Proposed EQS for Water Framework Directive Annex VIII substances: copper (saltwater) *(For consultation)*

by

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Executive summary

The Water Framework Directive requires EU Member States to ensure that all inland and coastal waters achieve 'good' water quality status by 2015. This goal will be realised through a range of measures, including the use of environmental quality standards (EQS) for a number of individual chemicals. Significant effort has been targeted at delivering implementable metrics by which to assess the quality of freshwaters. For metals, this has included the development of practical methods to account for (bio)availability and ambient background concentrations.

For marine systems there is generally less fate and behaviour data available for metals. Nevertheless, implementable EQS still need to be derived and therefore limitations in the data may present a range of challenges. This report deals with the first of those challenges through a review of the recent Voluntary Risk Assessment of Copper carried out by the European Copper Institute (ECI) in which relevant marine toxicity data were collated for the derivation of a predicted no effect concentration. Any relevant and reliable data that have been produced subsequently have also been reviewed.

The marine dataset for copper (Cu) has both the taxonomic breadth and relevant end points to allow the use of a species sensitivity distribution (SSD). Due to the quality and quantity of fate and behaviour data on Cu in the marine environment it is possible for an assessment to be made on the influence of water characteristics upon Cu availability. Increasing dissolved organic carbon (DOC) has been shown to significantly reduce the ecotoxicity of Cu in freshwater and marine water. A relationship was developed between Cu ecotoxicity to marine organisms and DOC in order to give a biologically relevant environmental metric of Cu exposure. Using the DOC correction described in this report each individual NOEC/L(E)C10 value was normalised to a predefined DOC concentration of 0.5 mg l^{-1} active DOC (equivalent to 1 mg l^{-1} measured DOC in natural seawater) and used to construct a reference species sensitivity distribution. An HC5 of 2.64µg Cu l^{-1} was generated through our reassessment of the available data.

The reference PNEC is derived by the application of an assessment factor to the HC5 from the reference SSD. In addition to the species covered in the single species studies, additional species and taxonomic groups were evaluated in a mesocosm study. An assessment factor of 1 is recommended for use with this revised DOC correction.

A reference EQS of 2.64 µg Cu l^{-1} is therefore considered protective of marine taxa. This value is greater than the estimated ambient background concentrations set at the 5th percentile of the frequency distribution of measured Cu concentrations for all regions in England and Wales for estuaries and coastal waters. This EQS is then adjusted to ambient conditions through the use of a bioavailability correction based on the concentration of DOC.

An indicative compliance assessment was performed for 63 sites on the basis of mean measured DOC concentrations and mean measured dissolved copper concentrations. Six of these sites fail the proposed EQS, these failures are relatively marginal with risk characterisation ratios of less than 1.5.

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1 Introduction

1.1 Report Structure

This report provides an in-depth assessment of a recent review of data and subsequent predicted no effect concentration (PNEC) derivation undertaken by the European Copper Institute (ECI). The approach and outcomes that have been followed in the ECI assessment need to be acceptable to regulatory agencies responsible for implementing the Water Framework Directive (WFD) and REACH. This report addresses the following objectives:

- Review the recent data and reports produced by ECI on the derivation of a marine PNEC for copper and evaluate the weight of evidence for determining the assessment factor used. This includes a review of the physicochemical factors that may mitigate copper toxic effects in coastal saltwater, including an assessment of the validity of the relationship on which the correction for dissolved organic carbon (DOC) was made.

- Estimate ambient background concentrations (ABC) for Cu for waters in England and Wales.

- Provide compliance assessments for a range of Cu EQS with monitoring data from marine sites around the coast of England and Wales to give an indication of the potential benefits of the derived environmental quality standard (EQS) and the water quality correction.

In this introductory section of the report we briefly describe the background and need for a marine EQS for Cu in the UK.

In Section 2 we provide a review of the chronic marine ecotoxicity data for Cu and the derivation process used by ECI to establish a PNEC.

The relationship between marine water physico-chemistry, specifically dissolved organic carbon (DOC), and Cu marine ecotoxicity is discussed in Section 3. In this section a correction to Cu exposure has been developed based on DOC.

