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Ecotoxicological assessment of a polyelectrolyte flocculant

AJ Harford, AC Hogan & RA van Dam

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Executive summary

Flocculant blocks are commonly used in water treatment processes to reduce suspended sediment loads of the water column. The mining industry has been increasingly interested in the application of flocculant blocks, with the aim of improving the quality of water released into the environment. Energy Resources of Australia Ltd (ERA) implemented the use of flocculant blocks during the 2006-07 wet season to reduce suspended sediment (and associated adsorbed metal) concentrations in a number of its on-site water bodies. To ensure appropriate on-site water management, ERA required information on whether the use of the flocculant blocks would introduce unacceptable toxicity to the Pond Waters at Ranger.

This study investigated the biological impacts of a flocculant block that contained an anionic polyacrylamide (PAM) active ingredient and a polyethylene glycol (PEG) based carrier. The toxicity of the whole flocculent block was assessed and the individual components of the block were also tested separately. Previous studies using acute tests with Northern hemisphere species indicated that the toxicity of the flocculant block was relatively low. However, this study used primarily chronic, sub-lethal tests to assess toxicity. Five Northern Australian tropical freshwater species (ie *Chlorella* sp, *Lemna aequinoctialis*, *Hydra viridissima* *Moinodaphnia macleayi* and *Mogurnda mogurnda*) were exposed to various concentrations of the flocculent block, PAM and PEG. The test solutions were measured for total organic carbon (TOC) as indicators of the total amount of PAM and PEG present, while total nitrogen (N) was measured as an indicator of the concentration of PAM alone. Viscosity was measured to provide a metric for assessing the physical effects of the medium itself on the organisms.

The results showed an extremely wide range of 'toxicity', with the flocculant blocks being essentially non-toxic to the duckweed, fish and algae (IC_{50}>3600 mg L^{-1}, IC_{10}>780 mg L^{-1}, nominal concentrations), slightly 'toxic' to the hydra (IC_{50}=1180-4250 mg L^{-1}, IC_{10}=120-160 mg L^{-1}, nominal concentrations) and highly 'toxic' to the cladoceran (IC_{50}=10 mg L^{-1}, IC_{10} = 5 mg L^{-1}, nominal concentrations). Investigation of the individual components indicated that the PAM was the primary 'toxicant' in the flocculant blocks. Increased viscosity at higher concentrations of the product was one of the possible contributing factors to the adverse effects observed in the cladocerans.

Water quality trigger values were calculated using species sensitivity distributions. In the event that 95 or 99% species protection levels (equating to TVs of 0.05 and 2 mg L^{-1} TOC) were to be applied then it may not be possible to use measurements of TOC or N as surrogates for the flocculant block constituents. The reason for this is that such low concentrations are essentially at or below the effective detection limits for these methods of analysis. In the event that ERA wishes to monitor the presence of flocculant block constituents, a TV protection level of 80%, (ie 30 mg L^{-1} TOC) for mine site water bodies would make monitoring of TOC levels as a signal of flocculant block contamination achievable.

Acknowledgments

We would like to thank the technical staff at ***eriss*** ecotoxicology laboratory, Kim Cheng, Claire Costello and Melanie Houston, for assisting with the toxicity tests. We would also like to thank the technical staff at ***eriss*** ecotoxicology laboratory, Kim Cheng, Claire Costello and Melanie Houston, for assisting with the toxicity tests.

Ecotoxicological assessment of a polyelectrolyte flocculant

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1 Introduction

For many decades, high molecular weight water-soluble polymers have been used in water purification processes to coagulate and flocculate particles, aiding in their removal from the water column (Bolto & Gregory 2007). These polymers (or polyelectrolytes) have been classed by their ionic nature ie cationic, anionic or non-ionic. Anionic polymers acts as true flocculants and bind suspended particles together to form larger particles that settle out of solution more rapidly, while cationic polymers acts as coagulants through neutralising the surface charges of particles (Liber et al 2005). Mining operations have long recognised the usefulness of flocculating polymers to reduce suspended sediment loads of their effluents. As such, these polymers are viewed as a pollution control measure and have rarely been recognised as a potential pollutant themselves. Despite their common use, and large volumes of these chemicals being released into the environment, only a limited number of studies have investigated their toxicity (Liber et al 2005).

There is an extremely limited database concerning the toxicity of water treatment polymers to aquatic organisms, especially data reporting chronic toxicity. The existing aquatic toxicity information indicates that the anionic class has a relatively low toxicity to aquatic organisms, while the cationic class is at least 100 times more toxic (Hamilton et al 1994). Consequently, cationic polymers have been studied to a greater degree and are reported to be toxic at concentrations <1 mg L^{-1}. Studies have shown that the cationic polyelectrolytes affect cell membrane integrity and that the effect is dependent on the charge density and hydrophobicity of the polymer (Narita et al 2001). However, some species-specific mechanisms of action have been reported and the effects on phytoplankton appear to be dependent on the molecular weight of the polymers rather than charge density (de Rosemond & Liber 2004).

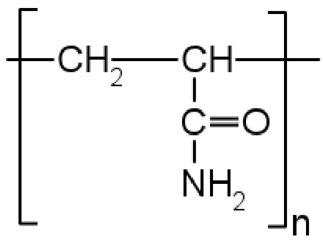
The primary mechanism of action of the anionic polymers is the binding of membranes (membranotrophic), which results in the inhibition of the cross-membrane transport of nutrients and essential elements. Consequently, the mechanism of anionic polymers is dependant on the chain length, with longer chains being more toxic (Bolto & Gregory 2007). A limited number of studies have reported that fish appear to be relatively tolerant of anionic polymer exposure with 100% survival commonly occurring at the highest concentrations tested, and with LC_{50}s of >20 mg L^{-1} – >1000 mg L^{-1} (Beim & Beim 1994, de Rosemond & Liber 2004). Cladocerans were commonly the most sensitive species tested and the reported acute toxicities (LC_{50}) of anionic polymers to *Ceriodaphnia dubia* (48 h) and *Daphnia magna* (96 h) were 218 mg L^{-1} and 14-17 mg L^{-1}, respectively (Biesinger et al 1976, Beim & Beim 1994, de Rosemond & Liber 2004). Beim and Beim (1994) also reported an LC_{50} of 2100 mg L^{-1} PAM for an amphipod, >100 mg L^{-1} PAM for a flatworm and >1000 mg L^{-1} PAM for an adult minnow (fish).

Energy Resources of Australia Ltd (ERA) implemented the use of flocculant blocks during the 2006–07 wet season to reduce suspended sediment (and associated adsorbed metal) concentrations in a number of its on-site water bodies. To ensure appropriate on-site water management, ERA required information on whether the use of the flocculant blocks would introduce unacceptable toxicity to the Pond Waters at Ranger. The flocculant blocks investigated in this study consisted of an anionic polyacrylamide (PAM, ~40%, Figure 1a) active ingredient and a polyethylene glycol (PEG, ~60%, Figure 1b) carrier compound. Ecotoxicological data provided in the Material Safety Data Sheets (MSDS) reported acute EC/LC_{50} values of 212 – >1000 mg L^{-1} for PAM (Table 1). A NOEC of 708 mg L^{-1} was also reported in documentation provided by the supplier (Environment Warehouse, unpublished data), however, it was unclear for which compound and aquatic species this value was derived. The above toxicity test data for the anionic polyacrylamides were derived from studies undertaken at the Société d'Ecotoxicologie et de Physico-Chimie (SEPC, Sarcey, France) and were used as supporting documentation for products assessed under European Union directive 67/548/EEC (ie REACH program, packaging and labelling of dangerous substances, Vehaar (2002)). The SEPC studies are also cited in National Industrial Chemicals Notification and Assessment Scheme (NICNAS) assessments of products containing anionic polyacrylamides (NICNAS 2005).

The carrier agent in the flocculant blocks, poly ethylene glycol (PEG) is known for its very low toxicity and a limited number of studies have reported no adverse responses in fish and phytoplankton following exposures up to 5 g L^{-1} (Wildish 1974, Bridié et al 1979, Chan et al 1981). Indeed, many studies use PEG as an inert carrier agent or negative control (eg Wildish 1974, Harford et al 2007), and its function in the flocculant block is to increase the solubility of the PAM.

The aims of this study were to determine the toxicity of (i) flocculant block, as a whole (ie dissolved), and (ii) the two individual ingredients, ie PAM and PEG, to five local freshwater species, and derive site-specific water quality trigger values for each.

a)



b)

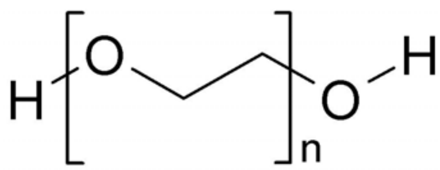


Figure 1 The chemical structures of a) polyacrylamide and b) polyethylene glycol (www.wikipedia.com)

Table 1 Ecotoxicological data provided with the flocculant block product (Environment Warehouse, unpublished data)

Species	Test duration	Endpoint	Anionic polyacrylamides LC_{50}/EC_{50}
Brachydanio rerio	96 h	Survival	357[a]
Daphnia magna	98 h	Immobilisation	212
Pseudomonas putida	24 h	Respiration	892
Chlorella vulgaris	72 h	Growth rate	>1000

a LC_{50} reported for *Brachydanio rerio*

2 Methods

2.1 Diluent

Natural Magela Creek water (NMCW) was used for the control treatment and dilution of the test solutions in the all tests. It was collected by SSD staff from Bowerbird Billabong (latitude 12° 46’ 15’’, longitude 132° 02’ 20’’) during the dry season and Georgetown Billabong (latitude 12° 40’ 28’, longitude 132° 55’ 52) during the wet season. The water was collected in 20 L acid-washed plastic containers and placed in storage at 4 ± 1°C within 1 h of collection. The water was then transported to ***eriss*** Ecotoxicology Laboratory in Darwin in an air-conditioned vehicle. At the laboratory, it was stored at 4 ± 1°C and filtered through Whatman #42 (2.5 µm pore size) filter paper within 4 d of collection. Throughout the testing period the NMCW had a pH of 6.1 - 6.8 units, a conductivity of 10–22 µS/cm and dissolved oxygen of >90%.

2.2 Chemistry

Chemistry samples were taken to ensure that; (i) the dilutions undertaken for the tests were accurate, (ii) no chemical contaminants were introduced to the test solutions during preparation, and (iii) the nutrient additions to the solutions used for the *Chlorella* sp and *L. aequinoctialis* tests were accurate. Due to the nature of the flocculant block constituents, Total Organic Carbon (TOC) was measured and used as a quantitative indicator of the PAM and PEG concentrations in flocculant block, while total nitrogen was measured and used as a quantitative indicator of the active ingredient (PAM) concentration. A metals analysis was conducted on the Magela Creek water controls, Milli-Q water blanks and procedural blanks for quality control of contaminants. However, a metals analysis could not be conducted on the treatment samples because the presence of the polyacrylamide meant that high concentrations of acid were needed to digest the chemical, resulting in impractically high detection limits.

TOC analyses were conducted at ***eriss*** (Shimadzu TOC-V CSH), while total nitrogen was analysed by flow injection analysis (Lachat 8000 series) at the Northern Territory Environmental Laboratories (NTEL, Berrimah, Northern Territory, Australia). Samples to be analysed for soluble metals were acidified to approximately 0.7% HNO_3 by adding 10 µL of 69% Aristar HNO_3 (BDH) for every mL of sample (determined by weighing sample bottles before and after sample addition). Samples for metals were stored at 4 ± 1°C until being sent to NTEL for analysis. Samples to be analysed for nutrients (NO_3 and PO_4) were then sealed with no head-space and frozen, before sending for analysis at NTEL.

The Brookfield and Funnel methods for viscosity analyses were conducted on surrogate test solutions by Mintech Chemical Industries (Berrimah, Northern Territory, Australia).

Viscosities of the solutions were measured to determine if a physical effect of the test solutions could be separated from the chemical effects.

2.3 Toxicity tests

The tests undertaken to assess the toxicity of flocculant block and its constituents, PAM and PEG, are listed in Table 2. The whole flocculant block (DamClear, Environmental Warehouse, Swansea, NSW, Australia), PAM (MAGNAFLOC® 1011, Ciba, Kwinana, WA) and PEG (DPW-1-1111, Ciba, Kwinana, WA) were all dissolved in NMCW and serially diluted to the concentrations shown in Table 2. Standardised ecotoxicological tests for five local freshwater species were conducted at the Environmental Research Institute of the Supervising Scientist laboratories between 10 September 2007 and 22 May 2008. Unless specified, all experiments were conducted in accordance with standardised protocols as detailed by Riethmuller et al (2003). The details of the test protocols are shown in Table 3.

Table 2 Details of each of the tests undertaken to assess the toxicity of flocculant block, PAM and PEG[a]

Test organism	Acute/ Chronic	Test code	Date	Concentrations tested (mg L^{-1})[b]
Flocculant block (commercial product)				
Chlorella sp. (unicellular alga)	Chronic	866G	10/09/07	0, 313, 625, 1250, 2500, 5000
Lemna aequinoctialis (duckweed)	Chronic	865L	09/09/07	0, 313, 625, 1250, 2500, 5000
Hydra viridissima (green hydra)	Chronic	862B	10/09/07	0, 313, 625, 1250, 2500, 5000
	Chronic	873B	01/10/07	0, 39, 78, 156, 313, 625, 1250
	Chronic	877B	15/10/07	0, 39, 78, 156, 313, 625, 1250
Moinodaphnia macleayi (cladoceran)	Chronic	863D	21/09/07	0, 39, 78, 156, 313, 625, 1250, 2500
	Chronic	874D	03/10/07	0, 1.22, 2.44, 4.88, 9.75, 19.5, 39, 78
Mogurnda mogurnda (fish)	Acute	864E	12/09/07	0, 313, 625, 1250, 2500, 5000
Polyacrylamide (PAM – Active ingredient)				
Chlorella sp. (unicellular alga)	Chronic	888G	20/11/07	0, 125, 250,500, 1000, 2000
Lemna aequinoctialis (duckweed)	Chronic	887L	19/11/07	0, 125, 250,500, 1000, 2000
Hydra viridissima (green hydra)	Chronic	883B	12/11/07	0, 16, 32, 63, 125, 250,500
	Chronic	892B	03/12/07	0, 16, 32, 63, 125, 250,500
Moinodaphnia macleayi (cladoceran)	Chronic	884D	12/11/07	0, 0.5, 1, 2, 4, 8, 16, 32
Mogurnda mogurnda (fish)	Acute	891E	01/12/07	0, 125, 250,500, 1000, 2000
Polyethylene glycol (PEG – Carrier)				
Chlorella sp (unicellular alga)	Chronic	913G	26/02/08	0, 750, 1500, 3000, 6000,12000
Lemna aequinoctialis (duckweed)	Chronic	919L	31/03/08	0, 750, 1500, 3000, 6000,12000
Hydra viridissima (green hydra)	Chronic	920B	08/04/08	0, 750, 1500, 3000, 6000,12000
Moinodaphnia macleayi (cladoceran)	Chronic	927D	16/05/08	0, 750, 1500, 3000, 6000,12000
Mogurnda mogurnda (fish)	Acute	923E	16/04/08	0, 750, 1500, 3000, 6000,12000

[a] Refer to Table 3 for toxicity test details

[b] All treatments were prepared using Magela Creek water as the diluent.

Table 3 Details of toxicity tests for five Australian tropical freshwater species used to assess the toxicity of flocculant blocks, PAM and PEG. Full details are provided in Riethmuller et al (2003)

Species (common name)	Test duration and endpoint	Control response acceptability criterion	Temperature, light intensity, photoperiod	Feeding/ nutrition	Culture medium	No. replicates (Individuals per replicate)	Test volume (mL)	Static/daily renewals
Chlorella sp. (unicellular green alga)	72-h population growth rate	1.4 ± 0.3 doublings day^{-1}; % CV[a] <20%	29 ± 1°C 100-150 μmol m^{-2} sec^{-1} 12:12h	14.5 mg/L NO_3 0.14 mg/L PO_4	Modified MBL medium	3 (3 × 10^4 cells)	50	Static
Lemna aequinoctialis (tropical duckweed)	96-h growth rate	Mean growth rate (k) ≥0.27; % CV <20%	29 ± 1°C 100-150 μmol m^{-2} sec^{-1} 12:12h	3 mg/L NO_3 0.3 mg/L PO_4	25% modified Hoagland's E and K medium	3 (4)	100	Static
Hydra viridissima (green hydra)	72-h population growth rate	Mean growth rate (k) ≥0.27; % CV <20%	27 ± 1°C 30-100 μmol m^{-2} sec^{-1} 12:12h	3 - 4 *Artemia* nauplii per hydra per day [c]	Natural Magela Creek water	3 (10)	30	Daily renewals
Moinodaphnia macleayi (cladoceran)	3 brood (120-144 h) reproduction	Mean adult survival ≥80%; mean neonates per adult ≥30; % CV <20%	27 ± 1°C 30-100 μmol m^{-2} sec^{-1} 12:12h	30 μl FFV[b] and 6 × 10^6 cells of *Chlorella* sp. per day	Natural Magela Creek water	10 (1)	30	Daily renewals
Mogurnda mogurnda (Northern trout gudgeon)	96-h survival	Mean larval survival ≥80%; % CV <20%	27 ± 1°C 30-100 μmol m^{-2} sec^{-1} 12:12h	Nil	Darwin filtered tap water	3 (10)	30	Daily renewals

[a] % CV: Percent co-efficient of variation

[b] FFV: fermented food with vitamins. Represents an organic and bacterial suspension prepared by method described in Riethmuller et al (2003).

[c] For all hydra tests, the amount of *Artemia* nauplii provided was the same but the delivery method differed between some tests. See text for details.

For the flocculant block testing, the *M. macleayi* and *H. viridissima* tests were repeated due to unexpected sensitive responses, where a No-Observed-Effect-Concentration (NOEC) could not be determined. A third *H. viridissima* test (877B) was conducted due to significantly different results between the first two tests (826B and 873B), which arose following the identification of unusual issues associated with the hydra feeding procedure in the viscous test solutions (see Results and Discussion for further explanation). Accordingly, the final hydra test involved a variation to the daily feeding method in the standard protocol, whereby 3-4 *Artemia* nauplii per hydra were added to the test solutions and briefly mixed, rather than being placed directly in the hydra's tentacles. This variation of the test protocol was repeated for the PAM tests (ie test 892B) but was not deemed necessary for the PEG tests because of the low viscosity and toxicity of PEG.

2.4 Quality Assurance and Quality Control

2.4.1 Chemistry

For each test, Milli-Q and procedural blanks were analysed as described above. Chemistry data for the blanks were initially assessed by searching for analyte concentrations higher than detection limits. Where these concentrations were greater than 2 μg/L and higher than background levels of NMCW, duplicate blank samples were re-analysed and/or the control water concentrations were compared with those in tests without blank contamination to determine if the contamination was limited to the one sample bottle or experienced throughout the test. Consequences of any contamination were then investigated and discussed on a case-by-case basis.

2.4.2 Water quality

For each test, data were considered free of confounding factors if: the recorded temperature of the incubator remained within the prescribed limits; the recorded pH in 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% throughout the test.

2.4.3 Control responses

Tests were considered valid if the control organisms met the following criteria:

Chlorella sp cell division rate test

- The growth rate of the control algae is within the range 1.4 ± 0.3 doublings day^{-1};
- There is <20% variability (ie co-efficient of variation, CV <20%) in the control growth rate.

L. aequinoctialis plant growth test

- The average increase in frond number in any control flask at test conclusion was at least four times that at test start (ie increase to 48 fronds/flask); and
- There was <20% variability (CV < 20%) in the control growth rate.

M. macleayi 3-brood reproduction test

- 80% or more of the cladocera are alive, female and have produced three broods at the end of the test period;
- Reproduction in the control averages 30 or more neonates surviving per female over the test period; and
- There was <20% variability (CV <20%) in control neonate production

H. viridissima population growth test

- More than 30 healthy hydroids (ie $k > 0.27$ day^{-1}) remain in each control dish at the end of the test period; and
- There was <20% variability (CV <20%) in the control growth rate.

M. mogurnda larval fish survival test

- The mean mortality and presence of fungus on the control fish does not exceed 20%;
- There was <20% variability (CV <20%) in control survival; and

2.5 Statistics

One way analysis of variance (ANOVA) and appropriate post-hoc tests were performed (using ToxCalc 5.0) on the results to determine NOECs and Lowest-Observed-Effect-Concentrations (LOECs). For the chronic tests linear interpolation was used to determine point estimates of Inhibitory Concentrations (ICs) that reduced endpoint responses by 10% and 50% (ie IC_{10} and IC_{50}) relative to the control response. For the *M. mogurnda* acute tests, maximum likelihood logit analysis was used to determine the 5% and 50% lethal concentrations (ie LC_{05} and LC_{50}). The IC_{10} (LC_{05} for *M. mogurnda*) toxicity estimates were used to construct a species sensitivity distribution (SSD) based on a Burr Type III distribution, from which Trigger Values (TVs) were calculated that would be protective of 80, 90, 95 and 99% of species (using BurrliOZ© 1.0.14, CSIRO, as detailed in ANZECC/ARMCANZ (2000)). The 95% confidence limits (CLs) of the TVs were estimated using the bootstrapping feature of BurrliOZ©, as described by Hose and van den Brink (2005). Toxicity results for the flocculant block formulation were reported in terms of nominal concentration (mg/L), TOC (mg/L) and total N (mg/L). For PAM, toxicity results were reported as nominal concentration (mg/L) only due to issues with the analysis of TOC and total N. For PEG, toxicity results were reported as nominal concentration (mg/L) and TOC (mg/L).

3 Results and discussion

3.1 Quality Assurance and Quality Control (QA/QC)

A summary of the QA/QC performance of the tests is presented in Table 4.

3.1.1 Flocculant block

All the toxicity tests were considered valid and control performance rates were at an acceptable level for all tests except for the 3rd *H. viridissima* test (877B, Table 4). However, this test used a modified protocol whereby the *Artemia* food was not given directly to the individual hydra (see section 3.2 for details). Consequently, this test was not expected to meet the same growth rates as the standard protocol and was considered valid.

Analysis of the blank, procedural blank and control (NMCW) chemistry samples showed that the test solutions were free of contamination by metals and that the correct amounts of nutrients were added to the *Chlorella* sp and *L. aequinoctialis* test. Control growth rates and physicochemical parameters were all with the specified ranges, except for *Chlorella* sp and *M. macleayi* (see below). It is also interesting to note that the flocculant block had the effect of slightly increasing the conductivity of the test solutions.

There were pH increases of up to 2.0 units measured in the *Chlorella* sp test (Appendix A). This was attributed to higher than normal growth rates of 1.9 doublings d^{-1} in the control, which indicates that the initial cell density may have been too high. It is unclear how such pH changes would affect the toxicity of the flocculant blocks. Taylor et al (2002) have reported that an increase in pH from 4.5 to 8.5 results in the dissociation of carboxyl functional groups, which increases the negativity of the polyelectrolyte and results in a conformation change of the PAM molecules (ie from coiled to extended). This increase in negative charge and change in conformation may have reduced the adsorption capacity of sediment particles for PAM. Whilst it does not necessarily follow that the pH rise of 2 units in the Chlorella toxicity test would reduce the toxicity of PAM to algal cells, at this pH the algal surface would be expected to have a net negative charge (Han et al 2006). Subsequently, a general observation is that increasing the charge density in the same direction of a molecule and a potential substrate will reduce the likelihood of binding of that molecule.

Table 4 QA/QC performance of the tests

Test species	Control performance criterion	Test Code	Toxicant	Mean control response ± %CV	Water quality [a]
Chlorella sp	1.4 ± 0.3 doublings day^{-1}	866G	Floc Block	1.9 ± 2	pH increase
		888G	PAM	1.6 ± 6	acceptable
		913G	PEG	1.6 ± 4	elevated Zn
Lemna aequinoctialis	48 fronds flask^{-1}	865L	Floc Block	59 ± 12	acceptable
		887L	PAM	57 ± 10	elevated Zn
		919L	PEG	36 ± 27	elevated Zn
Hydra viridissima	k > 0.27 day^{-1}	862B	Floc Block	0.33 ± 3.1	acceptable
		873B	Floc Block	0.33 ± 12.8	acceptable
		877B	Floc Block	0.26 ± 0.9	acceptable
		883B	PAM	0.28 ± 1.6	acceptable
		892B	PAM	0.29 ± 1.6	elevated Zn
		920B	PEG	0.34 ± 1.7	elevated Zn
Moinodaphnia macleayi	80% survival and >30 neonates adult^{-1}	874D	Floc Block	37 ± 5	pH increase
		884D	PAM	38 ± 21	pH increase
		927D	PEG	32 ± 17	elevated Zn
Mogurnda mogurnda	80% survival	864E	Floc Block	100 ±0	acceptable
		891E	PAM	93 ± 7	acceptable
		923E	PEG	100 ± 0	elevated Zn

[a] The acceptable water quality criteria are described in section 2.4.2

In some of the flocculant block treatment groups of the *M. macleayi* test (874D) there were pH changes of over 1.0 units, although the control groups did not vary by more than 0.55 units (Appendix A). These pH increases occurred in the higher-concentration treatments and were probably due to flocculant-induced inhibition of cladoceran feeding on the algae cells, which remove carbon dioxide and hydrogen ions from the test solutions during photosynthesis (Stumm & Morgan 1981). As discussed above, an increase in pH may have reduced the binding capacity of the PAM in the flocculant block formulation.

Strong linear relationships were observed between nominal flocculant block concentrations and 1) measured TOC (r^2=0.98, measured TOC (mg L^{-1}) =0.46 × nominal (mg L^{-1})), 2) total nitrogen (r^2=0.98, measured total nitrogen (mg L^{-1}) =0.08 × nominal (mg L^{-1})) and 3) viscosity (r^2=0.98, viscosity cPs = 0.02 nominal mg L^{-1}) (Appendix B). However, it is important to note that due to technical difficulties in the measurement of TOC (ie the viscosity of the test solutions clogged the analyser), the results presented are from surrogate test solutions not the actual solutions used in the tests, ie a new batch of solutions needed to be produced for the TOC analyses. The results presented for total nitrogen are based on measurements for sub-samples of the actual solutions used in the tests.

