Technical Report TR175, Initial investigation of hydrazine toxicity to selected marine species

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Executive summary

Hydrazine is a reducing agent which to prevent corrosion is used in very low concentrations in the boiler water of Nuclear Power Plants (NPP) and consequently a liquid effluent containing residual hydrazine may be released periodically to the environment through the cooling water system.

The fate of hydrazine in the aquatic environment is dependent on dilution/dispersion, chemical and biological degradation as well as processes such as volatilisation and sedimentation with hydrazine ultimately degrading to form nitrogen.

There is evidence that hydrazine is harmful to aquatic organisms at low concentrations with the lowest acute six-day EC_{50} of 0.4 μg l^{-1} for growth inhibition of a marine alga, *Dunaliella tertiolecta* (Dixon et al, 1979). The persistence of hydrazine is low to moderate dependent upon water quality.

To supplement hydrazine toxicity data for the marine environment for which there are few existing data, this report presents additional data for the toxicity of hydrazine to a range of marine species.

A preliminary test on a marine microalgal species, *Isochrysis galbana* indicates the likelihood of particular sensitivity to hydrazine (72 hour EC_{50} <10 μg l^{-1} hydrazine). Data from the literature indicate that other marine microalgae are also sensitive to hydrazine. The data for macroalgae indicates that one species tested, a marine red macroalga, *Ceramium tenuicorne* may be of a similar sensitivity but the growth of a brown algal species, *Fucus vesiculosus* appears to be two orders of magnitude less sensitive.

Data for two marine invertebrates indicate that a representative of the marine crustacea has a similar sensitivity to hydrazine to that of some freshwater crustacean species and that marine mollusc embryos are at least an order of magnitude more sensitive.

Therefore, the toxicity data generated in this study and the reviewed literature indicate that the lowest chronic data reported is for the marine microalgal species *Dunaliella tertiolecta* with 50% growth inhibition at 0.4 μg l^{-1} hydrazine (Dixon *et al*., 1979). For three species tested in this study the 50% effect concentration was indicated to be <10 μg l^{-1}. However, for the other toxicity studies 50% effect concentrations are all >10 μg l^{-1}.

More recent assessments used in support of Canadian Federal Water Quality Guidelines for hydrazine indicate concentrations below 0.2 μg l^{-1} have a low probability of adverse effects for marine life, whilst a freshwater threshold of 2.6 μg l^{-1} is applied based on a greater availability of data in the freshwater environment (Environment Canada, 2013).

Recent modelling data for predicted hydrazine discharge concentrations during operation and commissioning for Hinkley Point (BEEMS TR445) and for Sizewell (ES Appendix 21 E, BEEMS TR306) indicate that a potential operational discharge of residual hydrazine would be ca., 69 ng l^{-1} and so below the Canadian marine standard at the point of discharge.

Much lower, acute, and chronic PNEC values of 4 and 0.4 ng l^{-1} were generated based on a subset of toxicity data (BEEMS TR193) and these are considered precautionary triggers for further ecological evaluation. For Sizewell C the areas affected by operational discharge concentrations above these more precautionary values are small at ca., 14 ha at the surface for the 4 ng l^{-1} acute value (as a 95th percentile) and 158 ha at the surface for the chronic value of 0.4 ng l^{-1}. Areas affected at the bed are considerably smaller at 0.22 and 0.56 ha for acute and chronic values, respectively. The areas influenced by hydrazine concentrations above these precautionary trigger values at Sizewell C are likely to be further reduced as the modelling uses a precautionary decay rate, and the intermittent nature of the discharge will also reduce the

length of exposure and potentially further reduce the likelihood of chronic effects. Where chlorination of cooling water occurs, this will further reduce (ca., 80%) the residual hydrazine concentration discharged.

At Hinkley Point C the modelling of the operational discharges (TR445) also indicates relatively small areas affected by hydrazine above the acute, and chronic PNEC values: For the 69 ng l^{-1} release concentration, the chronic and acute PNEC concentrations at the surface are exceeded over an area of 47.26 ha and 1.96 ha, respectively. At the seabed, the chronic and acute PNEC concentrations are exceeded over an area of 0.52 ha and 0.1 ha, respectively. As the release concentration is well below the Canadian standard of 200 ng/l, hydrazine does not exceed 200 ng l^{-1} at the surface or seabed as a 95th percentile or a maximum.

For the commissioning discharges at Hinkley Point C (TR445) and Sizewell C (TR494) the potential discharge concentrations evaluated are 10, 15 with an additional higher value 30 μg l^{-1} included for HPC. The modelled discharge rate in both cases was 83.3 l/s^{-1}. The low discharge rate and the intermittent nature of the discharge (ca., 5h per day) results in predicted rapid initial dilution at both sites. For Sizewell C the area of discharged hydrazine with a concentration greater than 200 ng l^{-1} is limited to the immediate vicinity around the construction discharge outlet (0.34 ha as a 95th percentile for the 15 μg l^{-1} release scenario, TR494). For Hinkley Point C commissioning discharge in the context of the Canadian Federal Water Quality Guidelines for hydrazine, 200 ng l^{-1}, there is no exceedance in terms of 95th percentile concentrations at the surface or bed. If maximum values are considered, then concentrations do briefly exceed 200 ng/l over an area 5.37 ha at the surface but with no exceedance at the seabed.

1 Background

Hydrazine (N_2H_4) is highly reactive, weak base and reducing agent which to prevent corrosion is used in very low concentrations in the boiler water of Nuclear Power Plants (NPP), most commonly in the form of Hydrazine hydrate ($N_2H_4.H_2O$). Cooling water circuits of boilers of nuclear reactor facilities generate a liquid effluent containing residual hydrazine, which may be released periodically to the environment through the cooling water system.

The fate of hydrazine in the aquatic environment is dependent on dilution/dispersion and chemical and biological degradation as well as processes such as volatilisation and sedimentation (Kuch, 1996). In water, hydrazine reacts with dissolved oxygen to form gaseous nitrogen (N_2), following the reaction:

$$N_2H_4 + O_2 = N_2 + 2H_2O$$

Studies on the degradation rate of hydrazine in seawater collected from Hinkley point indicate a half-life value around 8.5 hours for an initial concentration of 50 $\mu g\ l^{-1}$ but the rate of degradation also appears to increase at lower concentrations (BEEMS Technical Report TR146).

Residual hydrazine may be present in the cooling water of HPC dependent upon how the station is operated (BEEMS Technical Report TR186) and at Sizewell C (BEEMS Technical Report TR494) but due to the periodic nature of hydrazine release in boiler water its presence in the discharge will be intermittent.

There is evidence that hydrazine is harmful to aquatic organisms at low concentrations with the lowest acute six day EC_{50} of 0.4 $\mu g\ l^{-1}$ for growth inhibition of a marine alga, *Dunaliella tertiolecta* (Dixon et al, 1979) and although its persistence is low to moderate this is dependent upon water quality.

Where a substance present in the discharge has the potential to harm marine habitats a Predicted No Effect Concentration (PNEC) is usually derived so that this may be compared to the Predicted Exposure Concentration with the resulting PEC/PNEC comparison indicating the potential areas over which effects may occur. Derivation of substance PNECs is based on available toxicity data for relevant species and the application of safety factors that are consistent with the nature and quality of the available data and the guidance given in section 3.3.1 of Part II of 'Technical guidance document in support of Commission Directive 93/67/EEC (TGD, 2003) on risk assessment for new notified substances and Commission Regulation (EC) No 1488/94 on risk assessment for existing substances'. The PNEC values derived from acute short term test data are applied as 95th percentiles and chronic PNECs based on longer term chronic toxicity test data are expressed as an annual average value providing protection against long-term exposure.

An evaluation of available toxicity data for hydrazine (ELIER0600773) applied the lowest acute data for *D.tertiolecta* to derive an acute PNEC of 0.004 $\mu g\ l^{-1}$ (4 $ng\ l^{-1}$) and a chronic value of 0.0004 $\mu g\ l^{-1}$ (0.4$ng\ l^{-1}$).

1.1 Aims and Scope

The PNEC for hydrazine derived by INERIS (French National Institute for Industrial Environment and Risks) is based on a limited base set of marine data. To strengthen confidence in the risk assessment of a cooling water discharge to the marine environment that potentially contain residual hydrazine this report provides preliminary data that includes the assessment of sensitive and relevant test endpoints for selected marine species including marine molluscs and marine macroalgae. Extending the dataset for the toxicity of hydrazine to a wider range of marine species is in keeping with the recommendations of the EU technical guidance for risk assessment of chemicals (TGD, 2003) which recommends the incorporation of taxa not included in base set freshwater hazard assessments (typically algae, crustaceans, and fish).

This report therefore assesses both the acute and chronic toxicological effects of hydrazine upon marine species that are representative of different taxonomic groups to strengthen the risk assessment for the potential discharge of residual hydrazine in power station cooling water systems.

For a full evaluation of the potential impact of chemical contaminants on marine ecosystems it is necessary to consider longer-term adverse effects upon ecologically important species. It is also necessary to consider sublethal effects of contaminant exposure on sensitive life-stages. As there are few established test procedures for many groups of marine species some initial trials were necessary for the procedures described before definitive tests could be conducted.

The objective of aquatic toxicity tests is to estimate the "safe" or "no effect" concentration for a given test compound or complex effluent, which is defined as the concentration that will allow normal behaviour and functioning for exposed organisms in the receiving waters. The endpoints that have been considered include death and survival, decreased reproduction and growth, various behavioural, physiological, cellular, biochemical, and genetic changes. Since it is not feasible to detect and/or measure all of these (and other possible) effects of toxic substances on a routine basis, observations in toxicity tests are more commonly limited to only a few effects, such as mortality, growth, and reproduction.

Current assessments of toxic effects are based on convenient endpoints from laboratory studies that are relatively easy to determine e.g. the 'no observed effect concentration' which is the highest concentration tested at which no statistically detectable difference to the control value can be determined. Or if regression type analyses are applied the concentration affecting a given percentage of organisms can be derived e.g. the EC_x where x maybe 50 or 10% of the organisms tested.

Acute lethality is generally easy to evaluate and is relevant and important to population level impacts. The results of this type of test are usually expressed as the concentration lethal to 50% of test organisms (LC_{50}) over relatively short exposure periods of 1– 4 days.

By increasing exposure periods and/or observing sublethal effects on sensitive life stages (e.g. embryos and juveniles) of different species more accurate, direct, estimates of the threshold or safe concentration of the toxicant could be obtained. Where a test endpoint is not mortality but some other effect usually affecting 50% of the exposed organism these may be termed an 'effect concentration' or EC_{50}. When growth is the effect being investigated the inhibitory concentration is the term applied to observed reductions in growth, hence IC_{50}.

The use of short-term toxicity tests including subchronic and chronic tests in risk assessment is especially attractive because they provide a more direct estimate of the safe concentrations of chemicals in receiving waters than is provided by acute toxicity tests, at an only slightly increased level of effort, compared to full life-cycle chronic tests.

In environmental risk assessment of chemicals, the prediction of true no-effect or safe concentrations is based on laboratory derived NOEC (No Observed Effect Concentration) values. However, the use of these values has been widely criticised (*e.g.* Isnard et. al., 2001; Kooijman et. al., 1996; Van der Hoeven, 2004). The main criticisms are that the NOEC increases with fewer replicates (e.g. as experimental design becomes less robust) and in addition it is dependent on experimental design and can only take the values of the tested concentrations and confidence limits cannot be applied. Concern regarding the use of NOECs has led to a preference for regression-based techniques with some authors (e.g. Isnard et. al., 2001) suggesting that the lower confidence limit of the 10% effect concentration may be an alternative to the use of NOECs and that does not result in major changes to the risk assessment procedure.

