (Note: } \log\frac{2\cancel{N_0}}{\cancel{N_0}} = \log 2) \quad (4)$$

Now, "rate of doubling" is $\frac{1}{t_{2x}}$ therefore using equation (4)

$$\text{"Rate of doubling"} = \frac{1}{t_{2x}} = \mu \div \ln 2 \quad (5)$$

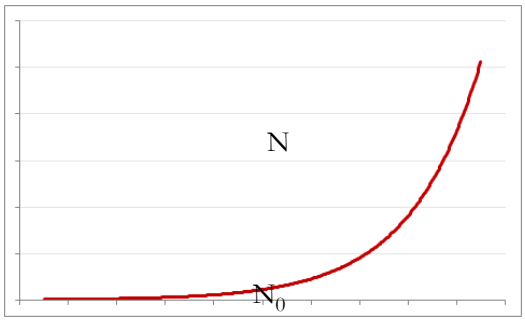
And since $\mu = \frac{1}{t}\log\frac{N}{N_0}\ 2.303$ (eq. 2.)

$$\text{Then "rate of doubling"} = \frac{1}{t}\log\frac{N}{N_0}\ 2.303 \div \ln 2 \quad (6)$$

Then rate of doubling per day $= \frac{1}{t}\log\frac{N}{N_0}\ 3.32 \text{ x } 24\text{h/day}$

And say $\frac{1}{t}\log\frac{N}{N_0} = \mu$, μ is the slope of log N vs. t(hrs)

Therefore rate of doubling per day = μ x 3.32 x 24



At any time *t*, the number of cells (*N*) is expressed as

$$N = N_0 e^{\mu t} \quad (1)$$

where μ is the specific rate constant for the cell division

The actual rate is given by

$$\text{Rate} = \frac{dN}{dt} \quad (2)$$

i.e. the change in number of cells, *dN*, over a period of time, *dt*

Taking the log of eq. 1 and further simplifying gives:

$$\mu = \frac{1}{t}\ln\frac{N}{N_0} = \frac{1}{t}\left(\log\frac{N}{N_0}\right) 2.303 \quad (3)$$

i.e. 2.303 comes about due to a change from natural log (ln) to $\log_{10}$

Doublings per day

Using equation 3 - $\mu = \frac{1}{t}\left(\log\frac{N}{N_0}\right)2.303$

i.e. $t = \frac{1}{\mu}\left(\log\frac{N}{N_0}\right)2.303$ (4)

the time for the number of cells to double yield is (t_{2x})

$$t_{2x} = \frac{1}{\mu}\left(\log\frac{2N_0}{N_0}\right)2.303$$

$$= \frac{1}{\mu}(\log 2)2.303 \quad (5)$$

Convert back to natural logs (ln) then;

$$t_{2x} = \frac{1}{\mu}\ln 2 \quad (6)$$

Now, "Rate of doubling" = $\frac{1}{t_{2x}}$ therefore reciprocal of (6)

Rate of doubling = $\mu\frac{1}{\ln 2}$ (7)

And from equation 3

$$\mu = \frac{1}{t}\left(\log\frac{N}{N_0}\right)2.303$$

∴ rate of doubling = $\frac{1}{t}\log\frac{N}{N_0}$ x $\frac{2.303}{\ln 2}$ 24h/day

$$= \mu\, x\, 3.32 \text{ x } 24\text{h/day}$$

$$\mu = \frac{1}{t} log\ \frac{N}{N_0}$$

Specific growth rate (μ)

$$\mu = \frac{1}{t}\ln\frac{N}{N_0} \quad (1)$$

Convert to log;

$$\mu = \frac{1}{t}\log\frac{N}{N_0}2.303 \quad (2)$$

Meanwhile, using eq. 2, calculate doublings.

i.e. time for the number of cells to double yield ($2N_0$)

$$\mu = \frac{1}{t_{2x}}\log\frac{2N_0}{N_0}\ 2.303 \quad (3)$$

Convert to natural log - $\mu = \frac{1}{t_{2x}}\ln 2$ (4)

Since $\log\frac{2\cancel{N_0}}{\cancel{N_0}} = \log 2$

Now, "rate of doubling" is $\frac{1}{t_{2x}}$ therefore using equation 4

'Rate of doubling' = $\frac{1}{t_{2x}} = \ \mu\ \div \ln 2$ (5)

And since $\mu = \ \frac{1}{t}\log\frac{N}{N_0}2.303$ (eq. 2)

Then "rate of doubling" = $\frac{1}{t}\log\frac{N}{N_0}2.303\ \div \ln 2$ (6)

And say; $\frac{1}{t}\log\frac{N}{N_0} = \mu$, then μ is the slope of log N vs. t(h)

$\mu t = \log N - \log N_0$

$\log N = \ \mu t + \log N_0$

Therefore 'rate of doubling' = $\mu\ x\ \frac{2.303}{\ln 2}$

Therefore doublings per day = $\mu\ x\ \frac{2.303}{\ln 2}$ x 24h/day

and hence doublings/day = μ x 3.32 x 24 where μ = slope of log N v t(h).

Appendix G
Example end of test worksheet

Figure G1 Algal population growth rate calculation table

	Day 0	Day 2	Day 3	Slope	Growth Rate (dblngs/d		Pearson	% Control	Mean %
Flask No.	All cell counts in (cells/mL) by 104				Mean				
A	0.28	1.73	1.73	0.01177	0.94		89%	74%	
SSW	0.28	2.55	2.52	0.01422	1.13	1.08	89%	89%	85%
1/12	0.28	2	2.98	0.01477	1.18		97%	92%	
	Avrg	**2.09**	**2.41**	***0.01358***					
B	0.28	2.52	3.56	0.01599	1.27		96%	100%	
SSW	0.28	3.03	4.73	0.01769	1.41	1.35	97%	111%	106%
1/10	0.28	3.31	4.03	0.01698	1.35		93%	106%	
	Avrg	**2.95**	**4.11**	***0.01689***					
C	0.28	3.25	5.16	0.01823	1.45		97%	114%	
SSW	0.28	3.59	8.55	0.02097	1.67	1.57	99%	131%	123%
1/8	0.28	3.44	7.16	0.02000	1.59		99%	125%	
	Avrg	**3.43**	**6.96**	***0.01974***					
D	0.28	3.23	8.24	0.02065	1.65		100%	129%	
SSW	0.28	3.87	9.78	0.02177	1.73	1.65	99%	136%	129%
1/6	0.28	3.41	6.63	0.01959	1.56		98%	123%	
	Avrg	**3.50**	**8.22**	***0.02067***					
E	0.28	3.06	7.2	0.01988	1.58		99%	124%	
SSW	0.28	3.92	9.93	0.02186	1.74	1.69	99%	137%	132%
1/5	0.28	3.79	9.82	0.02176	1.73		99%	136%	
	Avrg	**3.59**	**8.98**	***0.02117***					

	Log of Growth Rates			
	Day 0	Day 1	Day 2	Day 3	Below values for formula reference only			
	3.447158		4.2380461	4.2380461	0		48	72
	3.447158		4.4065402	4.4014005	0		48	72
	3.447158		4.30103	4.4742163	0		48	72
	3.447158		4.4014005	4.55145	0		48	72
	3.447158		4.4814426	4.6748611	0		48	72
	3.447158		4.519828	4.605305	0		48	72
	3.447158		4.5118834	4.7126497	0		48	72
	3.447158		4.5550944	4.9319661	0		48	72
	3.447158		4.5365584	4.854913	0		48	72
	3.447158		4.5092025	4.9159272	0		48	72
	3.447158		4.587711	4.9903389	0		48	72
	3.447158		4.5327544	4.8215135	0		48	72
	3.447158		4.4857214	4.8573325	0		48	72
	3.447158		4.5932861	4.9969492	0		48	72
	3.447158		4.5786392	4.9921115	0		48	72

		Growth Rate (dblngs/day)			
		Mean	Std dev	%CV	SE
A	0.08	0.94			
		1.13	1.08	0.13	11.75	0.07342
		1.18			
B	0.10	1.27			
		1.41	1.35	0.07	5.08	0.03945
		1.35			
C	0.13	1.45			
		1.67	1.57	0.11	7.04	0.06389
		1.59			
D	0.17	1.65			
		1.73	1.69	0.06	3.73	0.03642
		1.56			
E	0.20	1.58			
		1.74	1.66	0.11	6.72	0.06448
		1.73			

Appendix H
Lists of toxicity tests performed to determine nutrient concentrations and subsequent reference toxicity tests

Table H1 Details of each of the tests undertaken to determine the minimum amount of nutrients that can be added to a test and result in an acceptable population growth rate

Test code	Test start date	SSW production date		Concentrations tested (µg L^{-1})	Valid test? (Y/N)
1366G	05/11/13	16/10/13	N	453, 907, 1209, 1814, 3628, 7255, 14510	Y
			P	4.31, 8.63, 11.5, 17.3, 34.5, 69, 138	
1373G	25/11/13	15/11/13	N	907, 1209, 1814, 2418, 3628	Y
			P	8.63, 11.5, 17.3, 23, 34.5	
1376G	09/12/13	6/12/14	N	227, 453, 605, 907, 1209, 1814	Y
			P	2.16, 4.31, 5.75, 8.63, 11.5, 17.3	
1378G	13/01/14	10/1/14	N	907, 1209, 1451, 1814, 2418	Y
			P	8.63, 11.5, 13.8, 17.3, 23	
1380G	20/01/14	10/1/14	N	1209, 1451, 1814, 2418, 2902	Y
			P	11.5, 13.8, 17.3, 23, 27.6	

Table H2 Details of each of the reference toxicity tests performed with the reduced starting algal density and reduced nutrients