3.1.2 Polyacrylamide

Control performances for PAM toxicity tests were met and all the tests were considered valid (Table 4).

Analysis of the blank, procedural blank and control (NMCW) chemistry samples showed that the test solutions were relatively free of contaminant metals (Appendix B). However, elevated Zn levels were detected in the procedural blank of the *H. viridissima* test and in the procedural blank and control of the *L. aequinoctialis* test (8.6, 8.2 and 6.6 µg L^{-1} Zn, respectively). Although the Zn concentrations were above background concentrations, they did not appear to affect the control responses of the test and were unlikely to confound the results. Analysis of the nutrient concentrations suggested that less than the optimal amount of nutrients were added to the *Chlorella* sp tests (1.92 and 0.03 as opposed to 3.28 and 0.046 mg L^{-1} N & P, respectively, as stipulated in the test protocol); Appendix B). Nevertheless, excellent growth rates were achieved for both these tests. Control growth rates and physicochemical parameters were all with the specified ranges, except for the *M. macleayi* test, in which a pH increase was observed. This was similar to the pH changes in the flocculant block toxicity tests and was likely to be for the same reasons, ie a reduction in cladoceran algal feeding.

Chemical analysis of the test solutions for TOC and total N indicated that there might have been issues with preparing accurate dilutions of the solutions for analysis or indeed problems with dilution of the stock concentrates to prepare the test solutions. These issues likely arose from the extremely viscous nature of the stock concentrate, which appeared not to be a homogenous solution. In particular, the PAM did not necessarily mix completely with the water and appeared to settle on the bottom of containers whilst stored. It was also very difficult to pipette the PAM solutions for sub-sampling and dilution for analysis of TOC. These factors may have caused errors whilst diluting the test solutions and/or possibly while sub-sampling for chemical analysis. Alternatively, the viscosity of the test solutions may have also caused some issues during the chemical analysis techniques, as the instruments required substantial dilution of the test solution to be able to analyse the samples. A detailed discussion of each of the analyses follows.

- The tests solutions for the *L. aequinoctialis* and *Chlorella* sp tests showed strong linear relationships between nominal dilutions and TOC measurements. Furthermore, the proportions of TOC in the test solutions were near the expected concentrations, ie ~50% of the nominal concentration of PAM. The total N concentrations of the *L. aequinoctialis* test solutions showed a good linear relationship with nominal concentrations and were near expected ranges, which indicate that the test solutions were appropriately diluted for *L. aequinoctialis*. However, the total N of *Chlorella* sp in the 500 and 1000 mg/L test solutions appeared lower that expected, but as this result is in contrast to the TOC measurement it may have been due to issues with the chemical analysis rather than the dilution of the test solution.

- One treatment group from each of the *H. viridissima* tests (ie the 125 mg L^{-1} and 62 mg L^{-1} nominal PAM concentration groups for test 1 and 2, respectively) was removed from the derivation of the toxicity estimates due to seemingly anomalous elevated TOC and total N measurements. All other treatments showed a good relationship between nominal, TOC and total N concentrations, indicating appropriate dilution of these test solutions.

- Analysis of the *M. mogurnda* test solutions showed that, except for the highest concentration of 2000 mg L^{-1}, the TOC and total N concentrations of test solutions were much lower than

- The cladoceran test solutions showed low resolution of TOC and total N measurements between treatments at the lower concentrations (ie 0.5 – 4 mg L^{-1} nominal concentration, which is approaching the detection limit of these methods). All were around the NMCW background concentration of 4 mg L^{-1} and 0.3 mg L^{-1} for TOC and total N, respectively. This result highlights an issue of the technical limitations of these analyses at concentrations that display toxic effects.

The measurement of Brookfield viscosity was reliable at high concentrations but for the lowest concentrations the method lacked sensitivity. In the lowest concentration groups concentration-viscosity relationship was not observed, which is probably due to the fact that 1 cPs is the detection limit of the method. Consequently, the lowest four PAM concentrations all measured 1.28 cPs, which resulted in a sharp threshold in the viscosity-response curve. The method of Funnel viscosity was not sensitive even at the highest concentrations (Appendix B; Table B4) and, as a result, these data were not of use for this work.

The difficulties highlighted above with the reliability of the measured TOC and total N data for PAM meant that these data could not be used as metrics against which to assess the results from the toxicity tests. Consequently, the results and trigger values for PAM presented in this report are based only on the nominal concentrations used. However, it should be noted that the use of nominal concentrations is not the preferred approach as the concentrations of toxicant in the test system are assumed and not measured. The aforementioned difficult nature of the PAM might have resulted in errors in dilution of test solution, which introduces some uncertainty in the toxicity estimates and TVs for the PAM. Thus, these values should be only considered with these issues in mind. Ultimately, this situation is of no practical consequence for interpreting the toxicity test results since TOC and N concentration data were not needed to establish that PAM is the primary toxicant of the flocculant block (see Results and Discussion). Moreover, it is important to note that issues encountered with the chemical analyses of PAM where not encountered during the analysis the flocculant block product. Ultimately, PAM has not been deployed into the environment with out the PEG carrier and the TVs for the whole product are the most relevant.

3.1.3 Polyethylene glycol

All the toxicity tests were considered valid and control performances were at an acceptable level for all tests except for the *L. aequinoctialis* (919L, Table 4). However, as no signs of toxicity were observed up to the highest concentration tested, the test data were considered reliable.

Analysis of the blank, procedural blank and control (NMCW) chemistry samples showed that the correct amounts of nutrients were added to the *Chlorella* sp and *L. aequinoctialis* tests. Zinc concentrations of 4.6, 3.2, 5.2, 2.3 and 9.8 µg L^{-1} were also measured in the control NMCW of the *Chlorella* sp., *L. aequinoctialis*, *H. viridissima*, *M. macleayi* and *M. mogurnda* tests, respectively, which are higher than typical levels of Zn in NMCW (ie ~1 µg L^{-1}). Analysis of the procedural blanks suggest the majority of the Zn came from contamination of the plastic-ware used for the tests, which was probably due to the poor performance of the washing procedures in the laboratory at the time (see Appendix B). Procedural blank Zn concentrations were 2–3 µg L^{-1} compared with normal concentrations of <0.5 µg L^{-1}. However, these species showed no or very little reduction in growth rates following exposure to the test solution, which indicated that the measured levels of metals did not confound the test results. Control growth rates and physicochemical parameters were all within the specified ranges for all tests and these tests were considered valid.

Analysis of the highest concentration (ie 12 g L^{-1}) of PEG showed that there was significant levels of Al (96 ± 28 µg L^{-1}), Cr (22 ± 3 µg L^{-1}), Cu (2 ± 1 µg L^{-1}), Fe (205±39), Mn (2 ± 1 µg L^{-1}) and Zn (4 ± 0.6 µg L^{-1}). Thus, it is possible that the toxic effects observed following exposure to high concentrations of PEG may have been due in part to the contribution of co-occurring metals.

3.2 Toxicity of flocculant block formulation

While the term 'toxicity' has been used to describe the effects of the flocculant block formulation (40% PAM and 60% PEG), it should be noted that a significant contribution to the observed effects could be due to physical factors resulting from the viscosity of the dissolved flocculant block, rather than chemical toxicity per se. While it is not possible to quantify the relative proportions of effect due to physical and chemical factors, the results from the *M. macleayi* and *H. viridissima* tests provide some insights (see below).

The flocculant block formulation exhibited an extremely wide range of toxicity, from very toxic to the cladoceran, *M. macleayi* (IC_{50} = 10 mg L^{-1}, nominal concentration), to effectively non-toxic to the duckweed, *L. aequinoctialis* (IC_{50} >> 5000 mg L^{-1}, nominal concentration) (Figure 2 & Table 5). It was moderately toxic to *H. viridissima* (IC_{50} = 4250 mg L^{-1}, nominal concentration) and *Chlorella* sp (IC_{50} = 3690 mg L^{-1}, nominal concentration) and of low toxicity to *M. mogurnda* (IC_{50} = 6450 mg L^{-1}, nominal concentration). The order of sensitivity (from highest to lowest based on IC_{10} values) of the five species assessed was (noting that the *M. mogurnda* test is an acute survival test): *M. macleayi* >> *H. viridissima* >> *Chlorella* sp. > *M. mogurnda* > *L. aequinoctialis* (Figure 2 and Table 5). *M. macleayi* was by far the most sensitive organism and, in addition to the observed reproductive impairment, an inhibition of growth rate was evident. Furthermore, although 70% of the individuals exposed to 39 mg L^{-1} flocculant block survived the full duration of the test (ie 6 days), their average reproductive output was only 0.8 neonates per adult compared with 36.9 neonates per adult in the NMCW controls.

The flocculant block formulation could have affected organism response in two ways. Firstly, for the aquatic animals, the additional energy demands associated with swimming and feeding in the more viscous medium, especially at the upper end of the concentration range, could have contributed to the observed inhibitions of reproduction and growth. Secondly at the lower and less viscous concentrations of flocculant block, the effects observed for *M. macleayi* could be more attributable to direct chemical toxicity. Chemical analysis of the flocculant block formulation detected numerous metals, some of which where at concentrations that would be toxic to the organisms tested. Noteworthy, Al, Cr, Cu and Zn were detected at concentrations 3, 4, 12 and 5 times than the 95% default TVs, respectively, and mixtures of such metals would be likely to have at least an additive effect. However, the bioavailability of these metals was questionable as it was likely that at least some of the metals were strongly bound to the flocculant block matrix.

In addition to direct effects, the response of *M. macleayi* may have been partially a result of indirect effects via the food source. At the highest flocculant block concentrations tested, the algal feed did not disperse evenly, forming clumps and strings in the flocculant block solution. This effect possibly reduced the ability of *M. macleayi* to filter and ingest sufficient food. These observations, along with the fact that *M. macleayi* in the second highest concentration group showed delayed growth and reproduction, but did survive the testing period, suggests that either energy intake may have been reduced or extra energy expended for movement, or both.

Table 5 Summary of toxicity of flocculant block to the five tropical freshwater species tested

Species	Test ID	Measurement	Toxicity (mg per litre or cPs)[a]			
			$IC_{10\ (or\ 5)}$ (95%CL)	IC_{50} (95%CL)	NOEC	LOEC
Chlorella sp	866G	Nominal	960 (160-1690)	3690 (3110-4460)	310	630
		TOC	460 (60-810)	1880 (1540-2280)	160	300
		Total N	60 (10-100)	210	20	40
		Viscosity	30 (10-40)	90 (70-100)	12	20
L. aequinoctialis	865L	Nominal	>5000	>5000	>5000	5000
		TOC	>2590	>2590	>2590	2590
		Total N	120	>290	290	>290
		Viscosity	50	>120	120	>120
H. viridissima [b]	862B	Nominal	120 (60-330)	4250	<310	310
		TOC	60 (30-170)	2180	<160	160
		Total N	7 (5-20)	240	<20	20
		Viscosity	5 (3-10)	100	<10	10
	873B	Nominal	>1250	>1250	>1250	>1250
		TOC	>620	>620	>620	>620
		Total N	>130	>130	130	130
		Viscosity	>30	>30	30	>30
	877B	Nominal	160 (0-270)	1180	160	310
		TOC	80 (0-150)	610	80	170
		Total N	10 (0-30)	50	10	30
		Viscosity	8 (0-10)	30	7	10
M. macleayi	874D	Nominal	5 (5-6)	10 (5-15)	5	10
		TOC	4 (4-5)	10	4	7
		Total N	1 (0.5-1)	1.5 (1-2)	1	1.4
		Viscosity	1.5 (1.5-1.6)	2 (1.5-2.0)	1.5	2
M. mogurnda	864E	Nominal	780 (30-1510)[b]	6450 (4470-24800)	1250	2500
		TOC	350 (10-700)[b]	3440 (2310-13900)	600	1190
		Total N	150 (10-240) [b]	600 (440-2670)	130	290
		Viscosity	20 (0-40) [b]	>120	30	60

[a] Nominal values, TOC and Total N expressed at mg L^{-1}, while viscosity expressed as cPs.

[b] Three different feeding methods were used. See text for details

[c] Value reported for *M. mogurnda* represents the LC_{05} (ie concentration lethal to 5% of individuals relative to the controls).

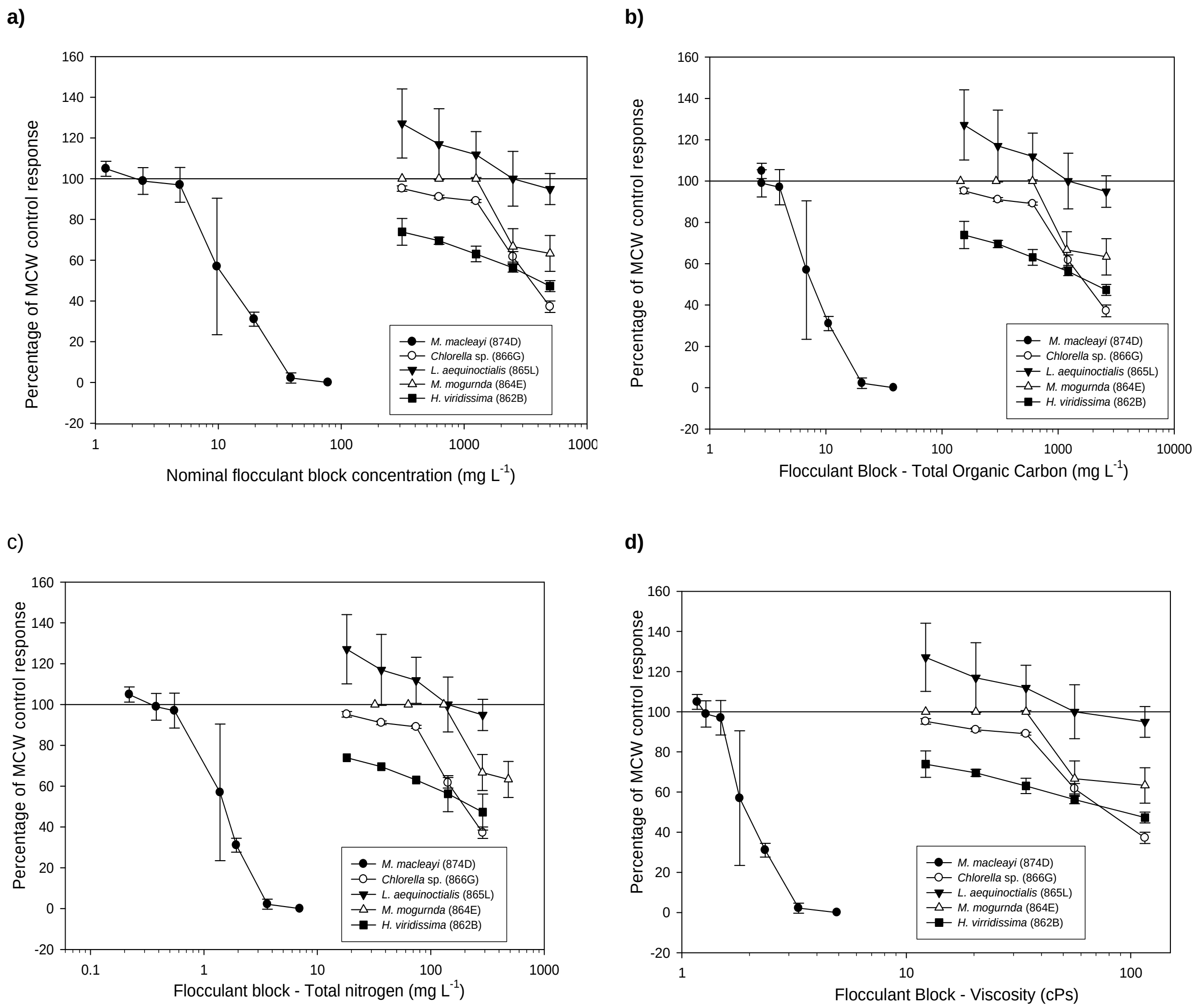


Figure 2 Effect of flocculant block on five local freshwater species. a) nominal concentrations b) measured TOC concentrations c) measured total nitrogen d) measured viscosity. The *H. viridissima* test shown corresponds to the first test conducted (862B). Data points represent the mean ± standard error (n = 3, except for *M. macleayi* where n = 10). Control responses are shown in Table 4.

Table 6 Key metals detected in flocculant block formulation

Metal	Al (µg/L)	Cr (µg/L)	Cu (µg/L)	Zn (µg/L)
Blank	8	<0.1	1	3
Flocculant block	168	3.6	17	42.5
95% Trigger values	55	1.0	1.4	8.0

Also noteworthy was the response of *H. viridissima* to different feeding regimes (Figure 3). The observed reduction in population growth was thought to be due to a feeding issue. Feeding of *Artemia* nauplii to *H. viridissima* is carried out once daily during the four day test period, and involves directly presenting each hydra with a standard amount (ie 3-4 individual specimens) of *Artemia*. In test 1 (862B), the hydra were fed using an equal amount of effort across all treatments (ie based on the standard protocol), such that, where hydra in higher flocculant block treatments were observed having difficulty capturing *Artemia*, no additional effort was used to facilitate the capture. The observed growth inhibition following flocculant block exposure in test 1 was originally attributed to an inability of the hydra to discharge its nematocysts in order to capture *Artemia*, or difficulties experienced by the test operator in presenting the *Artemia* to the hydra due to the viscous nature of the flocculant block, or a combination of both. Because a significant effect was observed at the lowest flocculant block treatment (313 mg L^{-1}), the test was repeated with lower treatment concentrations. This provided the opportunity to further investigate the hydra response.

In test 2, hydra were subjected to a different feeding regime, whereby they were fed until they had all received an approximately equal amount of food (ie where hydra were observed to have difficulty capturing *Artemia*, additional effort was used to facilitate the capture). This test demonstrated that the hydra exposed to flocculant block could in fact capture (and subsequently feed on) *Artemia* if sufficient direct contact occurred between *Artemia* and the hydras' tentacles. The 'facilitated feeding' method adopted in test 2 resulted in flocculant block having no adverse effect on *H. viridissima* population growth up to the highest test concentration of approximately 1250 mg L^{-1}. This indicated there was no direct toxic effect of flocculant block to *H. viridissima*. However, it was evident that the viscosity of the higher flocculant block concentrations (i) made it difficult for the hydras' tentacles to make contact with *Artemia*, and (ii) reduced the swimming ability of *Artemia*.

These observations led to test 3 (877B), which used a different feeding regime (which was evaluated prior to the toxicity test being conducted), whereby an approximately equal quantity of *Artemia* (4 *Artemia* per individual hydra) was dispensed into the test dish as one addition, and the test dish stirred to distribute the *Artemia* through the test dish. This 'passive' feeding regime enabled an assessment of the extent to which *H. viridissima* could encounter and capture its food source. The results of test 3 were similar to those of test 1, and confirmed that the effects of flocculant block on *H. viridissima* were due to its viscosity, which reduced the likelihood and ability of *H. viridissima* to encounter, and therefore capture, sufficient food (see Section 3.4 for a description of how the toxicity estimates were incorporated into the TV.

M. mogurnda, *L. aequinoctialis* and *Chlorella* sp were all very tolerant to flocculant block exposure. However, some interesting observations were made during these tests. For example, *M. mogurnda* survived high concentrations of the dissolved flocculant block but their movement was greatly inhibited and their operculum movement was increased (indicating a higher respiratory rate), although this could not be quantified. *L. aequinoctialis* growth rate increased by 27% in the presence of 155 mg L^{-1} flocculant block, which suggested nutrient availability may have been increased in this treatment group. *Chlorella* sp could not be counted with an electronic particle counter due to the viscosity of the solutions, even when diluted for counting. Therefore, microscope counts were required. The appearance of the algal cells' in the all the flocculant block treatment groups suggested that they were in a flocculated state (ie they were observed in aggregates) in the test. This observation was supported by the similar appearance of the algal food source at high flocculant block concentrations in the cladoceran test. Nevertheless, algal growth was not inhibited by high concentrations of the flocculant block, which suggests they were able to successfully absorb nutrients and grow in the viscous matrix.

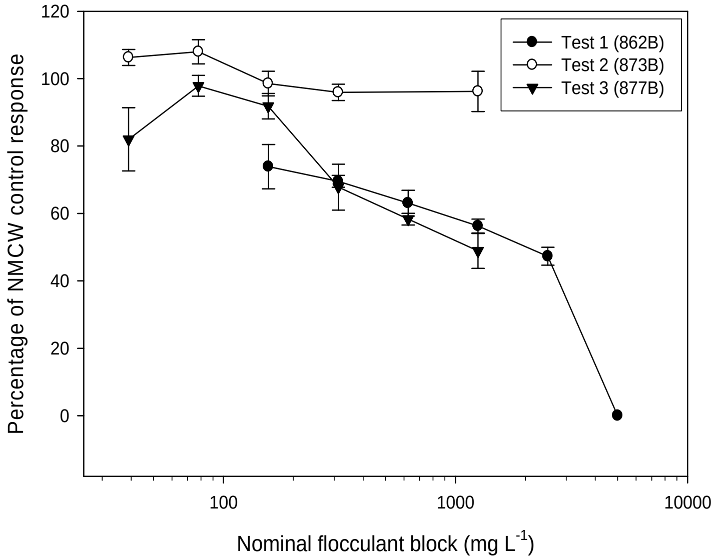


Figure 3 Effect of flocculant block on *H. viridissima* growth rates using three different feeding regimes. Data points represent the mean ± standard error (*n* = 3). Control responses are shown in table 4. Test 1 (862B) - Three to four *Artemia* nauplii presented directly to each hydra using an equal amount of effort across all treatment groups. Test 2 (873B) – Three to four *Artemia* nauplii presented directly to each hydra with additional effort provided to ensure all hydra captured the same amount of food. Test 3 (877B) – Four *Artemia* nauplii per hydra per day added to, and mixed through the test container in one addition.

3.3 Toxicity of PAM

Exposure to PAM alone elicited very similar responses to the flocculant block formulation (Table 7 & Figure 4). For example, *M. macleayi* showed a full toxic effect at 32 mg L^{-1} PAM and 78 mg L^{-1} flocculant block, both of which correspond to the same PAM concentration (ie the flocculant block contains 40% PAM). The reproductive, growth and survival responses were also similar to those observed for the flocculant block. The results strongly indicate that PAM was the primary toxicant in the flocculant block. As with the flocculant block, observations in the toxicity tests indicated that the flocculation of food contributed to the effects. However, metal analysis of the PAM showed that the metal content of the PAM solutions was very similar to the flocculant block (Table 8). This indicates that a direct chemical effect may have also been possible in treatments where viscosity was low.

The remaining species were an order of magnitude less sensitive to PAM than *M. macleayi* (Table 7 & Figure 4). *H. viridissima* exposed to concentrations of 500 mg L^{-1} showed an 82% reduction in population growth, while the growth rates of *Chlorella* sp and *L. aequinoctialis* were reduced by 88 and 96% following exposure to 2000 mg L^{-1} PAM (Figure 4). However, there were notable differences in the sensitivity of some species to PAM concentrations compared with the dilution of the flocculant block formulation containing equivalent PAM concentrations (based on a 40% assumed PAM content). For example, the growth rate of the duckweed, *L. aequinoctialis*, was almost completely inhibited at 2000 mg L^{-1} PAM but showed no response when exposed to 5000 mg L^{-1} flocculant block.

Conversely, the fish, *M. mogurnda*, was tolerant to the PAM displaying no significant effects following exposure to 2000 mg L^{-1} PAM. However, it is important to note that although the fish exposed to 2000 mg L^{-1} PAM survived the testing period their behaviour and condition was similar to that of surviving larvae at high flocculant block concentrations, in that they could not swim in the medium and appeared stressed (eg. rapid gill movement).

Table 7 Summary of toxicity of PAM to the five tropical freshwater species tested

Species	Test ID	Measurement	Toxicity (mg per litre)[a]			
			$IC_{10\ (or\ 5)}$ (95%CL)	IC_{50} (95%CL)	NOEC	LOEC
Chlorella sp	888G	Nominal	80 (20–190)	440 (280–580)	125	250
L. aequinoctialis	887L	Nominal	130 (0–400)	380 (50–580)	125	250
H. viridissima [b]	883B	Nominal	70 (0–100)	340 (300–390)	60	125
	892B	Nominal	20 (0–30)	>500	20	30
M. macleayi	884D	Nominal	1 (1–2)	6 (5–6)	1	2
M. mogurnda [a]	891E	Nominal	>2000	>2000	2000	>2000

[a] Value reported for *M. mogurnda* represents the LC_{05} (ie concentration lethal to 5% of individuals relative to the controls). [b] Two different feeding methods were used. See text for details.

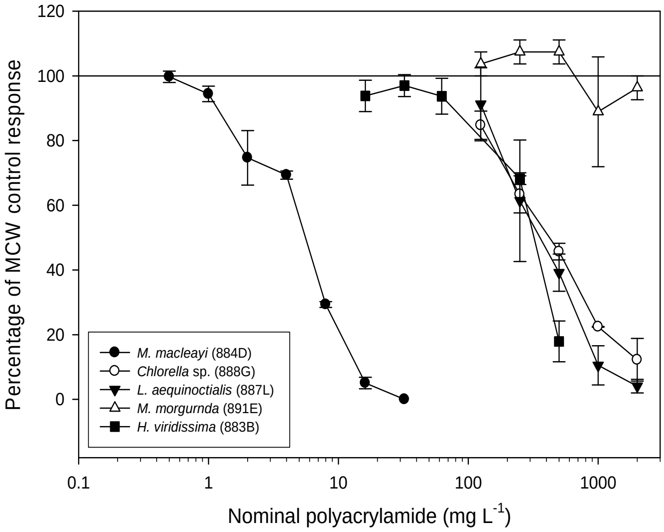


Figure 4 Effect of PAM on five local freshwater species. Results show nominal concentrations and data points represent the mean ± standard error (n = 3, except for *M. macleayi* where n = 10). Control responses are shown in Table 4.