Due to the delay in re commissioning sea water test systems at the Cefas laboratory in Lowestoft after a major refit, preliminary studies were conducted using a static replacement test design. As hydrazine degrades relatively rapidly in natural sea water (half-life approximately 8 hours) except for the micro algal

test the test solutions were renewed every 24 hours using artificial sea water as the dilution medium as hydrazine degradation was found to be reduced relative to the rate observed in natural sea water.

2 Methods

2.1 Preparation of the hydrazine dilution series for toxicity testing

All toxicity tests were conducted using static replacement of test solutions every 24 hours. A primary hydrazine stock solution (Stock A, 1 $g.l^{-1}$) was produced by diluting 0.165g of hydrazine dihydrochloride (H_4N_2 - 2HCl, 104.97g.mol, CAS number 5341-61-7 Sigma-Aldrich) in 50ml of artificial seawater (Table 1). A working stock (Stock B, 0.1$g.l^{-1}$) was then produced by diluting 5mls of stock A with 45mls of artificial seawater. The working stock solution was further diluted using artificial sea water (Table 1) to produce a dilution series of hydrazine concentrations for each test. Test organisms were exposed to hydrazine solutions in polystyrene well plates (details as described for each species). A series of parallel test solutions were also held in glass beakers to enable sufficient test solution to be sampled for chemical analysis. Chemical analysis was also conducted on samples within the polystyrene plates using a less sensitive method to enable some comparison of relative degradation rates between solution renewal for the higher concentration test solutions in the plates and in the glass beakers. Test plates were held in a constant temperature room (15± 2°C) for the duration of each test unless stated otherwise in the individual test descriptions.

In all experiments there was a control treatment (<0.010 $\mu g.l^{-1}$ hydrazine) and 5 nominal concentrations of hydrazine (see Table 4 for nominal and measured hydrazine concentrations in each test). Hydrazine was measured in the initial test solutions just after make-up and after 24 hours using the method described in BEEMS TR146 (detection limit 10 $\mu g.l^{-1}$ hydrazine).

Water quality was recorded daily (temperature, dissolved oxygen, pH and salinity) using a calibrated water quality meter. Observations were also made of each replicate in terms of mortality or growth as relevant (see details in methods sections for each specific test). All tests with the exception of the oyster *Crassostrea gigas* were performed in a constant temperature room maintained at 15 ± 2°C, the oyster test was run at 24 ± 2°C. Salinity and pH in the artificial sea water media were 29 - 34 ppt and 7.6 - 8.4 respectively. Dissolved oxygen was above 80% saturation in all of the tests.

For preparation of the artificial seawater a sodium bicarbonate ($NaHCO_3$) stock solution was made up with 5.04g dissolved in 200mls Reverse Osmosis water and then 0.2mls of this stock solution was added per 100mls (2ml per L) of artificial seawater. The salinity of the solution is high and therefore needs to be diluted, once fully mixed – so 85.7mls (per 1l) of the solution is replaced with Reverse Osmosis water. After preparation the artificial sea water was filtered through a 0.2µm Millipore filter into an appropriately sized schott bottle and autoclaved and stored ready for use.

Table 1 Artificial sea water composition

Chemical	Formula	Amount (mg) to add per 1litre Reverse Osmosis (RO) water
Strontium chloride	$SrCl_2$	20
Boric acid	H_3BO_3	30
Potassium bromide	KBr	100
Potassium chloride	KCl	700
Calcium chloride	$CaCl_2.2H_20$	1,470
Sodium sulphate	Na_2SO_4	4,000
Magnesium chloride	$MgCl_2.6H_20$	10,800
Sodium chloride	NaCl	23,500

2.2 Tisbe battagliai

Copepods are small crustaceans that are frequently dominant secondary producers in marine zooplankton (Hart, 1990) and are accordingly important to the marine foodweb. Copepods belonging to the genus Tisbe are particularly useful for risk assessment due to their small size, relatively short lifecycle and the ease of continuous culture (e.g. Williams, 1972 and Hutchinson et al.,1994). Sublethal tests (historically termed 'subchronic tests') at sensitive life-stages have been used to improve prediction of chronic effects compared with acute lethality tests (Hutchinson et al.,1994); in the case of Tisbe, these life-stages are usually neonate nauplii or adult females.

Tisbe used for the toxicity studies were obtained from a Cefas culture (obtained from an original stock supplied by Guernsey Sea Farms). A standard 48 hour exposure study was conducted with adult copepods (Figure 1). Observations on copepod mortality in each test well were made daily using a binocular stereomicroscope (with darkfield illumination and a magnification of six to eight times).

Figure 1 Adult Tisbe battagliai

Daily the adults were observed in the cell well plates using a low power stereo microscope. Adult Tisbe were assessed as dead if after 20 seconds of gentle agitation of the test plate no movement is observed in accordance with Cefas standard operating procedure (SOP 1575).

For statistical analysis average measured concentrations over the test period were used.

The test endpoint used for statistical analysis was adult survival after 24 and 48 hours exposure.

2.3 Pacific Oyster *Crassostrea gigas* (development test)

- **Test organisms**

Conditioned Pacific oysters were purchased from Guernsey Sea Farms Limited and were sexed by the suppliers. Male and female gametes were obtained by stripping the gonads as per the method of Thain (1991).

- **Static toxicity test procedure**

The oyster embryo development test was adapted from the method of Thain (1991) and run over 24 h at target temperature 24±1°C. The test was carried out using 4.5 ml samples in polystyrene 12-well cell well plates. Test solutions in each well had a density of ≅50 oyster embryos per ml (at the 16-32 cell stage of development). After 24 hours the test was terminated by adding 0.5 ml of a 10 % buffered formalin solution and the number of normally developed larvae was subsequently analysed using a high-power light microscope (Figure 2).

The data were analysed using ToxCalc™ v5.0 (Tidepool Scientific, USA) based on the percentage inhibition at each concentration. The growth inhibition test Effect Concentration (EC) values are calculated using linear interpolation, while the NOEC and LOEC values were determined using hypothesis testing.

At the beginning and end of the test, physico-chemical measurements (dissolved oxygen, pH, salinity and temperature) were taken from the water quality beakers that have undergone the same physical conditions as for the test plates and recorded in the test sheet.

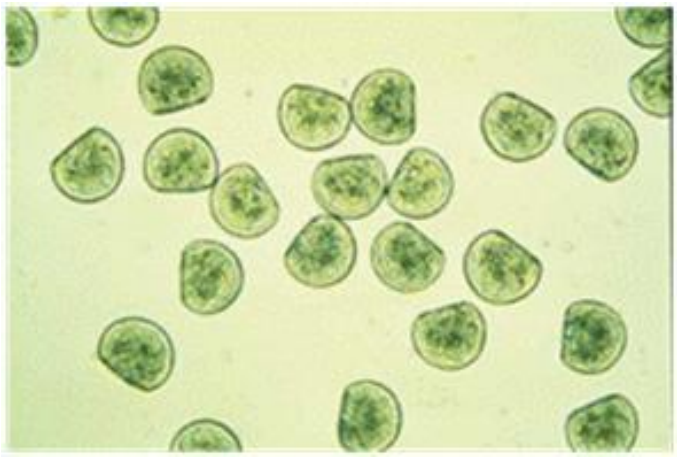


Figure 2 Normal 'D-shaped' oyster larvae after 24 hours.

2.4 Ceramium tenuicorne

Ceramium tenuicorne is a filamentous red macro algal species that can grow up to 10cm in length. It is widely distributed in temperate waters and is found in both brackish and marine waters (Ekelund, 2005). Growth is an overall expression of inhibiting effects and has been used as an endpoint by several authors both on green (Fletcher, 1989), brown (Thompson and Burrows, 1984) and red algae (Boney and Corner, 1962; Bruno and Eklund, 2003).

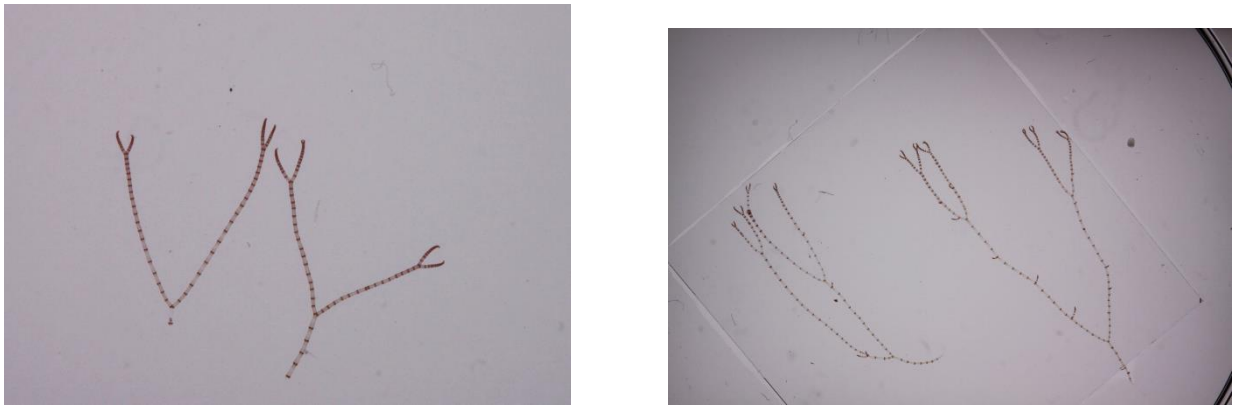
The basic approach in the test uses algal tips from Cefas monocultures adapted from salinity 20 - 30 (provided by Dr Britta Eklund at the University of Stockholm, Sweden) of *Ceramium* female gametophytes grown in defined test conditions and in a defined medium containing a range of concentrations of the test sample. The algal tips are incubated for a period of 7 d after which the increase in length is measured and the growth rate is calculated. The growth inhibition is determined as a reduction in growth rate, relative to control cultures grown under identical conditions. The growth inhibition assay was conducted according to the main principles of the international standard guideline (ISO 10710) with modifications (Eklund 2005; ISO 2010).

The growing tips (female gametophytes) of *C. tenuicorne* were cut aseptically to approx 1-2 mm long. Two pieces of *C. tenuicorne* were added to each of four test pods per 1 litre test vessel supplied with either chlorinated sea water at a range of concentrations or not chlorinated control sea water. The starting lengths of the *C. tenuicorne* were measured using light microscopy and image analysis (ImageJ program: Rasband, 97-09 http://rsb.info.nih.gov/ij/).

The *C. tenuicorne* female gametophytes were exposed for 7 days. They were kept at a light level of approximately 20 μmol (constant light) and target temperature of 15 ± 2°C. After 7 days, the test was

terminated, and the final gametophyte lengths were measured using light microscopy and image analysis (Figure 3). The EC50 was calculated using female gametophyte growth inhibition as the endpoint.

The data were analysed using ToxCalc™ v5.0 (Tidepool Scientific, USA) which then calculates the percentage inhibition at each concentration and generates the toxicity statistics. The growth inhibition test IC values are calculated using linear interpolation, while the NOEC and LOEC values were determined using hypothesis testing.



(A) (B)

Figure 3 Two specimens of C. tenuicorne female gametophytes before (A) and (B) following a 7 days exposure period

2.5 Fucus vesiculosus

As primary producer's macro algae are at the base of the marine food web but plants in the coastal ecosystem also play a key part in creating habitat for other species including important nursery areas for various fish species (Pihl et al., 1995). A number of studies have used macroalgae such as the seaweed *Fucus serratus* to look at chemical effects upon growth, photosynthesis and reproduction e.g. the movement of spermatozoa or percentage fertilisation success (Scanlan and Wilkinson, 1987), as well as the growth of Fucus germlings exposed to contaminants (Braithwaite and Fletcher, 2005, Brooks et al., 2008).

The test described here uses the growth, and germination of gametophytes of the brown macroalgae *Fucus vesiculosus* (bladder wrack), to determine chemical toxicity of test waters as per the method of Brooks et al. (2008). *Fucus vesiculosus* fronds with swollen reproductive receptacles at their ends were collected from Lowestoft beach.