Test Code	Test start date	SSW production date	U concentrations tested (µg L^{-1})	Valid test? (Y/N)
1383G	03/02/14	31/01/14	0, 5, 10, 20, 40, 80, 160	Y
1389G	18/02/14	17/02/14	0, 5, 10, 20, 40, 80, 160	Y
1404G	13/05/14	11/05/14	0, 5, 10, 20, 40, 80, 160	Y but temp problems
1415G	05/08/14	22/07/14	0, 5, 10, 20, 40, 80, 160	Y
1431G	09/12/14	04/12/14	0, 5, 10, 20, 40, 80, 160	Y
1455G	17/03/15	07/03/15	0, 0 no HEPES, 20, 40, 80, 160	Y
1466G	09/06/15	28/05/15	0, 0 no HEPES, 20, 40, 80, 160	Y
1468G	22/06/15	12/06/15	0, 0 no HEPES, 20, 40, 80, 160	Y
1479G	07/09/15	04/09/15	0, 20, 40, 80, 160	Y

Appendix I Water Quality measurements

Table I1 Nutrient reduction tests

1366G Alg_@3x10^3_03

Treatment (%)	0.031		0.063		0.125		0.250		0.5		1	
Parameter	0h	72h	0h	72h	0h	72h	0h	72h	0h	72h	0h	72h
pH	6.1	6.3	6.1	6.3	6.1	6.3	6.2	6.3	6.1	6.3	6.2	6.4
EC (μS cm^{-1})	22	22	22	22	24	24	27	27	34	34	48	48
DO (%)	112	94	110	93	109	95	110	95	107.5	95	108.1	94
Temp (°C)	22.6	24	22.4	24.3	22.1	24.4	22.1	24.6	22	24.9	21.8	24.9

1373G Alg_@3x10^3_04

Treatment (%)	0.063		0.083		0.125		0.167		0.25	
Parameter	0h	72h	0h	72h	0h	72h	0h	72h	0h	72h
pH	6.1	6.2	6.1	6.2	6.1	6.2	6.1	6.2	6.1	6.2
EC (μS cm^{-1})	21	21	22	22	23	22	24	23	26	26
DO (%)	103	91	109	93	108	93	106	93	106.7	91.5
Temp (°C)	22.4	24	22.5	24.3	22.4	24	22.1	24.2	21.9	24.7

1376G Alg_@3x10^3_05

Treatment (%)	0.016		0.031		0.042		0.063		0.083		0.125	
Parameter	0h	72h	0h	72h	0h	72h	0h	72h	0h	72h	0h	72h
pH	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.1	6.2
EC (µS cm^{-1})	20	20	20	20	20	20	21	21	22	22	23	22
DO (%)	110	90	111	92	108	89	104	91	104.5	89.7	104.9	91.3
Temp (°C)	24	24.3	24	24.9	23.7	25.1	23.1	25.3	22.5	24.3	22	24.6

1378G Alg_@3x10^3_06

Treatment (%)	0.063		0.083		0.100		0.125		0.167	
Parameter	0h	72h	0h	72h	0h	72h	0h	72h	0h	72h
pH	6.0	6.0	6.0	6.1	6.0	6.1	6.0	6.0	6.0	6.0
EC (µS cm^{-1})	21	21	23	23	22	22	23	22	24	24
DO (%)	109	96	114	92	110	88	103	93	107.8	92.5
Temp (°C)	25	22.9	25.2	23.4	24.7	23.5	23.1	25	22.8	24.4

1380G Alg_@3x10^{3}_07

Treatment (%)	0.083		0.100		0.125		0.167		0.2	
Parameter	0h	72h	0h	72h	0h	72h	0h	72h	0h	72h
pH	6.0	6.1	6.0	6.1	5.9	6.1	5.9	6.1	5.9	6.0
EC (µS cm^{-1})	22	24	22	22	23	23	24	24	25	24
DO (%)	104	85	108	85	105	91	103	89	102.5	89
Temp (°C)	26.4	25.4	25.9	25.4	25.5	25.7	25.5	24.9	24.2	24.9

Table I2 Reference toxicity tests

1383G Alg_Reftox_01

Treatment (µg L^{-1})	0		5		10		20		40		80		160	
Parameter	0h	72h	0h	72h	0h	72h	0h	72h	0h	72h	0h	72h	0h	72h
pH	6.1	6.3	6.0	6.2	6.1	6.2	6.0	6.2	6.0	6.1	6.0	6.1	6.0	6.1
EC (µS cm^{-1})	27	26	27	26	26	26	26	25	26	26	26	26	27	26
DO (%)	113.1	94.6	115.6	90.8	109.1	92.8	110	93.5	106.3	90.1	107.4	93	104.9	90.7
Temp (°C)	25.5	24.4	25.1	24.8	24.8	23.7	23.8	25.1	23.6	24.1	23.2	25.5	22.9	24.9

1389G Alg_Reftox_02

Treatment ($\mu g\ L^{-1}$)	0		5		10		20		40		80		160	
Parameter	0h	72h	0h	72h	0h	72h	0h	72h	0h	72h	0h	72h	0h	72h
pH	6.0	6.2	6.0	6.2	6.0	6.2	6.0	6.1	6.0	6.1	5.9	6.0	6.0	6.0
EC ($\mu S\ cm^{-1}$)	26	25	27	26	26	25	26	26	26	26	27	27	26	27
DO (%)	104.8	94.5	105.5	91.4	105.6	92.1	98.7	93	106.0	95.2	105.1	91.1	103.8	93.3
Temp (°C)	25.4	25.4	25.6	25.7	25.3	25.8	20.7	25.7	24.7	25.8	25.2	24.7	24.8	25.8

1404G Alg_Reftox_03

Treatment ($\mu g\ L^{-1}$)	0		5		10		20		40		80		160	
Parameter	0h	72h	0h	72h	0h	72h	0h	72h	0h	72h	0h	72h	0h	72h
pH	6.1	6.2	6.1	6.2	6.1	6.2	6.1	6.2	6.1	6.2	6.1	6.1	6.1	6.2
EC ($\mu S\ cm^{-1}$)	29	26	26	26	26	26	26	26	26	26	26	26	26	27
DO (%)	95.2	88.8	101.1	91.4	100.7	91.9	97.3	92.3	100.5	90.3	99.8	90.3	93.9	92.2
Temp (°C)	27	24.9	25	26.1	25	25.3	24.1	26.5	24.1	26.2	23.8	25.2	23.7	25.8

1415G Alg_Reftox_04

Treatment (µg L^{-1})	0		5		10		20		40		80		160	
Parameter	0h	72h	0h	72h	0h	72h	0h	72h	0h	72h	0h	72h	0h	72h
pH	5.8	6.0	5.8	6.0	5.8	6.0	5.9	6.1	5.9	5.9	5.9	5.9	5.9	5.8
EC (µS cm^{-1})	24	24	24	24	24	24	24	25	24	24	24	25	24	25
DO (%)	111.7	91.7	114.3	91.8	112.5	92.6	110.7	92.3	102.9	88.4	114.6	88.2	111.8	83.1
Temp (°C)	26.6	25.5	26.6	25.5	26.4	25.4	26.2	23.8	25.8	24.8	25.5	25.4	25.1	25.1

1431G Alg_Reftox_05

Treatment (µg L^{-1})	0		5		10		20		40		80		160	
Parameter	0h	72h	0h	72h	0h	72h	0h	72h	0h	72h	0h	72h	0h	72h
pH	6.0	6.1	6.0	6.1	5.9	6.1	6.0	6.1	6.0	6.1	6.1	6.0	6.0	6.0
EC (µS cm^{-1})	28	27	28	28	28	27	28	26	27	26	27	27	27	28
DO (%)	104.9	92.2	106.9	92.4	108.8	95.9	102.9	94.4	103.3	95.3	102.3	92.4	101.1	94.9
Temp (°C)	26.9	23	24.6	24.8	24.3	26.1	23.8	25.5	23.8	25.4	22.4	25.7	22	26.2

1455G Alg_Reftox_06

Treatment (µg L^{-1})	0		0 – No HEPES		20		40		80		160	
Parameter	0h	72h	0h	72h	0h	72h	0h	72h	0h	72h	0h	72h
pH	6.0	6.3	5.9	6.4	6.0	6.2	5.9	6.1	5.9	6.1	5.9	6.1
EC (µS cm^{-1})	26	27	23	23	26	26	26	26	26	27	26	27
DO (%)	106.6	93.2	113.9	93.7	107.2	92.5	116.2	93.5	116.9	92.5	109.9	93.2
Temp (°C)	25.2	24.8	26.2	25.4	25	25.8	26.5	25.4	26.3	25.1	25.6	25.7

1466G Alg_reftox_07

Treatment (µg L^{-1})	0		0 – No HEPES		20		40		80		160	
Parameter	0h	72h	0h	72h	0h	72h	0h	72h	0h	72h	0h	72h
pH	6.0	6.1	6.1	6.6	5.9	6.0	6.0	6.0	5.9	6.0	6.0	6.0
EC (µS cm^{-1})	26	25	23	22	26	26	26	26	26	26	26	26
DO (%)	114.6	95.1	119.5	95.8	115.9	94.2	115.9	93.6	113.3	93.4	106.1	23.3
Temp (°C)	25.7	26.2	26	26	26.2	26	26.2	26.1	26.1	25.8	25.7	26.1

1468G Alg_reftox_08

Treatment (µg L^{-1})	0		0 – No HEPES		20		40		80		160	
Parameter	0h	72h	0h	72h	0h	72h	0h	72h	0h	72h	0h	72h
pH	6.2	6.6	6.2	6.3	6.1	6.2	6.1	6.2	6.1	6.1	6.1	6.1
EC (µS cm^{-1})	24	23	27	26	27	27	27	27	27	27	27	27
DO (%)	105	95.5	106.7	95.7	105.8	95.3	103.4	93.4	104.7	94.4	104.9	94
Temp (°C)	26.3	24.9	26.3	25.5	26.7	25.7	26.7	25.7	26.1	26	26.3	26.2

1479G Alg_reftox_09

Treatment (µg L^{-1})	0		20		40		80		160	
Parameter	0h	72h	0h	72h	0h	72h	0h	72h	0h	72h
pH	6.1	6.2	6.1	6.1	6.1	6.0	6.1	6.0	6.1	6.0
EC (µS cm^{-1})	27	26	27	26	27	27	27	27	27	27
DO (%)	100.4	93.5	106.4	95.2	107.6	92.9	94.8	91.9	103.1	91.6
Temp (°C)	25.7	25.1	27.6	25.5	27.7	25.3	27.7	25.5	26.9	25.6

Appendix J Chemical analyses

Table J1 Metal and major ion analyses of Quality Control (QC) waters for nutrient reduction trials.