In Section 4 we independently assess the ecotoxicity data presented in Section 2 and calculate a PNEC on the basis of the available evidence. A brief description at the end of this section makes reference to the choice of assessment factors.

In Section 5 we give an indicative compliance assessment for Cu in marine waters in England and Wales using monitoring data. This compliance assessment has been undertaken using several EQS options and has also applied a correction for DOC. Estimates of the ambient background Cu concentrations are also given in order to contextualise the findings of this exercise.

Finally, in Section 6 conclusions and recommendations are provided in regard to the derivation and implementation of a marine EQS for Cu. Appendices are also provided summarising the ecotoxicity data used and individual study summary reports.

1.2 Background

A draft report proposing freshwater and marine EQS for copper was completed for the Environment Agency in 2005. This report referred extensively to the voluntary risk

assessment (VRAR 2005) on copper metal and copper compounds being carried out within the framework of EU Existing Substance Regulation 793/93, which was also in draft form at that time. The draft VRAR has been updated since then (ECI, 2008) and has been subject to considerable peer review therefore no further consideration of the reliability of the Q1 (high quality) data used in the VRAR has been carried out.

The draft EQS did not provide either a short- or long-term EQS for marine water bodies. At the time the EQS was drafted none of the wide range of saltwater toxicity data had been evaluated or selected in the context of the ongoing VRAR. In addition, the understanding of the fate and behaviour of Cu in marine waters was still relatively limited. As a consequence it was not considered appropriate to derive PNECs for the protection of the marine environment at that time.

Freshwater EQS derivation for metals has rightly received a significant amount of regulatory and industry attention over the last five years (EC 2010). However, as many of the challenges associated with the development of implementable evidence-based metrics for metals in freshwaters are met, focus has begun to turn to the marine environment. Now that the Cu VRAR has been completed the Environment Agency wishes to update the draft WFD EQS to include PNECs for the marine environment. In order to address this, the saltwater toxicity data used in the VRAR have been assessed and any relevant and reliable data that have been produced subsequently have also been included.

Short-term standards have not been derived in the current report because maximum allowable concentrations for metals are not considered a priority as it is the long-term standards that are used for compliance purposes in the UK.

2 Review of Cu marine ecotoxicity data for the derivation of a Cu EQS_{marine}

The regulatory requirements under both REACH and the WFD when deriving a PNEC for chronic marine exposure are effectively the same. Recent draft guidance from Working Group E of the EC Water Framework Directive's Expert Group does provide useful reference to the derivation of a marine PNEC (EQS) for metals (EC 2010). Both WFD and REACH (ECHA 2008b) related sources of information have been considered in the following review.

2.1 Data Sources

The saltwater dataset listed in the CuVRAR (ECI 2008) was derived from original papers published in peer-reviewed international journals. [Additional data specifically developed for inclusion into the dataset based upon relevant testing guidelines was also included, and has since been published in the open literature (Arnold et al. 2010a)]. The date of the last literature search quoted in the VRAR is 2007. A further literature search for saltwater chronic data was carried out for this report [ScienceDirect®[1] ,TOXNET®[2]].

2.2 Data Reliability and Relevance

The following is a summary of the specific items considered by the ECI when selecting data and which have also been used to assess the more recently published studies.

Type of test

Determination of what constitutes chronic exposure has been reviewed with respect to the sensitivity of the endpoint and the duration of the life stage under assessment. For unicellular algae and certain invertebrates (e.g. rotifers) an exposure time of four days or less covers one or more generations allowing chronic NOEC values to be derived from experiments of four or less days duration. Tests on the embryo-larval stages of organisms or germination of plants characterise effects on potentially sensitive life stages of these organisms. Where abnormal development within a 24 to 48 hour exposure period is indicative of effects such that continuation of these tests would derive no additional information which could provide protection for the environment a developmental NOEC value has been derived and included in the dataset.

Type of test material and methods

Tests should be performed according to standard operational procedures and sufficient detailed description should be given to assess that test validity criteria have been met.