Table 8 Metal analyses of flocculant block and polyacrylamide

Element	Al (µg/L)	Cd (µg/L)	Cu (µg/L)	Zn (µg/L)
Blank	8	0.06	1	3
Polyacrylamide	216	1.02	15.5	55.5
95% Trigger values	55	1.0	1.4	8.0

The results from the *H. viridissima* test show that the standard feeding method (Test 1) and the modified feeding method (Test 2; ie the passive feeding described in flocculant block section above), resulted in different concentration-responses (Figure 5). The modified feeding method resulted in a 20% effect at the second lowest treatment group of 63 mg L^{-1} but only a 42% reduction in the highest PAM treatment of 500 mg L^{-1}. The toxicity test using the standard feeding method showed no significant difference from the control in the 63 mg L^{-1} treatment but exposure to 500 mg L^{-1} resulted in a 82% reduction in hydra population growth rate. Consequently, there are significant differences in IC_{10} and IC_{50} between the two methods. It is unclear why the standard feeding method resulted in a higher reduction of growth at 500 mg L^{-1}. However, it should be noted that these values are based on nominal concentrations and TOC analysis of the test solutions indicated that PAM concentrations might have been higher in Test 1 than they should have been (Appendix C).

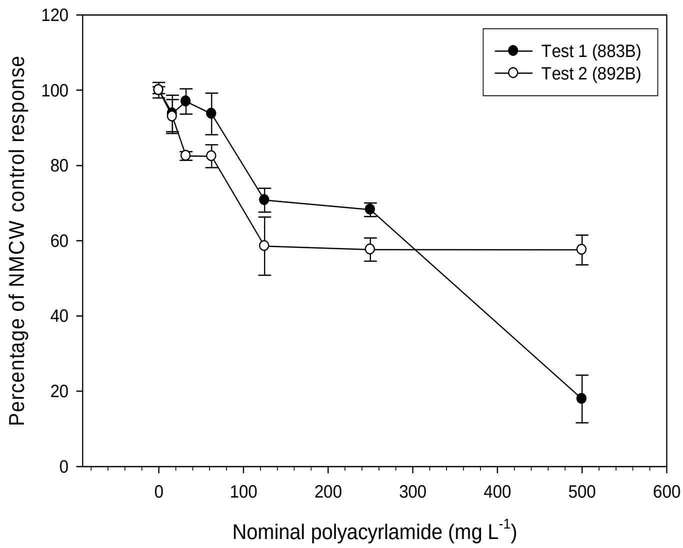


Figure 5 Effect of polyacrylamide on *H. viridissima* growth rates using two different feeding regimes. Data points represent the mean ± standard error (*n* = 3). Control responses are shown in table 4. Test 1 (883B) - Three to four *Artemia* nauplii presented directly to each hydra using an equal amount of effort across all treatment groups. Test 2 (892B) – Four *Artemia* nauplii per hydra per day added to, and mixed through the test container in one addition.

3.4 Toxicity of PEG

PEG was much less toxic compared with PAM and the flocculant block formulation (Figure 6 & Table 9). At high concentrations of up to 12 000 mg L^{-1} (nominal) the PEG produced a slightly ‘foamy’ solution, which is indicative of its surfactant-like properties (Wildish 1974). Both *Chlorella* sp and *L. aequinoctialis* were extremely tolerant of PEG, with concentrations ≤12 000 mg L^{-1} (nominal) having no effect on these species. Indeed, the duckweed’s growth rate was slightly higher following exposure to PEG, as it was for the flocculant block and PAM. *M. mogurnda* and *H. viridissima* exhibited partial inhibitory responses (~60% and 25% relative to the control response, respectively) following exposure to 12 000 mg L^{-1} PEG (nominal).

As was the case for the flocculant block and PAM, *M. macleayi* was the most sensitive species. Uniquely for this test, exposure to PEG resulted in a high, concentration-dependent mortality of offspring neonates (Figure 7). This response was not observed following exposure of *M. macleayi* to the flocculant block formulation or to PAM alone. Significant offspring mortality is rarely observed during chronic endpoint toxicity tests for *M. macleayi* and indicates that PEG may have directly affected the embryos in the brood sac.

Surprisingly, the usually tolerant fish species, *M. mogurnda*, was the second most sensitive species to PEG exposure, with an LC_{50} of 9330 mg L^{-1} and an LC_{05} of 1940 mg L^{-1}. The LC_{05} value is lower than the PAM LC_{05} of >2000 mg/L and suggests that, in the case of the fish, the primary toxicant in the flocculant block may have been PEG, and that a different mechanism of action is resulting in fish mortality following exposure to high concentrations of flocculant block.

Table 9 Summary of toxicity of polyethylene glycol (PEG), expressed as mg per litre (nominal and TOC), to the five tropical freshwater species tested

Species	Test ID	Measurement	Toxicity (mg per litre)			
			$IC_{10\ (or\ 5)}$ (95%CL)	IC_{50} (95%CL)	NOEC	LOEC
Chlorella sp.	913G	Nominal	>12000	>12000	12000	>12000
		TOC	>7000	>7000	7000	>7000
L. aequinoctialis	919L	Nominal	>12000	>12000	12000	>12000
		TOC	>7000	>7000	7000	>7000
H. viridissima	920B	Nominal	788 (512-2669)	>12000	750	1500
		TOC	470 (310-1640)	>7000	1750	3440
M. macleayi	927D	Nominal	790 (250-1170)	1990 (1420-2310)	750	1500
		TOC	470 (140-670)	1170 (850–1350)	450	890
M. mogurnda	923E	Nominal	1940 (0-3940)	9330 (5640-57950)	3000	6000
		TOC	1370 (780-1910)	5670 (4160–9410)	890	1750

[a] Value reported for *M. mogurnda* represents the LC_{05} (ie concentration lethal to 5% of individuals relative to the controls)

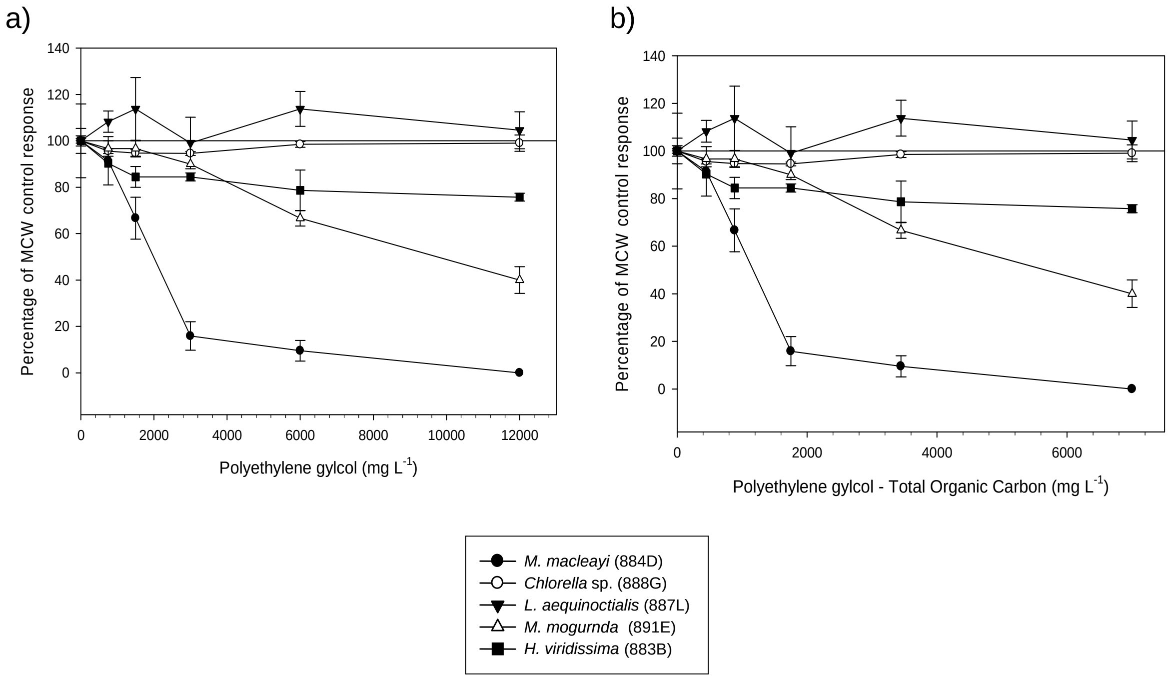


Figure 6 Effect of PEG on five local freshwater species. a) nominal concentrations and b) measured TOC concentrations. Data points represent the mean ± standard error (*n* = 3, except for *M. macleayi* where *n* = 10). Control responses are shown in table 4.

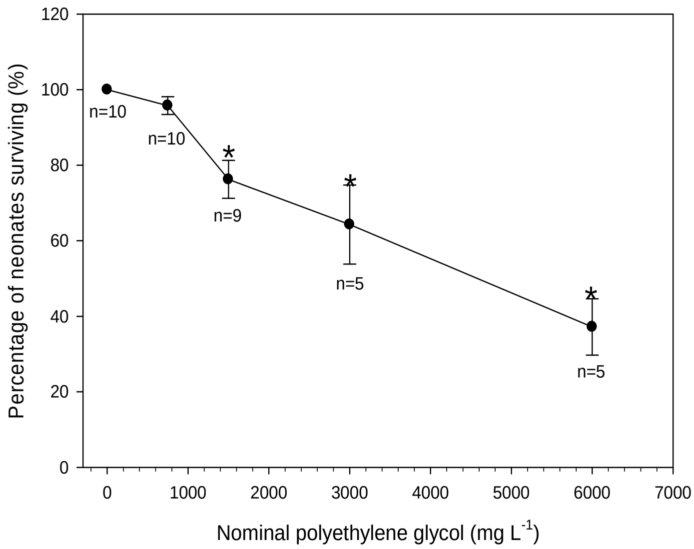


Figure 7 Effect of polyethylene glycol on neonate survival. Data points represent the mean ± standard error of *n* successful broods (as indicated on the chart) in the treatment group. Asterisks indicate that the group is significantly different from the control (P<0.05, Wilcoxon Rank Sum test)

3.5 Trigger values

The Species Sensitivity Distributions (SSDs) based on the flocculant block, PAM and PEG IC_{10}/LC_{05} values are shown in Figure 8. The associated TVs corresponding to the 80, 90, 95 and 99% species protection levels for the flocculant block, PAM (nominal concentrations only) and PEG (nominal and TOC concentrations) are presented in Table 10, respectively. Issues relating to the TVs and their application are discussed in the general discussion section of this report.

For the flocculant block and PAM toxicity tests using *H. viridissima*, the IC_{10} values from the standard feeding method and the passive feeding method were averaged because both these methods provided similar concentration-response relationships, and there was no justification to prefer one set of data over the other. Furthermore, neither method showed a tendency to produce more sensitive toxicity estimates. Compared with the standard feeding method the IC_{10} for the passive feeding method was higher for the flocculant block tests but lower for the PAM tests. Using the geometric mean of multiple IC_{10} values from the same endpoint is also consistent with the guidance provided in ANZECC/ARMCANZ (2000). However, no advice is provided when using data from slightly different methods. This is probably because all protocols are likely to differ to various degrees, eg. in the water quality used between laboratories. Thus, pooling the data in this case was considered valid.

Trigger values for PAM based on measured TOC and nitrogen are not shown due to the unreliable chemical analysis results, especially for the fish and cladoceran. Furthermore, the TV calculating for nominal PAM concentrations should be viewed with some caution, as there might have been errors in the dilution of the PAM treatments in the toxicity tests. It is also important to note that the model fits for the PEG SSDs were poorer than those for the flocculant block and PAM SSDs, due to two species showing no effect at the highest concentrations and the two most sensitive species having very similar toxicity estimates (Figure 8e & f). Consequently, the SSD for PEG would probably benefit from more definitive toxicity estimates for *Chlorella* sp and *L. aequinoctialis*. However, for this study this was deemed unnecessary as it is highly unlikely that the toxicity values for PEG represent environmentally-relevant concentrations.

The TVs are accompanied by very wide 95% confidence limits (CLs). This is primarily due to the low sample size of five, and the wide spread of toxicity values, which spans three orders of magnitude (ie from the very high sensitivity of *M. macleayi* to the very low sensitivity of *L. aequinoctialis*).

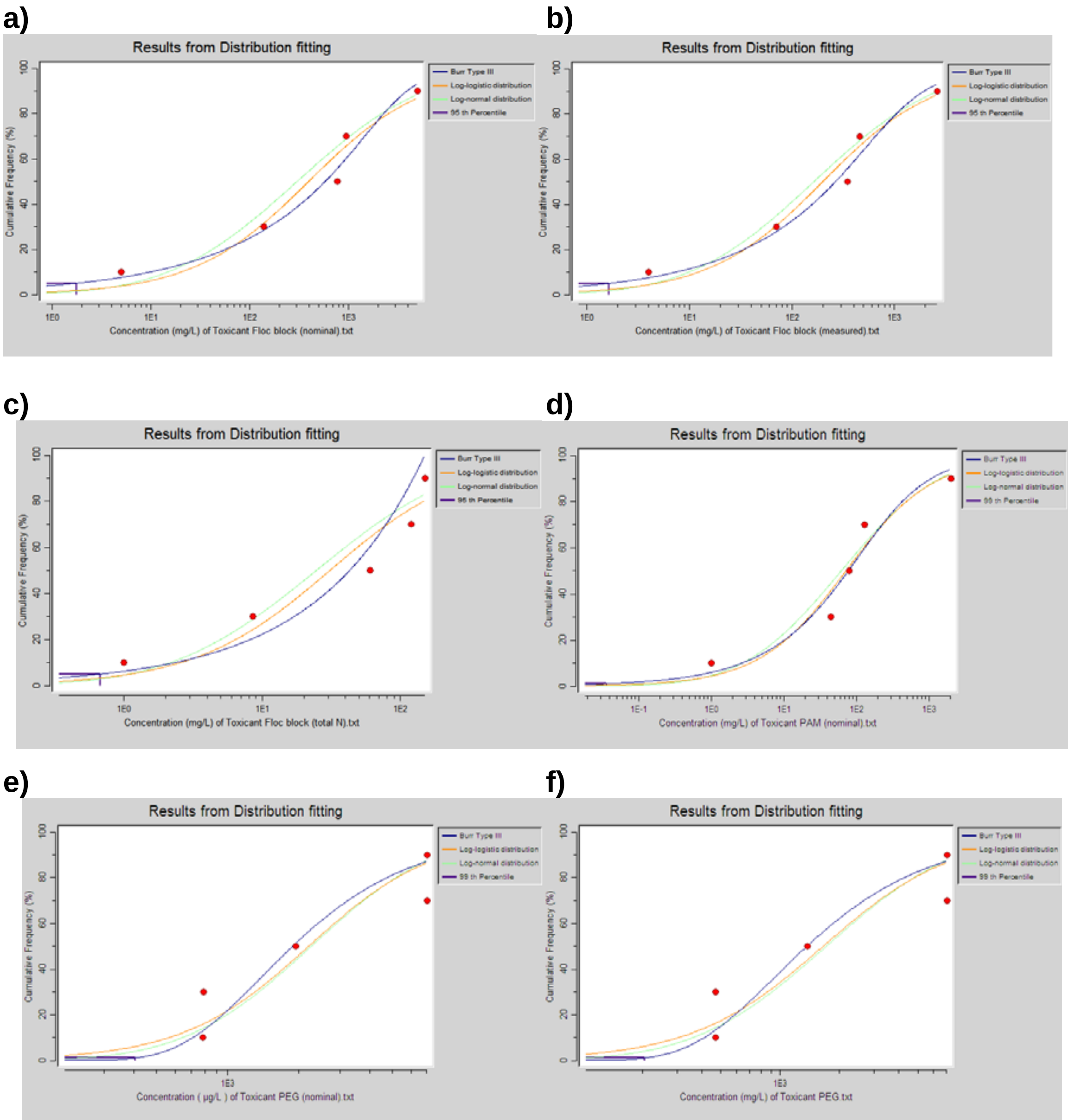


Figure 8 Species sensitivity distributions (from BurrliOZ©) for flocculant block, based on IC_{10}/LC_{05} values expressed as: a) flocculant block nominal concentration (mg L^{-1}); b) flocculant block total organic carbon (TOC) measured concentration (mg L^{-1}); c) flocculant block total nitrogen measured concentration (mg L^{-1}); d) polyacrylamide nominal concentration; e) polyethylene glycol nominal concentration (mg L^{-1}); and f) polyethylene glycol total organic carbon (TOC) measured concentration (mg L^{-1}).

Table 10 Calculated Trigger Values for the flocculant block and its two constituents, polyacrylamide and polyethylene glycol

Species protection level	Concentration (95% CL)		
	Nominal concentration (mg L^{-1})	Total organic carbon (mg L^{-1} Measured)	Total Nitrogen (mg L^{-1} Measured)
Flocculant Block			
99%	0.03 (0-400)	0.05 (0-180)	0.04 (0-20)
95%	2 (0-530)	2 (0-260)	1 (0-40)
90%	10 (0-670)	8 (0-310)	2 (0-60)
80%	60 (5-840)	30 (4-400)	8 (1-80)
Polyacrylamide (PAM)			
99%	0.4 (0-40)	N.A.[1]	N.A
95%	1 (0-50)	N.A.	N.A
90%	3 (0-70)	N.A.	N.A
80%	10 (0-110)	N.A.	N.A
Polyethylene glycol (PEG)			
99%	410 (280-1110)	210 (30-780)	N.A
95%	580 (430-1380)	320 (110-1270)	N.A
90%	710 (550-1690)	410 (200-1310)	N.A
80%	950 (720-2040)	590 (430-1670)	N.A

[1] Not applicable

3.6 General discussion

This study found that the sensitivity of the test organisms to the flocculant blocks varied considerably. It was also clear from the study that the active ingredient, PAM, was the primary toxicant to most species. The carrier, PEG, was relatively innocuous even at extremely high concentrations of grams per litre (Table 11). However, it should be noted that for *M. mogurnda* the IC_{10} for PEG (1940 mg L^{-1}) was marginally lower than the IC_{10} for PAM (>2000 mg L^{-1}), which suggests that PEG may be the primary fish toxicant in the flocculant block, albeit of low toxicity (Table 11). The low toxicity of PEG found in this study concurs with a limited number of studies that have used concentrations up to 5 g L^{-1} and that have rarely reported an adverse response in aquatic organisms (Wildish 1974, Bridie´ et al 1979, Chan et al 1981).

There are some notable differences between the results of the current study and the PAM toxicity data provided by the manufacturer. Of particular interest are the PAM toxicity estimates reported for the green alga, *Chlorella vulgaris*, and the zebrafish, *Brachydanio rerio*. The current study found an EC_{50} of 440 mg L^{-1} PAM for *Chlorella* sp., while the EC_{50} data provided by the manufacturer for *C. vulgaris* was >1000 mg L^{-1}. Unfortunately, no details of the *C. vulgaris* test are available and hence it is difficult to identify the reasons for the difference in observed toxicity. The difference is unlikely to be species-related as these two species are very similar phylogenetically. The toxicity tests in the present study were likely conducted at a higher temperature (ie 27°C compared to 25°C or lower for the other reported toxicity tests), which may account for the differences in the toxicity estimates reported. However, it might be expected that the higher temperatures would result in a less viscous solution and thus a lower toxicity, but this was not the case.

Table 11 Comparison of the polyacrylamide and polyethylene glycol toxicity

Species	Toxicity (mg per litre)			
	Flocculant Block IC/LC$_{50}$ (95%CL)	**PAM IC/LC$_{50}$ (95%CL)**	**PEG IC/LC$_{50}$ (95%CL)**	**Comment**
Chlorella sp	3690 (3110-4460)	440 (280-580)	>12000	PAM more toxic than PEG
L. aequinoctialis	>5000	380 (50-580)	>12000	PAM more toxic than PEG
H. viridissima [a]	4250	340 (300-390)	>12000	PAM more toxic than PEG
M. macleayi	10 (5-15)	6 (5-6)	1990 (1420-2310)	PAM more toxic than PEG
M. mogurnda [b]	6450 (4470-24800)	>2000	9330 (5640-57950)	PEG more toxic than PAM [c]

[a] Results for the standard protocol feeding method shown.

[b] LC$_{50}$ values shown for *M. mogurnda*

[c] Based on LC$_{05}$ values for >2000 and 1940 mg L^{-1} for PAM and PEG respectively.

The results of this study also found that PAM was markedly less toxic to the Australian tropical fish, *M. morgurnda* (LC$_{50}$ >2000 mg L^{-1}), compared with the northern hemisphere tropical fish, *B. rerio* (LC$_{50}$ 357 mg L^{-1}). However, *B. rerio* was tested using a non-renewal test design, compared to the daily renewals for *M. mogurnda* used here. It is unclear whether such a difference in test design could account for the difference in toxicity of PAM. Such a difference may be more attributable to inter-species differences in sensitivity.

M. macleayi was by far the most sensitive organism tested in this study. This finding concurs with the available literature, which reports that cladocerans are the most sensitive taxa to polyelectrolyte exposure, albeit from a limited number of species that have been used in toxicity test for these compounds. The results from this study are comparable with studies by Beim and Beim (1994) and Biesinger et al (1976), who reported 96-h LC$_{50}$s of 14 mg L^{-1} and 17 mg L^{-1} for *Daphnia magna*, respectively. The reasons for the observed effects on *M. macleayi* may be several-fold. Flocculation by the PAM of the cladocerans' food source may have made it more difficult for them to access food, while the higher viscosity of the test solutions may have increased the energy demands for locomotion and filter feeding, reducing the energy available for reproduction and growth. Moreover, at low concentrations of flocculant block and PAM, a chemical effect (cf. the above physical effects) may have contributed to the response. Metal analyses of However, it is important to note that the concentration of residual acrylamide monomer was not measured because it was likely to be present only in low concentrations and is not highly toxic to aquatic organisms (LC$_{50}$ ranges from ~100-200 mg L^{-1} for *D. magna* and fish (various species)).

The issue of a specific physical effect (as distinct from chemical toxicity) associated with each of the components individually and in a mixture (the flocculant block formulation) was investigated by plotting the response (neonates per adult) of the *M. macleayi* test against the measured viscosity for each of the three test solutions (Figure 9). The toxic effect of PEG was clearly unrelated to viscosity. High concentrations of PEG were only slightly more viscous than controls, but did appear 'soapy' suggesting surfactant properties. In contrast, the test response for the flocculant block and PAM displayed a dependence on both viscosity and concentration, albeit not to the same degree. The solutions containing PAM alone showed a greater response at lower viscosities compared to the flocculant block, which may indicate a chemical effect, in addition to viscosity, was contributing to toxicity (Figure 9). However, it is necessary to note that the measured viscosity of PAM solutions was not linear in the low concentration range of interest and at higher concentrations the PAM was more viscous than the flocculant block. (Figure 10). This may have been due to PAM's tendency to form non-homogenous solutions resulting in lower measurements because of areas of lower resistance in samples. However, these observations may also be related to reports that PAM solutions behave as psuedoplastic non-Newtonian fluids ie, viscosity decreases when increasing shear rates are applied (Autio & Houska 1991), although studies have reported that in the low concentration range of interest for the cladoceran study (ie >400 mg L-1) PAM has Newtonian fluid properties (Figure 9, Bjorneberg 1998). Moreover, the rheology of PEG-PAM mixtures could not be found in the literature and it is clear that the PEG 'carrier' significantly changes the PAM solution's properties. Finally, it should be noted that the reported accuracy of these measurements was ±1 cPs, with the total range of the measurements spanning only 1-5 cPs. Thus the indicated slight dependence on viscosity of PAM in Figure 9 is likely within the bounds of measurement precision, especially given the issues of solution heterogeneity noted above. Ultimately, the ability of the Brookfield viscosity measurement to inform the assessment of effects of viscosity is limited by its precision at the lower end of the viscosity scale.

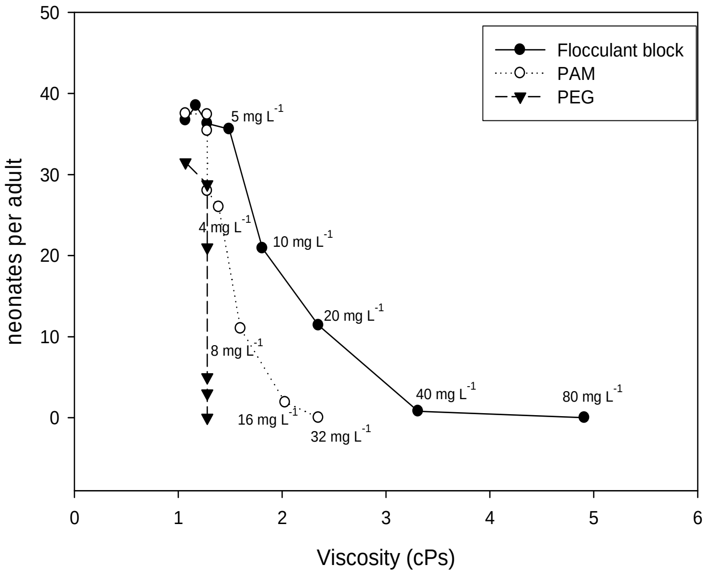


Figure 9 Response of *M. macleayi* reproduction to flocculant block, PAM and PEG, plotted against solution viscosity. The concentrations adjacent to the data points for flocculant block and PAM show the nominal concentration of the test solution measured.