Analyte	Al	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Se	U	Zn	Ca	Mg	Na	SO_4
Units	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}
PQL	0.1	0.02	0.01	0.1	0.01	1	0.01	0.01	0.01	0.2	0.001	0.1	0.1	0.1	0.1	0.5
1366G SSW	50	<0.02	0.01	<0.1	0.8	350	10	0.04	0.2	<0.2	0.2	0.8	0.5	0.6	1.3	3
1373G SSW	10	<0.02	0.02	<0.1	0.7	77	11	0.06	0.04	<0.2	0.06	0.7	0.4	0.6	2.4	96
1376G SSW	20	<0.02	0.02	<0.1	0.4	120	10	0.05	0.2	<0.2	0.05	0.8	0.5	0.7	2.4	110
1378G SSW	20	<0.02	0.02	<0.1	0.4	120	9	0.06	0.08	<0.2	0.06	0.7	0.4	0.6	2.6	110
1380G SSW	16	<0.02	0.02	<0.1	0.59	130	9.5	0.05	0.08	<0.2	0.051	0.7	0.4	0.6	2.7	110
1366G Blank	<0.1	<0.02	<0.01	<0.1	0.02	<1	<0.01	0.03	<0.01	<0.2	<0.001	<0.1	<0.1	<0.1	<0.1	<0.5
1373G Blank	<0.1	<0.02	<0.01	<0.1	0.09	<1	<0.01	<0.01	<0.01	<0.2	<0.001	<0.1	<0.1	<0.1	<0.1	0.5
1376G Blank	0.2	<0.02	<0.01	<0.1	0.05	<1	<0.01	<0.01	0.01	<0.2	<0.001	<0.2	<0.1	<0.1	<0.1	<0.5
1378G Blank	1	<0.02	<0.01	<0.1	0.02	<1	<0.01	<0.01	<0.01	<0.2	0.009	0.2	<0.1	<0.1	<0.1	<0.5
1380G Blank	0.1	<0.02	<0.01	<0.1	0.03	<1	<0.01	<0.01	<0.01	<0.2	<0.001	<0.1	<0.1	<0.1	<0.1	<0.5
1366G Pro Blank	0.1	<0.02	<0.01	<0.1	0.03	<1	<0.01	0.05	0.01	<0.2	0.005	<0.1	<0.1	<0.1	<0.1	<0.5
1373G Pro Blank	0.1	<0.02	<0.01	<0.1	0.04	<1	<0.01	0.01	<0.01	<0.2	0.02	<0.1	<0.1	<0.1	<0.1	0.5
1376G Pro Blank	0.2	<0.02	<0.01	<0.1	0.05	<1	<0.01	<0.01	0.01	<0.2	0.02	<0.2	<0.1	<0.1	0.1	<0.5
1378G Pro Blank	1	<0.02	<0.01	<0.1	0.02	<1	<0.01	<0.01	<0.01	<0.2	0.009	<0.1	<0.1	<0.1	<0.1	<0.5
1380G Pro Blank	1.6	<0.02	<0.01	<0.1	0.03	<1	2.7	<0.01	0.02	<0.2	0.020	<0.1	<0.1	<0.1	<0.1	<0.5

*Highlighted numbers are higher than would be expected in normal synthetic soft water

Table J2 Measured nitrate and phosphate concentrations in QC waters in nutrient reduction trials.

Test code	Proportion of current nutrients	Measured nutrient concentrations	
		Nitrate as N (mg L^{-1})	Phosphate as P (mg L^{-1})
1366G	1/32	0.070	<0.005
	1/16	0.30	<0.005
	1/8	0.25	<0.005
	1/4	0.53	<0.005
	1/2	0.70	0.006
	1	1.3	0.016
1373G	1/16	0.11	<0.005
	1/12	0.17	<0.005
	1/8	0.20	<0.005
	1/6	0.22	<0.005
	1/4	0.47	0.005
1376G	1/64	0.18	<0.005
	1/32	0.16	<0.005
	1/24	0.23	<0.005
	1/16	0.22	<0.005
	1/12	0.27	<0.005
	1/8	0.32	<0.005
1378G	1/16	0.16	<0.005
	1/12	0.12	<0.005
	1/10	0.098	<0.005
	1/8	0.20	<0.005
	1/6	0.31	<0.005
1380G	1/12	0.12	<0.005
	1/10	0.21	<0.005
	1/8	0.21	<0.005
	1/6	0.14	<0.005
	1/5	0.34	<0.005

Table J3 Metal and major ion analyses of QC waters for reference toxicity tests

Analyte	Al	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Se	U	Zn	SO_4	Ca	Na	Mg
Units	**µg L^{-1}**	**µg L^{-1}**	**µg L^{-1}**	**µg L^{-1}**	**µg L^{-1}**	**µg L^{-1}**	**µg L^{-1}**	**µg L^{-1}**	**µg L^{-1}**	**µg L^{-1}**	**µg L^{-1}**	**µg L^{-1}**	**mg L^{-1}**	**mg L^{-1}**	**mg L^{-1}**	**mg L^{-1}**
PQL	**0.1**	**0.02**	**0.01**	**0.1**	**0.01**	**1**	**0.01**	**0.01**	**0.01**	**0.2**	**0.001**	**0.1**	**0.5**	**0.1**	**0.1**	**0.1**
1383G Blk	0.3	<0.02	<0.01	<0.1	0.02	<1	<0.01	<0.01	<0.01	<0.2	<0.001	<0.1	<0.5	<0.1	<0.1	<0.1
1389G Blk	0.3	<0.02	<0.01	<0.1	0.03	<1	<0.01	<0.01	<0.01	<0.2	0.006	1.6	<0.5	<0.1	<0.1	<0.1
1404G Blk	0.1	<0.02	<0.01	<0.1	0.02	<1	<0.01	<0.01	<0.01	<0.2	<0.001	<0.1	<0.5	<0.1	<0.1	<0.1
1451G Blk	0.4	<0.02	<0.01	<0.1	<0.01	<1	<0.01	<0.01	<0.01	<0.2	<0.001	<0.1	<0.5	<0.1	<0.1	<0.1
1431G Blk	0.2	<0.02	<0.01	<0.1	0.07	<1	<0.01	<0.01	<0.01	<0.2	<0.001	<0.1	<1	<0.1	<0.1	<0.1
1455G Blk	0.5	<0.02	<0.01	<0.1	0.08	<1	<0.01	<0.01	<0.01	<0.2	<0.001	<0.1	<0.5	<0.1	<0.1	<0.1
1466G Blk	0.2	<0.02	<0.01	<0.1	0.06	<1	<0.01	<0.01	<0.01	<0.2	<0.001	<0.1	<0.5	<0.1	<0.1	<0.1
1468G Blk	0.3	<0.02	<0.01	<0.1	0.06	<1	<0.01	<0.01	0.01	<0.2	<0.001	<0.1	<0.5	<0.1	<0.1	<0.1
1479G Blk	<0.1	<0.02	<0.01	<0.1	<0.01	<1	<0.01	<0.01	0.01	<0.2	<0.001	<0.1	<0.5	<0.1	<0.1	<0.1
1383G Pro Blk	0.3	<0.02	<0.01	<0.1	0.02	<1	<0.01	<0.01	<0.01	<0.2	<0.001	<0.1	<0.5	<0.1	<0.1	<0.1
1389G Pro Blk	0.4	<0.02	<0.01	<0.1	0.03	<1	0.01	<0.01	<0.01	<0.2	0.009	3.1	<0.5	<0.1	<0.1	<0.1
1404G Pro Blk	0.9	<0.02	<0.01	<0.1	0.03	<1	<0.01	<0.01	0.02	<0.2	0.001	<0.1	<0.5	<0.1	<0.1	<0.1
1415G Pro Blk	9	<0.02	<0.01	<0.1	<0.01	<1	<0.01	<0.01	<0.01	<0.2	0.008	<0.1	<0.5	<0.1	<0.1	<0.1
1431G Pro Blk	0.2	<0.02	<0.01	<0.1	0.08	<1	<0.01	<0.01	<0.01	<0.2	<0.001	0.3	<1	<0.1	<0.1	<0.1
1455G Pro Blk	1	<0.02	<0.01	<0.1	0.07	<1	<0.01	0.01	<0.01	<0.2	<0.001	0.4	<0.5	<0.1	<0.1	<0.1
1466G Pro Blk	0.7	<0.02	0.01	<0.1	0.07	<1	<0.01	0.01	0.03	<0.2	0.2	0.2	<0.5	<0.1	<0.1	<0.1
1468G Pro Blk	0.9	<0.02	<0.01	<0.1	0.08	<1	<0.01	<0.01	0.04	<0.2	0.03	<0.1	<0.5	<0.1	<0.1	<0.1
1479G Pro Blk	2	<0.02	<0.01	<0.1	0.07	2	0.1	0.3	0.05	<0.2	0.003	0.4	<0.5	<0.1	<0.1	<0.1
1383G A	19	<0.01	0.02	<0.1	0.35	130	8.9	0.05	0.08	<0.2	0.04	0.9	110	0.4	3.6	0.6

Analyte	Al	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Se	U	Zn	SO_4	Ca	Na	Mg
Units	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	µg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}
PQL	0.1	0.02	0.01	0.1	0.01	1	0.01	0.01	0.01	0.2	0.001	0.1	0.5	0.1	0.1	0.1
1389G A	18	<0.01	0.02	<0.1	0.35	150	11	0.04	0.09	<0.2	0.052	4.2	93	0.4	6.8	0.5
1404G A	22	<0.02	0.02	<0.1	0.44	160	10	0.05	0.09	<0.2	0.072	0.7	100	0.4	3.5	0.6
1415G A	19	<.0.02	0.02	<0.1	0.6	120	10	0.04	0.08	<0.2	0.06	0.7	100	0.5	1.1	0.6
1431G A	10	<0.02	<0.01	<0.1	0.4	89	9	0.04	0.09	<0.2	0.03	0.8	84	0.4	3.6	0.5
1455G A	10	<0.02	<0.01	0.1	0.5	78	9	0.1	0.08	<0.2	0.02	0.8	110	0.5	3.6	0.6
1466G A	8	<0.02	<0.01	<0.1	0.5	50	9	0.1	0.2	<0.2	0.8	1	110	0.4	3.7	0.6
1468G A	12	<0.02	<0.01	<0.1	0.6	74	9	0.02	0.1	<0.2	0.07	2	3	0.5	2.5	0.6
1479G A	12	<0.02	<0.01	<0.1	0.7	68	9	0.2	0.1	<0.2	0.05	1	110	0.5	0.6	3.4

*Highlighted numbers are higher than would be expected in normal synthetic soft water

Table J4 Measured uranium concentrations in reference toxicity tests.