[1] http://www.sciencedirect.com/

[2] http://toxnet.nlm.nih.gov/

In the reported data, Cu^{2+} has been used as the test material with several salts being used as the precursor. It is generally recognized that under laboratory conditions almost all the copper is present in the dissolved fraction therefore the results can be regarded as being dissolved copper concentrations. For 51 out of the 56 high quality datapoints (Q1 data), retained in this report, the authors have reported that the seawater was filtered. Ahsanullah, 1995 (data for *Penaeus mergulensis* and *Penaeus monodon*) and Roeisjade 1980 (data for *Prothotheca staminea*) did not report if the seawater was filtered. Only Young, 1979 (data for *Panadlus danae*) reported that unfiltered seawater was used for the ecotoxicity tests. Evaluation of some other tests with reported measured total and total dissolved (filtered) values show good similarities between total and total dissolved values: e.g. the data from sea urchins (Hurd 2006), sheepheads minnow (Hurd 2006) and *Tisbe battagliai* (Williams 2006) show that the measured total dissolved concentrations were close to the measured total concentrations. The data for *Penaeus mergulensis*, *Penaeus monodon, Prothotheca staminea* and *Panadlus danae* were therefore retained for the evaluation. Therefore, the data included in the chronic effects database could be considered to be measured dissolved Cu concentrations. If it is not mentioned whether the NOEC/L(E)C_{10} values are based on measured or nominal concentrations, they were considered as nominal concentrations.

Chelating agents are compounds that exhibit a strong affinity towards divalent and trivalent metal ions, due to formation of multiple bonds between the ligands of the complexing agent and the metal ion. The presence of chelators in the test medium has the potential to affect the toxicity of copper. Where the test media contained EDTA (ethylene diamine tetra-acetic acid) or NTA (nitrilotriacetic acid) results were regarded as unreliable and excluded from the dataset.

Test medium – background concentrations

Any study not supported by analytical data was automatically excluded from the high quality studies (Q1 data). All other studies were rated as Q2 or Q3, depending on the data quality as well as the availability of copper background levels in the test media.

For metals where the EQS is within the range of likely background concentrations there is a need to consider how the standards can be reasonably and practically implemented (EC 2010). The recent draft guidance on derivation of EQS for the WFD comprehensively considers ambient background concentrations and suggests several methods for how they may be derived (including selection of a low percentile of a frequency distribution).

The Scientific Committee for Toxicology, Ecotoxicology and Environment have recommended that background levels of copper are not taken into account and that a ‘total risk’ approach is appropriate. The total risk approach accounts for the total dissolved amount of a metal in a water body. This means that no distinction is made between the fraction of a metal that is present in a water body for natural reasons and the fraction added due to anthropogenic activities. If no correction for water quality parameter dependent toxicity for the metal in question can be made this will, for metals with a significant natural background concentration in relation to the estimated effects threshold value (e.g. PNEC or quality standard), frequently result in the calculation of threshold values below local natural background concentrations.

Test medium – DOC concentrations

Where DOC and/or background concentrations were not determined, the following assumptions were made;

- Artificial seawater; Background Cu; 0.5 µg l^{-1}, Background DOC 0.3 mg/l (these are average values from Arnold et al. 2007)
- Natural seawater; Background Cu; 1.1 µg l^{-1} ([3]); Background DOC 2.0 mg l^{-1}
- Filtered natural seawater;Background Cu; 1.1 µg l^{-1}; Background DOC 2.0 mgl^{-1}
- Diluted natural seawater; Natural seawater × dilution factor
- Reconstituted natural seawater; as artificial seawater

Test medium – salinity

Marine waters are characterised by high pH (typically around 8.3), high salinity (35‰) and high ionic strength. Unlike the VRAR we have used a cut-off of 20‰ salinity for data to be used in the SSD. The effect of this is to remove one high quality data point for *Eurytemora affinis*. *E. affinis* is an estuarine copepod and experimental data suggests that the optimum salinity condition for its survival and reproduction is close to 10 ppt (Hall et al. 2008). The salinity of seawater is generally considered to be around 35 parts per thousand, although local conditions may result in differences due to dilution by freshwater inputs or evaporation due to high temperatures. UK coastal waters can often tend to have lower salinities due to reduced evaporation and dilution by freshwater inputs, and typical values for coastal waters may be closer to 32 parts per thousand.