Between 60 -100 receptacles are cut from the main fronds, (Figure 4) and after being rinsed in 0.2 µm filtered seawater, they are placed in a tray and covered with sea water dampened tissue and held for approximately 18 hours in a refrigerator at 2-8 ^{o}C.

Figure 4 Fucus fronds with swollen receptacles at their tips

The receptacles are then placed in filtered (0.2um) seawater and held at room temperature in the laboratory. There should be approximately 60 fronds per 200mls of seawater. To help aid spawning the beaker is placed in natural light (near a window). After 2 to 4 hours the zoospores are released, and the solution is cloudy (Figure 5). At this stage, the zoospores are collected. Using 0.2 µm filtered seawater at target temperature 15 ± 2°C, the fertilized eggs are then filtered through a 90µm mesh and onto a 25µm mesh sieve. The retained zygotes are then rinsed and diluted into a 100ml beaker to achieve a density of around 500 per ml.

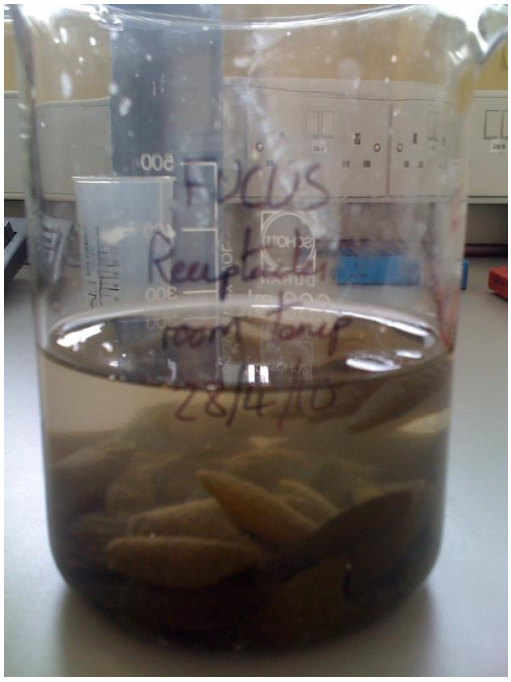


Figure 5 Fucus fronds following release of zoospores

Circular cover slips (20mm) were placed in mesh retainers (75µm) which were then placed within well plates containing natural seawater and left overnight at 20°C for the zoospores to adhere.

The following day the retainers were transferred to plates containing the test solutions, the zoospores were observed to ensure they had adhered to the cover slips. Every 24 hours the mesh retainers were transferred to a new plate with fresh hydrazine stock solution.

The study was carried out in a constant temperature room (15 ±2, with 20 µmole constant light).

Image analysis of preserved samples was carried out using Myrmica software and an Olympus S2X12 microscope. Viewing was at a magnification of 31.5x the Myrmica software was calibrated before use using a 10mm graticule.

Germlings were selected at random from within each replicate, only germlings with growth tubes longer than the diameter of the germling body were measured. In each case, photos of the slides were taken at beginning and at the end of the exposure period. For all treatments, germling tube length was measured and compared between treatments (Figure 6).

The data were analysed using ToxCalc™ v5.0 (Tidepool Scientific, USA) with percentage inhibition at each concentration calculated together with the endpoint toxicity statistics. The growth inhibition test IC values are calculated using linear interpolation, while the 96h NOEC and LOEC values were determined using hypothesis testing.

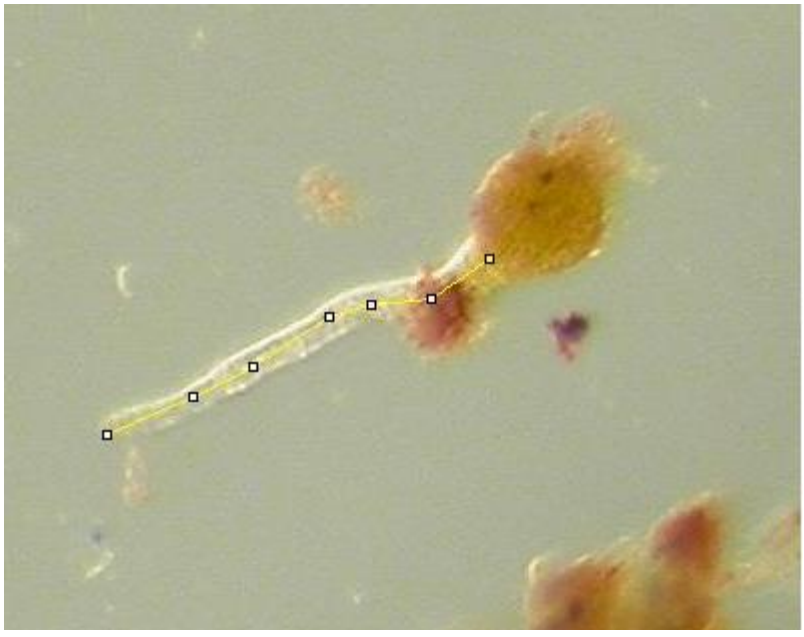


Figure 6 Normally developed germ tube on Fucus germling with point markers used in the calculation of germ tube length

- **Flagellate microalgal species (*Isochrysis galbana*) growth test**

  - **Culturing microalgae**

5 days before the beginning of the experiment the algae (*Isochrysis galbana*) was inoculated (5ml in 250ml) into artificial seawater (ASW) + Guillard's F2 medium. 24 hours before spiking test media with hydrazine, 3 ml of the algal inoculum described above was added to a new erlenmeyer flask with 124 ml of ASW + F2 medium to achieve an average concentration of $1.81E^{+4}$ cells.ml (sdve: $5.8E^{+2}$). The erlenmeyers flasks were placed in an incubator, set to shake continuously with constant light (20-30 umole) at a temperature of 20± 1°C for 72 hours.

  - **Static toxicity test procedure**

Samples for measurement of hydrazine concentration were taken at the same time as cell number evaluations and physiology were assessed: 15 minutes, 24, 48 and 72 hours.

2.6 Statistical Analyses

All statistical analyses of lethal and sublethal endpoints were performed in ToxCalc™ v5.0 (Tidepool Scientific, USA). When appropriate, survival and sub-lethal effects were analysed using linear (Probit model) or non-linear regression. Regression analyses were performed against the mean hydrazine concentration across the whole study period, including 'new' solutions just after they are made up and 'aged' solutions sampled from the test vessels after 24 hours.

The no observed effect concentration (NOEC), the lowest concentration tested that does not differ statistically from the control, was calculated for each dataset where possible. Either parametric or nonparametric hypothesis tests are recommended depending on the distribution of the data set (Weber et al., 1989). Since the derivation of the NOEC is based on a hypothesis testing procedure, it is highly dependent on the sample sizes (Oris and Bailer, 1993) and also depends on the design of the experiment because the NOEC can only be a value included in the tested concentrations.

In addition to calculation of NOECs, percentage of organisms affected by a given hydrazine concentration was calculated as either and effect concentration (EC) or inhibitory concentration (IC) based on a regression approach which involves the point estimation of an effect or inhibition concentration (e.g. IC_{50}) endpoint that causes a specified percent reduction (50%) in survival, growth or other parameter (Weber et al., 1989; Norberg-King, 1988). The 10% effect concentration with confidence limits was also derived as the lower confidence limit boundary for this value is considered to be an approximation to the NOEC (Isnard et al., 2001).

The Bartlett Homogeneity of Variance test and the Shapiro-Wilks Normality test respectively within the ToxCalc package were used to confirm if the data set met the assumptions required for a normal distribution and therefore identified appropriate tests of significance between the response of treatment groups and the control. All data analyses and outputs using ToxCalc™ v5.0 (Tidepool Scientific, USA) are shown in Appendix A.

3 Results

Of the five species tested, the filamentous red macro algal species *Ceramium tenuicorne*, and the flagellate microalga *Isochrysis galbana* were the most sensitive with 50% effect concentrations below the lowest nominal test concentration, 10 μg l^{-1}. This was the same for the oyster embryo. The brown macroalgae *Fucus vesiculosus* and *Tisbe battagliai* were the least sensitive species with the former having a 50% effect concentration >429 μg l^{-1} and the latter a value of 29 μg l^{-1} (95% Confidence limits 24-34 μg l^{-1}). Results of the toxicity tests are described in the following results section. Summaries of the statistical analyses can be found in Appendix A. Further details of the hydrazine concentration in fresh and aged test solutions measured in each of the studies are also provided in Appendix A.

3.1 *Tisbe battagliai*

The preliminary toxicity study with *T.battagliai* showed a clear dose-response relationship upon exposure to hydrazine (Table 2). The 24-h LC_{50} value for *T.battagliai* survival was >510 μg l^{-1} hydrazine with a NOEC of 51 μg l^{-1} hydrazine which is comparable to the L(E)C10 of 58 μg l^{-1} hydrazine (95% confidence limits 35 - 74 μg l^{-1}). The 48-h LC_{50} value for *T.battagliai* survival was 29 μg l^{-1} hydrazine with a NOEC of 17 μg l^{-1} hydrazine.

Table 2 Summary of 24 and 48 hour acute NOEC, L(E)C_{10} and L(E)C_{50} concentrations for *Tisbe battagliai*.

Test	NOEC	L(E)C_{10}	LCL	UCL	L(E)C_{50}	LCL	UCL
Study reference 16_03_11 (24h)	51	58	35	74	>510	-	-
Study reference 16_03_11 (48h)	17	-	-	-	29	24	34

Data are hydrazine concentrations (μg l^{-1}) with associated 95% confidence intervals (LCL – Lower Confidence Limit, UCL – Upper Confidence limit

3.2 Pacific Oyster *Crassostrea gigas* test results

Preliminary data for this species indicates that it is sensitive to hydrazine concentrations below the lowest nominal concentration tested (10 μg l^{-1}). As there were no normally developed larvae across the test concentration range no further analysis of these data was possible.

3.3 *Ceramium tenuicorne*

Growth of *C. tenuicorne* was significantly reduced relative to the control after 7 days exposure in all measured concentrations with only 20% of control growth at a nominal concentration of 10 μg l^{-1}(the lowest concentration tested).

3.4 Fucus vesiculosus

For fucus germling growth after 96 hours exposure to nominal concentrations of 10,25,50,100,250 and 500 μg l^{-1} hydrazine the IC_{50} calculated was >429 μg l^{-1} hydrazine and the IC_{10} was 49 μg l^{-1}. There was some variability in the response which meant that at a nominal concentration of 100 μg l^{-1} the germ tube length was not significantly reduced relative to the control.

Table 3 Summary of NOEC, IC_{10}, IC_{50}, Hydrazine concentrations for growth of *Fucus vesiculosus* germlings.