Test & treatment code	Nominal U concentration (µg L^{-1})	Measured U concentration (µg L^{-1})
1383G BLK	-	<0.001
1383G A	Bgd*	0.044
1383G B	5	3.8
1383G C	10	7.3
1383G D	20	16
1383G E	40	28
1383G F	80	56
1383G G	160	130
1389G BLK	-	0.006
1389G A	Bgd	0.052
1389G B	5	3.9
1389G C	10	7.4
1389G D	20	17
1389G E	40	31
1389G F	80	64
1389G G	160	130
1404G BLK	-	<0.001
1404G A	Bgd	0.072
1404G B	5	3.8
1404G C	10	7
1404G D	20	14
1404G E	40	27
1404G F	80	56
1404G G	160	110
1415G BLK	-	<0.001
1415G A	Bgd	0.06
1415G B	5	4.3
1415G C	10	8.6
1415G D	20	17
1415G E	40	34
1415G F	80	66
1415G G	160	140
1431G BLK	-	<0.001
1431G A	Bgd	0.03
1431G B	5	4.2
1431G C	10	8.4
1431G D	20	18
1431G E	40	35
1431G F	80	70
1431G G	160	140

Test & treatment code	Nominal U concentration ($\mu g\ L^{-1}$)	Measured U concentration ($\mu g\ L^{-1}$)
1455G BLK	-	<0.001
1455G A	Bgd	0.02
1455G B	Bgd – no HEPES	0.02
1455G C	20	15
1455G D	40	31
1455G E	80	62
1455G F	160	130
1466G BLK	-	<0.001
1466G A	Bgd	0.8
1466G B	Bgd – no HEPES	0.03
1466G C	20	15
1466G D	40	26
1466G E	80	54
1466G F	160	110
1468G BLK	-	<0.001
1468G A	Bgd	0.07
1468G B	Bgd – no HEPES	0.1
1468G C	20	14
1468G D	40	25
1468G E	80	48
1468G F	160	97
1479G BLK	-	<0.001
1479G A	0	0.05
1479G B	20	16
1479G C	40	30
1479G D	80	63
1479G E	160	140

*Bgd – indicates background uranium concentration in diluent waters

Table J5 Measured nitrate and phosphate concentrations in quality check waters in reference toxicity tests.

Test code and treatment	Measured nutrient concentrations	
	Nitrate as N (mg L^{-1})	Phosphate as P (mg L^{-1})
1383G BLK	<0.005	<0.005
1383G A	0.5	<0.005
1389G BLK	0.015	<0.005
1389G A	0.44	<0.005
1404G BLK	0.054	<0.005
1404G A	0.69	<0.005
1415G BLK	nm[a]	nm[a]
1415G A	nm[a]	nm[a]
1431G BLK	nm[a]	nm[a]
1431G A	nm[a]	nm[a]
1455G BLK	<0.005	<0.005
1455G A	0.77	0.008
1466G BLK	<0.005	<0.005
1466G A	0.32	0.006
1468G BLK	<0.005	<0.005
1468G A	0.4	0.005
1479G BLK	nm	nm
1479G A	nm	nm

[a] Samples lost due to power outage and defrosting of samples

Appendix K Analytical reports

Figure K1 Test raw data and analysis report for test 1366G

CETIS Analytical Report

Report Date: 05 Jan-16 14:13 (p 1 of 2)
Test Code: 1366G | 02-9019-0640

Algal Growth Inhibition Test — **eriss ecotoxicology lab**

Analysis ID:	02-3818-3741	**Endpoint:**	Growth rate (db/d)	**CETIS Version:**	CETISv1.8.7
Analyzed:	19 Nov-13 16:51	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	20-5562-4063	**Test Type:**	Algal growth inhibition	**Analyst:**	
Start Date:	19 Nov-13 16:41	**Protocol:**	Alga eriss tropical freshwater	**Diluent:**	Synthetic Soft Water
Ending Date:	19 Nov-13 16:41	**Species:**	Chlorella sp.	**Brine:**	Not Applicable
Duration:	n/a	**Source:**	eriss ecotoxicology lab	**Age:**	
Sample ID:	05-8494-4791	**Code:**	22DD8C97	**Client:**	Internal Lab
Sample Date:	19 Nov-13 16:41	**Material:**	Uranyl Sulphate	**Project:**	Special Studies
Receipt Date:	19 Nov-13 16:41	**Source:**	Reference Toxicant (U)		
Sample Age:	n/a	**Station:**	In House		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1255153	200	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value Test	1.947	2.652	0.7364	No Outliers Detected

Point Estimates

Level	µg/L	95% LCL	95% UCL
IC5	0.7717	0.6931	0.8255
IC10	0.5695	0.4316	0.6654
IC15	0.3903	0.2091	0.5187
IC20	0.2316	0.01979	0.3843
IC25	0.09103	n/a	n/a
IC40	>0.5	n/a	n/a
IC50	>0.5	n/a	n/a

Growth rate (db/d) Summary

			Calculated Variate						
Conc-µg/L	Code	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0.03		3	0.6167	0.54	0.74	0.06227	0.1079	17.49%	0.00%
0.06		3	0.9467	0.86	1.09	0.07219	0.125	13.21%	-53.51%
0.13		3	1.39	1.28	1.49	0.06083	0.1054	7.58%	-125.41%
0.25		3	1.663	1.62	1.71	0.02603	0.04509	2.71%	-169.73%
0.5		3	1.653	1.63	1.69	0.01856	0.03214	1.94%	-168.11%
1	SS	3	1.727	1.65	1.78	0.0393	0.06807	3.94%	-180.00%

Growth rate (db/d) Detail

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3
0.03		0.74	0.57	0.54
0.06		0.89	1.09	0.86
0.13		1.28	1.4	1.49
0.25		1.62	1.71	1.66
0.5		1.63	1.69	1.64
1	SS	1.65	1.75	1.78

Figure K1 continued

CETIS Analytical Report

Report Date: 05 Jan-16 14:13 (p 2 of 2)
Test Code: 1366G | 02-9019-0640

Algal Growth Inhibition Test **eriss ecotoxicology lab**

Analysis ID:	02-3818-3741	**Endpoint:**	Growth rate (db/d)	**CETIS Version:**	CETISv1.8.7
Analyzed:	19 Nov-13 16:51	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics

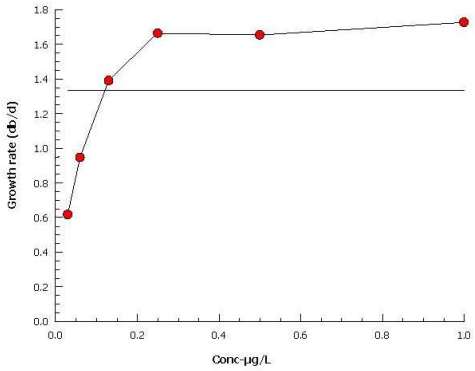


Figure K2 Test raw data and analysis report for test 1373G

Algal Growth Inhibition Test — **eriss ecotoxicology lab**

Analysis ID:	16-6491-8457	**Endpoint:**	Growth rate (db/d)	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Dec-13 11:53	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	08-7726-6263	**Test Type:**	Algal growth inhibition	**Analyst:**	
Start Date:	02 Dec-13 11:51	**Protocol:**	Alga eriss tropical freshwater	**Diluent:**	Synthetic Soft Water
Ending Date:	02 Dec-13 11:51	**Species:**	Chlorella sp.	**Brine:**	Not Applicable
Duration:	n/a	**Source:**	eriss ecotoxicology lab	**Age:**	
Sample ID:	14-2901-8366	**Code:**	552D16FE	**Client:**	Internal Lab
Sample Date:	10 Jan-14 11:51	**Material:**	nutrient reduction	**Project:**	Protocol Development
Receipt Date:	10 Jan-14 11:51	**Source:**	NONE		
Sample Age:	n/a	**Station:**			

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1449832	200	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value Test	1.76	2.548	0.9856	No Outliers Detected

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	>0.25	n/a	n/a	<400	n/a	n/a
IC10	>0.25	n/a	n/a	<400	n/a	n/a
IC15	>0.25	n/a	n/a	<400	n/a	n/a
IC20	>0.25	n/a	n/a	<400	n/a	n/a
IC25	>0.25	n/a	n/a	<400	n/a	n/a
IC40	>0.25	n/a	n/a	<400	n/a	n/a
IC50	>0.25	n/a	n/a	<400	n/a	n/a

Growth rate (db/d) Summary

			Calculated Variate						
Conc-%	Code	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0.06	SS	3	1.347	1.286	1.415	0.03733	0.06467	4.80%	0.00%
0.08		3	1.5	1.446	1.58	0.04076	0.0706	4.71%	-11.34%
0.13		3	1.69	1.645	1.737	0.02653	0.04595	2.72%	-25.45%
0.17		3	1.786	1.747	1.809	0.0197	0.03412	1.91%	-32.59%
0.25		3	1.897	1.846	1.933	0.02602	0.04507	2.38%	-40.84%

Growth rate (db/d) Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3
0.06	SS	1.415	1.286	1.341
0.08		1.446	1.473	1.58
0.13		1.688	1.737	1.645
0.17		1.747	1.802	1.809
0.25		1.846	1.933	1.912

Figure K2 continued

Algal Growth Inhibition Test | **eriss ecotoxicology lab**

Analysis ID:	16-6491-8457	**Endpoint:**	Growth rate (db/d)	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Dec-13 11:53	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics

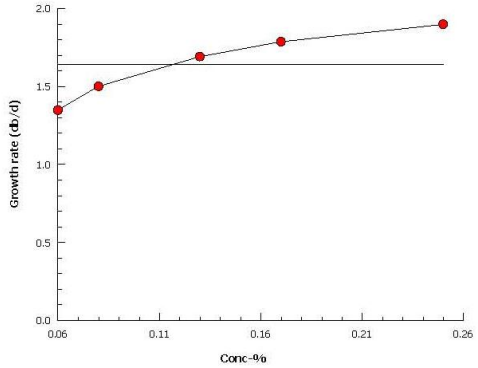


Figure K3 Test raw data and analysis report for test 1376G

Algal Growth Inhibition Test **eriss ecotoxicology lab**

Analysis ID:	19-6078-4249	**Endpoint:**	Growth rate (db/d)	**CETIS Version:**	CETISv1.9.0
Analyzed:	03 Mar-16 11:03	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	06-4853-9872	**Test Type:**	Algal growth inhibition	**Analyst:**	Ceiwen Pease
Start Date:	09 Dec-13 11:51	**Protocol:**	Alga eriss tropical freshwater	**Diluent:**	Synthetic Soft Water
Ending Date:	12 Dec-13 11:51	**Species:**	Chlorella sp.	**Brine:**	Not Applicable
Duration:	72h	**Source:**	eriss ecotoxicology lab	**Age:**	
Sample ID:	11-9927-2975	**Code:**	1376G	**Client:**	Internal Lab
Sample Date:	10 Jan-14 11:51	**Material:**	nutrient reduction	**Project:**	Protocol Development
Receipt Date:	10 Jan-14 11:51	**Source:**	NONE		
Sample Age:	n/a	**Station:**			

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1607622	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	>0.125	n/a	n/a	<800	n/a	n/a
IC10	>0.125	n/a	n/a	<800	n/a	n/a
IC15	>0.125	n/a	n/a	<800	n/a	n/a
IC20	>0.125	n/a	n/a	<800	n/a	n/a
IC25	>0.125	n/a	n/a	<800	n/a	n/a
IC40	>0.125	n/a	n/a	<800	n/a	n/a
IC50	>0.125	n/a	n/a	<800	n/a	n/a

Growth rate (db/d) Summary

			Calculated Variate						
Conc-%	Code	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0.02	SS	3	0.7143	0.6894	0.7348	0.0133	0.02303	3.22%	0.00%
0.03		3	0.7677	0.7311	0.7884	0.01836	0.0318	4.14%	-7.47%
0.04		3	0.7826	0.7144	0.828	0.03471	0.06012	7.68%	-9.56%
0.06		3	0.9037	0.859	0.9345	0.02286	0.0396	4.38%	-26.51%
0.08		3	1.116	1.063	1.148	0.02669	0.04623	4.14%	-56.28%
0.125		3	1.436	1.399	1.467	0.01993	0.03453	2.40%	-101.01%

Growth rate (db/d) Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3
0.02	SS	0.6894	0.7188	0.7348
0.03		0.7311	0.7837	0.7884
0.04		0.7144	0.8054	0.828
0.06		0.9177	0.859	0.9345
0.08		1.063	1.148	1.138
0.125		1.399	1.467	1.442

Figure K3 continued

Algal Growth Inhibition Test | **eriss ecotoxicology lab**

Analysis ID:	19-6078-4249	**Endpoint:**	Growth rate (db/d)	**CETIS Version:**	CETISv1.9.0
Analyzed:	03 Mar-16 11:03	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics

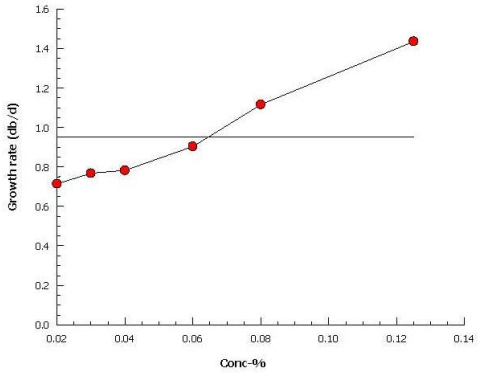


Figure K4 Test raw data and analysis report for test 1378G

Algal Growth Inhibition Test **eriss ecotoxicology lab**

Analysis ID:	16-6491-8457	**Endpoint:**	Growth rate (db/d)	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Dec-13 11:53	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	08-7726-6263	**Test Type:**	Algal growth inhibition	**Analyst:**	
Start Date:	02 Dec-13 11:51	**Protocol:**	Alga eriss tropical freshwater	**Diluent:**	Synthetic Soft Water
Ending Date:	02 Dec-13 11:51	**Species:**	Chlorella sp.	**Brine:**	Not Applicable
Duration:	n/a	**Source:**	eriss ecotoxicology lab	**Age:**	
Sample ID:	14-2901-8366	**Code:**	552D16FE	**Client:**	Internal Lab
Sample Date:	10 Jan-14 11:51	**Material:**	nutrient reduction	**Project:**	Protocol Development
Receipt Date:	10 Jan-14 11:51	**Source:**	NONE		
Sample Age:	n/a	**Station:**			

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1449832	200	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value Test	1.76	2.548	0.9856	No Outliers Detected

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	>0.25	n/a	n/a	<400	n/a	n/a
IC10	>0.25	n/a	n/a	<400	n/a	n/a
IC15	>0.25	n/a	n/a	<400	n/a	n/a
IC20	>0.25	n/a	n/a	<400	n/a	n/a
IC25	>0.25	n/a	n/a	<400	n/a	n/a
IC40	>0.25	n/a	n/a	<400	n/a	n/a
IC50	>0.25	n/a	n/a	<400	n/a	n/a

Growth rate (db/d) Summary

			Calculated Variate						
Conc-%	Code	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0.06	SS	3	1.347	1.286	1.415	0.03733	0.06467	4.80%	0.00%
0.08		3	1.5	1.446	1.58	0.04076	0.0706	4.71%	-11.34%
0.13		3	1.69	1.645	1.737	0.02653	0.04595	2.72%	-25.45%
0.17		3	1.786	1.747	1.809	0.0197	0.03412	1.91%	-32.59%
0.25		3	1.897	1.846	1.933	0.02602	0.04507	2.38%	-40.84%

Growth rate (db/d) Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3
0.06	SS	1.415	1.286	1.341
0.08		1.446	1.473	1.58
0.13		1.688	1.737	1.645
0.17		1.747	1.802	1.809
0.25		1.846	1.933	1.912

Figure K4 continued

Algal Growth Inhibition Test | **eriss ecotoxicology lab**

Analysis ID:	16-6491-8457	**Endpoint:**	Growth rate (db/d)	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Dec-13 11:53	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics

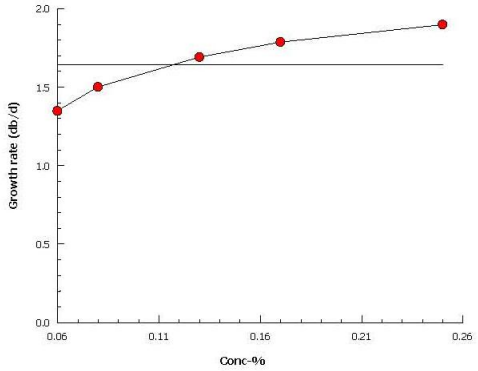


Figure K5 Test raw data and analysis report for test 1380G

CETIS Analytical Report

Report Date: 03 Mar-16 10:59 (p 1 of 1)
Test Code: 1380G | 19-6194-3511

Algal Growth Inhibition Test — **eriss ecotoxicology lab**

Analysis ID:	04-0352-0314	**Endpoint:**	Growth rate (db/d)	**CETIS Version:**	CETISv1.9.0
Analyzed:	03 Mar-16 10:59	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	18-0624-0162	**Test Type:**	Algal growth inhibition	**Analyst:**	Ceiwen Pease
Start Date:	20 Jan-14	**Protocol:**	Alga eriss tropical freshwater	**Diluent:**	Synthetic Soft Water
Ending Date:	23 Jan-14 11:51	**Species:**	Chlorella sp.	**Brine:**	Not Applicable
Duration:	84h	**Source:**	eriss ecotoxicology lab	**Age:**	
Sample ID:	16-4479-4161	**Code:**	1380G	**Client:**	Internal Lab
Sample Date:	10 Jan-14 11:51	**Material:**	nutrient reduction	**Project:**	Protocol Development
Receipt Date:	10 Jan-14 11:51	**Source:**	NONE		
Sample Age:	9d 12h	**Station:**			

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	419441	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	>0.2	n/a	n/a	<500	n/a	n/a
IC10	>0.2	n/a	n/a	<500	n/a	n/a
IC15	>0.2	n/a	n/a	<500	n/a	n/a
IC20	>0.2	n/a	n/a	<500	n/a	n/a
IC25	>0.2	n/a	n/a	<500	n/a	n/a
IC40	>0.2	n/a	n/a	<500	n/a	n/a
IC50	>0.2	n/a	n/a	<500	n/a	n/a

Growth rate (db/d) Summary

			Calculated Variate						
Conc-%	Code	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0.08	SS	3	1.082	0.9378	1.177	0.07342	0.1272	11.75%	0.00%
0.1		3	1.346	1.274	1.41	0.03945	0.06833	5.08%	-24.31%
0.125		3	1.573	1.453	1.671	0.06389	0.1107	7.04%	-45.29%
0.17		3	1.647	1.561	1.734	0.05	0.0866	5.26%	-52.15%
0.2		3	1.687	1.584	1.742	0.05136	0.08895	5.27%	-55.82%

Growth rate (db/d) Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3
0.08	SS	0.9378	1.133	1.177
0.1		1.274	1.41	1.353
0.125		1.453	1.671	1.594
0.17		1.645	1.734	1.561
0.2		1.584	1.742	1.734