The salinity of the North Sea has been monitored (Joyce 2006) along the ferry route between Harwich (UK) and Rotterdam (Netherlands). Salinity measurements were made at nine stations along the ferry route. An annual variability is observed in the salinity data, although variations are generally small at most of the stations. One station which is close to Rotterdam showed a relatively high frequency of low salinity measurements. This is presumed to be due to freshwater inputs and a similar situation may exist in other coastal areas where there are large riverine inputs. The 10th percentile of salinity measurements at this station is 20.6 parts per thousand, and the value of 20 parts per thousand is therefore considered to be an appropriate cut-off for the selection of data which are representative of truly marine conditions.

2.3 Derivation of effect concentrations

According to ECHA Guidance the NOEC is defined as “the highest concentration tested at which the measured parameter shows no significant inhibition” or the test concentration immediately below the LOEC (ECHA 2008a). There has to be a concentration-effect relationship. In the past, the NOEC was determined directly from the concentration-effect curve by consideration of the deviation of the control (e.g. 10%) or it was derived on the basis of ANOVA (analysis of variance) and a subordinate test (e.g. Dunett's). The preconditions for the use of ANOVA have to be fulfilled (normal

[3]the mean/median copper concentrations of the natural seawater control test media are 2.4/2.1 µg Cu l^{-1}, the median copper concentration in European marine waters was 0.6 µg Cu l^{-1}median (from exposure assessment); the median coastal zone PECvalue (from exposure assessment) was 1.1 µg Cu l^{-1},. Considering that the latter was still below 2.1 µg Cu l^{-1} (median value in test waters), this value was retained as default natural copper background value in the absence of measured data (VRAR 2008).

distribution, homogeneous variances). This method to derive the NOEC with the ANOVA has been criticised and the calculation of an EC10 is now considered as a preferable alternative, and current guidance allows the use of NOEC and EC10 values interchangeably.

In the VRAR the following approach was taken, and any deviations from this proposed in the current assessment are identified:

- If possible, "real" NOEC values were derived from the data reported, i.e. the NOEC is one of the concentrations actually used in the test. Preference was given to NOECs that were identified by statistical analysis as the highest concentration in a series of test concentrations showing no statistical effect compared to the control ($p \leq 0.05$). The NOEC was derived on the conditions that the LOEC results in > 10% effect (inhibition) compared to the control. The concentration range tested was also considered. Where the difference factor between tested concentrations was high (e.g. molar orders of magnitude), the NOEC was considered unreliable, and attempts were made to derive EC10 values, where appropriate.
- If a "real" NOEC could not be derived from the data reported, it was replaced by a E(L)C10 value calculated from the concentration-effect relationship.
- In this assessment, as enough reliable 'true' NOEC or EC10 data could be extracted from literature, it was decided not to derive NOEC values from LOEC or MATC in the Q1 assessment.
- Due to the fact that the Q1 analysis did not fully represent the diversity of taxa encountered in marine waters, additional Q2 data (Q1 data except that the copper levels were not measured, but background levels are known or could be estimated) was assessed and used in a second tier of PNEC determination.
- Since the publication of the VRAR further data have become available such that in this assessment only Q1 data have been used in the calculation of the HC5 presented in this report.

2.4 Chronic toxicity database

Chronic NOEC data of saltwater microalgae, higher plants, invertebrates and fish that were selected in the VRAR for PNEC derivation are summarized in Appendix 1 (the data reported in Tables A1-1 to A1-3 are those of Tables 3-6, 3-9 and 3-11 for Q1 data and 3-7 and 3-10 for Q2 data). New data identified since the VRAR is shown in Table 2.1.