Test	NOEC	IC_{10}	LCL	UCL	IC_{50}	LCL	UCL
Growth (96h) (Study 31/03/2011)	-	49	39	120	>429	-	-

Data are hydrazine concentrations (μg l^{-1}) with associated 95% confidence intervals (LCL – Lower Confidence Limit, UCL – Upper Confidence limit). The data that are underlined are values derived from extrapolation below the concentration range tested and hence of low reliability

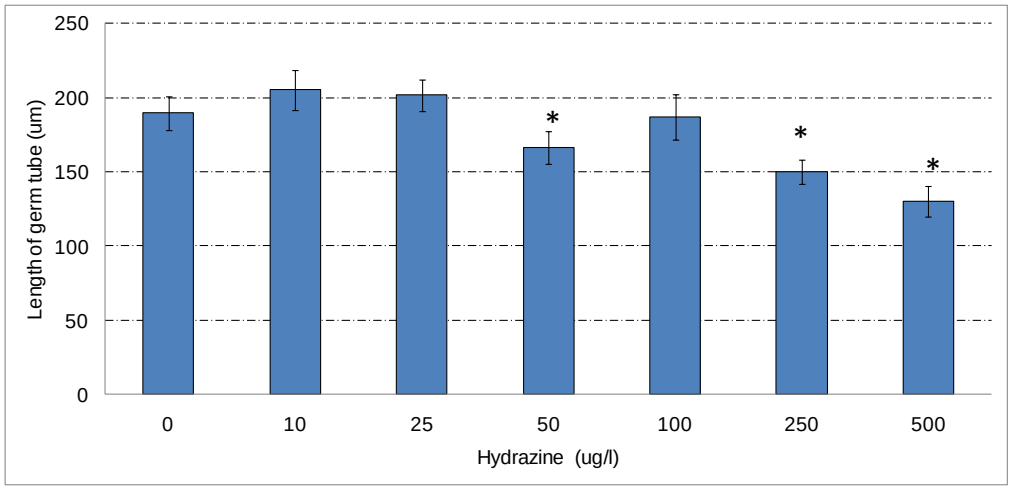


Figure 7 Growth of *F. vesiculosus* after 96 hours exposure to a range of hydrazine concentrations

Data are mean length (µm) ± 95% confidence limits (n=40) of germlings of *Fucus vesiculosus* after 96 hours of exposure to hydrazine. Asterisks indicate a statistically significant reduction in growth relative to the control using Wilcoxan Rank Sum Test (a; P < 0.05, n=40) compared to an artificial sea water alone control

3.5 Isochrysis galbana

Growth of *Isochrysis galbana* was low in all test concentrations including the control and was below the validity requirements for the test. Poor growth resulted from a failure in the lighting system which meant that light intensity was about a quarter of that recommended. There was low growth in the control group (based on the cell counts) but there was no increase in cell number in all hydrazine concentrations tested, nominally 10 - 300 µg l^{-1} hydrazine.

3.6 Quality Assurance

The measured mean hydrazine concentration in the initial test solutions were generally within 20% of the target concentration but after 24 hours at nominal concentrations of 10 µg l^{-1} there was a reduction in the test concentration of 20 - 80% of the initial measured concentration (Table 4). Actual measured concentrations averaged across the test period were used to derive EC_{50} and other effect data.

Control mortality was zero for the copepod *T.battagliai* lethal studies for this species.

Each of the tests was based on methodology from the scientific literature which has been developed into Cefas standard operating procedures. In the case of the red alga *C. tenuicorne* growth studies the approach was modified for flow through test systems from the international standard guideline (ISO 10710) with additional modification (Eklund 2005; ISO 2010) including constant lighting. The test results met the minimum growth required in the control; three times increase in seven days.

Water quality parameters measured during tests were within specified limits for each of the tests conducted except for temperature and these are detailed in Appendix A for each test. An error in the handling of water samples prior to temperature measurement has resulted in daily measurements recorded in the appendix showing higher temperatures than the specified range. As the survival and growth of species in control group was good in each of the tests (except for the failed isochrysis test) It is not considered that this deviation in the test protocol will affect the results of these studies as they were conducted in a constant temperature rooms or incubators which maintain air temperature within ±2°C.

Table 4 Nominal and mean measured hydrazine concentrations over the test period in each species test

Nominal Hydrazine Concentration ($\mu g.l^{-1}$)	***Tisbe Battaglai*** (study #2)	***Crasostrea gigas*** (study #3)	***Ceramium Ternuicorne*** (study #4)	***Fucus vesiculosus*** (study #5)	**Nominal Hydrazine Concentration ($ug.l^{-1}$)**	***Isochrysis glabana*** (study #1)
Control (<10)	<10	<10	<10	<10	Control (<10)	<10
10	18 ± 6	6 ± 3	9 ± 1	9 ± 2	5	<10
25	30 ± 4	15 ± 3	19 ± 4	20 ± 5	10	4 ± 3
50	49 ± 7	37± 4	53 ± 8	53 ± 8	30	23 ± 4
100	98 ± 6	87 ± 7	87 ± 5	87 ± 9	75	66 ± 5
250	240 ± 11	294 ± 24	210 ± 7	212 ± 11	150	138 ± 6
500	510 ± 13	500 ± 63	428 ± 13	429 ± 19	300	295 ± 27

Data are mean hydrazine concentrations ($\mu g\ l^{-1}$) ± 95% confidence limits (CL.). confidence limits were with the exception of the isochrysis test in which solutions were not changed, calculated based on the average hydrazine concentration in each of three replicate samples taken from freshly made up solutions and after 24 hours on every day on which the test was run. The same solutions were shared for the fucus and ceramium studies and hence same concentration dataset is used. Further detail on hydrazine addition, are provided in Appendix A

4 Discussion

To supplement hydrazine toxicity data for the marine environment for which there are few existing data this report presents additional preliminary data for the toxicity of hydrazine to a range of marine species.

Hydrazine degradation rate in natural sea water is rapid. Ideally toxicological studies maintain the substance to be tested within ±20% of the intended nominal concentration. To achieve this criterion for substances that degrade relatively rapidly it is necessary to maintain constant addition of the test substance to the sea water in which the test organisms are maintained. Due to the time required to get a constant dosing system prepared it was decided to conduct some preliminary static trials. To minimise degradation in these tests some initial trials were conducted to investigate hydrazine degradation in artificial sea water. The results of these trials indicated that hydrazine concentration could be maintained within 20% over 24 hours and therefore tests were conducted with replacement of test solutions every 24 hours. This test solution replacement regime was reasonably effective for hydrazine concentrations > 25 $\mu g\ l^{-1}$ but degradation in artificial sea water was higher in the lower concentration range, e.g. 50 – 60% over 24 hours. Despite the degree of degradation of hydrazine in the test solutions the toxicity data indicate that a number of the species tested are sensitive to hydrazine concentrations <10 $\mu g\ l^{-1}$. Figure 8 shows the spread of toxicity data for the species evaluated in this report. The sensitivity of micro algae to hydrazine is indicated by existing static test data e.g. an EC_{50} based on growth of 0.4 $\mu g\ l^{-1}$ for the marine green algal species *Dunaliella tertiolecta* (Dixon et al, 1979). In this study *Isochrysis galbana* had a 72 hour EC_{50} under 10 $\mu g\ l^{-1}$. Data for the red macro algae *Ceramium tenuicorne* indicates that this species may also have a 96 hour EC_{50} under 10 $\mu g\ l^{-1}$ which compares in sensitivity to data for the growth of brown algae gametophytes, 96 hour EC_{50} of 5 $\mu g\ l^{-1}$ (James, 1979). In contrast the growth rate of germlings of the brown macro algae *Fucus vesiculosus* was much less sensitive to hydrazine exposure with an EC_{50} >429 $\mu g\ l^{-1}$ hydrazine. Data for the two animal species tested, the marine copepod crustacean *Tisbe battagliai* and the oyster embryo *Crassostrea gigas* indicates a similar range of sensitivity to that of the plant species tested with a 48 hour EC_{50} of 29 $\mu g\ l^{-1}$ hydrazine for the copepod and an EC_{50} <10 $\mu g\ l^{-1}$ hydrazine for development of the oyster embryo. Literature data for freshwater crustacean species indicates that 48 hour exposure to hydrazine they are of a similar order of magnitude of sensitivity to the marine copepod e.g. 40 and 160 $\mu g\ l^{-1}$ hydrazine for *Hyallela azteca* (Fisher et al, 1980) and *Daphnia pulex* (Velte, 1984) respectively. No freshwater or marine data were available for molluscs to compare to the data in this report. The higher sensitivity of the oyster test is also influenced by the fact that it evaluates embryo development and juveniles may be more sensitive than adults.

Based on hydrazine toxicity data from the literature and on the preliminary results presented here the predicted discharge of residual hydrazine at Hinkley Point C (TR445) and Sizewell C (TR494) from operational and commissioning discharges, is likely to be well below a concentration that would be expected to have significant effects at the population level upon all the species tested to date (Figure 8). Effects may occur within the immediate vicinity of the commissioning discharge if exposure is prolonged beyond a few days based on the sensitivity range of some of the species tested but the area likely to be influenced is predicted to be very small e.g. a few hectares in the vicinity of the immediate discharge and the seabed is unlikely to be significantly affected. The intermittent nature of the discharge particularly during commissioning will also act to reduce the length of exposure and potentially further reduce any effects.

The preliminary toxicity results indicate a similar sensitivity range for marine organisms to that indicated by previous freshwater and limited marine data. Constant dosing studies are being evaluate for potential to confirm the sensitivity of some of the species tested under static conditions as hydrazine degradation in the test solution introduces more uncertainty in the calculation of definitive test endpoints.

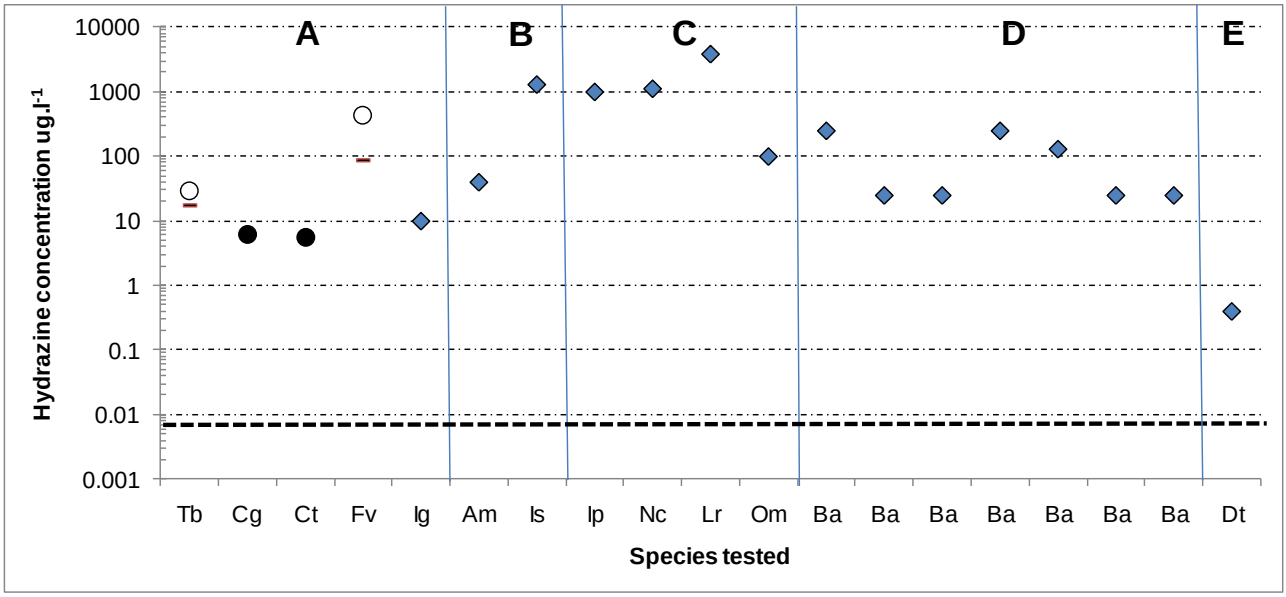


Figure 8 NOEC and equivalent values for the different species tests and endpoints in this report (covering exposure periods of 2 to 7 days). Data are shown on a logarithmic scale with the dotted line representing the predicted operational discharge concentration for hydrazine

Data are L(E)C_{50} values with NOECs (bars). All group 'A' Circle symbols are data from this study with black circles representing extrapolated values below the 10 ug.l^{-1} detection limit. Diamond symbols are literature data, group 'B' are freshwater crustacean; group 'C' are freshwater fish; group 'D' are marine macroalgal germlings and the data are lowest observed effect concentrations; and 'E' is a marine microalgae. Species abbreviations: Tisbe (Tb); Crassostrea (Cg); Ceramium(Ct); Fucus(Fv); Isochrysis (Ig); Amphipod crustacean (Am); Isopod crustacean (Is); catfish, *Ictalurus punctatus* (IP); fish, golden shiner *Notemigonus crysoleucas* (Nc); fish, guppy, *Lebistes reticulates* (Lr); rainbow trout, *Oncorhynchus mykiss* (Om); Marine brown macroalgal species (Ba); Marine microalgae, *Dunaliella tertiolecta* (Dt).