Graphics

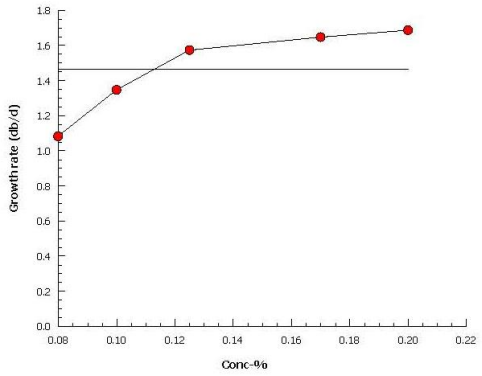


Figure K6 Test raw data and analysis report for test 1383G

CETIS Analytical Report

Algal Growth Inhibition Test — **eriss ecotoxicology lab**

Analysis ID:	15-3530-3030	**Endpoint:**	Growth rate (db/d)	**CETIS Version:**	CETISv1.8.7
Analyzed:	17 Feb-14 15:29	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	17-7845-7038	**Test Type:**	Algal growth inhibition	**Analyst:**	
Start Date:	03 Feb-14	**Protocol:**	Alga eriss tropical freshwater	**Diluent:**	Synthetic Soft Water
Ending Date:	06 Feb-14	**Species:**	Chlorella sp.	**Brine:**	Not Applicable
Duration:	72h	**Source:**	eriss ecotoxicology lab	**Age:**	
Sample ID:	09-5088-1456	**Code:**	1383G	**Client:**	Internal Lab
Sample Date:	07 Feb-14 15:54	**Material:**	Uranyl Sulphate	**Project:**	Reference Toxicity Program
Receive Date:	07 Feb-14 15:54	**Source:**	Reference Toxicant (U)		
Sample Age:	NA	**Station:**	In House		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	890311	200	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	2.031	2.734	0.7044	No Outliers Detected

Point Estimates

Level	µg/L	95% LCL	95% UCL
IC5	10.82	N/A	25.71
IC10	18.02	N/A	25.23
IC15	21.76	11.67	30.47
IC20	26.24	15.42	32.02
IC25	29.17	23.14	32.44
IC40	35.2	31.03	38.82
IC50	39.88	35.14	45.09

Growth rate (db/d) Summary

			Calculated Variate						
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0.044	Synthetic Soft W	3	1.84	1.66	1.93	0.09	0.1559	8.47%	0.0%
3.8		3	1.757	1.68	1.86	0.05364	0.09292	5.29%	4.53%
7.3		3	1.807	1.74	1.88	0.04055	0.07024	3.89%	1.81%
16		3	1.713	1.65	1.8	0.04484	0.07767	4.53%	6.88%
28		3	1.44	1.4	1.46	0.02	0.03464	2.41%	21.74%
56		3	0.4167	0.29	0.58	0.0857	0.1484	35.62%	77.36%
130		3	0.08333	-0.01	0.2	0.06173	0.1069	128.3%	95.47%

Growth rate (db/d) Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3
0.044	Synthetic Soft Wa	1.93	1.93	1.66
3.8		1.86	1.68	1.73
7.3		1.74	1.88	1.8
16		1.8	1.69	1.65
28		1.46	1.46	1.4
56		0.58	0.38	0.29
130		0.06	0.2	-0.01

Figure K6 continued

Algal Growth Inhibition Test | **eriss ecotoxicology lab**

Analysis ID:	15-3530-3030	**Endpoint:**	Growth rate (db/d)	**CETIS Version:**	CETISv1.8.7
Analyzed:	17 Feb-14 15:29	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics

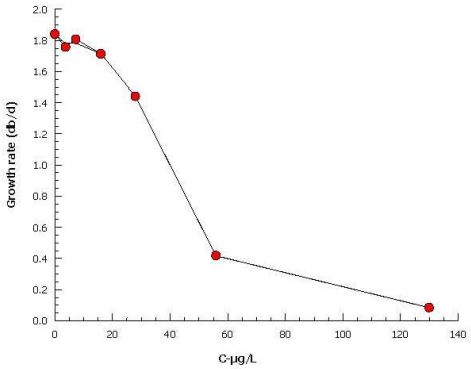


Figure K7 Test raw data and analysis report for test 1389G

Algal Growth Inhibition Test — **eriss ecotoxicology lab**

Analysis ID:	11-6683-2758	**Endpoint:**	Growth rate (db/d)	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Apr-14 15:13	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	09-3900-0997	**Test Type:**	Algal growth inhibition	**Analyst:**	
Start Date:	18 Feb-14	**Protocol:**	Alga eriss tropical freshwater	**Diluent:**	Synthetic Soft Water
Ending Date:	21 Feb-14	**Species:**	Chlorella sp.	**Brine:**	Not Applicable
Duration:	72h	**Source:**	eriss ecotoxicology lab	**Age:**	
Sample ID:	02-8526-7362	**Code:**	1100D5A2	**Client:**	Internal Lab
Sample Date:	27 Feb-14 08:58	**Material:**	Uranyl Sulphate	**Project:**	Reference Toxicity Program
Receive Date:	27 Feb-14 08:58	**Source:**	Reference Toxicant (U)		
Sample Age:	NA	**Station:**	In House		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	841137	200	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	2.316	2.734	0.2794	No Outliers Detected

Point Estimates

Level	µg/L	95% LCL	95% UCL
IC5	13.53	1.847	22.37
IC10	19.11	15.82	21.99
IC15	22.15	18.04	25.69
IC20	25.65	20.44	31.09
IC25	29.67	22.31	34.28
IC40	36.48	31.77	39.84
IC50	41.13	36.64	44.88

Growth rate (db/d) Summary

			Calculated Variate						
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0.052	Synthetic Soft W	3	1.873	1.84	1.92	0.02404	0.04163	2.22%	0.0%
3.9		3	1.807	1.77	1.84	0.02028	0.03512	1.94%	3.56%
7.4		3	1.853	1.83	1.89	0.01856	0.03214	1.73%	1.07%
17		3	1.76	1.7	1.8	0.03055	0.05291	3.01%	6.05%
31		3	1.377	1.26	1.45	0.05897	0.1021	7.42%	26.51%
64		3	0.2433	0.16	0.37	0.06438	0.1115	45.82%	87.01%
130		3	0.05667	0.04	0.07	0.008819	0.01528	26.96%	96.98%

Growth rate (db/d) Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3
0.052	Synthetic Soft Wa	1.84	1.92	1.86
3.9		1.81	1.77	1.84
7.4		1.84	1.83	1.89
17		1.78	1.8	1.7
31		1.45	1.42	1.26
64		0.37	0.16	0.2
130		0.06	0.07	0.04

Figure K7 continued

Algal Growth Inhibition Test | **eriss ecotoxicology lab**

Analysis ID:	11-6683-2758	**Endpoint:**	Growth rate (db/d)	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Apr-14 15:13	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics

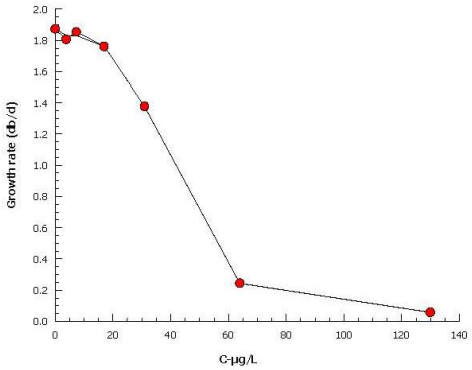


Figure K8 Test raw data and analysis report for test 1404G

CETIS Analytical Report

Report Date: 12 May-15 16:15 (p 1 of 2)
Test Code: 1404G | 09-8747-0228

Algal Growth Inhibition Test — **eriss ecotoxicology lab**

Analysis ID:	12-3760-1597	**Endpoint:**	Growth rate (db/d)	**CETIS Version:**	CETISv1.8.7
Analyzed:	10 Jun-14 11:24	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	10-5277-5855	**Test Type:**	Algal growth inhibition	**Analyst:**	Tom Mooney
Start Date:	13 May-14	**Protocol:**	Alga eriss tropical freshwater	**Diluent:**	Synthetic Soft Water
Ending Date:	16 May-14	**Species:**	Chlorella sp.	**Brine:**	Not Applicable
Duration:	72h	**Source:**	eriss ecotoxicology lab	**Age:**	
Sample ID:	18-3172-2648	**Code:**	1404G	**Client:**	Internal Lab
Sample Date:	21 May-14 13:38	**Material:**	Uranyl Sulphate	**Project:**	Reference Toxicity Program
Receive Date:	21 May-14 13:38	**Source:**	Reference Toxicant (U)		
Sample Age:	NA	**Station:**	In House		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	140311	200	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	2.451	2.734	0.1696	No Outliers Detected

Point Estimates

Level	µg/L	95% LCL	95% UCL
IC5	11.78	9.812	14.57
IC10	17.6	15.97	19.56
IC15	23.86	20.51	30.23
IC20	27.88	26.95	29.14
IC25	29.44	28.46	30.64
IC40	34.63	33.43	35.93
IC50	38.58	37.13	40.29

Growth rate (db/d) Summary

			Calculated Variate						
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0.072	Synthetic Soft W	3	1.857	1.85	1.86	0.00333	0.005768	0.31%	0.0%
3.8		3	1.83	1.81	1.85	0.01155	0.02	1.09%	1.44%
7		3	1.837	1.82	1.86	0.01202	0.02081	1.13%	1.08%
14		3	1.74	1.73	1.76	0.01	0.01732	1.0%	6.28%
27		3	1.54	1.51	1.58	0.02082	0.03605	2.34%	17.06%
56		3	0.2833	0.23	0.35	0.03528	0.0611	21.57%	84.74%
110		3	0.04	0	0.06	0.02	0.03464	86.6%	97.85%

Growth rate (db/d) Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3
0.072	Synthetic Soft Wa	1.85	1.86	1.86
3.8		1.85	1.81	1.83
7		1.86	1.82	1.83
14		1.73	1.73	1.76
27		1.58	1.53	1.51
56		0.35	0.23	0.27
110		0.06	0.06	0