New data

Baumann et al (2009) investigated the effects of copper on photosynthetic activity, measured as pulse amplitude modulation (PAM) chlorophyll fluorescence yield in seven species of green, red and brown macroalgae over a 14-day period. The data were assigned as Q3 data as the difference factor between tested concentrations was high (0, 0.1, 1 and 10 µmol l^{-1}) and there was only one replicate per concentration containing 3 plants. There was insufficient data presented in the paper to allow for the calculation of a notional EC10. There was no significant effect of different copper treatments on the yield of *Ascophyllum nodosum*, *Fucus vesiculosus*, *Cladophora rupestris*, *Ulva intestinalis* and *Polysiphonia lanosa* over the 14-day period. The 10µmol l^{-1} (63.5 µg l^{-1}) treatment significantly reduced yield in *Chondrus crispus* by day 7 and post day 7 for *Palmaria palmata*.

Five marine microalgae *Tetraselmis chuii*, *Rhodomonas salina*, *Chaetoceros* sp., *Isochrysis galbana* (*T-iso*) and *Nannochloropsis gaditana* were exposed to nine different copper concentrations, between 5 and 600 µg l^{-1} and a control (Debelius et al. 2009). EC50 values are reported in the paper but no reliable NOEC/EC10 data could be calculated. NOEC values were estimated graphically and assigned as Q3 data. The EC50 values were 48, 58, 88, 137 and 330 µg l^{-1} for *R. salina*, *I. galbana*, *Chaetoceros* sp., *N. gaditana* and *T. chuii*, respectively.

Complete life-cycle toxicity tests of copper chloride to marine copepod, *Tigropus japonicus*, were conducted using a single test concentration of 10µg l^{-1} and a control (Kwok et al. 2008). Overall mortality (mean ± 1SD; n=10) of *T. japonicus* in the control and treatment groups was 10.4 ± 4% and 13.1 ± 4%, respectively. At 10µg l^{-1}development slowed down significantly, time to first reproduction was delayed by approximately a half-day and the intrinsic rate of population increase was significantly lowered. No reliable NOEC data can be derived from this study as only a single copper concentration was tested. This study was assigned as Q3 data.

Hall et al. (2008) studied the effects of salinity at 2.5, 5, 15 and 25 ppt at dissolved organic carbon (DOC) concentrations of between 1.3 and 3.3 mg l^{-1} and DOC concentrations of 2, 4, 6 and 8 mg l^{-1} at a fixed salinity of 10 ppt on the acute toxicity of copper to the estuarine copepod, *Eurytemora affinis*. The 96-hour LC50 values were 71, 104, 68 and 58 µg l^{-1}at 2.5, 5, 15 and 25 ppt salinity, respectively. The 96-hour LC50s for *E. affinis*, increased from 76 to 166µg l^{-1}as DOC concentrations increased from 2 to 8 mg l^{-1}. The EC10 values were 52, 72.5, 13.2 and 25.7 µg l^{-1}at 2.5, 5, 15 and 25 ppt salinity, respectively and 63.9, 62.5, 22.6, and 87.6 µg l^{-1}at DOC concentrations of 2, 4, 6 and 8 mg l^{-1}, respectively. *E. affinis* is an estuarine copepod and the results suggest that there is a salinity effect on the organism. Optimal condition for its survival and reproduction is close to 10 ppt. The results of this study therefore were not included in the SSD derivation.

A 96-hour static test design was used to determine the chronic effects of Cu on the intrinsic population growth rates of the euryhaline rotifer, *Brachionus plicatilis* ("L" strain (Arnold et al. 2010b).The test was performed over a measured concentration range between 0.9 (control) and 22.7 µg dissolved Cu l^{-1} at 15 ppt salinity and a DOC concentration < 1.0 mg C l^{-1}.The intrinsic growth rate EC10 was 8.8µg dissolved Cu l^{-1}. This test was carried out at 15 ppt salinity which is below the cut-off point of 20 ppt therefore this result was not included in the SSD derivation.