5 References

BEEMS Technical Report TR146 (2010). *Hinkley Point: Laboratory studies of the decay of Hydrazine measured in sea water samples*.

BEEMS Technical Report TR186. Predicted effects of New Nuclear Build on Water Quality at Hinkley Point. Cefas, Lowestoft

BEEMS. Technical Report TR445: Hinkley Point C Modelling of hydrazine commissioning discharge plume during commissioning and operation. Cefas, Lowestoft.

BEEMS. Technical Report TR494: Sizewell C Hydrazine Commissioning Discharge Plume Modelling. Cefas, Lowestoft.

Boney, A.D., and Corner, E.D.S. On the effects of some carcinogenic hydrocarbons on the growth of sporelings of marine red algae, Journal of the Marine Biological Association of the UK 42 (1962), pp. 579–585.

Braithwaite, R.A., Fletcher, T.R.L., 2005. The toxicity of Irgarol 1051 and Sea-Nine 211 to the non-target macroalga Fucus serratus Linnaeus, with the aid of an image capture and analysis system Journal of Experimental Marine Biology and Ecology 322, 111– 121

Brooks, S.J., Bolam T., Tolhurst, L., Bassett, J., Roche, J.L., Waldock, M., Barry, J., Thomas, K.V. Dissolved organic carbon reduces the toxicity of copper to germlings of the macroalgae, Fucus vesiculosus, Ecotoxicology and Environmental Safety, 70, pp. 88-98, 2008.

Bruno, E., and B. Eklund, Two new growth inhibition tests with the filamentous algae Ceramium strictum and C. tenuicorne (Rhodophyta), Environmental Pollution 125 (2003), pp. 287–293.

Dixon, P.S., Scherfig, J. and Justice, C.A. Use of unicellular algae for evaluation of potential aquatic contaminants. Air Force aerospace Med.Res.Lab. Report No. AMRL-TR-79-90.

Ekelund, B. Development of a growth inhibition test with the marine and brackish water red alga *Ceramium tenuicorne*. Marine Pollution Bulletin 50 (2005) 921–930

ELIER0600773, CIDEN. Summary sheets of PNEC baseline values used in the environmental impact studies for "Waste" projects, Revision No B. October 2008

Environment Canada (2011). Screening Assessment for the Challenge. Hydrazine. Chemical Abstracts Service Registry Number 302-01-2. Environment Canada/Health Canada, January 2011, pp. 1-96.: http://www.ec.gc.ca/ese-ees/17647095-B851-46F4-A4BB-79F887D84666/batch10_302-01-2_en.pdf Accessed 28th October 2011

Fletcher, R.L. A bioassay technique using the marine fouling green alga Enteromorpha, International Biodetermination 25 (1989), pp. 407–422.

Hutchinson, T.H., Williams, T.D., Eales, G.J., 1994. Toxicity of cadmium, hexavalent chromium and copper to marine fish larvae (Cyprinodon variegatus) and copepods (Tisbe battagliai). Mar. Environ. Res. 38, 275–290.

Isnard, P.,Flammarion,P.,Roman,G.,Babut,M.,Bastien,P.,Bintein,S.,Essermeant,L., Ferard, F.J.,Gallotti,S.S.,Saouter,E.,Saroli,M.,Thiebaud,H.,Tomassone,R., Vindimian, E.,2001.Statistical analysis of regulatory ecotoxicity tests.Chemosphere 45,659–669.

ISO (2010) Water quality — Growth inhibition test with the marine and brackish water macro alga Ceramium tenuicorne. ISO International Standard reference ISO 10710:2010(E), International Organization for Standardization, Geneva, Switzerland

Kooijman, S.A.L.M.,Hanstveit,A.,Nyholm,N.,1996.No-effect concentration of algal growth inhabition tests. WaterRes.30,1625–1632.

Kuch DJ. 1996. Bioremediation of hydrazine: a literature review. Report No. AL/EQ-TR-1994-0055. Armstrong Laboratory/Environics Directorate, Tyndall Air Force Base, FL 32403-5323.

Norberg-King, T.J. 1988. An interpolation estimate for chronic toxicity: The ICp approach. National effluent toxicity assessment center technical report 05-88. U.S. Environmental Protection Agency, Duluth, MN

Oris, J.T. and Bailer, A.J. 1993. Statistical analysis of the Ceriodaphnia toxicity test: sample size determination for reproductive effects. Environmemtal Toxicology and Chemistry 12, 85-90

Pihl, L., Isaksson, I., Wennhage, H., Moksnes, P.-O., 1995. Recent increase of filamentous algae in shallow Swedish bays: effects on the community structure of epibenthic fauna and fish. Netherlands Journal of Aquatic Ecology 29, 249–358.

Scanlan, C. M. and Wilkinson, M., 1987. The Use of Seaweeds in Biocide Toxicity Testing. Part 1. The Sensitivity of Different Stages in the Life-history of Fucus, and of Other Algae, to Certain Biocides. Marine Environmental Research 21, 11-29

TGD. Technical Guidance Document in support of Commission Directive 93/67/EEC on risk assessment of new notified chemicals and Commission Regulation (EC) No. 1488/94 on risk assessment of existing chemicals (1996). Luxembourg: Office for Official Publications of the European Communities; 2003. http://ecb.jrc.ec.europa.eu/home.php?CONTENU=/DOCUMENTS/TECHNICAL_GUIDANCE_DOCUMENT/EDITION_2/, accessed April 2011.

Thain J (1991) Biological effects of contaminants: the oyster (Crassostrea gigas) embryo bioassay. ICES Techniques in Marine Environmental Sciences number 11. 12 pp.

Thompson, R.S., and Burrows, E.M. The toxicity of copper, zinc, and mercury to the brown macroalga Laminaria saccharina. In: G. Persoone, E. Jaspers and C. Claus, Editors, Ecotoxicological Testing for the Marine Environment vol. 2, State Univ. Ghent and Inst. Mar. Scient. Res., Bredene, Belgium (1984), pp. 259–268.

Van der Hoeven,N.,2004.Current issues in statistics and models for ecotoxicological risk assessment. Acta Bio theor.53,201–217.

Vevers, W.F., Dixon, D.R.. and Dixon, L.R.J. 2010. The role of hydrostatic pressure on developmental stages of Pomatoceros lamarcki (Polychaeta: Serpulidae) exposed to water accommodated fractions of crude oil and positive genotoxins at simulated depths of 1000–3000 m Environmental Pollution 158 (2010) 1702–1709

Water Framework Directive (WFD), European Commission. 2003. Common implementation strategy for the water framework directive (2000/60/EC): Transitional and coastal waters – Typology, Reference conditions and classification systems, Guidance Document No 5. Office for official publications of the European Communities.
http://circa.europa.eu/Public/irc/env/wfd/library?l=/framework_directive/guidance_documents&vm=detailed&sb=Title

Weber, C.I., Peltier, W.H., Norberg-King, T.J., Horning, W.B., Kessler, F.A., Menkedick, J.R., Neiheisel, T.W., Lewis, P.A., Klemm, D., Pickering, Q.H., Robinsin, E.L., Lazorchak, J.M., Wymer, L.J., and Freyberg,

R.W. 1989. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, 2nd ed. Cincinnati, Ohio: U.S. Environmental Protection Agency.

Williams, T.D., 1992. Survival and development of copepod larvae Tisbe battagliai in surface microlayer, water and sediment elutriates from the German Bight. Mar. Ecol. Prog. Ser. 91, 221–228.

6 Appendix A: Toxicity test data analysis, hydrazine concentration and water quality data for each study reported

Table 5 Acute Toxicity Test data analysis for *Tisbe battagliai* (24 h) using ToxCalc™ v5.0 (Tidepool Scientific, USA). (Study Reference #2)

BEEMS tisbe-24 Hr Survival												
Start Date:	16/03/2011		Test ID:	C5045G			Sample ID:		Hydrazine			
End Date:	18/03/2011		Lab ID:	CEFAS-Cefas Lowestoft			Sample Type:		EFF3-Power Plant			
Sample Da			Protocol:	TB-Tisbe ISO protocol			Test Species:		TB-Tisbe Battagliai			
Comments												

Conc-ug/L	1	2	3	4
B-Control	1.0000	1.0000	1.0000	1.0000
16.88	1.0000	1.0000	1.0000	1.0000
28.48	1.0000	1.0000	1.0000	1.0000
51.32	1.0000	0.8000	1.0000	1.0000
98.23	0.4000	0.8000	0.4000	0.6000
241.57	0.8000	1.0000	0.6000	0.4000
509.59	1.0000	0.6000	0.2000	0.4000

Conc-ug/L	Mean	N-Mean		Resp	Not Resp	Total	N	Fisher's Exact P	1-Tailed Critical		Isotonic Mean	Isotonic N-Mean
B-Control	1.0000	1.0000		0	20	20	4				1.0000	1.0000
16.88	1.0000	1.0000		0	20	20	4	1.0000	0.0500		1.0000	1.0000
28.48	1.0000	1.0000		0	20	20	4	1.0000	0.0500		1.0000	1.0000
51.32	0.9500	0.9500		1	19	20	4	0.5000	0.0500		0.9500	0.9500
*98.23	0.5500	0.5500		9	11	20	4	0.0006	0.0500		0.6250	0.6250
*241.57	0.7000	0.7000		6	14	20	4	0.0101	0.0500		0.6250	0.6250
*509.59	0.5500	0.5500		9	11	20	4	0.0006	0.0500		0.5500	0.5500

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Fisher's Exact Test	51.32	98.23	71.00115	
Treatments vs B-Control				

Linear Interpolation (200 Resamples)

Point	ug/L	SD	95% CL(Exp)		Skew
IC05	51.32	7.09	26.96	64.98	-0.5835
IC10	58.54	6.15	34.81	74.30	-0.3063
IC15	65.75	13.81	42.66	87.69	10.1020
IC20	72.97	15.51	57.09	102.66	10.1706
IC25	80.19				
IC40	330.91				
IC50	>509.59				

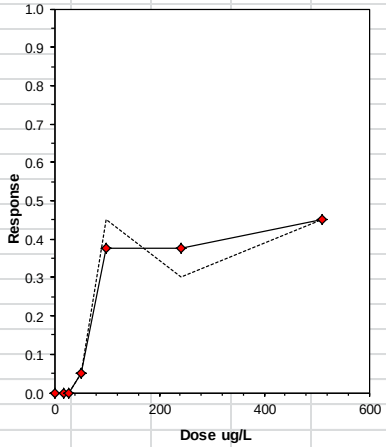


Table 6 Acute Toxicity Test data for *Tisbe battagliai* (48 h) using ToxCalc™ v5.0 (Tidepool Scientific, USA). (Study Reference #2)

BEEMS tisbe-48 Hr Survival												
Start Date:	16/03/2011		Test ID:	C5045G			Sample ID:		Hydrazine			
End Date:	18/03/2011		Lab ID:	CEFAS-Cefas Lowestoft			Sample Type:		EFF3-Power Plant			
Sample Da			Protocol:	TB-Tisbe ISO protocol			Test Species:		TB-Tisbe Battagliai			
Comments												

Conc-ug/L	1	2	3	4
B-Control	1.0000	1.0000	1.0000	1.0000
16.88	1.0000	1.0000	1.0000	1.0000
28.48	0.8000	0.4000	0.0000	0.0000
51.32	0.0000	0.0000	0.0000	0.2000
98.23	0.0000	0.0000	0.0000	0.0000
241.57	0.2000	0.2000	0.0000	0.0000
509.59	0.0000	0.0000	0.0000	0.0000