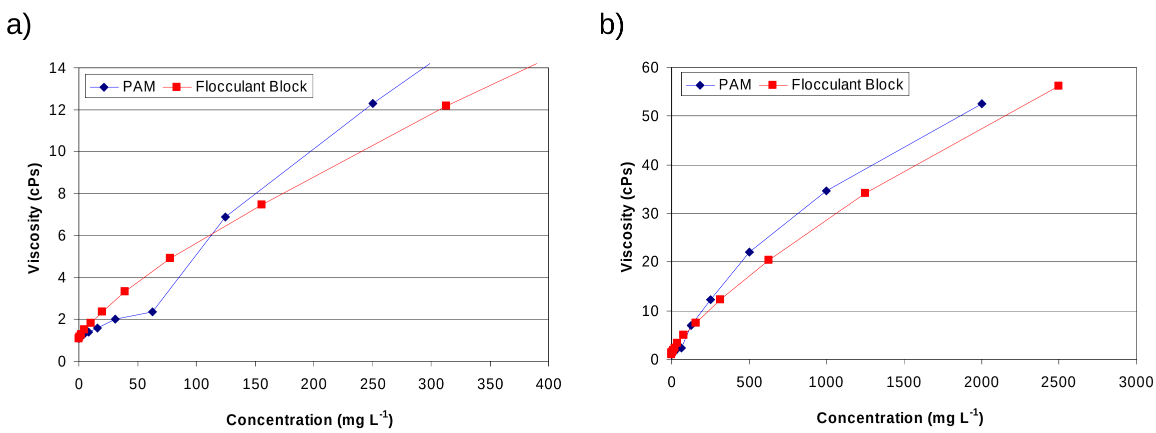


Figure 10 Relationship between nominal concentration and measured viscosity for flocculant block and PAM. a) low concentration range b) the full concentration range of test solutions

Although, it is reasonable to question the environmental relevance of the *M. macleayi* finding in light of the fact that the flocculants would also be bound to suspended sediment in the field and thereby removed from the water column. However, it is logical to conclude from the observations made during testing that these products also flocculate algae and organic particulates that are necessary for the growth of filter feeding organisms. Subsequently, any reduction in the availability of food would reduce the overall energy available to cladocerans and thus their reproductive output. Nonetheless, additional environmental factors will also influence the concentration and environmental fate of the flocculant blocks in the mine waters (eg flow rates).

It was unfortunate that the measured TOC and total N results were unreliable to the point that TVs based on these parameters could not be derived with confidence. The issues associated with the chemical analyses of the PAM were due to the extremely difficult properties of the PAM at the concentrations tested in this study. In the absence of the PEG carrier, the PAM solutions comprised a heterogeneous gel-like solution. The PAM appeared to settle-out with the gel sitting on the bottom of the test containers and was extremely difficult to pipette. Consequently, obtaining a representative sub-sample of the test solution was difficult. In addition, the viscosity of the PAM was a challenge for the analytical instruments. However, the test results clearly implicated PAM as the primary toxicant, which was the main aim of testing the PAM solution alone.

Finally, the limitations, and hence the appropriate use of the TVs derived in this study need to be emphasised. If a Magela Creek TV was required, the typically adopted level of species protection of 99% for this system would equate to a TV of 0.02 mg L^{-1} (as DOC), which is far below the background level of DOC in Magela Creek (ie ~1-5 mg L^{-1}). Inputs of flocculant block components at these levels could not therefore be detected by measuring TOC, the most practicable method for screening. It is possible that a method for specifically measuring these types of organics at such low concentrations could be developed but that option would need to be specifically investigated.

For management of waters on-site, a level of species protection of 99 or 95% would be inappropriate. In this case, a lower level of protection (say 90 or 80%) would be more fitting for water bodies already impacted from mining (ANZECC/ARMCANZ 2000). This would be especially the case for runoff from waste rock stockpiles wherein the objective is to reduce the loading of fine particulates as soon as possible after these waters enter sediment traps and/or polishing wetlands. The flocculant block products will readily bind to the suspended solids in the mine waters causing aggregation of the particles (the role of a flocculant) and sedimentation to the base of the pond, where they would most likely be retained on the mine site. The 90 and 80% TVs based on TOC (8 and 30 mg L^{-1}, respectively) are likely to be sufficiently above 'background' TOC in Ranger mine waters (1–3 mg L^{-1}; ERA, pers comm.) for practical quantification of flocculants in Ranger water bodies. Thus, it may be possible using analysis of TOC to detect the inputs of dissolved flocculant block components.

5 Conclusions

To date, flocculant blocks, and specifically their anionic polymer ingredients, have been considered relatively non-toxic. Consequently, limited studies have focused on the environmental impacts of these products. Indeed, compared with their cationic counterparts, previous toxicity test work has indicated that anionic polymers are much less toxic.

However, the present study has demonstrated that sensitivity of different freshwater species to anionic polymers can vary considerably. More importantly, the value of measuring chronic sub-lethal endpoints has been demonstrated through the low toxicity estimate derived from the 6 day cladoceran reproduction test. Measurement of the low viscosity and high metal concentrations in the test solutions indicate that some of the effects of PAM may have been due to direct toxic effects rather than indirect effects due to viscosity. Nevertheless, this issue should not greatly alter how the results of the study are applied because the mechanism of action is secondary to the importance of the toxicity estimates derived.

A key challenge with respect to the application of the water quality guideline values derived from this study is the ability of a standard water quality monitoring program to measure and detect concentrations of dissolved flocculant block components in waters downstream of where such products have been deployed. Depending on the agreed level of species protection, there may be substantial practical difficulty in using TOC as a surrogate to monitor levels of flocculant block components in mine waters due to relatively high background concentrations of TOC. The measurement of nitrogen as a surrogate for PAM is also unlikely to be practical, as the proportion of nitrogen in the flocculant block is even lower than the carbon content of the product. Furthermore, nitrogen levels in water bodies on the Ranger mine site may also be elevated due to inputs from explosives residues.

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Appendix A Physicochemical data for the toxicity tests

Appendix A1 Physicochemical data for the flocculant block toxicity tests

Chlorella sp test (866G)

		Flocculant Block Concentration (mg L^{-1})											
		0		313		625		1250		2500		5000	
Day	Parameter	0 h	72 h	0 h	72 h	0 h	72 h	0 h	72 h	0 h	72 h	0 h	72 h
0	pH	6.72	7.91	6.62	8.65	6.49	8.77	6.50	8.65	6.38	7.91	6.42	7.07
	Conductivity[1]	23	47	28	46	34	51	47	63	71	85	123	141
	DO[2]	97	99	98	100	100	97	102	90	102	82	84	87

[1] Conductivity units are in µS cm^{-1}

[2] DO: Dissolved oxygen. Measurements are expressed as percent saturation

Lemna aequinoctialis test (865L)

		Flocculant Block Concentration (mg L^{-1})											
		0		313		625		1250		2500		5000	
Day	Parameter	0 h	96 h	0 h	96 h	0 h	96 h	0 h	96 h	0 h	96 h	0 h	96 h
0	pH	6.72	7.20	6.25	7.30	6.49	7.27	6.50	7.21	6.38	6.73	6.42	6.68
	Conductivity[1]	23	19	28	23	34	31	47	43	71	77	123	120
	DO[2]	98	94	98	85	100	84	102	74	102	69	84	78

[1] Conductivity units are in µS cm^{-1}

[2] DO: Dissolved oxygen. Measurements are expressed as percent saturation

Hydra viridissima test (862B)

		Flocculant Block Concentration (mg L^{-1})											
		0		313		625		1250		2500		5000	
Day	Parameter	0 h	24h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h
0	pH	6.66	6.79	6.61	6.82	6.52	6.79	6.46	6.79	6.45	6.67	6.37	6.60
	Conductivity[1]	16	30	21	25	27	42	40	44	65	71	115	127
	DO[2]	106	94	96.6	93	103	82	101	86	95	74	85	81
1	pH	6.50	6.61	6.58	6.60	6.53	6.66	6.45	6.63	6.41	6.61	6.40	6.59
	Conductivity	16	17	21	22	27	30	40	42	66	66	114	118
	DO	108	93	92	86	93	83	85	69	95	82	77	81
2	pH	6.44	6.70	6.49	6.72	6.51	6.69	6.56	6.70	6.51	6.67	6.46	6.60
	Conductivity	16	17	21	22	27	29	40	43	65	66	115	119
	DO	106	92	103	89	97	78	97	70	97	82	80	75
3	pH	6.46	6.60	6.75	6.80	6.55	6.89	6.61	6.93	6.51	6.91	6.47	6.87
	Conductivity	16	17	21	22	27	28	39	42	64	68	115	118
	DO	106	93	101	90	106	85	90	72	97	83	93	90

[1] Conductivity units are in µS cm^{-1}

[2] DO: Dissolved oxygen. Measurements are expressed as percent saturation

Hydra viridissima test (873B)

		Flocculant Block Concentration (mg L^{-1})											
		0		39		78		156		313		1250	
Day	Parameter	0 h	24h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h
0	pH	6.66	6.69	6.80	6.70	6.68	6.71	6.68	6.70	6.64	9.79*	6.63	6.79
	Conductivity[1]	16	19	17	19	19	21	21	22	26	26	57	50
	DO[2]	106	97	104	97	106	94	105	91	109	91	82	84
1	pH	6.44	6.63	6.43	6.71	6.48	6.65	6.51	6.72	6.53	6.76	6.44	6.79
	Conductivity	19	19	18	20	19	20	21	22	25	27	56	58
	DO	113	96	115	92	106	92	111	87	110	86	104	85
2	pH	6.55	6.73	6.52	6.76	6.49	6.78	6.54	6.79	6.58	6.85	6.56	6.91
	Conductivity	17	20	17	21	19	21	21	23	25	28	56	60
	DO	118	93	115	94	117	93	114	95	117	93	112	79
3	pH	6.46	6.70	6.56	6.74	6.58	6.75	6.54	6.83	6.64	6.88	6.55	6.95
	Conductivity	17	18	18	19	19	20	21	21	26	26	56	58
	DO	115	100	118	98	117	96	109	96	108	95	92	84

* indicates likely erroneous reading

[1] Conductivity units are in µS cm^{-1}.

[2] DO: Dissolved oxygen. Measurements are expressed as percent saturation

Hydra viridissima test (877B)

		Flocculant Block Concentration (mg L^{-1})													
		0		39		78		156		313		625		1250	
Day	Parameter	0 h	24h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h
0	pH	6.54	6.68	6.59	6.76	6.56	6.75	6.60	6.79	6.53	6.78	6.58	6.80	6.58	6.75
	Conductivity[1]	18	20	19	21	20	21	21	23	25	26	32	32	47	49
	DO[2]	105	92	98	93	99	90	100	87	106	85	96	80	99	73
1	pH	6.61	6.75	6.65	6.80	6.61	6.79	6.63	6.82	6.54	6.83	6.58	6.83	6.60	6.94
	Conductivity	18	20	22	20	20	21	21	23	25	27	33	33	47	49
	DO	113	93	106	91	109	95	103	86	106	82	99	75	92	71
2	pH	6.57	6.75	6.72	6.77	6.64	6.77	6.66	6.82	6.63	6.84	6.68	6.86	6.70	6.93
	Conductivity	18	20	19	20	20	21	21	23	25	27	32	33	47	49
	DO	113	93	109	91	106	90	110	90	114	85	102	81	103	78
3	pH	6.54	6.70	6.60	6.78	6.63	6.81	6.65	6.85	6.61	6.85	6.59	6.90	6.59	6.90
	Conductivity	20	22	19	20	20	20	21	21	25	26	31	32	45	48
	DO	112	91	105	93	105	92	112	91	110	89	97	76	105	77

[1] Conductivity units are in µS cm^{-1}

[2] DO: Dissolved oxygen. Measurements are expressed as percent saturation

Moinodaphnia macleayi **(863D)**

		Flocculant Block Concentration (mg L^{-1})															
		0		39		78		156		313		625		1250		2500	
Day	Parameter	0 h	24h	0 h	24h	0 h	24h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h
0	pH	5.39*	6.94	6.56	7.19	6.57	7.88	6.62	8.58	6.62	8.57	6.60	8.26	6.60	7.71	6.52	7.30
	Conductivity[1]	17	20	17	19	18	20	19	22	22	24	30	32	43	46	73	77
	DO[2]	106	97	103	99	107	96	110	100	103	95	99	91	104	87	100	85
1	pH	6.55	6.80	6.57	7.11	6.59	7.03	6.61	7.09	6.52	7.14	6.59	7.11	6.54	7.03	6.49	6.85
	Conductivity	19	19	18	19	18	21	20	21	23	24	30	32	45	45	75	76
	DO	111	93	115	96	111	92	116	91	108	94	115	80	108	81	98	85
2	pH	6.50	7.04	6.55	7.09	6.55	8.05	6.58	8.49	6.55	8.66						
	Conductivity	21	19	17	20	17	20	20	22	23	25	NA	NA	NA	NA	NA	NA
	DO	114	101	114	98	116	108	116	106	113	98						
3	pH	6.59	6.90	6.68	7.15	6.69	7.10										
	Conductivity	17	20	18	20	18	20	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	DO	108	100	105	102	112	103										
4	pH	6.57	6.85	6.65	6.99	6.65	7.00										
	Conductivity	20	20	18	21	18	21	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	DO	111	96	115	97	114	98										

* indicates likely erroneous reading

[1] Conductivity units are in $\mu S\ cm^{-1}$.

[2] DO: Dissolved oxygen. Measurements are expressed as percent saturation.

N/A = Not analysed due to 100% mortality in treatment

Moinodaphnia macleayi (874D)

		Flocculant Block Concentration (mg L^{-1})															
		0		1.2		2.4		5		10		20		39		78	
Day	Parameter	0 h	24h	0 h	24h	0 h	24h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h
0	pH	6.54	6.93	6.62	6.83	6.66	6.92	6.67	6.93	6.69	7.95	6.73	7.76	6.68	7.83	6.68	7.95
	Conductivity[1]	17	20	17	20	17	20	17	20	17	20	17	20	18	21	17	21
	DO[2]	109	94	107	100	104	99	107	98	109	103	112	100	110	103	105	102
1	pH	6.63	6.90	6.58	7.01	6.59	7.01	6.63	7.06	6.68	6.96	6.73	7.09	6.63	7.04	6.80	7.00
	Conductivity	24	20	19	20	17	20	17	21	17	20	18	20	18	21	19	21
	DO	111	98	104	98	115	90	110	98	112	92	112	97	109	95	106	95
2	pH	6.78	6.81	6.72	6.88	6.67	6.96	6.72	7.01	6.61	6.89	6.65	7.25	6.65	7.16	6.62	7.26
	Conductivity	17	20	17	20	16	20	17	20	17	20	18	20	18	20	19	22
	DO	109	97	108	97	108	96	111	97	108	98	107	95	110	101	103	98
3	pH	6.51	6.92	6.58	7.48	6.51	7.31	6.54	7.42	6.64	7.25	6.68	7.62	6.68	8.32		
	Conductivity	17	19	17	20	18	20	17	19	17	20	17	20	18	21	NA	NA
	DO	108	97	114	101	108	99	115	101	116	101	109	101	109	103		
4	pH	6.62	7.00	6.70	7.45	6.70	7.34	6.72	7.37	6.73	7.62	6.72	8.29	6.72	8.29		
	Conductivity	17	19	17	19	17	19	17	19	17	19	17	20	18	20	NA	NA
	DO	106	94	111	94	109	95	113	97	111	98	112	102	111	104		

[1] Conductivity units are in $\mu S\ cm^{-1}$

[2] DO: Dissolved oxygen. Measurements are expressed as percent saturation

N/A = Not analysed due to 100% mortality in treatment

Mogurnda mogurnda **test (864E)**

		Flocculant Block Concentration (mg L^{-1})											
		0		313		625		1250		2500		5000	
Day	Parameter	0 h	24h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h
0	pH	6.57	6.80	6.56	6.73	6.46	6.72	6.56	6.71	6.37	6.59	6.39	6.46
	Conductivity[1]	16	20	25	28	34	37	56	59	97	98	169	163
	DO[2]	103	92	106	84	95	79	90	82	95	75	78	73
1	pH	6.57	6.59	6.68	6.64	6.62	6.66	6.55	6.60	6.50	6.52	6.28	6.29
	Conductivity	15	16	24	25	35	36	55	58	96	98	169	177
	DO	100	101	104	104	91	78	92	83	96	80	93	77
2	pH	6.59	6.79	6.75	6.79	6.74	6.79	6.74	6.71	6.66	6.59	6.65	6.45
	Conductivity	16	17	27	28	38	39	58	60	99	100	178	180
	DO	94	92	85	85	78	82	83	79	80	81	76	87
3	pH	6.55	6.74	6.69	6.73	6.78	6.78	6.62	6.73	6.46	6.74	6.36	6.59
	Conductivity	16	20	25	29	35	40	54	57	90	99	177	181
	DO	115	88	117	82	115	84	114	71	113	77	105	80

[1] Conductivity units are in $\mu S\ cm^{-1}$

[2] DO: Dissolved oxygen. Measurements are expressed as percent saturation

N/A = Not analysed due to 100% mortality in treatment

Appendix A2 Physicochemical data for the PAM toxicity tests

Hydra viridissima test (883B)

		PAM (mg L^{-1})													
		0		16		32		63		125		250		500	
Day	Parameter	0 h	24h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h
0	pH	6.65	6.75	6.69	6.85	6.62	6.90	6.65	7.00	6.80	7.02	6.86	7.14	7.39	7.27
	Conductivity[1]	21	21	28	23	24	25	26	32	28	36	30	70	79	95
	DO[2]	104	91	103	91	106	91	107	86	106	87	101	80	101	74
1	pH	6.61	6.55	6.65	6.70	6.72	6.73	6.80	6.80	6.90	6.91	6.93	7.01	7.40	7.28
	Conductivity	20	22	21	24	23	25	26	29	42	35	31	53	143	152
	DO	114	94	116	92	114	92	105	93	99	83	99	89	89	80
2	pH	6.90	6.61	6.84	9.76	6.82	6.78	6.83	6.81	7.09	7.00	6.92	6.88	7.63	7.31
	Conductivity	21	23	22	24	23	26	26	29	43	50	32	38	169	174
	DO	114	94	109	93	113	93	110	91	93	77	104	89	92	83
3	pH	6.81	6.86	6.81	6.93	6.76	6.96	6.80	7.02	6.99	7.23	6.83	7.10	7.53	7.39
	Conductivity	20	21	21	23	23	24	26	27	39	42	32	33	112	152
	DO	119	94	123	91	117	92	118	87	104	82	118	89	93	84

[1] Conductivity units are in µS cm^{-1}

[2] DO: Dissolved oxygen. Measurements are expressed as percent saturation

Hydra viridissima test (892B)

		PAM (mg L^{-1})													
		0		16		32		63		125		250		500	
Day	Parameter	0 h	24h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h
0	pH	6.55	6.58	6.65	6.75	6.69	6.85	6.69	6.96	6.93	7.04	6.88	7.31	7.51	7.45
	Conductivity[1]	20	22	22	23	24	25	25	33	32	33	30	83	106	118
	DO[2]	111	93	106	92	116	87	114	80	90	79	85	73	79	79
1	pH	6.39	6.81	6.59	6.89	6.64	6.89	6.68	6.90	6.91	7.15	7.05	7.32	7.32	7.23
	Conductivity	21	21	22	23	24	25	24	26	36	42	41	76	84	110
	DO	115	95	111	91	111	96	118	89	98	73	101	77	87	75
2	pH	6.66	6.82	6.70	6.89	6.75	6.95	6.78	6.98	7.01	7.18	7.13	7.24	7.32	7.28
	Conductivity	20	22	22	23	24	25	24	26	37	40	49	58	84	96
	DO	115	92	112	92	116	95	114	88	105	82	110	81	91	78
3	pH	6.67	6.83	6.69	6.92	6.72	6.99	6.73	7.04	7.01	7.26	7.07	7.29	7.32	7.39
	Conductivity	20	21	22	22	24	25	25	25	38	39	47	51	94	100
	DO	113	93	107	92	115	92	119	93	94	79	101	86	98	72

[1] Conductivity units are in $\mu S\ cm^{-1}$

[2] DO: Dissolved oxygen. Measurements are expressed as percent saturation

Moinodaphnia macleayi (884D)

		PAM (mg L^{-1})															
		0		0.5		1		2		4		8		16		32	
Day	Parameter	0 h	24h	0 h	24h	0 h	24h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h
0	pH	6.53	8.60	6.60	7.80	6.66	8.10	6.78	7.96	6.73	7.57	6.72	7.85	6.73	7.94	6.78	8.72
	Conductivity[1]	22	24	19	21	19	22	21	22	20	24	20	23	21	25	23	25
	DO[2]	105	100	114	99	107	103	106	100	103	101	111	101	106	100	94	101
1	pH	6.63	7.60	6.67	8.41	6.65	8.39	6.72	7.61	6.70	8.06	6.70	8.73	6.71	9.25*	6.77	8.79
	Conductivity	21	23	19	22	20	22	20	22	20	23	20	24	21	26	23	25
	DO	110	100	115	102	112	102	108	99	112	101	116	104	109	109	101	104
2	pH	6.51	7.19	6.49	7.36	6.56	7.25	6.64	7.17	6.61	7.35	6.61	8.23	6.62	8.18	6.62	8.11
	Conductivity	24	23	20	22	20	22	20	23	20	22	21	23	22	23	23	25
	DO	108	99	112	100	111	98	110	98	114	101	110	103	107	104	105	101
3	pH	6.52	7.75	6.58	7.33	6.55	7.34	6.65	7.56	6.60	7.91	6.58	8.28	6.60	8.37	6.68	
	Conductivity	23	23	20	22	20	22	20	23	20	22	20	23	21	24	23	NA
	DO	113	95	113	93	112	94	115	97	114	98	103	99	111	100	105	
4	pH	6.53	8.72	6.57	7.70	6.60	7.59	6.61	7.56	6.62	7.76	6.62	7.73	6.64	8.06		
	Conductivity	20	33	20	23	19	23	20	23	20	23	20	23	21	24	NA	NA
	DO	116	92	117	94	111	93	112	96	112	96	113	94	110	98		

* Indicates likely erroneous reading

[1] Conductivity units are in µS/cm

[2] DO: Dissolved oxygen. Measurements are expressed as percent saturation

N/A = Not analysed due to 100% mortality in treatment

Lemna aequinoctialis test (887L)

		PAM (mg L^{-1})											
		0		125		250		500		1000		2000	
Day	Parameter	0 h	96 h	0 h	96 h	0 h	96 h	0 h	96 h	0 h	96 h	0 h	96 h
0	pH	6.73	7.13	7.03	7.22	7.26	7.24	7.39	7.35	7.60	7.74	7.62	7.75
	Conductivity[1]	31	23	44	45	75	71	101	111	268	272	350	388
	DO[2]	106	93	78	84	94	86	97	78	74	81	84	74

[1] Conductivity units are in μS cm^{-1}.

[2] DO: Dissolved oxygen. Measurements are expressed as percent saturation.

Chlorella sp test (888G)

		PAM (mg L^{-1})											
		0		125		250		500		1000		2000	
Day	Parameter	0 h	72 h	0 h	72 h	0 h	72 h	0 h	72 h	0 h	72 h	0 h	72 h
0	pH	6.37	6.65	6.86	6.63	6.94	6.84	6.85	7.07	6.99	7.18	7.13	7.20
	Conductivity[1]	56	48	142	61	202	104	237	205	303	308	386	382
	DO[2]	100	95	97	85	88	74	98	70	88	71	93	75

[1] Conductivity units are in μS cm^{-1}

[2] DO: Dissolved oxygen. Measurements are expressed as percent saturation

***Mogurnda mogurnda* test (891E)**

		PAM (mg L^{-1})											
		0		125		250		500		1000		2000	
Day	Parameter	0 h	24h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h
0	pH	6.67	6.86	6.83	6.80	6.74	6.97	6.73	7.05	6.83	7.48	7.62	7.49
	Conductivity[1]	22	27	24	28	24	29	25	34	31	138	121*	360
	DO[2]	101	93	104	84	114	89	107	91	108	82	78	84
1	pH	6.62	6.77	6.75	6.90	6.70	7.13	6.70	7.34	7.81	7.43	7.64	7.53
	Conductivity	22	24	24	27	26	50	27	77	164	238	354	376
	DO	119	93	115	92	118	87	120	81	90	72	86	67*
2	pH	6.58	6.83	6.72	6.97	6.73	7.18	6.79	7.38	7.31	7.42	7.65	7.58
	Conductivity	22	24	25	32	27	52	29	53	163	251	362	361
	DO	116	95	113	93	117	90	114	83	88	81	87	73
3	pH	6.58	6.88	7.09	7.11	7.37	7.16	7.57	7.28	7.49	7.41	7.57	7.50
	Conductivity	22	25	45	49	74	75	140	132	217	201	329	306
	DO	118	99	95	86	109	89	96	77	94	87	72	85

*Indicates likely erroneous reading

[1] Conductivity units are in µS/cm

[2] DO: Dissolved oxygen. Measurements are expressed as percent saturation

N/A = Not analysed due to 100% mortality in treatment

Appendix A3 Physicochemical data for the PEG toxicity tests

Chlorella sp test (913G)

		PEG (mg L^{-1})											
		0		750		1500		3000		6000		12000	
Day	Parameter	0 h	72 h	0 h	72 h	0 h	72 h	0 h	72 h	0 h	72 h	0 h	72 h
0	pH	6.07	6.40	6.09	6.42	6.08	6.43	6.11	6.47	6.13	6.58	6.13	6.72
	Conductivity[1]	43	37	41	39	42	39	42	39	44	40	49	42
	DO[2]	97	96	102	92	106	96	96	94	98	93	102	92

[1] Conductivity units are in µS cm^{-1}

[2] DO: Dissolved oxygen. Measurements are expressed as percent saturation

Lemna aequinoctialis test (864E)

		PEG (mg L^{-1})											
		0		750		1500		3000		6000		12000	
Day	Parameter	0 h	96 h	0 h	96 h	0 h	96 h	0 h	96 h	0 h	96 h	0 h	96 h
0	pH	6.74	6.60	6.41	6.67	6.49	6.74	6.40	6.77	6.38	6.84	6.35	7.04
	Conductivity[1]	19	24	19	17	21	16	21	19	23	19	27	24
	DO[2]	94	89	99	88	97	89	92	86	96	87	95	87

[1] Conductivity units are in µS cm^{-1}

Hydra viridissima test (920B)