Arnold et al. (2010c) were able to show that the organic matter content of the sample affects copper toxicity to *B. plicatilis* more than other water chemistry parameters. At DOC concentrations of 1.7 and 4.1 mg C l^{-1} the 24-h LC50 values increased by approximately a factor of ten (368 – 442 µg l^{-1}) and 48-h LC50 increased by more than a factor of eleven (45.5 – 173.4 µg l^{-1}) over LC50 values measured in reconstituted salt water samples at similar salinity. No EC10 or NOEC values were reported, although the study indicates that the protective effect of DOC is similar for both the 24 and 48 hour time points.

The effects of copper on early life stages of the blue mussel, *Mytilus trossulus*, were assessed using the following endpoints: sperm swimming speed and fertilization capacity, egg viability and embryo development (Fitzpatrick et al. 2008). Sperm, eggs and embryos were exposed to six copper concentrations between 0.32 and 100µg l^{-1} plus a control. The most sensitive endpoint was embryo development. A significant increase (~50-60%) in abnormal development was observed between the 3.2 and 10µg l^{-1} copper treatments, with 100% abnormal embryo development occurring in the 100µg l^{-1} treatment. Embryos developed in unamended seawater (controls) exhibited 20 ± 3.5% abnormal embryos and a similar percentage of abnormal embryos were observed in the three lowest test concentrations. Test validity criteria are not mentioned in the

paper, however, US EPA Guidelines recommend that the percent normal embryos should be at least 90% in the surviving controls and that the minimum significant difference is < 25% relative to control. For this reason the embryo toxicity data has been evaluated as Q3 data. A decrease in sperm swimming speed and fertilization success was only evident in the highest test concentration and no effect on egg viability was apparent at any concentration tested. The results presented in the paper are based on nominal values however mean measured concentrations are available and have been reported in Table 2.1.

Manyin and Rowe (2010) carried out a life cycle exposure using *Palaemonetes pugio* larvae. The larvae were exposed at nominal free divalent cation concentrations of 9 and 26 µgCu^{2+} l^{-1}. During the full life cycle exposure there were no significant effects of Cu on survival of larval, juvenile and adult life stages. Although many shrimp in the Cu treatments became gravid no larvae were produced from any of the clutches. The concentration of free copper ions was buffered by adding nitrilotracetic acid (NTA) at 5 x 10^{-5} M. Water samples were analyzed for total dissolved copper using ICP-MS and AA. The total Cu concentrations were very similar for the two treatments (3200 and 3270µg l^{-1}) and as a result of variation in samples and limits of analytical resolution, concentrations in the two treatments could not be reliably distinguished. The exposure medium was tap water to which Instant Ocean sea salts had been added to produce salinity of 10 ppt. The use of NTA and a salinity level below the cut-off threshold means that the data from this study were evaluated as Q3 data.

Nadella et al. (2009) assessed the toxicity of dissolved Cu in the mussel *Mytilus trossolus*, at various salinity and DOC levels using a 48-hour embryo developmental test. A salinity threshold of > 20 ppt was required for normal control development. Experimental addition of DOC, from three freshwater sources, reduced Cu toxicity. The protective effect of two of the DOC sources was only evident at DOC concentrations < 9 mg C l^{-1}. The natural organic matter from these two sources had a high humic acid content and at DOC concentrations > 10 - 20 mg Cl^{-1} reduced embryo survival was observed even in the absence of added Cu. The background DOC concentration in control seawater samples was 4.0 mg Cl^{-1}. The data from this study were evaluated as Q2.

Rosen et al. (2008) investigated embryo-larval development in the mussel, *Mytilus galloprovincialis* and the sea urchin, *Strongylocentrotus purpuratus*, when exposed to seven copper concentrations bracketing the expected LC50 in natural seawaters with different DOC concentrations. NOEC values ranged from 4.1 to 7.08 µg dissolved Cu $l^{-1}$for *M. Galloprovincialis* as DOC increased from 1.3 to 2.24 mg $l^{-1}$and for *S. purpuratus* NOECs were 9.1 and 14.1 µg dissolved Cu l^{-1} at DOC concentrations of 2.47 and 3.43 mg l^{-1}, respectively. These data were evaluated as Q1 and were taken forward for use in the derivation of the SSD.