Conc-ug/L	Mean	N-Mean	Resp	Not Resp	Total	N	Fisher's Exact P	1-Tailed Critical	Number Resp	Total Number
B-Control	1.0000	1.0000	0	20	20	4			0	20
16.88	1.0000	1.0000	0	20	20	4	1.0000	0.0500	0	20
*28.48	0.3000	0.3000	14	6	20	4	0.0000	0.0500	14	20
*51.32	0.0500	0.0500	19	1	20	4	0.0000	0.0500	19	20
*98.23	0.0000	0.0000	20	0	20	4	0.0000	0.0500	20	20
*241.57	0.1000	0.1000	18	2	20	4	0.0000	0.0500	18	20
*509.59	0.0000	0.0000	20	0	20	4	0.0000	0.0500	20	20

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Fisher's Exact Test	16.88	28.48	21.92584	
Treatments vs B-Control				

Trimmed Spearman-Karber			
Trim Level	EC50	95% CL	
0.0%	28.942	24.428	34.291
5.0%	25.935	22.754	29.561
10.0%	25.533	22.139	29.447
20.0%	24.857	21.157	29.205
Auto-0.0%	28.942	24.428	34.291

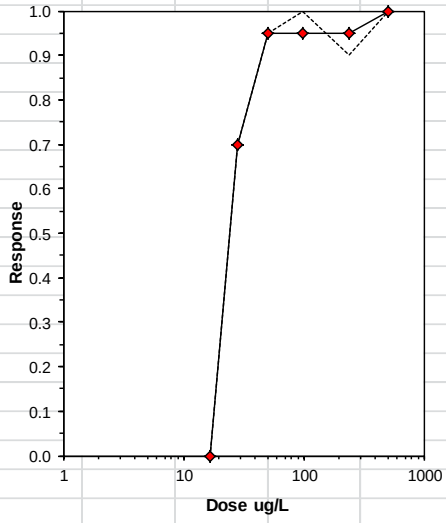


Table 7 Water quality data for *Tisbe battagliai* (24 and 48 h) (Study Reference #2)

Concentration µg l^{-1}	Time	Temperature	pH	Salinity	DO saturation%
Control	0 hrs	24	8.3	30	97
Control	24 hrs	23	8.1	30	92
10	0 hrs	22	8.4	30	93
	24 hrs (fresh)	23	8.2	30	92
	48 hrs (aged)	18	7.7	29	101
25	0 hrs	22	8.4	30	95
	24 hrs (aged)	19	5.8	30	93
	24 hrs (fresh)	23	8.3	30	92
	48 hrs (aged)	18	7.8	30	102
50	0 hrs	22	8.4	30	94
	24 hrs (aged)	17	7.7	30	102
	24 hrs (fresh)	23	8.3	30	102
	48 hrs (aged)	18	8.0	30	103
100	0 hrs	22	8.3	30	94
	24 hrs (aged)	17	7.8	30	103
	24 hrs (fresh)	23	8.3	30	103
	48 hrs (aged)	18	7.9	30	103
250	0 hrs	23	8.3	30	93
	24 hrs (aged)	17	7.8	30	103
	24 hrs (fresh)	23	8.2	30	91
	48 hrs (aged)	18	7.9	30	104
500	0 hrs	23	8.3	30	94
	24 hrs (aged)	17	7.6	30	104
	24 hrs (fresh)	23	8.2	30	92
	48 hrs (aged)	18	7.9	30	104

(Temperatures reflect initial and final temperatures prior to and following introduction to the constant temperature room and therefore do not necessarily represent the temperature maintained during the exposure period)

Table 8 Hydrazine concentration data (ug.l^{-1}) for *Tisbe battagliai* (24 and 48 h) (Study Reference #2)

Nominal Concentration	Day 1 Fresh (0h) measured	Day 1 Fresh (0h) measured	Day 1 Fresh (0h) measured	Day 1 Aged (24h) measured	Day 1 Aged (24h) measured	Day 1 Aged (24h) measured
0	4.78	21.44	3.67	2.56	3.67	3.67
10	12.56	43.67	11.44	8.11	10.33	8.11
25	28.11	35.89	42.56	21.44	24.78	23.67
50	50.33	53.67	51.44	37.00	33.67	34.78
100	104.78	99.22	104.78	84.78	85.89	82.56
250	249.22	243.67	250.33	209.22	215.89	204.78
500	532.56	494.78	549.22	490.33	489.22	477.00

Table 9 Hydrazine concentration data (ug.l^{-1}) for *Tisbe battagliai* (24 and 48 h) (Study Reference #2)

Nominal Concentration	Day 2 Fresh (0h) measured	Day 2 Fresh (0h) measured	Day 2 Fresh (0h) measured	Day 2 Aged (24h) measured	Day 2 Aged (24h) measured	Day 2 Aged (24h) measured
0	17.00	12.56	10.33	11.44	14.78	8.11
10	18.11	12.56	13.67	19.22	37.00	20.33
25	30.33	29.22	25.89	27.00	24.78	40.33
50	77.00	51.44	60.33	49.22	45.89	47.00
100	114.78	104.78	110.33	97.00	97.00	94.78
250	254.78	267.00	261.44	249.22	241.44	232.56
500	528.11	525.89	518.11	522.56	503.67	482.56

Table 10 Water quality data for *Crassostera gigas* 24h test (Study Reference #3)

Concentration µg l^{-1}	Time	Temperature	pH	Salinity	DO saturation%
Control	0 hrs	23	8.2	31	98
Control	24 hrs	25	7.8	31	103
10	0 hrs	23	8.0	31	94
	24 hrs	25	7.6	31	99
25	0 hrs	23	8.2	31	96
	24 hrs	25	7.8	31	100
50	0 hrs	23	8.2	31	97
	24 hrs	25	7.8	31	100
100	0 hrs	23	8.2	31	97
	24 hrs	25	7.8	32	99
250	0 hrs	23	8.2	31	97
	24 hrs	24	7.8	31	99
500	0 hrs	23	8.2	31	97
	24 hrs	24	7.8	31	100

Table 11 Hydrazine concentration data (ug.l^{-1}) for *Crassostera gigas* (24h) (Study Reference #3)

Nominal Concentration	Day 1 Fresh (0h) measured	Day 1 Fresh (0h) measured	Day 1 Fresh (0h) measured	Day 1 Aged (24h) measured	Day 1 Aged (24h) measured	Day 1 Aged (24h) measured
0	-9.26	-10.37	-10.37	-9.26	-8.14	-7.03
10	0.74	2.97	2.97	11.86	8.52	7.41
25	18.52	18.52	14.08	12.97	10.74	12.97
50	42.97	40.74	40.74	36.30	32.97	30.74
100	100.74	96.30	87.41	81.86	79.63	78.52
250	314.08	324.08	322.97	285.19	264.08	256.30
500	572.97	585.19	552.97	444.08	432.97	411.86

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NOT PROTECTIVELY MARKED

Table 12 7 day Growth Inhibition Toxicity Test data for hydrazine, analysis for *Ceramium tenuicorne* (168 h) using ToxCalc™ v5.0 (Tidepool Scientific, USA). (Study Reference #4)

-7 day growth inhibition												
Start Date:	30/03/2011		Test ID:	C5045G			Sample ID:		Hydrazine			
End Date:	06/04/2011		Lab ID:	CEFAS-Cefas Lowestoft			Sample Type:		EFF3-Power Plant			
Sample Da			Protocol:	OECD Ceramium			Test Species:		Ceramium tenuicorne			
Comments												

Conc-ug/L	1	2	3	4	5	6	7	8
B-Control	1.0351	0.9123	1.1053	1.0702	1.0351	1.0877	0.7193	1.0351
8.71	0.2632	0.1404	0.1754	0.1930	0.3158	0.0877	0.2105	0.1053
19.64	0.1053	0.0526	0.0877	0.0526	0.0000	0.0702	0.0702	0.0877
52.76	0.0175	0.0526	0.0702	0.0175	0.1228	0.0877	0.0000	0.0000
87.1	0.0000	0.0526	0.0702	0.0000	0.0702	0.0000	0.0000	0.0351
212.34	0.0000	0.0877	0.0175	0.0000	0.0702	0.0526	0.0526	0.0175
429.24	0.0175	0.0000	0.0000	0.0000	0.0000	0.2105	0.0000	0.0000

			Transform: Untransformed					Rank	1-Tailed	Isotonic	
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
B-Control	1.0000	1.0000	1.0000	0.7193	1.1053	12.755	8			1.0000	1.0000
*8.71	0.1864	0.1864	0.1864	0.0877	0.3158	41.466	8	36.00	46.00	0.1864	0.1864
*19.64	0.0658	0.0658	0.0658	0.0000	0.1053	48.860	8	36.00	46.00	0.0658	0.0658
*52.76	0.0461	0.0461	0.0461	0.0000	0.1228	97.524	8	36.00	46.00	0.0461	0.0461
*87.1	0.0285	0.0285	0.0285	0.0000	0.0702	113.650	8	36.00	46.00	0.0329	0.0329
*212.34	0.0373	0.0373	0.0373	0.0000	0.0877	88.710	8	36.00	46.00	0.0329	0.0329
*429.24	0.0285	0.0285	0.0285	0.0000	0.2105	258.875	8	36.00	46.00	0.0285	0.0285

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.05)	1.146776	0.895	-0.9289	6.240104
Bartlett's Test indicates unequal variances (p = 4.29E-04)	24.46466	16.81189		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	<8.71	8.71		
Treatments vs B-Control				

Linear Interpolation (200 Resamples)					
Point	ug/L	SD	95% CL		Skew
IC05*	0.5353	0.0190	0.5036	0.5781	0.2772
IC10*	1.0706	0.0379	1.0073	1.1561	0.2772
IC15*	1.6058	0.0569	1.5109	1.7342	0.2772
IC20*	2.1411	0.0758	2.0146	2.3123	0.2772
IC25*	2.6764	0.0948	2.5182	2.8904	0.2772
IC40*	4.2822	0.1516	4.0292	4.6246	0.2772
IC50*	5.3528	0.1895	5.0365	5.7807	0.2772

* indicates IC estimate less than the lowest concentration

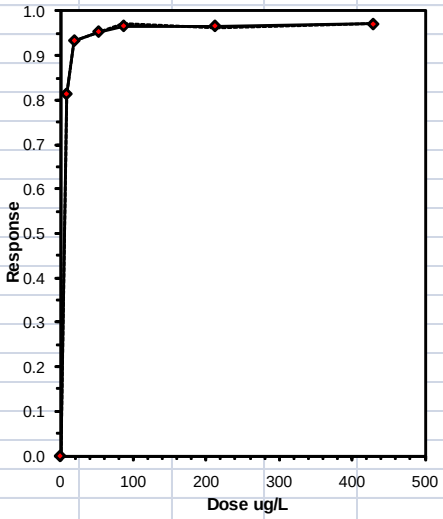

Table 13 Water quality data for *Ceramium tenuicorne* (168 h) (Study Reference #4) Control

Concentration µg l-1	Time	Temperature	pH	Salinity	DO saturation%
Control	0 hrs	16.8	7.8	34	100
Control day 1	Fresh stock	18.8	8.0	29	98
Day2	Aged stock	17	7.5	29	97
	Fresh stock	18.1	7.9	31	98
Day 3	Aged stock	16.9	8.0	31	94
	Fresh stock	20.1	7.8	31	100
Day 4	Aged stock	16.8	7.9	30	99
	Fresh stock	20.5	7.8	30	98
Day 5	Aged stock	15.2	7.8	30	99
	Fresh stock	21.5	8.0	33	99
Day 6	Aged stock	12.4	2.0	34	77
	Fresh stock	24.1	7.9	31	99
Day 7	Aged stock	20.9	7.6	31	101
	Fresh stock	17	7.5	29	97