Figure K8 continued

CETIS Analytical Report

Report Date: 12 May-15 16:15 (p 2 of 2)
Test Code: 1404G | 09-8747-0228

Algal Growth Inhibition Test **eriss ecotoxicology lab**

Analysis ID:	12-3760-1597	**Endpoint:**	Growth rate (db/d)	**CETIS Version:**	CETISv1.8.7
Analyzed:	10 Jun-14 11:24	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics

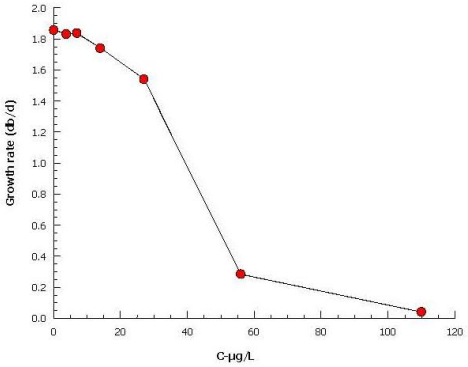


Figure K9 Test raw data and analysis report for test 1415G

CETIS Analytical Report

Report Date: 12 May-15 16:21 (p 1 of 2)
Test Code: 1415G | 20-5213-3272

Algal Growth Inhibition Test — **eriss ecotoxicology lab**

Analysis ID:	01-6080-8538	**Endpoint:**	Growth rate (db/d)	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Aug-14 16:40	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	15-4240-7443	**Test Type:**	Algal growth inhibition	**Analyst:**	Ceiwen Pease
Start Date:	05 Aug-14	**Protocol:**	Alga eriss tropical freshwater	**Diluent:**	Synthetic Soft Water
Ending Date:	08 Aug-14	**Species:**	Chlorella sp.	**Brine:**	Not Applicable
Duration:	72h	**Source:**	eriss ecotoxicology lab	**Age:**	
Sample ID:	00-4198-1989	**Code:**	2809825	**Client:**	Internal Lab
Sample Date:	08 Aug-14 14:25	**Material:**	Uranyl Sulphate	**Project:**	Reference Toxicity Program
Receive Date:	08 Aug-14 14:25	**Source:**	Reference Toxicant (U)		
Sample Age:	NA	**Station:**	In House		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1235553	200	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	2.457	2.734	0.1654	No Outliers Detected

Point Estimates

Level	µg/L	95% LCL	95% UCL
IC5	20.13	16.61	20.84
IC10	23.8	20.46	25.48
IC15	28.11	24.23	31.09
IC20	33.17	28.32	36
IC25	35.9	33.76	37.94
IC40	43.45	40.51	47.14
IC50	49.33	45.58	55.34

Growth rate (db/d) Summary

			Calculated Variate						
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0.06	Synthetic Soft W	3	1.738	1.694	1.806	0.03451	0.05978	3.44%	0.0%
4.3		3	1.71	1.664	1.743	0.02354	0.04077	2.38%	1.62%
8.6		3	1.712	1.677	1.741	0.01856	0.03216	1.88%	1.5%
17		3	1.868	1.709	1.982	0.0819	0.1419	7.6%	-7.45%
34		3	1.392	1.365	1.418	0.01528	0.02647	1.9%	19.89%
66		3	0.4735	0.3754	0.5947	0.06434	0.1114	23.53%	72.76%
140		3	-0.00074	-0.02913	0.05283	0.0268	0.04641	-6313.0%	100.0%

Growth rate (db/d) Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3
0.06	Synthetic Soft Wa	1.714	1.694	1.806
4.3		1.664	1.723	1.743
8.6		1.677	1.718	1.741
17		1.982	1.709	1.911
34		1.418	1.395	1.365
66		0.5947	0.3754	0.4505
140		0.05283	-0.0259	-0.02913

Figure K9 continued

Algal Growth Inhibition Test | **eriss ecotoxicology lab**

Analysis ID:	01-6080-8538	**Endpoint:**	Growth rate (db/d)	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Aug-14 16:40	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics

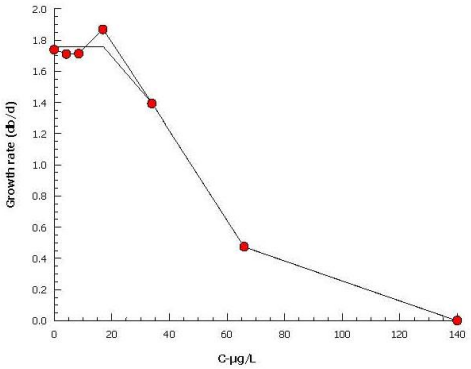


Figure K10 Test raw data and analysis report for test 1431G

CETIS Analytical Report

Report Date: 12 May-15 16:22 (p 1 of 2)
Test Code: 1431G | 02-9764-2702

Algal Growth Inhibition Test — **eriss ecotoxicology lab**

Analysis ID:	20-5085-6867	**Endpoint:**	Growth rate (db/d)	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Jan-15 8:50	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	20-5293-4847	**Test Type:**	Algal growth inhibition	**Analyst:**	Ceiwen Pease
Start Date:	09 Dec-14	**Protocol:**	Alga eriss tropical freshwater	**Diluent:**	Synthetic Soft Water
Ending Date:	12 Dec-14	**Species:**	Chlorella sp.	**Brine:**	Not Applicable
Duration:	72h	**Source:**	eriss ecotoxicology lab	**Age:**	
Sample ID:	18-6729-6881	**Code:**	6F4CB071	**Client:**	Internal Lab
Sample Date:	16 Dec-14 13:07	**Material:**	Uranyl Sulphate	**Project:**	Reference Toxicity Program
Receive Date:	16 Dec-14 13:07	**Source:**	Reference Toxicant (U)		
Sample Age:	NA	**Station:**	In House		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1060309	200	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	2.657	2.734	0.0714	No Outliers Detected

Point Estimates

Level	µg/L	95% LCL	95% UCL
IC5	8.214	3.497	65.34
IC10	28.1	N/A	47.6
IC15	36.34	11.21	42.95
IC20	38.38	27.66	45.83
IC25	40.53	32.32	48.85
IC40	47.71	37.37	62.43
IC50	53.17	41.22	85.67

Growth rate (db/d) Summary

			Calculated Variate						
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0.03	Synthetic Soft W	3	1.781	1.755	1.811	0.0163	0.02823	1.59%	0.0%
4.2		3	1.952	1.9	2.001	0.02907	0.05035	2.58%	-9.58%
8.4		3	1.77	1.632	1.95	0.09428	0.1633	9.23%	0.63%
18		3	1.739	1.661	1.815	0.04439	0.07688	4.42%	2.4%
35		3	1.651	1.481	1.972	0.1609	0.2786	16.88%	7.33%
70		3	0.4586	0.207	0.922	0.232	0.4018	87.62%	74.26%
140		3	-0.1106	-0.2762	0.06479	0.09854	0.1707	-154.4%	106.2%

Growth rate (db/d) Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3
0.03	Synthetic Soft Wa	1.777	1.755	1.811
4.2		1.9	2.001	1.956
8.4		1.95	1.632	1.729
18		1.74	1.661	1.815
35		1.972	1.499	1.481
70		0.2467	0.922	0.207
140		0.06479	-0.1203	-0.2762

Figure K10 continued

Algal Growth Inhibition Test | **eriss ecotoxicology lab**

Analysis ID:	20-5085-6867	**Endpoint:**	Growth rate (db/d)	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Jan-15 8:50	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics

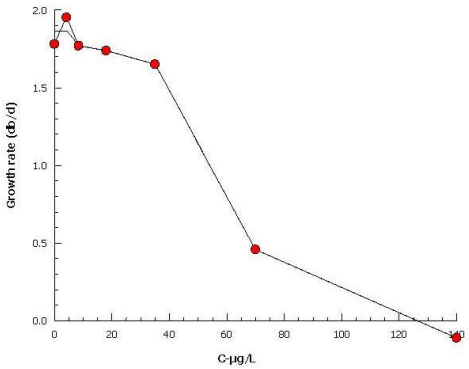


Figure K11 Test raw data and analysis report for test 1455G

CETIS Analytical Report

Algal Growth Inhibition Test — **eriss ecotoxicology lab**

Analysis ID:	05-6851-8384	**Endpoint:**	Growth rate (db/d)	**CETIS Version:**	CETISv1.8.7
Analyzed:	01 May-15 14:17	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	18-9563-0164	**Test Type:**	Algal growth inhibition	**Analyst:**	Ceiwen Pease
Start Date:	17 Mar-15	**Protocol:**	Alga eriss tropical freshwater	**Diluent:**	Synthetic Soft Water
Ending Date:	20 Mar-15	**Species:**	Chlorella sp.	**Brine:**	Not Applicable
Duration:	72h	**Source:**	eriss ecotoxicology lab	**Age:**	
Sample ID:	18-6347-5920	**Code:**	1455G	**Client:**	Internal Lab
Sample Date:	24 Mar-15 10:16	**Material:**	Uranyl Sulphate	**Project:**	Reference Toxicity Program
Receive Date:	24 Mar-15 10:16	**Source:**	Reference Toxicant (U)		
Sample Age:	NA	**Station:**	In House		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	869780	200	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	2.853	2.548	0.0070	Outlier Detected

Point Estimates

Level	µg/L	95% LCL	95% UCL
IC5	1.918	0.7135	7.162
IC10	7.347	1.133	24.78
IC15	16	6.308	47.79
IC20	18.93	14.2	47.73
IC25	22.37	14.97	47.11
IC40	33.01	20.34	45.37
IC50	37.3	27.93	48.13

Growth rate (db/d) Summary

			Calculated Variate						
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0.02	Synthetic Soft W	3	2.11	2.093	2.122	0.008827	0.01529	0.72%	0.0%
15		3	1.834	1.761	1.945	0.05648	0.09783	5.33%	13.1%
31		3	1.374	1.14	1.838	0.232	0.4018	29.24%	34.88%
62		3	0.171	0.04736	0.2434	0.06213	0.1076	62.92%	91.9%
130		3	0.012	-0.02872	0.06548	0.02793	0.04838	403.1%	99.43%

Growth rate (db/d) Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3
0.02	Synthetic Soft Wa	2.116	2.093	2.122
15		1.761	1.945	1.796
31		1.838	1.14	1.144
62		0.2223	0.2434	0.04736
130		-0.02872	0.06548	-0.00075