The studies carried out in support of the derivation of a DOC bioavailability correction factor are discussed in sections 3.1 and 3.2. The endpoints used in the SSD derivation are shown in Table 2.1 with the exception of those for *M. galloprovincialis*. The spread of the data for this species is shown in Figure 3.8. The relevant study summaries are provided in Appendix 2.

Table 2.1 Overview of new data for saltwater organisms published since completion of the VRAR

Chemical form	Species	Taxo-nomic group	Age and/ or size of test organism	Test duration	Effect	End-point	Value (µg/l)	Chemical Analysis	Exposure	Cu back-ground (µg l^{-1})	DOC	Temp (°C)	pH	Salinity (g/l)	Test water	Reference and data quality code
Algae and higher plants																
Copper chloride	*Ascophyllum nodosum*	Macro-algae	40-60mm	14 days	Chlorophyll fluorescence	NOEC	635	measured	Static renewal	0.6 m	NR	10±1	NR	NR	un-enriched sterile seawater	Baumann et al. 2009 Q3
Copper chloride	*Fucus vesiculosus*	Macro-algae	Apical plant parts	14 days	Chlorophyll fluorescence	NOEC	635	measured	Static renewal	0.6 m	NR	10±1	NR	NR	un-enriched sterile seawater	Baumann et al. 2009 Q3
Copper chloride	*Ulva intestinalis*	Macro-algae	40-60mm	14 days	Chlorophyll fluorescence	NOEC	635	measured	Static renewal	0.6 m	NR	10±1	NR	NR	un-enriched sterile seawater	Baumann et al. 2009 Q3
Copper chloride	*Cladophora rupestris*	Macro-algae	Up to 100mm	14 days	Chlorophyll fluorescence	NOEC	635	measured	Static renewal	0.6 m	NR	10±1	NR	NR	un-enriched sterile seawater	Baumann et al. 2009 Q3
Copper chloride	*Polysiphonia lanosa*	Macro-algae	40-60mm	14 days	Chlorophyll fluorescence	NOEC	635	measured	Static renewal	0.6 m	NR	10±1	NR	NR	un-enriched sterile seawater	Baumann et al. 2009 Q3
Copper chloride	*Chondrus crispus*	Macro-algae	40-60mm	14 days	Chlorophyll fluorescence	NOEC	63.5	measured	Static renewal	0.6 m	NR	10±1	NR	NR	un-enriched sterile seawater	Baumann et al. 2009 Q3
Copper chloride	*Palmaria palmata*	Macro-algae	40-60mm	14 days	Chlorophyll fluorescence	NOEC	63.5	measured	Static renewal	0.6 m	NR	10±1	NR	NR	un-enriched	Baumann et al. 2009

Chemical form	Species	Taxo-nomic group	Age and/ or size of test organism	Test duration	Effect	End-point	Value (µg/l)	Chemical Analysis	Exposure	Cu back-ground (µg l^{-1})	DOC	Temp (°C)	pH	Salinity (g/l)	Test water	Reference and data quality code
															sterile seawater	Q3
Copper	*Tetraselmis chuii*	Micro-algae	10^4 cells ml^{-1}	72 hours	Growth inhibition (cell density)	NOEC	120	measured	static	<1.0	NR	20±1	8	NR	Enriched sterile filtered seawater	Debelius et al. 2009 Q3
Copper	*Rhodomonas salina*	Micro-algae	1.5 x 10^4 cells ml^{-1}	72 hours	Growth inhibition (cell density)	NOEC	5	measured	static	<1.0	NR	20±1	8	NR	Enriched sterile filtered seawater	Debelius et al. 2009 Q3
Copper	*Chaetoceros* sp.	Micro-algae	4.2 x 10^4 cells ml^{-1}	72 hours	Growth inhibition (cell density)	NOEC	40	measured	static	<1.0	NR	20±1	8	NR	Enriched sterile filtered seawater	Debelius et al. 2009 Q3
Copper	*Isochrysis galbana (T-iso)*	Micro-algae	6.6 x 10^4 cells ml^{-1}	72 hours	Growth inhibition (cell density)	NOEC	40	measured	static	<1.0	NR	20±1	8	NR	Enriched sterile filtered seawater	Debelius et al. 2009 Q3
Copper	*Nanno-chloropsis gaditana*	Micro-algae	42 x 10^4 cells ml^{-1}	72 hours	Growth inhibition (cell density)	NOEC	80	measured	static	<1.0	NR	20±1	8	NR	Enriched sterile filtered seawater	Debelius et al. 2009 Q3
Invertebrates																
Copper chloride	*Eurytemora affinis*	CRU	~ 24 hours	96 hours	Mortality	EC10	22.6	nominal	static	< 2.0 m	6.0	25±1	7.8-8.74	10	Sterile natural estu-arine water	Hall et al. 2008 Pacific EcoRisk 2010 Q3
Copper chloride	*Eurytemora affinis*	CRU	~ 24 hours	96 hours	Mortality	EC10	13.2	nominal	static	< 2.0 m	1.34 m	25±1	7.8-8.74	15	Sterile natural	Hall et al. 2008