Table 14 Water quality data for *Ceramium tenuicorne* (168 h) (Study Reference #4) nominal 10 μg l^{-1}

Concentration μg l^{-1}	Time	Temperature	pH	Salinity	DO saturation%
Control	0 hrs	17.1	7.9	34	97
Control day 1	Fresh stock	18.6	8.0	29	93
Day2	Aged stock	16.9	7.5	29	97
	Fresh stock	19.4	7.9	31	98
Day 3	Aged stock	16.9	8.0	31	94
	Fresh stock	20.1	7.8	31	99
Day 4	Aged stock	16.8	7.9	30	99
	Fresh stock	20.5	7.9	31	95
Day 5	Aged stock	15.2	7.7	31	100
	Fresh stock	21.5	7.8	33	97
Day 6	Aged stock	10.7	2.0	35	71
	Fresh stock	24.1	7.9	31	97
Day 7	Aged stock	20.7	7.5	31	101

Table 15 Water quality data for *Ceramium tenuicorne* (168 h) (Study Reference #4) nominal 25 μg l^{-1}

Concentration μg l^{-1}	Time	Temperature	pH	Salinity	DO saturation%
Control	0 hrs	17.2	8.0	34	10
Control day 1	Fresh stock	18.6	8.0	29	93
Day2	Aged stock	16.9	7.6	29	96
	Fresh stock	19.8	8.0	31	95
Day 3	Aged stock	16.9	8.0	31	98
	Fresh stock	20.1	7.8	31	99
Day 4	Aged stock	16.8	7.9	30	99
	Fresh stock	20.5	7.9	31	93
Day 5	Aged stock	15.2	7.8	31	102
	Fresh stock	21.5	7.8	33	97
Day 6	Aged stock	10.5	2.0	35	68
	Fresh stock	24.1	7.9	31	97
Day 7	Aged stock	-	-	-	-

Table 16 Water quality data for *Ceramium tenuicorne* (168 h) (Study Reference #4) nominal 50 μg l^{-1}

Concentration μg l^{-1}	Time	Temperature	pH	Salinity	DO saturation%
Control	0 hrs	17.3	8.0	34	96
Control day 1	Fresh stock	18.6	8.0	29	93
Day2	Aged stock	16.9	7.7	29	96
	Fresh stock	19.8	7.9	31	95
Day 3	Aged stock	16.9	8.0	31	98
	Fresh stock	20.1	7.9	31	99
Day 4	Aged stock	16.8	7.9	30	100
	Fresh stock	20.5	8.0	31	93
Day 5	Aged stock	15.2	7.7	30	102
	Fresh stock	21.5	7.8	33	95
Day 6	Aged stock	10.4	2.0	35	68
	Fresh stock	24.2	7.9	31	98
Day 7	Aged stock	20.7	7.5	31	100

Table 17 Water quality data for *Ceramium tenuicorne* (168 h) (Study Reference #4) nominal 100 μg l^{-1}

Concentration μg l^{-1}	Time	Temperature	pH	Salinity	DO saturation%
Control	0 hrs	17.3	8.0	34	96
Control day 1	Fresh stock	18.6	8.0	29	93
Day2	Aged stock	17	7.7	29	96
	Fresh stock	19.5	7.9	31	94
Day 3	Aged stock	16.9	8.0	31	99
	Fresh stock	20.1	7.9	31	97
Day 4	Aged stock	16.8	7.9	30	99
	Fresh stock	20.5	8.0	31	92
Day 5	Aged stock	15.2	7.8	32	94
	Fresh stock	21.5	7.8	32	94
Day 6	Aged stock	10.4	2.0	34	63
	Fresh stock	24.2	7.9	31	98
Day 7	Aged stock	20.8	7.4	31	101

Table 18 Water quality data for *Ceramium tenuicorne* (168 h) (Study Reference #4) nominal 250 μg l^{-1}

Concentration μg l^{-1}	Time	Temperature	pH	Salinity	DO saturation%
Control	0 hrs	17.3	8.0	33	95
Control day 1	Fresh stock	18.6	8.0	29	92
Day2	Aged stock	17	7.7	29	97
	Fresh stock	19.5	7.9	31	95
Day 3	Aged stock	16.9	8.0	31	99
	Fresh stock	20.1	7.9	31	97
Day 4	Aged stock	16.8	7.8	30	99
	Fresh stock	20.5	8.0	31	93
Day 5	Aged stock	15.2	7.9	30	91
	Fresh stock	21.5	7.9	30	91
Day 6	Aged stock	10.9	2.0	33	66
	Fresh stock	24.2	7.9	31	97
Day 7	Aged stock	20.9	7.5	31	104

Table 19 Water quality data for *Ceramium tenuicorne* (168 h) (Study Reference #4) nominal 500 μg l^{-1}

Concentration μg l^{-1}	Time	Temperature	pH	Salinity	DO saturation%
Control	0 hrs	17.3	8.0	33	97
Control day 1	Fresh stock	18.5	8.0	29	91
Day2	Aged stock	17	7.6	29	98
	Fresh stock	19.5	7.9	31	93
Day 3	Aged stock	16.9	8.1	31	98
	Fresh stock	20.1	7.8	31	98
Day 4	Aged stock	16.8	7.8	30	99
	Fresh stock	20.5	8.0	31	93
Day 5	Aged stock	15.2	8.0	30	92
	Fresh stock	21.5	8.0	30	92
Day 6	Aged stock	11.2	2.0	32	67
	Fresh stock	24.2	7.8	31	97
Day 7	Aged stock	21.3	7.4	31	104

(Temperatures reflect initial and final temperatures prior to and following introduction to the constant temperature room and therefore do not necessarily represent the temperature maintained during the exposure period the low pH values resulted from an error in which sample preservation occurred prior to physical readings being taken)

Table 20 Hydrazine concentration data for *Ceramium tenuicorne* (168 h) (Study Reference #4) Day 1

Nominal Concentration	Day 1 Fresh measured	Day 1 Fresh measured	Day 1 Fresh measured	Day 1 Aged measured	Day 1 Aged measured	Day 1 Aged measured
0	0.78	-0.33	0.78	-0.33	6.33	0.78
10	9.67	8.56	11.89	0.78	3.00	1.89
25	20.78	21.89	20.78	11.89	10.78	11.89
50	45.22	53.00	41.89	34.11	34.11	38.56
100	89.67	88.56	87.44	73.00	68.56	64.11
250	210.78	218.56	225.22	171.89	174.11	157.44
500	433.00	435.22	459.67	338.56	345.22	324.11

Table 21 Hydrazine concentration data for *Ceramium tenuicorne* (168 h) (Study Reference #4) continued Day 2

Nominal Concentration	Day 2 Fresh measured	Day 2 Fresh measured	Day 2 Fresh measured	Day 2 Aged measured	Day 2 Aged measured	Day 2 Aged measured
0	3.00	1.89	-1.44	33.00	-2.56	20.78
10	0.78	3.00	3.00	9.67	13.00	13.00
25	0.78	3.00	1.89	20.78	20.78	25.22
50	43.00	49.67	45.22	40.78	39.67	40.78
100	95.22	90.78	91.89	88.56	85.22	77.44
250	228.56	224.11	219.67	184.11	184.11	177.44
500	428.56	451.89	439.67	395.22	408.56	471.89

Table 22 Hydrazine concentration data for *Ceramium tenuicorne* (168 h) (Study Reference #4) continued Day 3

Nominal Concentration	Day 3 Fresh measured	Day 3 Fresh measured	Day 3 Fresh measured	Day 3 Aged measured	Day 3 Aged measured	Day 3 Aged measured
0	-0.33	0.78	3.00	1.89	0.78	1.89
10	11.89	10.78	11.89	6.33	5.22	1.89
25	24.11	28.56	19.67	1.89	3.00	4.11
50	49.67	77.44	47.44	48.56	44.11	114.11
100	96.33	109.67	93.00	85.22	87.44	93.00
250	221.89	219.67	225.22	184.11	205.22	205.22
500	436.33	430.78	427.44	441.89	446.33	441.89

Table 23 Hydrazine concentration data for *Ceramium tenuicorne* (168 h) (Study Reference #4) continued Day 4

Nominal Concentration	Day 4 Fresh measured	Day 4 Fresh measured	Day 4 Fresh measured	Day 4 Aged measured	Day 4 Aged measured	Day 4 Aged measured
0	7.44	0.78	-0.33	1.89	-2.56	3.00
10	8.56	10.78	24.11	11.89	11.89	9.67
25	24.11	74.11	24.11	19.67	23.00	19.67
50	46.33	47.44	51.89	50.78	41.89	46.33
100	88.56	97.44	93.00	88.56	84.11	97.44
250	233.00	251.89	239.67	201.89	189.67	203.00
500	439.67	436.33	487.44	406.33	428.56	414.11

Table 24 Hydrazine concentration data for *Ceramium tenuicorne* (168 h) (Study Reference #4) continued Day 5

Nominal Concentration	Day 5 Fresh measured	Day 5 Fresh measured	Day 5 Fresh measured	Day 5 Aged measured	Day 5 Aged measured	Day 5 Aged measured
0	1.89	-1.44	1.89	1.89	-2.56	3.00
10	10.78	11.89	9.67	11.89	11.89	9.67
25	31.89	25.22	24.11	19.67	23.00	19.67
50	49.67	46.33	53.00	50.78	41.89	46.33
100	109.67	117.44	119.67	88.56	84.11	97.44
250	247.44	241.89	248.56	201.89	189.67	203.00
500	489.67	475.22	497.44	406.33	428.56	414.11

Table 25 Hydrazine concentration data for *Ceramium tenuicorne* (168 h) (Study Reference #4) continued Day 6

Nominal Concentration	Day 6 Fresh measured	Day 6 Fresh measured	Day 6 Fresh measured	Day 6 Aged measured	Day 6 Aged measured	Day 6 Aged measured
0	-0.33	-1.44	0.78	0.78	4.11	0.78
10	7.44	7.44	5.22	8.56	11.89	10.78
25	24.11	19.67	19.67	23.00	16.33	15.22
50	45.22	176.33	36.33	81.89	80.78	78.56
100	80.78	87.44	87.44	44.11	46.33	37.44
250	218.56	228.56	206.33	227.44	209.67	210.78
500	491.89	456.33	428.56	423.00	425.22	431.89

Table 26 Hydrazine concentration data for *Ceramium tenuicorne* (168 h) (Study Reference #4) continued Day 7

Nominal Concentration	Day 7 Fresh measured	Day 7 Fresh measured	Day 7 Fresh measured	Day 7 Aged measured	Day 7 Aged measured	Day 7 Aged measured
0	-2.56	-0.33	4.11	Not measured	Not measured	Not measured
10	9.67	3.00	9.67	Not measured	Not measured	Not measured
25	25.22	20.78	15.22	Not measured	Not measured	Not measured
50	40.78	30.78	40.78	Not measured	Not measured	Not measured
100	91.89	89.67	87.44	Not measured	Not measured	Not measured
250	197.44	210.78	197.44	Not measured	Not measured	Not measured
500	405.22	375.22	356.33	Not measured	Not measured	Not measured

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NOT PROTECTIVELY MARKED

Table 27 Growth Inhibition Toxicity Test data for hydrazine, analysis for *Fucus vesiculosus* (96 h) using ToxCalc™ v5.0 (Tidepool Scientific, USA). (Study Reference #5)

-Frond growth										
Start Date:	31/03/2011		Test ID:	C5045G		Sample ID:		Hydrazine		
End Date:	04/04/2011		Lab ID:	CEFAS-Cefas Lowestoft		Sample Type:		EFF3-Power Plant		
Sample Da			Protocol:	BEEMS		Test Species:		Fucus serratus		
Comments										