Figure K11 continued

Algal Growth Inhibition Test | **eriss ecotoxicology lab**

Analysis ID:	05-6851-8384	**Endpoint:**	Growth rate (db/d)	**CETIS Version:**	CETISv1.8.7
Analyzed:	01 May-15 14:17	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics

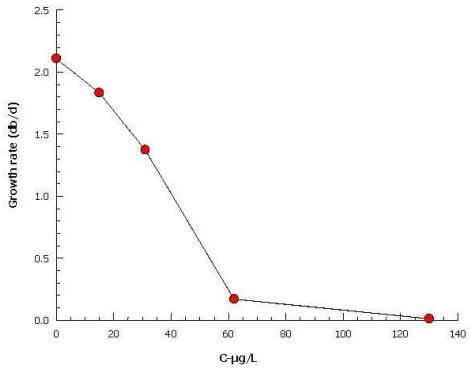


Figure K12 Test raw data and analysis report for test 1466G

CETIS Analytical Report

Report Date: 05 Jan-16 14:11 (p 1 of 2)
Test Code: 1466G | 18-7341-3790

Algal Growth Inhibition Test — **eriss ecotoxicology lab**

Analysis ID:	05-5467-3369	**Endpoint:**	Growth rate (db/d)	**CETIS Version:**	CETISv1.8.7
Analyzed:	13 Jul-15 13:08	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	18-0764-0017	**Test Type:**	Algal growth inhibition	**Analyst:**	Ceiwen Pease
Start Date:	09 Jun-15	**Protocol:**	Alga eriss tropical freshwater	**Diluent:**	Synthetic Soft Water
Ending Date:	12 Jun-15	**Species:**	Chlorella sp.	**Brine:**	Not Applicable
Duration:	72h	**Source:**	eriss ecotoxicology lab	**Age:**	
Sample ID:	08-8034-3788	**Code:**	1466G	**Client:**	Internal Lab
Sample Date:	12 Jun-15 15:06	**Material:**	Uranyl Sulphate	**Project:**	Reference Toxicity Program
Receipt Date:	12 Jun-15 15:06	**Source:**	Reference Toxicant (U)		
Sample Age:	n/a	**Station:**	In House		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	362909	200	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value Test	2.607	2.548	0.0362	Outlier Detected

Point Estimates

Level	µg/L	95% LCL	95% UCL
IC5	1.136	1.112	1.162
IC10	1.534	1.478	1.596
IC15	2.007	1.907	2.117
IC20	2.569	2.41	2.742
IC25	3.235	3	3.492
IC40	6.075	5.453	6.769
IC50	8.962	7.873	10.19

Growth rate (db/d) Summary

			Calculated Variate						
Conc-µg/L	Code	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0.8	SS	3	2.17	2.166	2.173	0.002351	0.004073	0.19%	0.00%
15		3	0.7847	0.7136	0.845	0.03831	0.06635	8.46%	63.85%
26		3	-0.01783	-0.2389	0.1012	0.1106	0.1916	-1074.76%	100.82%
54		3	-0.04485	-0.147	0.006276	0.0511	0.08851	-197.36%	102.07%
110		3	-0.07443	-0.1154	-0.04366	0.02131	0.03691	-49.60%	103.43%

Growth rate (db/d) Detail

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3
0.8	SS	2.173	2.166	2.172
15		0.7136	0.7955	0.845
26		0.1012	0.08421	-0.2389
54		0.00623	-0.147	0.006276
110		-0.1154	-0.06427	-0.04366

Figure K12 continued

Algal Growth Inhibition Test — **eriss ecotoxicology lab**

Analysis ID:	05-6851-8384	**Endpoint:**	Growth rate (db/d)	**CETIS Version:**	CETISv1.8.7
Analyzed:	01 May-15 14:17	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics

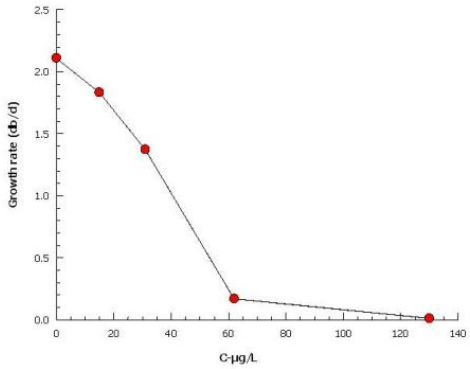


Figure K13 Test raw data and analysis report for test 1468G

CETIS Analytical Report

Algal Growth Inhibition Test — **eriss ecotoxicology lab**

Analysis ID:	00-8758-3715	**Endpoint:**	Growth rate (db/d)	**CETIS Version:**	CETISv1.8.7
Analyzed:	13 Jul-15 13:10	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	07-6817-6216	**Test Type:**	Algal growth inhibition	**Analyst:**	Ceiwen Pease
Start Date:	22 Jun-15	**Protocol:**	Alga eriss tropical freshwater	**Diluent:**	Synthetic Soft Water
Ending Date:	25 Jun-15	**Species:**	Chlorella sp.	**Brine:**	Not Applicable
Duration:	72h	**Source:**	eriss ecotoxicology lab	**Age:**	
Sample ID:	15-9956-0398	**Code:**	1468G	**Client:**	Internal Lab
Sample Date:	25 Jun-15 15:59	**Material:**	Uranyl Sulphate	**Project:**	Reference Toxicity Program
Receipt Date:	25 Jun-15 15:59	**Source:**	Reference Toxicant (U)		
Sample Age:	n/a	**Station:**	In House		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1420830	200	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value Test	2.408	2.548	0.1004	No Outliers Detected

Point Estimates

Level	µg/L	95% LCL	95% UCL
IC5	1.879	1.204	2.626
IC10	6.537	3.197	10.69
IC15	14.17	11.35	14.53
IC20	14.79	14.33	15.13
IC25	15.42	15	15.76
IC40	17.5	17.14	17.79
IC50	19.03	18.68	19.32

Growth rate (db/d) Summary

			Calculated Variate						
Conc-µg/L	Code	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0.1	SS	3	1.915	1.897	1.932	0.01029	0.01783	0.93%	0.00%
14		3	1.655	1.618	1.675	0.01824	0.0316	1.91%	13.58%
25		3	0.3285	0.3184	0.3478	0.009645	0.0167	5.08%	82.84%
48		3	-0.00116	-0.02907	0.01675	0.01414	0.02449	-2106.10%	100.06%
97		3	-0.1728	-0.2158	-0.1025	0.03544	0.06139	-35.53%	109.02%

Growth rate (db/d) Detail

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3
0.1	SS	1.932	1.897	1.915
14		1.671	1.618	1.675
25		0.3193	0.3184	0.3478
48		0.008826	0.01675	-0.02907
97		-0.2158	-0.1025	-0.2

Figure K13 continued

Analysis ID:	00-8758-3715	**Endpoint:**	Growth rate (db/d)	**CETIS Version:**	CETISv1.8.7
Analyzed:	13 Jul-15 13:10	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Graphics

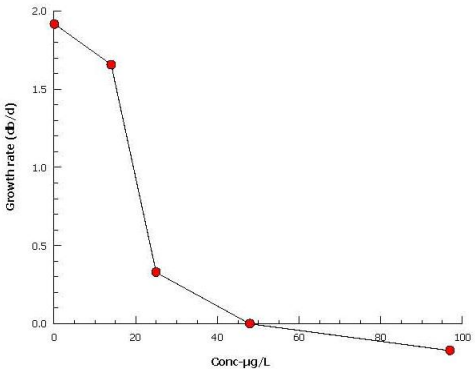


Figure K14 Test raw data and analysis report for test 1479G

CETIS Analytical Report

Report Date: 05 Jan-16 14:12 (p 1 of 1)
Test Code: 1479G | 12-0184-6999

Algal Growth Inhibition Test — **eriss ecotoxicology lab**

Analysis ID:	16-9035-6070	**Endpoint:**	Growth rate (db/d)	**CETIS Version:**	CETISv1.9.0
Analyzed:	23 Sep-15 15:20	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes
Batch ID:	12-7824-8524	**Test Type:**	Algal growth inhibition	**Analyst:**	Ceiwen Pease
Start Date:	07 Sep-15	**Protocol:**	Alga eriss tropical freshwater	**Diluent:**	Synthetic Soft Water
Ending Date:	10 Sep-15	**Species:**	Chlorella sp.	**Brine:**	Not Applicable
Duration:	72h	**Source:**	eriss ecotoxicology lab	**Age:**	
Sample ID:	21-2063-6292	**Code:**	1479G	**Client:**	Internal Lab
Sample Date:	25 Jun-15 15:59	**Material:**	Uranyl Sulphate	**Project:**	Reference Toxicity Program
Receipt Date:	25 Jun-15 15:59	**Source:**	Reference Toxicant (U)		
Sample Age:	73d 8h	**Station:**	In House		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	987894	200	Yes	Two-Point Interpolation

Point Estimates

Level	µg/L	95% LCL	95% UCL
IC5	1.304	0.8023	2.098
IC10	4.054	1.968	7.826
IC15	10.09	3.573	22.57
IC20	16.51	12.72	17.8
IC25	17.68	16.3	19.01
IC40	21.69	20	23.33
IC50	24.84	22.89	26.9

Growth rate (db/d) Summary

			Calculated Variate						
Conc-µg/L	Code	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0.05	SS	3	2.054	1.993	2.106	0.03298	0.05713	2.78%	0.00%
16		3	1.69	1.634	1.721	0.0279	0.04832	2.86%	17.72%
30		3	0.7383	0.6607	0.7979	0.04059	0.07031	9.52%	64.05%
63		3	0.07282	0.04318	0.1025	0.01714	0.02968	40.76%	96.45%
140		3	0.04349	0.02028	0.05601	0.01161	0.02012	46.26%	97.88%

Growth rate (db/d) Detail

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3
0.05	SS	1.993	2.062	2.106
16		1.634	1.721	1.715
30		0.7979	0.7561	0.6607
63		0.1025	0.04318	0.07275
140		0.05601	0.05416	0.02028

Graphics