		PEG (mg L^{-1})											
		0		750		1500		3000		6000		12000	
Day	Parameter	0 h	24h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h
0	pH	6.27	6.43	5.93	6.46	6.29	6.53	6.36	6.59	6.37	6.67	6.41	6.86
	Conductivity[1]	15	11	17	12	17	13	16	15	16	17	20	20
	DO[2]	103	85	105	89	107	89	103	89	109	89	114	85
1	pH	6.11	6.55	6.18	6.56	6.24	6.60	6.31	6.66	6.31	6.73	6.37	6.89
	Conductivity	12	12	11	12	12	14	14	16	16	17	20	21
	DO	105	90	101	91	105	91	108	89	107	88	112	85
2	pH	6.35	6.55	6.38	6.55	6.35	6.66	6.38	6.63	6.47	6.80	6.53	6.93
	Conductivity	11	12	10	12	12	14	14	16	16	18	19	21
	DO	119	92	118	91	112	92	115	91	113	91	116	89
3	pH	6.40	6.51	6.32	6.58	6.44	6.63	6.42	6.84	6.53	6.92	6.58	7.08
	Conductivity	10	11	11	11	12	12	14	15	16	17	19	20
	DO	118	96	117	96	115	94	115	94	119	95	118	95

[1] Conductivity units are in µS cm^{-1}

[2] DO: Dissolved oxygen. Measurements are expressed as percent saturation

***Moinodaphnia macleayi* (922D)**

		PEG (mg L^{-1})											
		0		750		1500		3000		6000		12000	
Day	Parameter	0 h	24h	0 h	24h	0 h	24h	0 h	24 h	0 h	24 h	0 h	24 h
0	pH	6.80	6.85	6.86	6.91	6.90	7.05	6.90	7.05	6.89	7.03	6.90	7.12
	Conductivity[1]	20	17	19	18	19	17	21	20	23	23	28	26
	DO[2]	95	88	93	91	94	94	96	92	99	90	107	92
1	pH	6.80	6.80	6.83	6.90	6.85	6.88	6.86	6.92	6.84	7.00	6.83	7.12
	Conductivity	18	18	16	18	17	19	18	20	21	23	25	27
	DO	102	91	105	91	102	92	102	89	104	93	101	94
2	pH	6.75	6.78	6.75	6.85	6.76	6.87	6.79	6.91	6.77	7.00	6.79	7.08
	Conductivity	17	18	16	19	16	19	18	21	21	23	25	27
	DO	109	87	111	87	109	93	110	94	110	90	119	92
3	pH	6.61	6.78	6.66	6.81	6.70	6.83	6.70	6.86	6.70	6.88	6.69	
	Conductivity	17	18	18	19	19	20	20	20	23	23	27	NA
	DO	109	92	112	95	110	95	111	91	114	93	111	
4	pH	6.55	6.88	6.53	6.92	6.67	6.94	6.79	7.01	6.67	7.04		
	Conductivity	17	20	19	18	19	19	21	21	21	23	NA	NA
	DO	115	95	115	94	117	89	108	90	115	90		

[1] Conductivity units are in µS cm^{-1}

[2] DO: Dissolved oxygen. Measurements are expressed as percent saturation

N/A = Not analysed due to 100% mortality in treatment

***Mogurnda mogurnda* test (923E)**

		PAM (mg L^{-1})											
		0		750		1500		3000		6000		12000	
Day	Parameter	0 h	24h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h
0	pH	6.46	6.72	6.37	6.77	6.35	6.73	6.33	6.83	6.37	6.91	6.44	7.06
	Conductivity[1]	10	16	10	16	11	16	13	17	15	20	20	25
	DO[2]	102	87	109	92	109	90	111	90	109	91	110	87
1	pH	6.24	6.56	6.30	6.60	6.29	6.61	6.31	6.66	6.37	6.82	6.42	6.94
	Conductivity	10	12	17	12	11	13	12	14	15	17	20	22
	DO	102	90	116	88	109	88	120	91	114	86	119	84
2	pH	6.40	6.59	6.29	6.53	6.32	6.62	6.34	6.70	6.39	6.77	6.41	6.90
	Conductivity	11	12	11	12	11	13	13	14	15	19	20	23
	DO	112	90	113	91	111	91	108	92	103	89	111	91
3	pH	6.58	6.73	6.35	6.65	6.26	6.63	6.24	6.65	6.39	6.80	6.37	6.96
	Conductivity	10	13	10	13	11	14	12	15	15	18	20	25
	DO	113	90	117	87	119	88	115	90	117	88	115	80

[1] Conductivity units are in $\mu S\ cm^{-1}$

[2] DO: Dissolved oxygen. Measurements are expressed as percent saturation

N/A = Not analysed due to 100% mortality in treatment

Appendix B Chemical analyses

Table B1 Nutrient analysis

Test code	Date	Sample type	Nitrate mg L^{-1}	Phosphate mg L^{-1}
865L & 866G	10/09/2007	Procedural blank	0.01	<0.005
865L	10/09/2007	Blank	<0.005	<0.005
865L	10/09/2007	NMCW	0.685	0.1
866G	10/09/2007	NMCW	2.97	0.045
887L	30/10/2007	Procedural blank	0.01	<0.005
887L	30/10/2007	Blank	<0.005	<0.005
887L	30/10/2007	NMCW	0.66	0.085
888G	20/11/2007	Procedural blank	0.005	<0.005
888G	20/11/2007	NMCW	1.92	0.03
913G	26/02/2008	Procedural blank	0.01	<0.005
913G	26/02/2008	NMCW	2.21	0.035
919L	31/03/2008	Procedural blank	0.03	<0.005
919L	31/03/2008	NMCW	0.605	0.055

Table B2 Metal analysis

Test code(s)	Date	Sample	Al µg L^{-1}	Ca mg L^{-1}	Cd µg L^{-1}	Co µg L^{-1}	Cr µg L^{-1}	Cu µg L^{-1}	Fe µg L^{-1}	Mg mg L^{-1}	Mn µg L^{-1}	Ca mg L^{-1}	Ni µg L^{-1}	Pb µg L^{-1}	SO_4 mg L^{-1}	Se µg L^{-1}	U µg L^{-1}	Zn µg L^{-1}
865L/862B/866G	10/09/07	Pro blank	0.1	<0.1	<0.02	<0.01	<0.1	<0.01	<20	<0.1	<0.01	<0.1	0.05	<0.01	<0.1	<0.2	0.006	0.2
865L/862B/866G	10/09/07	Blank	0.2	<0.1	<0.02	<0.01	<0.1	<0.01	<20	<0.1	<0.01	<0.1	0.03	<0.01	<0.1	<0.2	<0.001	<0.1
865L/862B/866G	10/09/07	NMCW	12.8	0.2	<0.02	0.05	<0.1	0.28	100	0.8	2.71	1.3	0.38	0.02	0.2	<0.2	0.012	0.8
864E	12/09/07	Pro blank	7.4	<0.1	<0.02	<0.01	<0.1	0.02	<20	<0.1	0.02	<0.1	0.38	0.01	<0.1	<0.2	0.033	0.2
864E	12/09/07	Blank	0.6	<0.1	<0.02	<0.01	<0.1	<0.01	<20	<0.1	<0.01	<0.1	0.15	<0.01	<0.1	<0.2	<0.001	<0.1
864E	12/09/07	NMCW	14.2	0.2	0.02	0.05	<0.1	0.21	100	0.8	3.22	1.2	0.39	0.02	0.2	<0.2	0.017	1.2
863D	20/09/07	Pro blank	0.5	<0.1	<0.02	<0.01	<0.1	<0.01	<20	<0.1	<0.01	<0.1	0.02	<0.01	<0.1	<0.2	0.001	<0.1
863D	20/09/07	Blank	0.3	<0.1	<0.02	<0.01	<0.1	<0.01	<20	<0.1	<0.01	<0.1	0.03	<0.01	<0.1	<0.2	<0.001	0.2
863D	20/09/07	NMCW	12.8	0.2	0.04	0.06	<0.1	0.25	120	0.8	3.83	1.3	0.23	0.05	0.3	<0.2	0.012	2.2
877B	15/10/07	Blank	<0.1	<0.1	<0.02	<0.01	<0.1	<0.01	<20	<0.1	<0.01	<0.1	0.08	<0.01	<0.1	<0.2	<0.001	<0.1
877B	15/10/07	Pro blank	0.6	<0.1	<0.02	<0.01	<0.1	0.02	<20	<0.1	0.01	<0.1	2.1	0.01	0.2	<0.2	0.005	0.3
877B	15/10/07	NMCW	13.5	0.3	0.06	0.06	<0.1	0.24	200	1	4.06	1.2	0.55	0.03	0.3	<0.2	0.015	1.6
873B	1/10/07	Blank	<0.1	<0.1	<0.02	<0.01	<0.1	<0.01	<20	<0.1	<0.01	<0.1	0.03	<0.01	<0.1	<0.2	<0.001	0.2
873B	1/10/07	Pro blank	0.4	<0.1	<0.02	<0.01	<0.1	0.04	<20	<0.1	<0.01	<0.1	0.03	0.01	<0.1	<0.2	0.004	0.2
873B	1/10/07	NMCW	12	0.2	<0.02	0.05	<0.1	0.18	140	0.9	3.19	1.3	0.15	0.02	0.3	<0.2	0.014	0.8
874D	3/10/07	Blank	<0.1	<0.1	<0.02	<0.01	<0.1	<0.01	<20	<0.1	<0.01	<0.1	1.76	<0.01	<0.1	<0.2	<0.001	0.1
874D	3/10/07	Pro blank	<0.1	<0.1	<0.02	<0.01	<0.1	0.01	<20	<0.1	0.03	<0.1	0.13	<0.01	0.2	<0.2	<0.001	0.2
874D	3/10/07	NMCW	20	0.3	0.04	0.08	<0.1	0.24	180	0.9	5.07	1.3	1.02	0.04	0.3	<0.2	0.02	0.9
887L	30/10/07	Blank	12.1	<0.1	<0.02	<0.01	<0.1	1.1	<20	<0.1	0.38	<0.1	0.17	0.07	<0.1	<0.2	0.013	8.2
887L	30/10/07	Pro blank	1	<0.1	<0.02	<0.01	0.1	0.78	<20	<0.1	0.44	<0.1	0.17	0.04	<0.1	<0.2	0.052	0.4
887L	30/10/07	NMCW	5.5	0.3	0.02	0.02	<0.1	1.17	60	1	0.92	1.3	0.76	0.05	0.4	<0.2	0.013	6.6
888G	20/11/07	Pro blank	0.2	<0.1	<0.02	<0.01	<0.1	0.06	<20	<0.1	0.27	<0.1	0.08	0.02	<0.1	<0.2	<0.001	0.3

Table B2 (continued) Metal analysis

Test code(s)	Date	Sample	Al µg L^{-1}	Ca mg L^{-1}	Cd µg L^{-1}	Co µg L^{-1}	Cr µg L^{-1}	Cu µg L^{-1}	Fe µg L^{-1}	Mg mg L^{-1}	Mn µg L^{-1}	Ca mg L^{-1}	Ni µg L^{-1}	Pb µg L^{-1}	SO4 mg L^{-1}	Se µg L^{-1}	U µg L^{-1}	Zn µg L^{-1}
888G	20/11/07	NMCW	1.4	0.3	<0.02	<0.01	<0.1	0.84	<20	1	0.35	7.9	0.42	0.05	101	<0.2	0.037	0.3
884D/883B	12/11/07	Pro blank	0.2	<0.1	<0.02	<0.01	<0.1	<0.01	<20	<0.1	<0.01	<0.1	0.01	<0.01	<0.1	<0.2	<0.001	<0.1
884D/883B	12/11/07	Blank	0.1	<0.1	<0.02	<0.01	<0.1	<0.01	<20	<0.1	<0.01	<0.1	0.07	0.01	<0.1	<0.2	0.028	0.1
884D/883B	12/11/07	NMCW	11.1	0.3	0.02	0.05	<0.1	0.31	120	1	2.71	1.4	0.21	0.03	0.3	<0.2	0.011	0.8
891E	1/12/07	Blank	0.7	<0.1	<0.02	<0.01	<0.1	0.04	<20	<0.1	0.03	<0.1	0.04	0.03	<0.1	<0.2	<0.001	1
891E	1/12/07	Pro blank	0.4	<0.1	<0.02	<0.01	<0.1	0.04	<20	<0.1	0.05	<0.1	0.03	0.03	<0.1	<0.2	<0.001	0.7
891E	1/12/07	NMCW	4.9	0.4	0.06	0.02	<0.1	0.4	60	1.1	0.57	1.7	0.4	0.08	0.5	<0.2	0.013	3
892B	3/12/07	Blank	0.4	<0.1	<0.02	<0.01	<0.1	<0.01	<20	<0.1	0.02	<0.1	0.03	0.02	<0.1	<0.2	<0.001	0.5
892B	3/12/07	Pro blank	1	<0.1	<0.02	<0.01	<0.1	0.03	<20	<0.1	0.08	<0.1	0.09	0.03	<0.1	<0.2	0.024	<0.1
892B	3/12/07	NMCW	9.5	0.3	0.02	0.03	<0.1	0.3	100	1	1	1.4	0.46	0.07	0.3	<0.2	0.029	8.6
913G	26/02/08	Pro blank	1.1	<0.1	<0.02	<0.01	<0.1	0.19	<20	<0.1	<0.01	<0.1	0.02	0.02	<0.1	<0.2	0.018	2.6
913G	26/02/08	NMCW	67.5	0.3	<0.02	0.14	0.5	0.53	240	0.4	6.38	7.3	0.65	0.18	96.7	<0.2	0.038	4.6
913G	26/02/08	PEG 12 g L^{-1}	159	0.3	<0.02	0.11	23.3	6.31	240	0.4	5.67	7.3	1.42	0.16	98.9	<0.2	0.036	5.6
919L	31/03/08	Pro blank	0.8	<0.1	<0.02	<0.01	0.1	0.04	<20	<0.1	<0.01	<0.1	1.25	0.01	<0.1	<0.2	0.029	2.1
919L	31/03/08	NMCW	45.7	0.3	<0.02	0.21	<0.1	0.3	160	0.3	15.1	1	3.77	0.07	0.2	<0.2	0.079	3.2
919L	31/03/08	PEG 12 g L^{-1}	103	0.2	<0.02	0.23	25.7	0.47	140	0.3	13.3	1.2	4.79	0.1	0.5	<0.2	0.072	4.9
920B	8/04/08	Pro blank	0.9	<0.1	<0.02	<0.01	0.1	0.11	<20	<0.1	<0.01	<0.1	0.05	0.02	<0.1	<0.2	0.021	2.9
920B	8/04/08	NMCW	49.6	0.3	<0.02	0.19	0.1	0.36	200	0.3	13.4	1	0.27	0.41	0.2	<0.2	0.061	5.2
923E	16/04/08	Pro blank	0.4	<0.1	<0.02	<0.01	0.1	0.04	<20	<0.1	<0.01	<0.1	0.09	0.01	<0.1	<0.2	0.007	<0.1
923E	16/04/08	NMCW	50	0.8	<0.02	0.2	0.2	3.44	180	0.4	14.8	1.1	0.5	0.11	0.3	<0.2	0.033	9.8
923E	16/04/08	PEG 12 g L^{-1}	102	0.3	<0.02	0.23	25.6	1.76	140	0.4	13.8	1.3	0.64	0.1	0.5	<0.2	0.071	2.9
927D	16/05/08	Pro blank	2.1	<0.1	<0.02	<0.01	<0.1	0.67	<20	<0.1	0.09	<0.1	0.24	0.07	<0.1	<0.2	0.007	1.5
927D	16/05/08	NMCW	14.7	0.4	<0.02	0.08	<0.1	0.44	240	0.8	3.57	1.2	0.34	0.07	0.2	<0.2	0.015	2.3
927D	16/05/08	PEG 12 g L^{-1}	21.4	0.4	<0.2	<0.1	13.5	0.54	300	0.8	5.02	1.3	0.89	0.17	0.4	<2	0.028	3.9

Table B3 Metal analyses of flocculant block (5 g/L) and polyacrylamide (2 g/L) solutions

Element	Ag	Al	As	Au	B	Ba	Br	Cd	Cr	Cu	Fe	Hg	I	In
Units	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Blank	0.05	8	0.3	0.51	4.5	1.16	17	0.06	<0.1	1	<20	0.04	<5	0.07
Flocculant block	1.25	168	5.15	11.5	31.5	24.4	424	0.6	3.6	17	80	0.6	10	1.06
Polyacrylamide	1.15	216	4.9	11.2	79	24.8	320	1.02	2.9	15.5	100	0.68	10	1.12

Table B3 (continued) Metal analyses of flocculant block and polyacrylamide

Element	Mg	Mn	Na	Ni	Pb	SO_4	Se	Sn	Sr	V	Zn
Units	mg/L	µg/L	mg/L	µg/L	µg/L	mg/L	µg/L	µg/L	µg/L	µg/L	µg/L
Blank	<0.1	0.2	0.2	0.15	0.25	<0.1	0.4	9.5	0.12	0.5	3
Flocculant block	1	3.95	83.8	2.42	0.8	4.3	8.8	85.3	3.94	9.9	42.5
Polyacrylamide	3.3	3.95	98.7	3	1.85	17.2	2.6	72.6	2.66	9.7	55.5

Table B4 Viscosity measurements

Sample	Nominal concentration (mg L^{-1})	Brookfield viscosity (cPs)	Funnel viscosity (sec)
PAM	2000	52.6	40
PAM	1000	34.6	33
PAM	500	22.1	18
PAM	250	12.3	18
PAM	125	6.89	18
PAM	63	2.35	18
PAM	31	2.03	18
PAM	16	1.6	17
PAM	8	1.39	17
PAM	4	1.28	17
PAM	2	1.28	17
PAM	1	1.28	17
PAM	0.5	1.28	17
PEG	12000	NA	12*
PEG	6000	1.49	17
PEG	3000	1.28	17
PEG	1500	1.28	17
PEG	750	1.28	17
PEG	375	1.28	17
PEG	188	1.28	17
PEG	94	1.28	17
Floc Block	5000	NA	34
Floc Block	2500	56.2	30
Floc Block	1250	34.1	27
Floc Block	625	20.4	25
Floc Block	313	12.2	24
Floc Block	156	7.47	23
Floc Block	78	4.91	21
Floc Block	39	3.31	18
Floc Block	20	2.35	17
Floc Block	10	1.81	17
Floc Block	5	1.49	17
Floc Block	2	1.28	17
Floc Block	1	1.17	17
NMCW	0	1.07	17
Milli Q water	0	1.07	17

NA = Not analysed

Appendix C Total Organic Carbon analyses

Table C1 Total Organic Carbon measurement of Flocculent Block test solutions

Nominal Floc Bloc (mg/L)	Measured Floc Block TOC (mg/L)						
	Clad	Algae	Lemna	Fry	Hydra 1	Hydra 2	Hydra 3
0	1.9	1.7	1.7	1.8	1.723	1.748	2.9
1.22	NA	NA	NA	NA	NA	NA	NA
2.44	2.8	NA	NA	NA	NA	NA	NA
4.88	2.8	NA	NA	NA	NA	NA	NA
9.75	4.0	NA	NA	NA	NA	NA	NA
19.5	6.8	NA	NA	NA	NA	NA	NA
39	10.5	NA	NA	NA	NA	NA	22.3
78	20.5	NA	NA	NA	NA	20.99	43.2
156.25	38.3	NA	NA	NA	NA	40.64	81.8
312.5	NA	155.4	155.4	144.4	155.4	79.28	173.6
625	NA	303.2	303.2	292.2	303.2	156.5	316.2
1250	NA	604.1	604.1	601.8	604.1	615.2	643.8
2500	NA	1222	1222	1186	1222	NA	NA
5000	NA	2593	2593	2600	2593	NA	NA

NA = Not analysed

Table C1 Total Organic Carbon measurement of PAM test solutions

Nominal PAM (mg/L)	Measured PAM TOC (mg/L)					
	Clad	Algae	Lemna	Fry	Hydra 1	Hydra 2
0	4.1	3.1	3.1	3.1	4.1	3.142
0.5	3.5	NA	NA	NA	NA	NA
1	3.8	NA	NA	NA	NA	NA
2	4.2	NA	NA	NA	NA	NA
4	4.8	NA	NA	NA	NA	NA
8	6.7	NA	NA	NA	NA	NA
16	10.2	NA	NA	NA	6.6	6.4
32	33.5	NA	NA	NA	13.8	12.3
62	NA	NA	NA	NA	61.5	24.0
125	NA	49.0	61.8	49.0	N.A.	49.0
250	NA	124.9	123.6	98.5	144.1	98.5
500	NA	245.7	253.8	198.1	226.9	198.1
1000	NA	415.9	415.9	415.9	NA	NA
2000	NA	1081	941	785.5	NA	NA

Table C1 Total Organic Carbon measurement of PEG surrogate solutions

Nominal PEG (mg/L)	Measured PEG TOC (mg/L)
0	0
375	170
750	448.5
1500	887
3000	1750
6000	3444.5
12000	7002

Appendix D Total Nitrogen analyses

Table D1 Total Nitrogen measurement of Flocculent Block test solutions

Nominal Floc Block (mg/L)	Measured Floc Block Total Nitrogen (mg/L)						
	Clad	**Algae***	**Lemna***	**Fry**	**Hydra 1**	**Hydra 2**	**Hydra 3**
0	0.11	0	0	0.07	0.08	0.14	0.16
1.22	0.22	NA	NA	NA	NA	NA	NA
2.44	0.38	NA	NA	NA	NA	NA	NA
4.88	0.55	NA	NA	NA	NA	NA	NA
9.75	1.39	NA	NA	NA	NA	NA	NA
19.5	1.93	NA	NA	NA	NA	NA	NA
39	3.63	NA	NA	NA	NA	3.83	3.06
78	7.00	NA	NA	NA	NA	7.29	6.01
156.25	NA	NA	NA	NA	NA	15.2	11.4
312.5	NA	27.7	17.8	32.1	18.2	30.6	25
625	NA	47	36.3	63.3	36.7	NA	45.9
1250	NA	90.1	74.3	130	74.7	125	47.9
2500	NA	189.1	141.6	285	142	NA	NA
5000	NA	361.1	286.6	483	287	NA	NA

* Adjusted for the addition of nutrients to the test. NA = not analysed

Table D2 Total Nitrogen measurement of PAM test solutions

Nominal (mg/L)	Measured Floc Block Total Nitrogen (mg/L)					
	Clad	**Hydra**	**Algae***	**Lemna***	**Fry**	**Hydra 2**
0	0.33	0.18	0	0	0.37	0.17
0.5	0.19	NA	NA	NA	NA	NA
1	0.29	NA	NA	NA	NA	NA
2	0.52	NA	NA	NA	NA	NA
4	0.84	NA	NA	NA	NA	NA
8	1.19	NA	NA	NA	NA	NA
16	2.62	2.57	NA	NA	NA	2.5
32	4.68	5.36	NA	NA	NA	5.4
62	NA	21.2	NA	NA	NA	44.9 [a]
125	NA	97.6 [a]	2.1	17.0	5.6	20.6
250	NA	54.6	4.2	40.0	5.7	88.8
500	NA	103	12.7	58.6	10.9	148
1000	NA	NA	36.9	114.6	13.8	NA
2000	NA	NA	320	273.6	320	NA

* Adjusted for the addition of nutrients to the test

[a] Removed from analysis NA = not analysd

Appendix E Statistical analyses (Nominal concentrations)

862B – Hyd_FBloc_01

Green Hydra Population Growth Test-Population growth rate (k

Start Date:	10/09/2007	Test ID:	862B	Sample ID:	FLOC BLOC
End Date:	14/09/2007	Lab ID:	ERISS-eriss ecotoxicology lal	Sample Type:	FLOC BLOC
Sample Date:		Protocol:	BTT B-eriss tropical freshwat	Test Species:	HV-Hydra viridissima
Comments:					

Conc-mg/L	1	2	3
0	0.3271	0.3202	0.3402
312.5	0.2829	0.2389	0.2082
625	0.2291	0.2189	0.2389
1250	0.2082	0.2291	0.1855
2500	0.1971	0.1733	0.1855
5000	0.1469	0.1469	0.1733

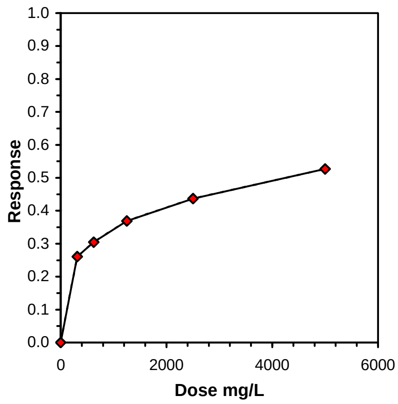
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
0	0.3292	1.0000	0.3292	0.3202	0.3402	3.089	3				0.3292	1.0000
*312.5	0.2433	0.7392	0.2433	0.2082	0.2829	15.416	3	5.195	2.500	0.0413	0.2433	0.7392
*625	0.2289	0.6955	0.2289	0.2189	0.2389	4.371	3	6.065	2.500	0.0413	0.2289	0.6955
*1250	0.2076	0.6306	0.2076	0.1855	0.2291	10.504	3	7.355	2.500	0.0413	0.2076	0.6306
*2500	0.1853	0.5629	0.1853	0.1733	0.1971	6.430	3	8.705	2.500	0.0413	0.1853	0.5629
*5000	0.1557	0.4731	0.1557	0.1469	0.1733	9.765	3	10.494	2.500	0.0413	0.1557	0.4731

Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.97073		0.858		0.2984	0.99384
Bartlett's Test indicates equal variances (p = 0.41)					5.08543		15.0863			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	<312.5	312.5			0.04133	0.12554	0.01073	0.00041	4.6E-06	5, 12
Treatments vs 0										