Conc-ug/L	1	2	3	4	5	6	7	8	9	10
B-Control	208.55	191.78	214.17	174.76	186.84	213.04	159.85	245.91	132.04	166.97
B-Control	148.03	187.86	187.54	166.83	170.06	268.57	161.89	108.87	210.97	198.04
B-Control	222.71	169.40	230.80	177.33	169.24	164.57	153.94	179.12	165.27	163.02
B-Control	156.58	213.50	149.19	247.11	188.63	169.59	257.96	202.51	266.75	230.40
8.71	324.54	213.58	218.64	162.76	180.99	174.84	218.52	160.99	265.00	177.16
8.71	219.52	158.10	223.53	207.90	299.07	225.63	214.11	210.71	213.13	257.57
8.71	216.79	193.21	214.83	207.07	186.13	202.47	163.56	170.56	157.25	210.05
8.71	205.85	227.10	228.70	105.08	169.53	249.43	191.01	234.48	253.85	95.96
19.64	193.60	271.70	230.76	220.72	225.07	170.83	240.74	185.78	240.54	170.32
19.64	226.59	204.13	134.39	191.57	217.65	236.96	204.32	192.42	214.06	176.38
19.64	184.64	199.68	129.47	190.51	216.26	223.17	170.20	196.55	167.60	109.79
19.64	209.10	240.94	215.43	247.16	202.24	235.67	203.35	141.97	250.54	177.63
52.76	177.78	180.67	107.02	138.61	211.70	144.22	122.29	145.64	134.73	135.54
52.76	140.64	167.05	131.88	234.39	184.43	146.40	203.13	231.71	179.68	150.37
52.76	149.16	194.26	172.94	186.08	177.62	146.08	197.70	235.32	148.23	169.28
52.76	197.32	159.19	105.42	180.77	235.17	186.78	170.44	131.60	103.54	145.73
87.1	224.98	214.36	241.12	236.86	324.67	230.64	221.10	262.93	225.73	206.97
87.1	210.67	207.18	137.80	108.07	203.42	206.31	146.47	210.19	201.21	121.46
87.1	109.44	208.20	167.66	168.97	196.30	141.94	137.68	223.24	159.78	188.12
87.1	276.92	153.55	149.20	135.19	123.67	121.87	167.82	164.42	143.83	200.85
212.34	111.53	141.79	125.09	103.46	153.59	170.73	101.00	140.24	129.90	139.97
212.34	143.02	144.07	97.25	161.05	203.02	170.68	120.98	161.53	147.09	107.94
212.34	144.54	187.64	146.31	186.07	158.87	165.64	164.95	152.56	172.17	171.92
212.34	166.12	178.17	137.61	179.14	177.87	118.63	161.06	107.41	168.38	178.57
429.24	83.02	129.27	109.24	222.58	90.48	128.70	105.08	94.43	96.98	100.25
429.24	135.60	87.12	90.76	100.25	178.96	120.41	97.72	145.87	83.27	143.97
429.24	123.19	212.22	116.54	153.94	145.44	143.63	130.72	120.13	133.18	175.97
429.24	163.94	175.54	143.02	134.56	147.99	108.34	110.51	144.47	155.75	118.78

			Transform: Untransformed					Rank	1-Tailed		Isotonic	
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical		Mean	N-Mean
B-Control	189.50	1.0000	189.50	108.87	268.57	19.424	40				198.75	1.0000
8.71	205.23	1.0830	205.23	95.96	324.54	21.347	40	1824.00	1371.00		198.75	1.0000
19.64	201.51	1.0634	201.51	109.79	271.70	17.421	40	1832.00	1371.00		198.75	1.0000
*52.76	166.51	0.8787	166.51	103.54	235.32	21.189	40	1347.00	1371.00		176.77	0.8894
87.1	187.02	0.9869	187.02	108.07	324.67	26.041	40	1574.00	1371.00		176.77	0.8894
*212.34	149.94	0.7912	149.94	97.25	203.02	17.855	40	1137.00	1371.00		149.94	0.7544
*429.24	130.04	0.6862	130.04	83.02	222.58	25.499	40	985.00	1371.00		130.04	0.6543

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.05)	0.764216	0.895	0.233566	0.668569
Bartlett's Test indicates unequal variances (p = 8.60E-03)	17.19254	16.81189		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Wilcoxon Rank Sum Test	87.1	212.34	135.9956	
Treatments vs B-Control				

Linear Interpolation (200 Resamples)					
Point	ug/L	SD	95% CL		Skew
IC05	34.61	8.56	22.19	49.45	4.3722
IC10	49.59	26.62	38.79	120.66	0.9191
IC15	123.66	25.74	48.59	158.71	-0.9977
IC20	170.05	20.47	128.88	206.43	-0.1137
IC25	221.91	34.34	177.36	299.68	0.5766
IC40	>429.24				
IC50	>429.24				

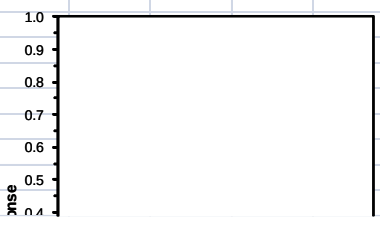

Table 28 Water quality data for *Fucus vesiculosus* (96 h) (Study Reference #5) Control

Concentration µg l-1	Time	Temperature	pH	Salinity	DO saturation%
Control	0 hrs	18.5	8.11	29	86.1
Control day 1	Fresh stock	16.8	7.89	29.1	95.5
Day2	Aged stock	20.4	8.14	29.1	92.5
	Fresh stock	16.9	8.02	29.9	97.9
Day 3	Aged stock	21.2	7.99	30.4	94.6
	Fresh stock	20.5	8.04	30.9	98.1
Day 4	Aged stock	16.8	8.09	31.2	98.9
	Fresh stock	15.4	8.04	30.2	97.5

Table 29 Water quality data for *Fucus vesiculosus* (96 h) (Study Reference #5) 10 µg l^{-1}

Concentration µg l-1	Time	Temperature	pH	Salinity	DO saturation%
0	Fresh stock	18.5	8.18	29	83.2
Day 1	Aged stock	17	7.95	29.1	91.4
	Fresh stock	20.2	8.13	29	92.6
Day2	Aged stock	16.9	8.01	29.9	98.4
	Fresh stock	21.2	8.01	30.4	97.2
Day 3	Aged stock	20.5	8.04	30.8	97.4
	Fresh stock	16.8	8.12	31.2	98.6
Day 4	Aged stock	15.4	8.02	30.5	96.2

Table 30 Water quality data for *Fucus vesiculosus* (96 h) (Study Reference #5) 25 μg l^{-1}

Concentration μg l^{-1}	Time	Temperature	pH	Salinity	DO saturation%
0	Fresh stock	18.4	8.22	29	81.1
Day 1	Aged stock	17	8.02	29.1	89.9
	Fresh stock	20.2	8.15	29	93.9
Day2	Aged stock	16.9	7.99	29.8	99.6
	Fresh stock	21.2	8.01	30.4	98.1
Day 3	Aged stock	20.5	8.04	30.7	96.2
	Fresh stock	16.8	8.14	31.2	98.4
Day 4	Aged stock	15.4	8.01	30.7	96.4

Table 31 Water quality data for *Fucus vesiculosus* (96 h) (Study Reference #5) 50 μg l^{-1}

Concentration μg l^{-1}	Time	Temperature	pH	Salinity	DO saturation%
0	Fresh stock	18.3	8.21	29	80
Day 1	Aged stock	17	8.02	29.1	90.3
	Fresh stock	20.3	8.14	29.1	90.9
Day2	Aged stock	16.9	8.04	29.8	95.2
	Fresh stock	21.2	8.02	30.3	98.2
Day 3	Aged stock	20.5	8.06	30.7	96.2
	Fresh stock	16.8	8.12	31.2	98.6
Day 4	Aged stock	15.4	8.04	29.9	97

Table 32 Water quality data for *Fucus vesiculosus* (96 h) (Study Reference #5) 100 μg l^{-1}

Concentration μg l^{-1}	Time	Temperature	pH	Salinity	DO saturation%
0	Fresh stock	18.3	8.21	29	80.1
Day 1	Aged stock	17.1	8.07	29.1	91.2
	Fresh stock	20.3	8.16	29.1	91.4
Day2	Aged stock	16.9	8.06	29.8	94.6
	Fresh stock	21.2	8.01	30.3	98.2
Day 3	Aged stock	20.5	8.07	30.7	96.9
	Fresh stock	16.8	8.12	30.9	98.9
Day 4	Aged stock	15.4	8.02	30.7	98.2

Table 33 Water quality data for *Fucus vesiculosus* (96 h) (Study Reference #5) 250 μg l^{-1}

Concentration μg l^{-1}	Time	Temperature	pH	Salinity	DO saturation%
0	Fresh stock	18.2	8.18	29	80.2
Day 1	Aged stock	17	8.03	29.1	91.6
	Fresh stock	20.3	8.18	29	91.5
Day2	Aged stock	16.9	8.02	29.8	94.6
	Fresh stock	21.2	8.01	30.3	98.4
Day 3	Aged stock	20.5	8.07	30.7	94.5
	Fresh stock	16.8	8.1	31.1	97.9
Day 4	Aged stock	15.4	7.98	31	99.5

Table 34 Water quality data for *Fucus vesiculosus* (96 h) (Study Reference #5) 500 μg l^{-1}

Concentration μg l^{-1}	Time	Temperature	pH	Salinity	DO saturation%
0	Fresh stock	18.2	8.16	29.1	80
Day 1	Aged stock	17	7.92	29	90.8
	Fresh stock	20.2	8.16	29.1	91.1
Day2	Aged stock	16.9	8.01	29.8	92.7
	Fresh stock	21.2	7.99	30.3	98.6
Day 3	Aged stock	20.5	8.08	30.6	96.2
	Fresh stock	16.8	8.1	31.1	96.4
Day 4	Aged stock	15.4	8.01	31	98.4

(Temperatures reflect initial and final temperatures prior to and following introduction to the constant temperature room and therefore do not necessarily represent the temperature maintained during the exposure period)

Table 35 Hydrazine concentration data for *Fucus vesiculosus* (96 h) (Study Reference #5) Day 1-2

Nominal Concentration	0hrs measured	Day 1 Fresh measured	Day 1 Aged measured	Day 2 Fresh measured	Day 2 Aged measured
0	-1.44	0.41	2.26	1.15	17.07
10	7.81	10.04	1.89	2.26	11.89
25	21.15	21.15	11.52	1.89	22.26
50	47.07	46.70	35.59	45.96	40.41
100	92.63	88.56	68.56	92.63	83.74
250	210.41	218.19	167.81	224.11	181.89
500	417.44	442.63	335.96	440.04	425.22

Table 36 Hydrazine concentration data for *Fucus vesiculosus* (96 h) (Study Reference #5) Day 3-5

Nominal Concentration	Day 3 Fresh measured	Day 3 Aged measured	Day 4 Fresh measured	Day 4 Aged measured	Day 5 Fresh measured	Day 5 Aged measured
0	1.15	1.52	2.63	0.78	0.78	146.33
10	11.52	4.48	14.48	11.15	10.78	11.15
25	24.11	3.00	40.78	20.78	27.07	21.52
50	58.19	68.93	48.56	46.33	49.67	47.44
100	99.67	88.56	93.00	90.04	115.59	88.93
250	222.26	198.19	241.52	198.19	245.96	228.56
500	431.52	443.37	454.48	416.33	487.44	450.41

Table 37 Hydrazine concentration data for *Fucus vesiculosus* (96 h) (Study Reference #5) Day 6-7

Nominal Concentration	Day 6 Fresh measured	Day 6 Aged measured	Day 7 Fresh measured
0	-0.33	1.89	0.41
10	6.70	10.41	7.44
25	21.15	18.19	20.41
50	85.96	80.41	37.44
100	85.22	42.63	89.67
250	217.81	215.96	201.89
500	458.93	426.70	378.93