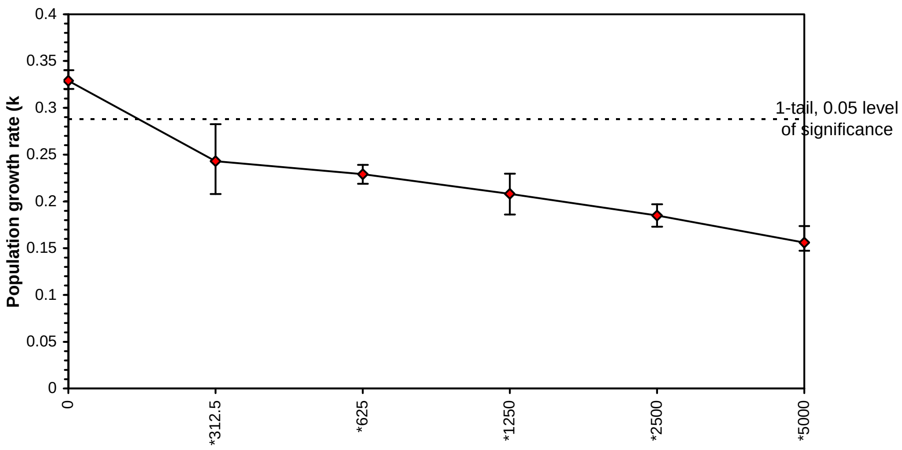
Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05*	59.9003	14.3874	31.831	167.219	1.9250
IC10*	119.801	28.7749	63.662	334.438	1.9250
IC15*	179.701	41.8926	95.493	495.604	1.7111
IC20*	239.601	55.3118	127.324	630.07	1.4756
IC25*	299.502	90.1062	159.155	765.942	0.8064
IC40	1815.34	415.551	184.67	3193.95	-0.1022
IC50	4250.32				

* indicates IC estimate less than the lowest concentration



Dose-Response Plot



863D – Clad_FBloc_01

Cladoceran Reproduction Test-Total neonates

Start Date:	21/09/2007	Test ID:	863D	Sample ID:	FLOC BLOC
End Date:	26/09/2007	Lab ID:	ERISS-eriss ecotoxicology la	Sample Type:	FLOC BLOC
Sample Date:		Protocol:	BTT G-eriss tropical freshwa	Test Species:	MOMA-Moinodaphnia macle
Comments:					

Conc-gm/L	1	2	3	4	5	6	7	8	9	10
MCW	40.000	33.000	38.000	38.000	36.000	30.000	37.000	40.000	39.000	38.000
0.039	6.000	5.000	5.000	5.000	6.000	5.000	5.000	7.000	5.000	7.000
0.078	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.156	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.313	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.625	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
1.25	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2.5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

			Transform: Untransformed					Rank	1-Tailed	Isot
Conc-gm/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean
MCW	36.900	1.0000	36.900	30.000	40.000	8.613	10			36.900
*0.039	5.600	0.1518	5.600	5.000	7.000	15.058	10	55.00	82.00	5.600
0.078	0.000	0.0000	0.000	0.000	0.000	0.000	10			0.000
0.156	0.000	0.0000	0.000	0.000	0.000	0.000	10			0.000
0.313	0.000	0.0000	0.000	0.000	0.000	0.000	10			0.000
0.625	0.000	0.0000	0.000	0.000	0.000	0.000	10			0.000
1.25	0.000	0.0000	0.000	0.000	0.000	0.000	10			0.000
2.5	0.000	0.0000	0.000	0.000	0.000	0.000	10			0.000

Auxiliary Tests	Statistic	Critical	Skew
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.84579	0.868	-1.5714
F-Test indicates unequal variances (p = 5.24E-04)	14.2031	6.54109	

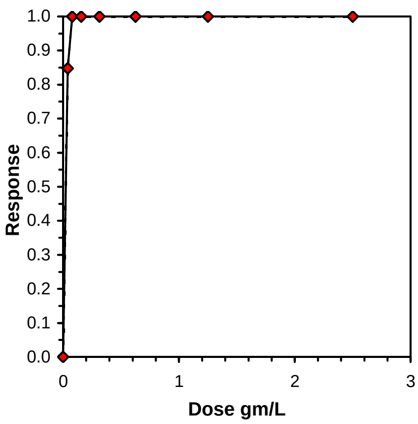
Hypothesis Test (1-tail, 0.05)

Wilcoxon Two-Sample Test indicates significant differences
Treatments vs MCW

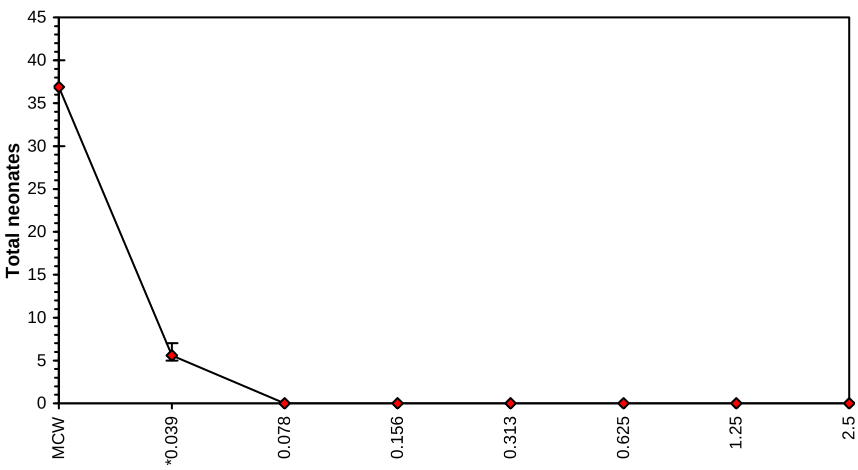
Linear Interpolation (200 Resamples)

Point	gm/L	SD	95% CL		Skew
IC05*	0.0023	0.0000	0.0023	0.0024	0.6128
IC10*	0.0046	0.0000	0.0045	0.0047	0.6128
IC15*	0.0069	0.0001	0.0068	0.0071	0.6128
IC20*	0.0092	0.0001	0.0090	0.0094	0.6128
IC25*	0.0115	0.0001	0.0113	0.0118	0.6128
IC40*	0.0184	0.0002	0.0181	0.0188	0.6128
IC50*	0.0230	0.0002	0.0226	0.0235	0.6128

* indicates IC estimate less than the lowest concentration



Dose-Response Plot



864E – Fry_FBloc_01

Gudgeon Sac Fry Survival Test-Number of fry					
Start Date:	12/09/2007	Test ID:	864E	Sample ID:	FBLOC
End Date:	16/09/2007	Lab ID:	ERISS-eriss ecotoxicology lal	Sample Type:	FLOC BLOC
Sample Date:		Protocol:	BTT E-eriss tropical freshwat	Test Species:	MMO-Mogurnda mogurnda
Comments:					

Conc-gm/L	1	2	3
0	10.000	10.000	10.000
312.5	10.000	10.000	10.000
625	10.000	10.000	10.000
1250	10.000	10.000	10.000
2500	5.000	8.000	7.000
5000	8.000	6.000	5.000

			Transform: Untransformed						1-Tailed			
Conc-gm/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
0	10.000	1.0000	10.000	10.000	10.000	0.000	3				10.000	0.0000
312.5	10.000	1.0000	10.000	10.000	10.000	0.000	3	0.000	2.500	1.800	10.000	0.0000
625	10.000	1.0000	10.000	10.000	10.000	0.000	3	0.000	2.500	1.800	10.000	0.0000
1250	10.000	1.0000	10.000	10.000	10.000	0.000	3	0.000	2.500	1.800	10.000	0.0000
*2500	6.667	0.6667	6.667	5.000	8.000	22.913	3	4.629	2.500	1.800	6.667	0.3333
*5000	6.333	0.6333	6.333	5.000	8.000	24.119	3	5.092	2.500	1.800	6.333	0.3667

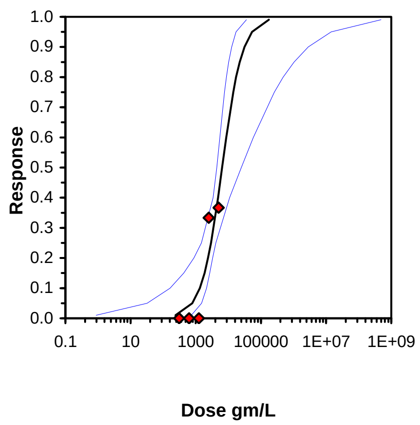
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.76171	0.858	-1E-16	2.44375
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	1250	2500	1767.77		1.80021	0.18002	9.83333	0.77778	1.9E-04	5, 12
Treatments vs 0										

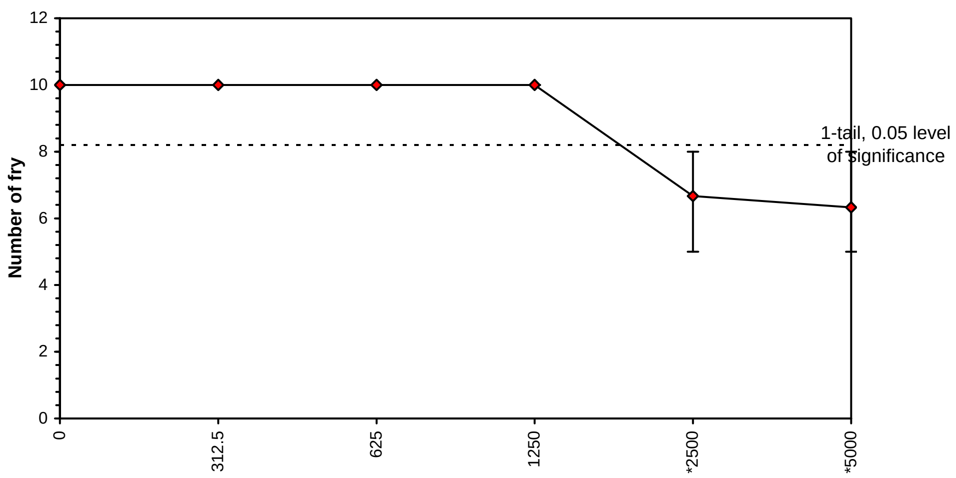
Maximum Likelihood-Logit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	3.2057	1.10027	1.04918	5.36222	0	1.28866	7.81473	0.73			9
Intercept	-12.211	3.91463	-19.884	-4.5388							
TSCR											

Point	Logits	gm/L	95% Fiducial Limits	
EC01	-4.595	237.617	0.88409	727.32
EC05	-2.944	777.675	32.3476	1511.92
EC10	-2.197	1330.11	162.23	2141.8
EC15	-1.735	1854.39	431.737	2709.59
EC20	-1.386	2381.51	876.531	3328.83
EC25	-1.099	2928.15	1498.07	4143.58
EC40	-0.405	4817.45	3454.94	11076.2
EC50	0.000	6446.14	4472.21	24796.1
EC60	0.405	8625.46	5528.75	58123.8
EC75	1.099	14190.8	7677.29	258045
EC80	1.386	17448	8750.92	481613
EC85	1.735	22407.8	10231.7	1027398
EC90	2.197	31240.1	12563.4	2817050
EC95	2.944	53432	17443.9	1.4E+07
EC99	4.595	174873	35763.1	4.9E+08



Dose-Response Plot



865L – Lem_FBloc_01

Lemna Growth Inhibition-Incr. in biomass

Start Date:	10/09/2007	Test ID:	865L	Sample ID:	FBLOC
End Date:	14/09/2007	Lab ID:	ERISS-eriss ecotoxicology la	Sample Type:	FLOC BLOC
Sample Date:		Protocol:	BTT D-eriss tropical freshwa	Test Species:	LAE-Lemna aequinoctialis
Comments:					

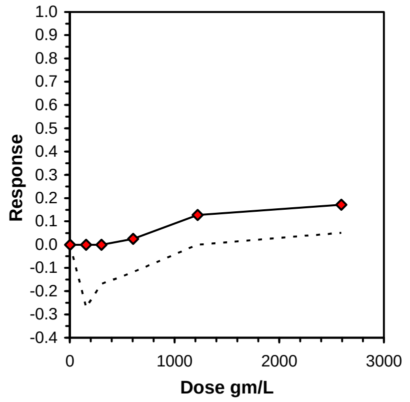
Conc-gm/L	1	2	3	percentage of control			mean	se
1.723	63.000	63.000	51.000	106.78	106.78	86.4407	100	6.77966
155.4	66.000	95.000	64.000	111.864	161.017	108.475	127.119	16.9774
303.2	83.000	75.000	49.000	140.678	127.119	83.0508	116.949	17.3953
604.1	65.000	78.000	55.000	110.169	132.203	93.2203	111.864	11.2853
1222	71.000	62.000	44.000	120.339	105.085	74.5763	100	13.453
2593	47.000	60.000	61.000	79.661	101.695	103.39	94.9153	7.6428

			Transform: Untransformed						1-Tailed		Isotonic	
Conc-gm/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
1.723	59.000	1.0000	59.000	51.000	63.000	11.743	3				67.667	1.0000
155.4	75.000	1.2712	75.000	64.000	95.000	23.132	3	-1.483	2.500	26.977	67.667	1.0000
303.2	69.000	1.1695	69.000	49.000	83.000	25.763	3	-0.927	2.500	26.977	67.667	1.0000
604.1	66.000	1.1186	66.000	55.000	78.000	17.474	3	-0.649	2.500	26.977	66.000	0.9754
1222	59.000	1.0000	59.000	44.000	71.000	23.301	3	0.000	2.500	26.977	59.000	0.8719
2593	56.000	0.9492	56.000	47.000	61.000	13.947	3	0.278	2.500	26.977	56.000	0.8276

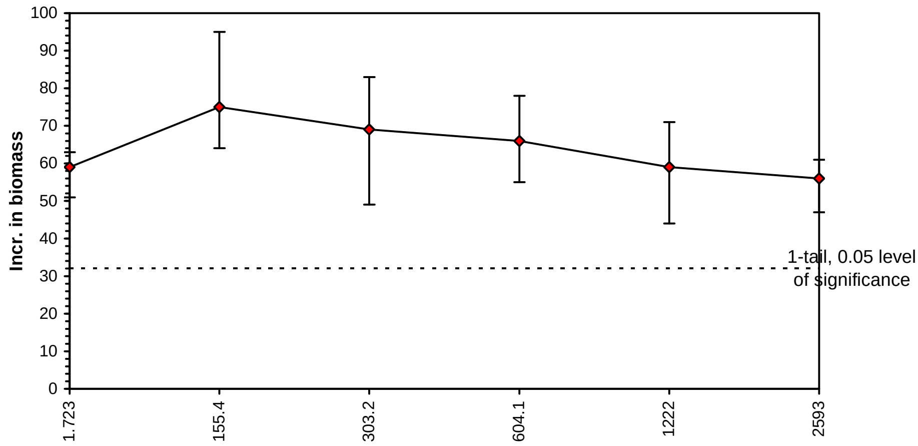
Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.95913		0.858		-0.0603	-0.8566
Bartlett's Test indicates equal variances (p = 0.80)					2.34461		15.0863			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	2593	>2593			26.9774	0.45724	158.4	174.667	0.50797	5, 12
Treatments vs 1.723										

Linear Interpolation (200 Resamples)

Point	gm/L	SD	95% CL(Exp)	Skew
IC05	755.633			
IC10	1054.28			
IC15	1899.88			
IC20	>2593			
IC25	>2593			
IC40	>2593			
IC50	>2593			



Dose-Response Plot



866G – Alg_FBloc_02

Algal Growth Inhibition Test-Growth rate

Start Date:	10/09/2007	Test ID:	866G	Sample ID:	FLOC BLOC
End Date:	13/09/2007	Lab ID:	ERISS-eriss ecotoxicology lal	Sample Type:	FLOC BLOC
Sample Date:		Protocol:	BTT G-eriss tropical freshwat	Test Species:	CH-Chlorella sp.
Comments:					

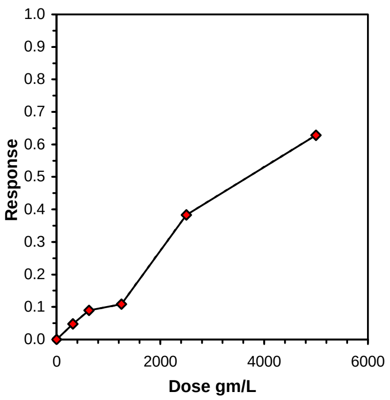
Conc-gm/L	1	2	3
0	1.8778	1.9484	1.9412
312.5	1.8809	1.7951	1.8146
625	1.7488	1.7210	1.7816
1250	1.7399	1.7025	1.6946
2500	1.2840	1.1310	1.1431
5000	0.8052	0.6191	0.7204

			Transform: Untransformed						1-Tailed		Isotonic	
Conc-gm/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
0	1.9225	1.0000	1.9225	1.8778	1.9484	2.020	3				1.9225	1.0000
312.5	1.8302	0.9520	1.8302	1.7951	1.8809	2.457	3	1.912	2.500	0.1206	1.8302	0.9520
*625	1.7505	0.9105	1.7505	1.7210	1.7816	1.735	3	3.565	2.500	0.1206	1.7505	0.9105
*1250	1.7123	0.8907	1.7123	1.6946	1.7399	1.414	3	4.354	2.500	0.1206	1.7123	0.8907
*2500	1.1861	0.6169	1.1861	1.1310	1.2840	7.170	3	15.260	2.500	0.1206	1.1861	0.6169
*5000	0.7149	0.3719	0.7149	0.6191	0.8052	13.038	3	25.023	2.500	0.1206	0.7149	0.3719

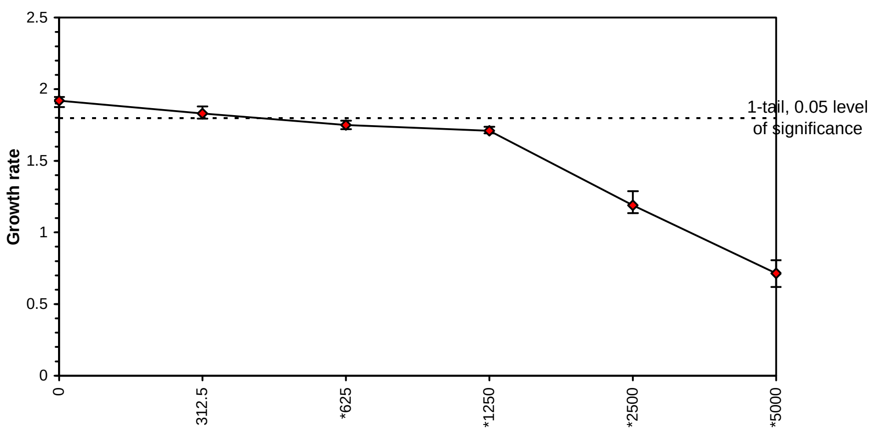
Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.97483		0.858		0.31768	0.11939
Bartlett's Test indicates equal variances (p = 0.44)					4.79589		15.0863			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	312.5	625	441.942		0.12064	0.06275	0.6648	0.00349	5.4E-11	5, 12
Treatments vs 0										

Linear Interpolation (200 Resamples)

Point	gm/L	SD	95% CL(Exp)		Skew
IC05	327.604	79.1031	95.2798	663.445	0.5636
IC10	956.789	254.362	107.935	1707.67	-0.0321
IC15	1435.84	46.8012	1266.08	1630.49	0.3151
IC20	1664.15	51.8217	1468.19	1899.16	0.3721
IC25	1892.46	63.33	1668.83	2195.88	0.5063
IC40	2672.85	184.879	2157.73	3433.97	0.4180
IC50	3692.96	163.501	3050.57	4321.62	0.1027



Dose-Response Plot



873B – Hyd_FBloc_02

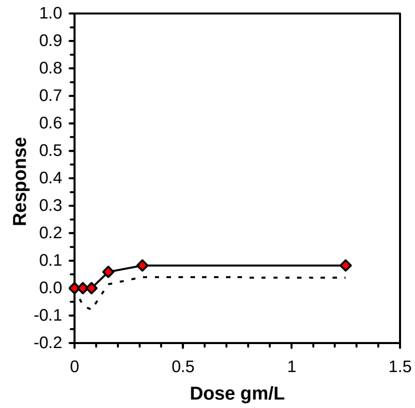
Green Hydra Population Growth Test-Population growth rate (k					
Start Date:	1/10/2007	Test ID:	873B	Sample ID:	FLOC BLOC
End Date:	5/10/2007	Lab ID:	ERISS-eriss ecotoxicology la	Sample Type:	FLOC BLOC
Sample Date:		Protocol:	BTT B-eriss tropical freshwa	Test Species:	HV-Hydra viridissima
Comments:					

Conc-gm/L	1	2	3
MCW	0.2908	0.3338	0.3760
0.039	0.3466	0.3704	0.3466
0.078	0.3588	0.3402	0.3815
0.156	0.3132	0.3202	0.3527
0.3125	0.3202	0.3059	0.3338
1.25	0.2908	0.3132	0.3588

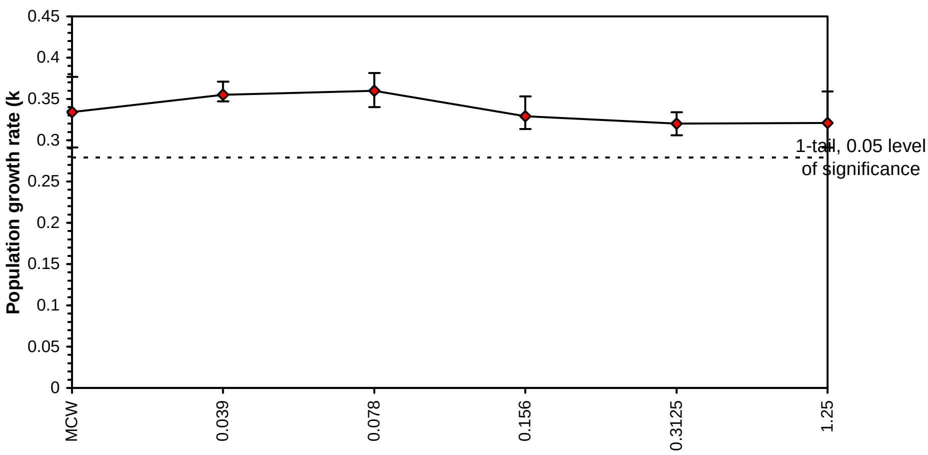
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-gm/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
MCW	0.3335	1.0000	0.3335	0.2908	0.3760	12.778	3				0.3494	1.0000
0.039	0.3545	1.0630	0.3545	0.3466	0.3704	3.880	3	-0.964	2.500	0.0545	0.3494	1.0000
0.078	0.3602	1.0799	0.3602	0.3402	0.3815	5.739	3	-1.224	2.500	0.0545	0.3494	1.0000
0.156	0.3287	0.9856	0.3287	0.3132	0.3527	6.419	3	0.220	2.500	0.0545	0.3287	0.9408
0.3125	0.3200	0.9594	0.3200	0.3059	0.3338	4.346	3	0.622	2.500	0.0545	0.3204	0.9171
1.25	0.3209	0.9622	0.3209	0.2908	0.3588	10.795	3	0.578	2.500	0.0545	0.3204	0.9171

Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.97067		0.858		0.23104	-0.1651
Bartlett's Test indicates equal variances (p = 0.61)					3.59897		15.0863			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	1.25	>1.25			0.05446	0.1633	0.00088	0.00071	0.35024	5, 12
Treatments vs MCW										

Linear Interpolation (200 Resamples)				
Point	gm/L	SD	95% CL(Exp)	Skew
IC05	0.1439			
IC10	>1.25			
IC15	>1.25			
IC20	>1.25			
IC25	>1.25			
IC40	>1.25			
IC50	>1.25			



Dose-Response Plot



874D – Clad_FBloc_02

Cladoceran Reproduction Test-Total neonates

Start Date:	3/10/2007	Test ID: 874D	Sample ID:	FLOC BLOC
End Date:	8/10/2007	Lab ID: ERISS-eriss ecotoxicology la	Sample Type:	FLOC BLOC
Sample Date:		Protocol: BTT D-eriss tropical freshwa	Test Species:	MOMA-Moinodaphnia macleayi
Comments:				

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
MCW	38.000	38.000	36.000	35.000	37.000	37.000	34.000	35.000	41.000	36.000
1.22	38.000	38.000	36.000	38.000	40.000	39.000	37.000	40.000	39.000	40.000
2.44	41.000	32.000	37.000	37.000	34.000	37.000	38.000	36.000	36.000	35.000
4.88	37.000	37.000	39.000	28.000	36.000	39.000	35.000	35.000	36.000	34.000
9.75	29.000	26.000	29.000	32.000	31.000	17.000	16.000	0.000	0.000	29.000
19.5	11.000	10.000	12.000	11.000	11.000	9.000	12.000	12.000	13.000	13.000
39	0.000	0.000	2.000	0.000	0.000	2.000	2.000	1.000	0.000	1.000
78	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

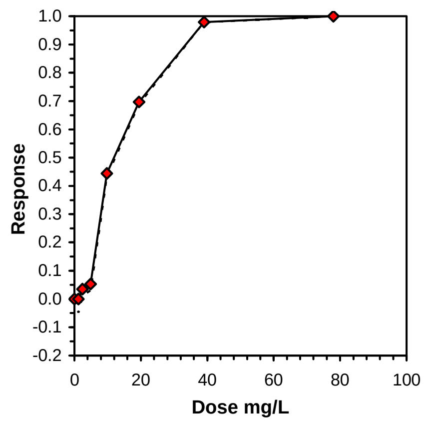
			Transform: Untransformed					Rank	1-Tailed	Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
MCW	36.700	1.0000	36.700	34.000	41.000	5.457	10			37.600	1.0000
1.22	38.500	1.0490	38.500	36.000	40.000	3.517	10	134.00	74.00	37.600	1.0000
2.44	36.300	0.9891	36.300	32.000	41.000	6.628	10	101.00	74.00	36.300	0.9654
4.88	35.600	0.9700	35.600	28.000	39.000	8.803	10	97.50	74.00	35.600	0.9468
*9.75	20.900	0.5695	20.900	0.000	32.000	58.836	10	55.00	74.00	20.900	0.5559
*19.5	11.400	0.3106	11.400	9.000	13.000	11.096	10	55.00	74.00	11.400	0.3032
*39	0.800	0.0218	0.800	0.000	2.000	114.867	10	55.00	74.00	0.800	0.0213
78	0.000	0.0000	0.000	0.000	0.000	0.000	10			0.000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.01)	1.84534	1.035	-1.9974	9.79344
Bartlett's Test indicates unequal variances (p = 1.32E-18)	96.5441	16.8119		

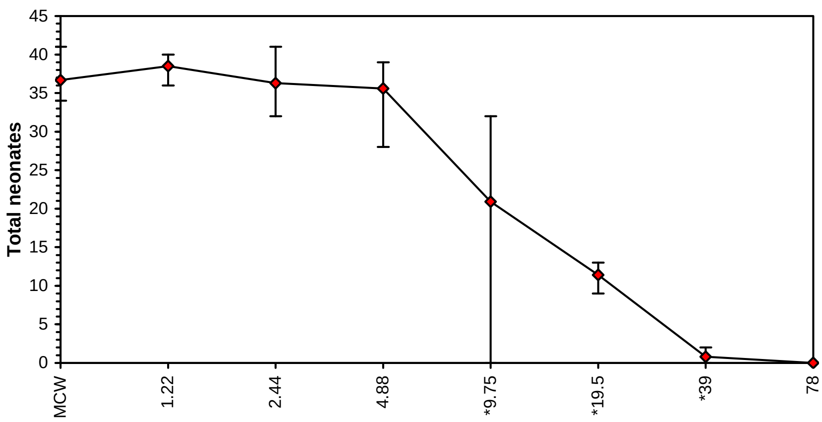
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	4.88	9.75	6.89783	
Treatments vs MCW				

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05	4.462	1.069	2.183	5.342	-0.7112
IC10	5.463	0.368	4.562	6.168	-0.5170
IC15	6.086	0.415	5.509	6.925	1.0539
IC20	6.709	0.568	6.040	7.994	1.2086
IC25	7.332	0.740	6.482	9.202	1.1367
IC40	9.200	1.399	7.866	12.824	0.6540
IC50	11.905	2.022	8.778	15.164	-0.2504



Dose-Response Plot



877B - Hyd_FBloc_03

Green Hydra Population Growth Test-Population growth rate (k

Start Date:	15/10/2007	Test ID:	877B	Sample ID:	FLOC BLOC
End Date:	19/10/2007	Lab ID:	ERISS-eriss ecotoxicology la	Sample Type:	FLOC BLOC
Sample Date:		Protocol:	BTT B-eriss tropical freshwa	Test Species:	HV-Hydra viridissima
Comments:					

Conc-gm/L	1	2	3
0	0.2574	0.2829	0.2389
39	0.2082	0.1733	0.2574
78	0.2662	0.2574	0.2389
156	0.2483	0.2483	0.2189
313	0.1733	0.1469	0.2082
625	0.1469	0.1469	0.1605
1250	0.1469	0.1327	0.1014

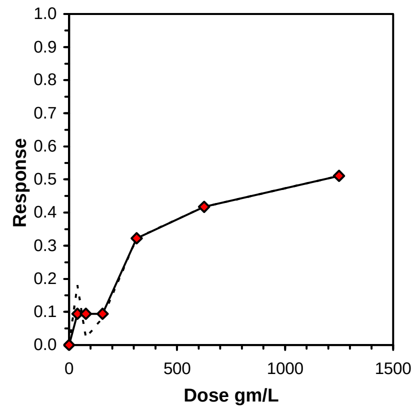
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-gm/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
0	0.2597	1.0000	0.2597	0.2389	0.2829	8.501	3				0.2597	1.0000
39	0.2130	0.8200	0.2130	0.1733	0.2574	19.843	3	2.308	2.530	0.0512	0.2352	0.9057
78	0.2542	0.9786	0.2542	0.2389	0.2662	5.484	3	0.274	2.530	0.0512	0.2352	0.9057
156	0.2385	0.9183	0.2385	0.2189	0.2483	7.128	3	1.048	2.530	0.0512	0.2352	0.9057
*313	0.1762	0.6783	0.1762	0.1469	0.2082	17.451	3	4.126	2.530	0.0512	0.1762	0.6783
*625	0.1515	0.5832	0.1515	0.1469	0.1605	5.153	3	5.346	2.530	0.0512	0.1515	0.5832
*1250	0.1270	0.4890	0.1270	0.1014	0.1469	18.358	3	6.554	2.530	0.0512	0.1270	0.4890

Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.98649		0.873		0.12918	-0.0551
Bartlett's Test indicates equal variances (p = 0.52)					5.1778		16.8119			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	156	313	220.971		0.05123	0.19727	0.00817	0.00062	4.5E-05	6, 14
Treatments vs 0										

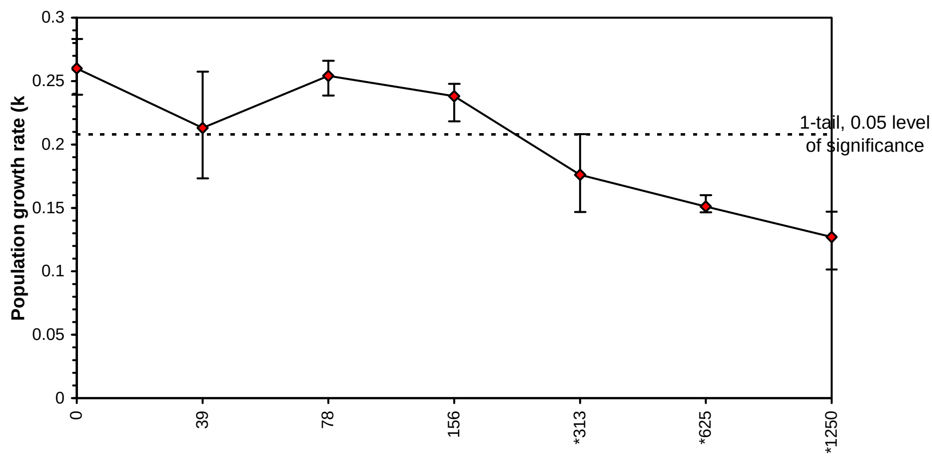
Linear Interpolation (200 Resamples)

Point	gm/L	SD	95% CL(Exp)		Skew
IC05*	20.6683	58.2368	0.88367	359.27	1.3754
IC10	159.903	74.2445	0	303.895	-0.1530
IC15	194.426	58.3926	0	351.459	-1.3387
IC20	228.949	41.0833	105.38	413.262	0.2724
IC25	263.472	49.958	141.616	553.179	1.2550
IC40	569.748	152.394	2.60248	1311.84	0.5560
IC50	1176.79				

* indicates IC estimate less than the lowest concentration



Dose-Response Plot



883B – Hyd_PAM_01

Green Hydra Population Growth Test-Population growth rate (k

Start Date:	12/11/2007	Test ID:	883B	Sample ID:	ST-Spiked Toxicant
End Date:	16/11/2007	Lab ID:	ERISS-eriss ecotoxicology lal	Sample Type:	PAM - Polyacrylamide
Sample Date:		Protocol:	BTT B-eriss tropical freshwat	Test Species:	HV-Hydra viridissima
Comments:					

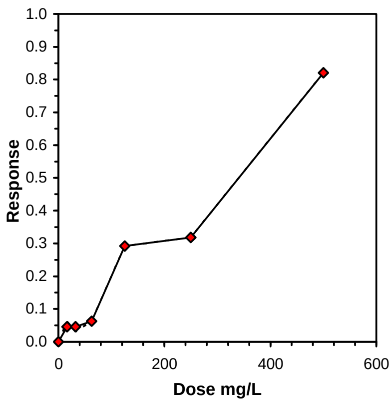
Conc-mg/L	1	2	3
MCW	0.3647	0.3588	0.3402
16.125	0.3466	0.3527	0.2985
32.25	0.3588	0.3202	0.3527
62.5	0.3059	0.3704	0.3202
125	0.2291	0.2662	0.2574
250	0.2291	0.2483	0.2483
500	0.0238	0.0656	0.1014

			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
MCW	0.3546	1.0000	0.3546	0.3402	0.3647	3.593	3				0.3546	1.0000
16.125	0.3326	0.9381	0.3326	0.2985	0.3527	8.932	3	1.046	2.530	0.0531	0.3383	0.9540
32.25	0.3439	0.9700	0.3439	0.3202	0.3588	6.028	3	0.507	2.530	0.0531	0.3383	0.9540
62.5	0.3322	0.9369	0.3322	0.3059	0.3704	10.190	3	1.065	2.530	0.0531	0.3322	0.9369
*125	0.2509	0.7076	0.2509	0.2291	0.2662	7.730	3	4.939	2.530	0.0531	0.2509	0.7076
*250	0.2419	0.6823	0.2419	0.2291	0.2483	4.592	3	5.367	2.530	0.0531	0.2419	0.6823
*500	0.0636	0.1794	0.0636	0.0238	0.1014	61.024	3	13.862	2.530	0.0531	0.0636	0.1794

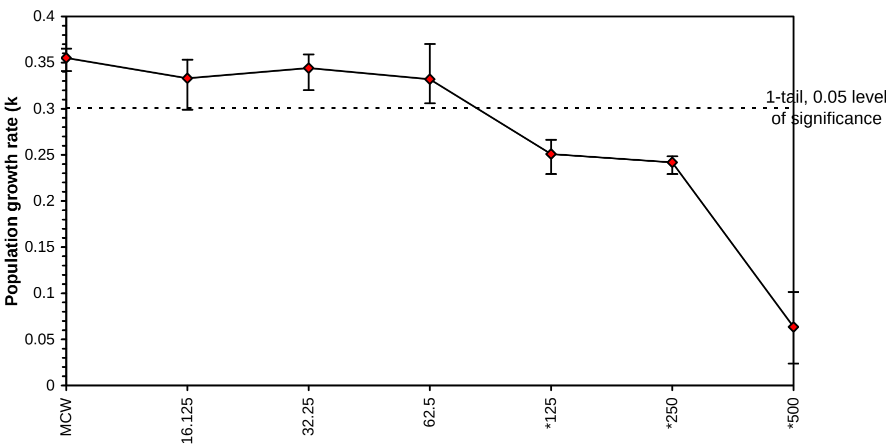
Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.95736		0.873		-0.1171	-0.5243
Bartlett's Test indicates equal variances (p = 0.67)					4.05402		16.8119			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test Treatments vs MCW	62.5	125	88.3883		0.05311	0.14978	0.03202	0.00066	1.4E-08	6, 14

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05	39.38	22.61	0.00	108.87	0.0198
IC10	72.56	13.57	0.00	95.40	-1.4683
IC15	86.19	8.45	41.74	106.37	-0.6989
IC20	99.82	7.45	64.43	124.35	-0.4180
IC25	113.44	12.16	85.57	186.66	2.6491
IC40	290.89	9.04	254.86	331.01	0.1738
IC50	340.61	11.28	297.56	385.38	0.4417



Dose-Response Plot



884D – Clad _PAM_01

Cladoceran Reproduction Test-Total neonates

Start Date:	12/11/2007	Test ID:	884D	Sample ID:	ST-Spiked Toxicant
End Date:	17/11/2007	Lab ID:	ERISS-eriss ecotoxicology la	Sample Type:	PAM - Polyacrylamide
Sample Date:		Protocol:	BTT D-eriss tropical freshwa	Test Species:	MOMA-Moinodaphnia macleayi
Comments:					

Conc-gm/L	1	2	3	4	5	6	7	8	9	10
MCW	40.000	41.000	25.000	44.000	39.000	40.000	21.000	43.000	43.000	39.000
0.5	37.000	39.000	37.000	33.000	40.000	38.000	36.000	37.000	37.000	40.000
1	36.000	39.000	31.000	34.000	36.000	34.000	31.000	38.000	37.000	38.000
2	32.000	30.000	33.000	32.000	31.000	27.000	31.000	0.000	33.000	31.000
4	27.000	24.000	25.000	27.000	26.000	24.000	28.000	25.000	26.000	28.000
8	10.000	10.000	10.000	13.000	11.000	12.000	12.000	11.000	10.000	11.000
16	0.000	0.000	3.000	3.000	3.000	5.000	5.000	0.000	0.000	0.000
32	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

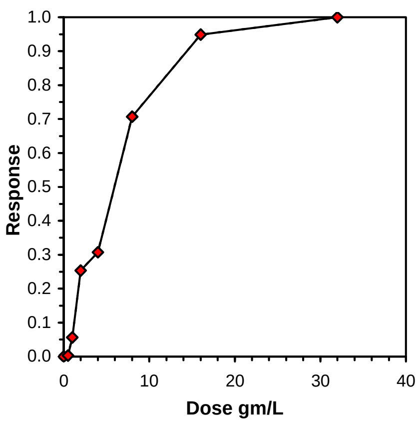
			Transform: Untransformed					Rank	1-Tailed	Isotonic	
Conc-gm/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
MCW	37.500	1.0000	37.500	21.000	44.000	21.044	10			37.500	1.0000
0.5	37.400	0.9973	37.400	33.000	40.000	5.523	10	82.00	74.00	37.400	0.9973
1	35.400	0.9440	35.400	31.000	39.000	8.012	10	76.00	74.00	35.400	0.9440
*2	28.000	0.7467	28.000	0.000	33.000	35.675	10	73.00	74.00	28.000	0.7467
*4	26.000	0.6933	26.000	24.000	28.000	5.734	10	72.00	74.00	26.000	0.6933
*8	11.000	0.2933	11.000	10.000	13.000	9.583	10	55.00	74.00	11.000	0.2933
*16	1.900	0.0507	1.900	0.000	5.000	112.198	10	55.00	74.00	1.900	0.0507
32	0.000	0.0000	0.000	0.000	0.000	0.000	10			0.000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.01)	2.15778	1.035	-3.5202	17.0352
Bartlett's Test indicates unequal variances (p = 3.75E-13)	70.1808	16.8119		

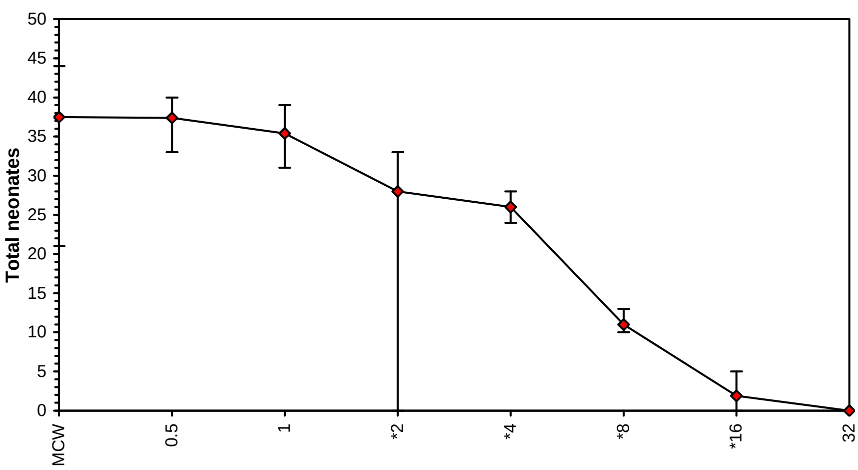
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1	2	1.41421	
Treatments vs MCW				

Linear Interpolation (200 Resamples)

Point	gm/L	SD	95% CL		Skew
IC05	0.9438	0.3437	0.2704	1.3654	-0.0754
IC10	1.2230	0.3366	0.5735	1.8381	0.2172
IC15	1.4764	0.3842	0.9528	2.4688	1.0085
IC20	1.7297	0.5353	1.2743	3.1550	1.0109
IC25	1.9831	0.7570	1.4863	3.8937	0.4310
IC40	4.9333	0.4261	4.1602	5.3289	-3.0504
IC50	5.9333	0.2670	5.3179	6.2421	-0.4458



Dose-Response Plot



887L – Lem_PAM_01

Lemna Growth Inhibition-Incr. in biomass					
Start Date:	19/11/2007	Test ID:	887L	Sample ID:	ST-Spiked Toxicant
End Date:	23/11/2007	Lab ID:	ERISS-eriss ecotoxicology la	Sample Type:	PAM-Polyacrylamide
Sample Date:		Protocol:	BTT D-eriss tropical freshwa	Test Species:	LAE-Lemna aequinoctialis
Comments:					

Conc-mg/L	1	2	3
MCW	51.000	57.000	63.000
125	62.000	40.000	54.000
250	21.000	28.000	56.000
500	16.000	24.000	27.000
1000	6.000	0.000	12.000
2000	0.000	3.000	4.000

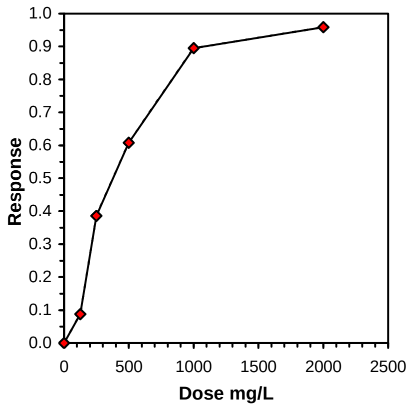
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
MCW	57.000	1.0000	57.000	51.000	63.000	10.526	3				57.000	1.0000
125	52.000	0.9123	52.000	40.000	62.000	21.414	3	0.625	2.500	19.994	52.000	0.9123
*250	35.000	0.6140	35.000	21.000	56.000	52.915	3	2.751	2.500	19.994	35.000	0.6140
*500	22.333	0.3918	22.333	16.000	27.000	25.461	3	4.335	2.500	19.994	22.333	0.3918
*1000	6.000	0.1053	6.000	0.000	12.000	100.000	3	6.377	2.500	19.994	6.000	0.1053
*2000	2.333	0.0409	2.333	0.000	4.000	89.214	3	6.835	2.500	19.994	2.333	0.0409

Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.95478		0.858		0.62911	1.38325
Bartlett's Test indicates equal variances (p = 0.17)					7.72766		15.0863			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	125	250	176.777		19.9942	0.35078	1580.09	95.9444	5.2E-05	5, 12
Treatments vs MCW										

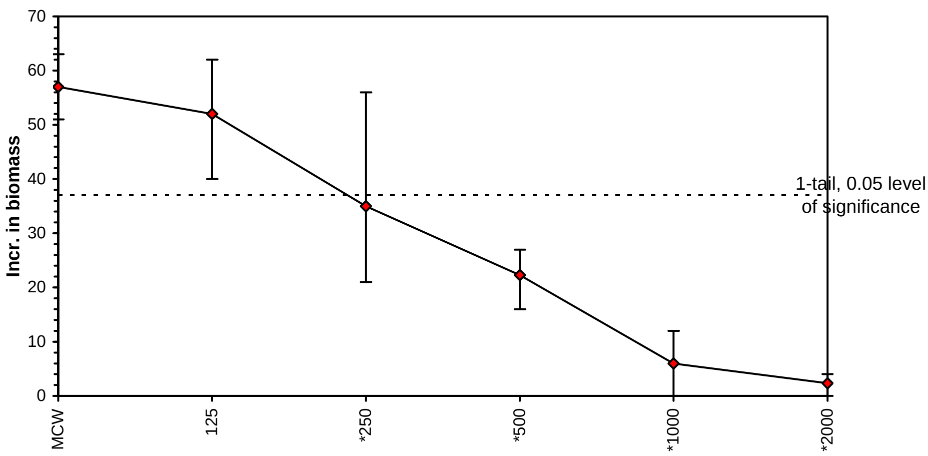
Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05*	71.25	51.34	0.00	265.51	0.8205
IC10	130.15	51.45	0.00	395.63	0.6453
IC15	151.10	51.41	0.00	417.78	0.7371
IC20	172.06	54.82	0.00	444.23	0.7258
IC25	193.01	57.75	17.61	474.68	0.6983
IC40	265.79	70.44	100.09	571.14	0.4728
IC50	378.29	85.16	46.53	576.39	-0.3159

* indicates IC estimate less than the lowest concentration



Dose-Response Plot



888G – Alg_PAM_01

Algal Growth Inhibition Test-Growth rate					
Start Date:	20/11/2007	Test ID:	888G	Sample ID:	ST-Spiked Toxicant
End Date:	23/11/2007	Lab ID:	ERISS-eriss ecotoxicology la	Sample Type:	PAM-Polyacrylamide
Sample Date:		Protocol:	BTT G-eriss tropical freshwa	Test Species:	CH-Chlorella sp.
Comments:					

Conc-mg/L	1	2	3
MCW	1.7283	1.5495	1.5603
125	1.2290	1.4085	1.4616
250	0.8979	0.9647	1.2032
500	0.8054	0.6627	0.7420
1000	0.3591	0.3611	0.3654
2000	0.0794	0.4102	0.1032

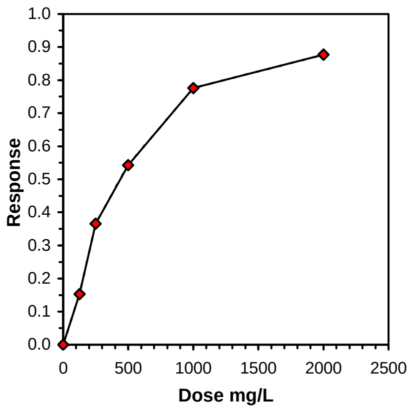
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
MCW	1.6127	1.0000	1.6127	1.5495	1.7283	6.218	3				1.6127	1.0000
125	1.3664	0.8473	1.3664	1.2290	1.4616	8.919	3	2.465	2.500	0.2498	1.3664	0.8473
*250	1.0219	0.6337	1.0219	0.8979	1.2032	15.706	3	5.913	2.500	0.2498	1.0219	0.6337
*500	0.7367	0.4568	0.7367	0.6627	0.8054	9.707	3	8.768	2.500	0.2498	0.7367	0.4568
*1000	0.3619	0.2244	0.3619	0.3591	0.3654	0.889	3	12.519	2.500	0.2498	0.3619	0.2244
*2000	0.1976	0.1225	0.1976	0.0794	0.4102	93.381	3	14.164	2.500	0.2498	0.1976	0.1225

Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.94355		0.858		0.633	-0.3549
Bartlett's Test indicates equal variances (p = 0.03)					12.3803		15.0863			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	125	250	176.777		0.24977	0.15488	0.92888	0.01497	3.7E-08	5, 12
Treatments vs MCW										

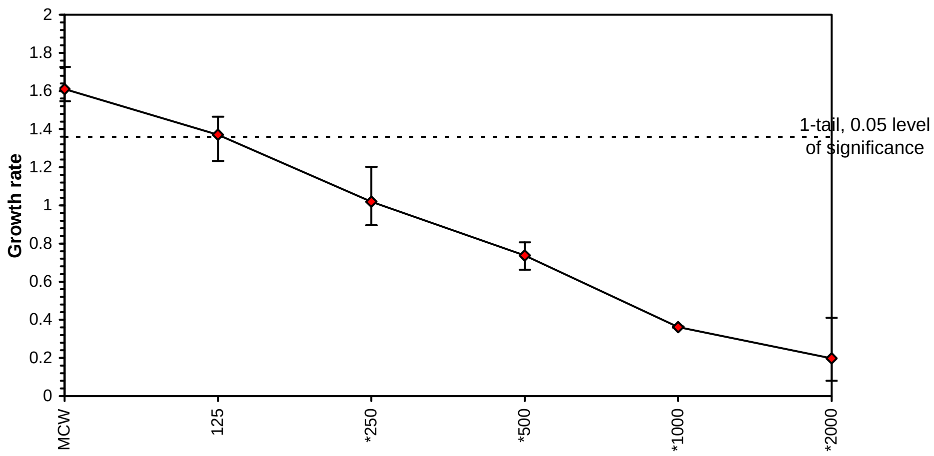
Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05*	40.92	12.84	10.16	111.60	1.2580
IC10*	81.85	22.12	20.31	188.87	0.3898
IC15*	122.77	22.63	30.47	196.15	-0.3285
IC20	152.67	22.61	52.76	237.36	-0.2419
IC25	181.93	23.26	90.05	274.80	0.3610
IC40	297.60	45.43	147.93	471.23	0.3030
IC50	438.94	37.81	276.76	581.95	-0.2479

* indicates IC estimate less than the lowest concentration



Dose-Response Plot



891E – Fry_PAM_01

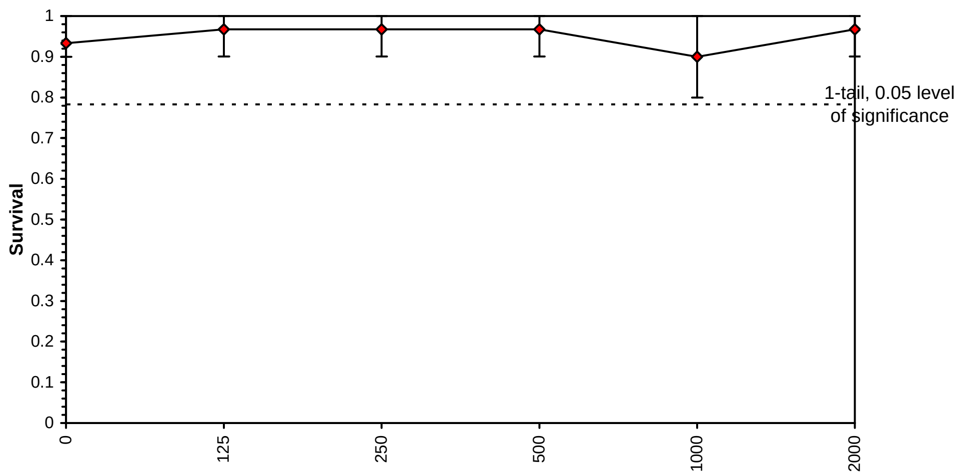
Gudgeon Sac Fry Survival Test-Survival					
Start Date:	1/12/2007	Test ID:	891E	Sample ID:	PAM
End Date:	5/12/2007	Lab ID:	ERISS-eriss ecotoxicology lal	Sample Type:	PAM
Sample Date:		Protocol:	BTT E-eriss tropical freshwat	Test Species:	MMO-Mogurnda mogurnda
Comments:					

Conc-mg/L	1	2	3
0	0.9000	1.0000	0.9000
125	0.9000	1.0000	1.0000
250	0.9000	1.0000	1.0000
500	1.0000	0.9000	1.0000
1000	0.8000	1.0000	0.9000
2000	0.9000	1.0000	1.0000

			Transform: Arcsin Square Root						1-Tailed			
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD		
0	0.9333	1.0000	1.3034	1.2490	1.4120	7.219	3				2	30
125	0.9667	1.0357	1.3577	1.2490	1.4120	6.930	3	-0.627	2.500	0.2166	1	30
250	0.9667	1.0357	1.3577	1.2490	1.4120	6.930	3	-0.627	2.500	0.2166	1	30
500	0.9667	1.0357	1.3577	1.2490	1.4120	6.930	3	-0.627	2.500	0.2166	1	30
1000	0.9000	0.9643	1.2561	1.1071	1.4120	12.145	3	0.546	2.500	0.2166	3	30
2000	0.9667	1.0357	1.3577	1.2490	1.4120	6.930	3	-0.627	2.500	0.2166	1	30

Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.88264		0.858		-0.2239	-1.1336
Bartlett's Test indicates equal variances (p = 0.98)					0.79451		15.0863			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	2000	>2000			0.14669	0.15771	0.00553	0.01126	0.77654	5, 12
Treatments vs 0										

Dose-Response Plot



892B – Hyd_PAM_02

Green Hydra Population Growth Test-Population growth rate (k

Start Date:	3/12/2007	Test ID:	892B	Sample ID:	PAM
End Date:	7/12/2007	Lab ID:	ERISS-eriss ecotoxicology la	Sample Type:	PAM
Sample Date:		Protocol:	BTT B-eriss tropical freshwa	Test Species:	HV-Hydra viridissima
Comments:					

Conc-mg/L	1	2	3
MCW	0.2829	0.2829	0.2908
16.125	0.2908	0.2483	0.2574
31.25	0.2291	0.2389	0.2389
62.5	0.2189	0.2389	0.2483
125	0.2082	0.1327	0.1605
250	0.1469	0.1733	0.1733
500	0.1605	0.1469	0.1855

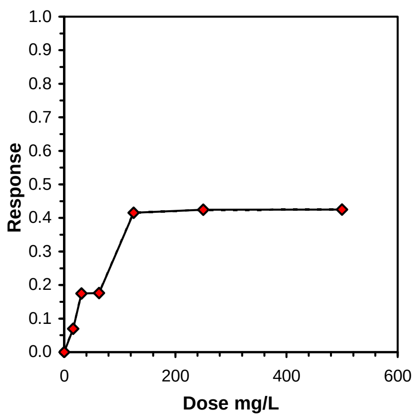
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
MCW	0.2855	1.0000	0.2855	0.2829	0.2908	1.605	3				0.2855	1.0000
16.125	0.2655	0.9300	0.2655	0.2483	0.2908	8.424	3	1.213	2.530	0.0417	0.2655	0.9300
*31.25	0.2356	0.8253	0.2356	0.2291	0.2389	2.403	3	3.026	2.530	0.0417	0.2356	0.8253
*62.5	0.2354	0.8244	0.2354	0.2189	0.2483	6.389	3	3.042	2.530	0.0417	0.2354	0.8244
*125	0.1671	0.5854	0.1671	0.1327	0.2082	22.871	3	7.181	2.530	0.0417	0.1671	0.5854
*250	0.1645	0.5762	0.1645	0.1469	0.1733	9.244	3	7.339	2.530	0.0417	0.1645	0.5762
*500	0.1643	0.5755	0.1643	0.1469	0.1855	11.901	3	7.352	2.530	0.0417	0.1643	0.5755

Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.97111		0.873		0.42001	0.86464
Bartlett's Test indicates equal variances (p = 0.18)					8.81876		16.8119			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	16.125	31.25	22.4479		0.04171	0.1461	0.00787	0.00041	5.0E-06	6, 14
Treatments vs MCW										

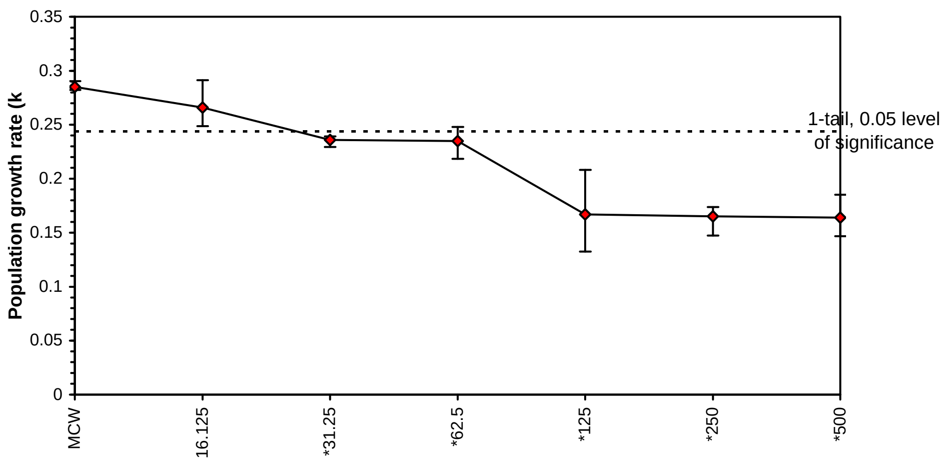
Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)		Skew
IC05*	11.51	4.81	0.32	30.43	0.4450
IC10	20.45	4.27	3.47	30.91	-0.2631
IC15	27.68	8.80	15.07	103.73	3.3418
IC20	68.87	7.88	23.06	90.52	-1.2049
IC25	81.95	7.62	62.29	122.21	1.4960
IC40	121.17				
IC50	>500				

* indicates IC estimate less than the lowest concentration



Dose-Response Plot



913G – Alg_PEG_01

Algal Growth Inhibition Test-Growth rate

Start Date:	26/02/2008	Test ID:	913G	Sample ID:	ST-Spiked Toxicant
End Date:	29/02/2008	Lab ID:	ERISS-eriss ecotoxicology lal	Sample Type:	AMB1-Ambient water
Sample Date:		Protocol:	BTT G-eriss tropical freshwat	Test Species:	CH-Chlorella sp.
Comments:					

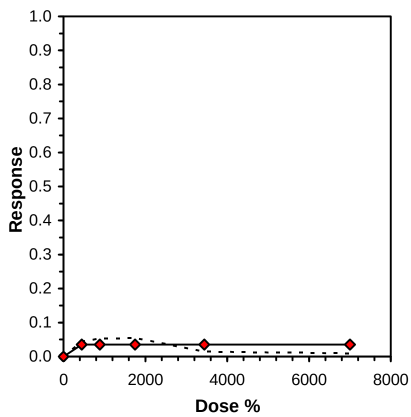
Conc-%	1	2	3
0	1.6524	1.6322	1.5366
448.5	1.5450	1.5033	1.5580
887	1.5579	1.5368	1.4720
1750	1.4937	1.5341	1.5312
3444.5	1.5715	1.6214	1.5553
7002	1.6756	1.4842	1.6155

			Transform: Untransformed						1-Tailed		Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
0	1.6071	1.0000	1.6071	1.5366	1.6524	3.847	3				1.6071	1.0000
448.5	1.5354	0.9554	1.5354	1.5033	1.5580	1.863	3	1.606	2.500	0.1116	1.5504	0.9647
887	1.5222	0.9472	1.5222	1.4720	1.5579	2.942	3	1.901	2.500	0.1116	1.5504	0.9647
1750	1.5197	0.9456	1.5197	1.4937	1.5341	1.483	3	1.958	2.500	0.1116	1.5504	0.9647
3444.5	1.5827	0.9849	1.5827	1.5553	1.6214	2.175	3	0.545	2.500	0.1116	1.5504	0.9647
7002	1.5918	0.9905	1.5918	1.4842	1.6756	6.151	3	0.343	2.500	0.1116	1.5504	0.9647

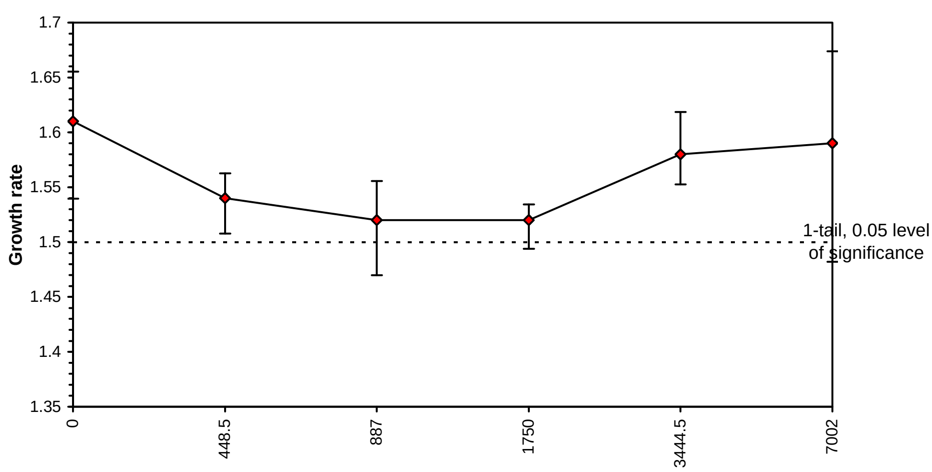
Auxiliary Tests					Statistic		Critical			Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.95521		0.858			-0.6557	0.6426
Bartlett's Test indicates equal variances (p = 0.42)					4.98722		15.0863				
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**	
Dunnett's Test	7002	>7002		0.01428	0.11157	0.06943	0.00444	0.00299	0.26515	5, 12	
Treatments vs 0											

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)	Skew
IC05	>7002			
IC10	>7002			
IC15	>7002			
IC20	>7002			
IC25	>7002			
IC40	>7002			
IC50	>7002			



Dose-Response Plot



919L – Lem_PEG_01

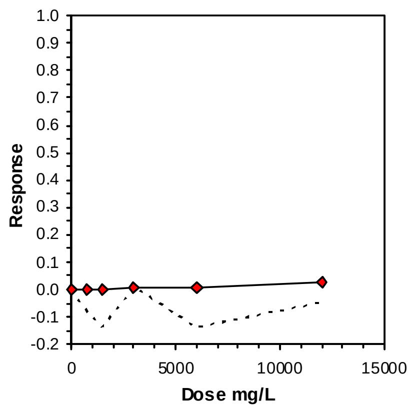
Lemna Growth Inhibition-Incr. in biomass										
Start Date:	31/03/2008		Test ID:	919L			Sample ID:		ST-Spiked Toxicant	
End Date:	4/03/2008		Lab ID:	ERISS-eriss ecotoxicology la			Sample Type:		PEG - Polyethylene gycol	
Sample Date:			Protocol:	BTT L-eriss tropical freshwat			Test Species:		LAE-Lemna aequinoctialis	
Comments:										
Conc-mg/L	1	2	3	:ntage co	1	2	3	mean	SE	
MCW	40.000	44.000	25.000		110.092	121.101	68.8073	100	15.9168	
750	41.000	41.000	36.000		112.844	112.844	99.0826	108.257	4.58716	
1500	33.000	50.000	41.000		90.8257	137.615	112.844	113.761	13.5146	
3000	39.000	28.000	41.000		107.339	77.0642	112.844	99.0826	11.1233	
6000	43.000	36.000	45.000		118.349	99.0826	123.853	113.761	7.5095	
12000	38.000	43.000	33.000		104.587	118.349	90.8257	104.587	7.94519	

			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
MCW	36.333	1.0000	36.333	25.000	44.000	27.569	3				39.000	1.0000
750	39.333	1.0826	39.333	36.000	41.000	7.339	3	-0.540	2.500	13.878	39.000	1.0000
1500	41.333	1.1376	41.333	33.000	50.000	20.576	3	-0.901	2.500	13.878	39.000	1.0000
3000	36.000	0.9908	36.000	28.000	41.000	19.444	3	0.060	2.500	13.878	38.667	0.9915
6000	41.333	1.1376	41.333	36.000	45.000	11.433	3	-0.901	2.500	13.878	38.667	0.9915
12000	38.000	1.0459	38.000	33.000	43.000	13.158	3	-0.300	2.500	13.878	38.000	0.9744

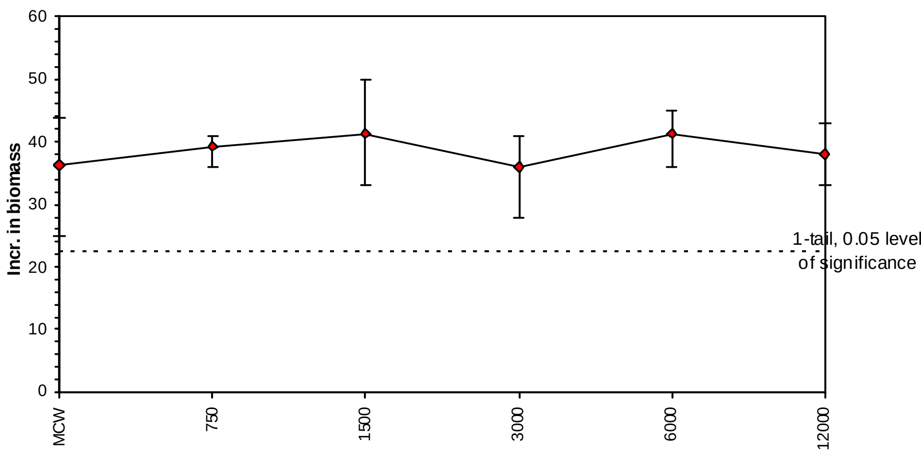
Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.95084		0.858		-0.4861	-0.6372
Bartlett's Test indicates equal variances (p = 0.70)					2.97032		15.0863			
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	12000	>12000			13.8778	0.38196	16.5889	46.2222	0.86684	5, 12
Treatments vs MCW										

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)	Skew
IC05	>12000			
IC10	>12000			
IC15	>12000			
IC20	>12000			
IC25	>12000			
IC40	>12000			
IC50	>12000			



Dose-Response Plot



920B– Hyd_PEG_01

Green Hydra Population Growth Test-Population growth rate (k										
Start Date:	8/04/2008		Test ID:	920B		Sample ID:		ST-Spiked Toxicant		
End Date:	12/04/2008		Lab ID:	ERISS-eriss ecotoxicology la		Sample Type:		PEG - Polyetheylene Gycol		
Sample Date:			Protocol:	BTT B-eriss tropical freshwa		Test Species:		HV-Hydra viridissima		
Comments:										
Conc-%	1	2	3	:ntage cc	1	2	3	mean	SE	
0	0.3400	0.3400	0.3500		99.0291	99.0291	101.942	100	0.97087	
750	0.3100	0.3100	0.3100		90.2913	90.2913	90.2913	90.2913	0	
1500	0.2800	0.2700	0.3200		81.5534	78.6408	93.2039	84.466	4.4491	
3000	0.3000	0.2900	0.2800		87.3786	84.466	81.5534	84.466	1.6816	
6000	0.3000	0.3000	0.2100		87.3786	87.3786	61.165	78.6408	8.73786	
12000	0.2600	0.2500	0.2700		75.7282	72.8155	78.6408	75.7282	1.6816	

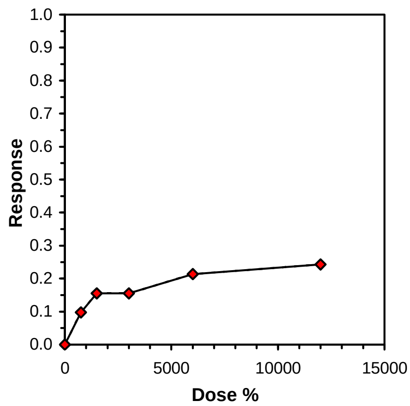
			Transform: Untransformed						1-Tailed		Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
0	0.3433	1.0000	0.3433	0.3400	0.3500	1.682	3				0.3433	1.0000
750	0.3100	0.9029	0.3100	0.3100	0.3100	0.000	3	1.659	2.500	0.0502	0.3100	0.9029
*1500	0.2900	0.8447	0.2900	0.2700	0.3200	9.123	3	2.654	2.500	0.0502	0.2900	0.8447
*3000	0.2900	0.8447	0.2900	0.2800	0.3000	3.448	3	2.654	2.500	0.0502	0.2900	0.8447
*6000	0.2700	0.7864	0.2700	0.2100	0.3000	19.245	3	3.650	2.500	0.0502	0.2700	0.7864
*12000	0.2600	0.7573	0.2600	0.2500	0.2700	3.846	3	4.148	2.500	0.0502	0.2600	0.7573

Auxiliary Tests					Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)					0.86223		0.858		-1.0766	3.5453
Equality of variance cannot be confirmed										
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	750	1500	1060.66	0.13333	0.05023	0.1463	0.00267	0.00061	0.01637	5, 12
Treatments vs 0										

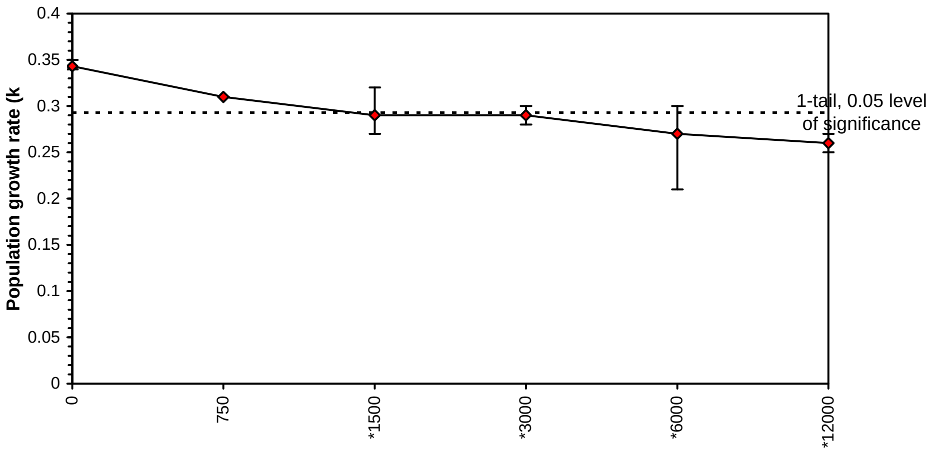
Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05*	386.25	32.1884	264.188	469.169	-0.0220
IC10	787.5	253.66	511.875	2668.98	2.5518
IC15	1431.25	1883.44	852.978	13152.3	1.2548
IC20	5300	2145.51	1597.31	15281	0.7257
IC25	>12000				
IC40	>12000				
IC50	>12000				

* indicates IC estimate less than the lowest concentration



Dose-Response Plot



923E – Fry_PEG_01

Gudgeon Sac Fry Survival Test-96 hr Survival				
Start Date: 16/04/2005	Test ID: 923E	Sample ID:	ST-Spiked Toxicant	
End Date: 20/04/2005	Lab ID: ERISS-eriss ecotoxicology la	Sample Type:	PEG - Polyetheylene Gycol	
Sample Date:	Protocol: BTT E-eriss tropical freshwa	Test Species:	MMO-Mogurnda mogurnda	
Comments:				

Conc-ug/L	1	2	3	›ntage cc	1	2	3	mean	SE
MCW	10.000	10.000	10.000		100	100	100	100	0
750	9.000	10.000	10.000		90	100	100	96.6667	3.33333
1500	9.000	10.000	10.000		90	100	100	96.6667	3.33333
3000	9.000	9.000	9.000		90	90	90	90	0
6000	7.000	6.000	7.000		70	60	70	66.6667	3.33333
12000	5.000	3.000	4.000		50	30	40	40	5.7735

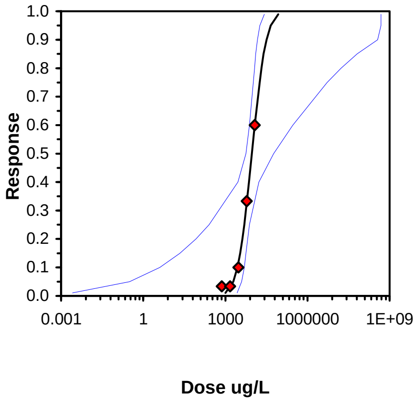
			Transform: Untransformed					1-Tailed			
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
MCW	10.000	1.0000	10.000	10.000	10.000	0.000	3				10.000	0.0000
750	9.667	0.9667	9.667	9.000	10.000	5.973	3	0.707	2.500	1.179	9.667	0.0333
1500	9.667	0.9667	9.667	9.000	10.000	5.973	3	0.707	2.500	1.179	9.667	0.0333
3000	9.000	0.9000	9.000	9.000	9.000	0.000	3	2.121	2.500	1.179	9.000	0.1000
*6000	6.667	0.6667	6.667	6.000	7.000	8.660	3	7.071	2.500	1.179	6.667	0.3333
*12000	4.000	0.4000	4.000	3.000	5.000	25.000	3	12.728	2.500	1.179	4.000	0.6000

Auxiliary Tests				Statistic		Critical		Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)				0.88473		0.858		-0.3865	0.425
Equality of variance cannot be confirmed									
Hypothesis Test (1-tail, 0.05)	**NOEC**	**LOEC**	**ChV**	**TU**	**MSDu**	**MSDp**	**MSB**	**MSE**	**F-Prob**	**df**
Dunnett's Test	3000	6000	4242.64		1.17851	0.11785	16.9	0.33333	1.2E-07	5, 12
Treatments vs MCW										

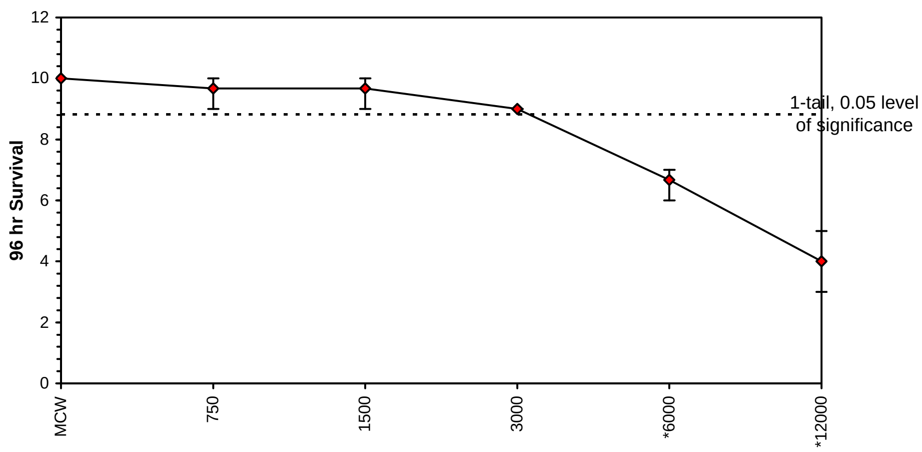
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	2.41475	1.06607	0.32525	4.50424	0	0.07487	7.81473	0.99	3.96993	0.41412	4
Intercept	-4.5864	4.13619	-12.693	3.52057							
TSCR											

Point	Probits	ug/L	95% Fiducial Limits	
EC01	2.674	1015.16	0.00258	2710.37
EC05	3.355	1944.26	0.31338	3943.49
EC10	3.718	2749.2	3.9901	4882.23
EC15	3.964	3473.08	21.9113	5714.61
EC20	4.158	4182.07	83.4317	6584.72
EC25	4.326	4904.61	256.313	7621.86
EC40	4.747	7328.39	2862.67	16687.5
EC50	5.000	9330.94	5640.64	57944.8
EC60	5.253	11880.7	7644.33	292539
EC75	5.674	17752	10467.4	5224133
EC80	5.842	20819	11621	1.7E+07
EC85	6.036	25068.9	13047.1	6.5E+07
EC90	6.282	31669.7	15006.1	3.7E+08
EC95	6.645	44781.2	18337	4.9E+08
EC99	7.326	85766.5	26411.3	4.9E+08



Dose-Response Plot



927D – Clad_PEG_01

Cladoceran Reproduction Test-Total neonates

Start Date:	16/05/2008	Test ID:	927D	Sample ID:	ST-Spiked Toxicant
End Date:	21/05/2005	Lab ID:	ERISS-eriss ecotoxicology la	Sample Type:	PEG - Polyethylene glycol
Sample Date:		Protocol:	BTT D-eriss tropical freshwa	Test Species:	MOMA-Moinodaphnia macleayi
Comments:					

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
MCW	32.000	37.000	21.000	32.000	32.000	34.000	24.000	36.000	37.000	30.000
750	32.000	33.000	27.000	35.000	33.000	31.000	0.000	34.000	32.000	34.000
1500	31.000	34.000	25.000	35.000	0.000	29.000	34.000	29.000	33.000	31.000
3000	0.000	16.000	0.000	0.000	0.000	28.000	29.000	0.000	27.000	4.000
6000	23.000	4.000	19.000	0.000	0.000	0.000	14.000	0.000	14.000	0.000
12000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

			Transform: Untransformed					Rank	1-Tailed	Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
MCW	31.500	1.0000	31.500	21.000	37.000	16.948	10			31.500	1.0000
750	29.100	0.9238	29.100	0.000	35.000	35.949	10	101.00	75.00	29.100	0.9238
1500	28.100	0.8921	28.100	0.000	35.000	36.733	10	93.00	75.00	28.100	0.8921
*3000	10.400	0.3302	10.400	0.000	29.000	125.958	10	61.00	75.00	10.400	0.3302
*6000	7.400	0.2349	7.400	0.000	23.000	123.394	10	56.00	75.00	7.400	0.2349
*12000	0.000	0.0000	0.000	0.000	0.000	0.000	10	55.00	75.00	0.000	0.0000

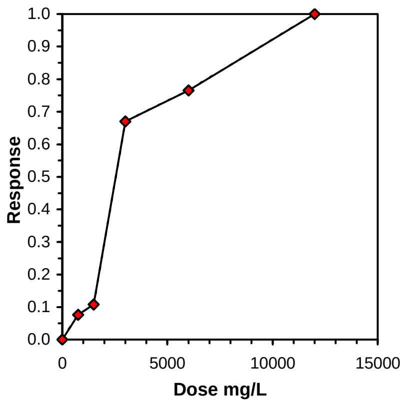
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.01)	1.43838	1.035	-0.8404	2.71049
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	1500	3000	2121.32	
Treatments vs MCW				

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL		Skew
IC05*	492.188	518.676	167.163	1633.67	0.3870
IC10	1312.5	490.972	334.325	1778.34	-0.3611
IC15	1612.29	427.064	501.488	1987.26	-0.9690
IC20	1745.76	370.974	668.65	2149.68	-1.2464
IC25	1879.24	308.166	1048.79	2312.1	-0.9164
IC40	2279.66	321.282	1713.83	2847.48	1.1971
IC50	2546.61	471.812	2092.75	3799.36	2.7237

* indicates IC estimate less than the lowest concentration



Dose-Response Plot