Chemical form	Species	Taxo-nomic group	Age and/ or size of test organism	Test duration	Effect	End-point	Value (µg/l)	Chemical Analysis	Exposure	Cu back-ground (µg l^{-1})	DOC	Temp (°C)	pH	Salinity (g/l)	Test water	Reference and data quality code
															estu-arine water	Pacific EcoRisk 2010 Q3
Copper chloride	*Tigropus japonicus*	CRU	< 24 hours	20-30 days	Reproduction	Effect	10	nominal	Static renewal	NR	NR	25±1	7.9-8.0	30	Artificial seawater	Kwok et al. 2008 Q3
	Palaemonetes pugio	CRU							Static renewal							Manyin and Rowe 2009
Copper	*Brachionus plicatilis*	ROT	< 4 hours	48 hours	Mortality	EC50	2	measured	static	<0.1 – 1.2 m	< 0.5 m	25±1	7.6	6	Artificial seawater	Arnold et al. 2010bQ3
Copper	*Brachionus plicatilis*	ROT	< 4 hours	48 hours	Mortality	EC50	173.4	measured	static	2.5 – 2.9 m	4.0 m	25±1	8.3	7	Artificial seawater	Arnold et al. 2010b Q3
Copper chloride	*Mytilus trossulus*	MOL	Sperm/ eggs	100 min	Sperm motility/ fertilization success	NOEC	27.4	measured	static	1.5 m	NR	NR	NR	NR	Filtered seawater	Fitzpatrick et al. 2008 Q2
Copper chloride	*Mytilus trossulus*	MOL	Sperm/ eggs	100 min ex-posure	Egg viability	NOEC	> 71	measured	static	1.5 m	NR	11	NR	NR	Filtered seawater	Fitzpatrick et al. 2008 Q2
Copper chloride	*Mytilus trossulus*	MOL	Embryo	48 hours	Embryo development	NOEC	4.6	measured	static	1.5 m	NR	20	NR	NR	Filtered seawater	Fitzpatrick et al. 2008 Q3
Cu in 2%HNO_3	*Crassostrea virginica*	MOL	Embryo	48 hours	Embryo development	EC10	7.8	measured	static	0.3 m	0.8	15	7.7	29.8	Filtered seawater	Arnold et al. 2010a Q1
Cu in 2%HNO_3	*Crassostrea virginica*	MOL	Embryo	48 hours	Embryo development	EC10	17.2	measured	static	1.8 m	2.6	15	7.8	29.6	Filtered seawater	Arnold et al. 2010a Q1
Cu in 2%HNO_3	*Crassostrea virginica*	MOL	Embryo	48 hours	Embryo development	EC10	28.3	measured	static	3.1 m	3.9	15	7.9	29.7	Filtered seawater	Arnold et al. 2010a Q1
Cu in 2%HNO_3	*Crassostrea virginica*	MOL	Embryo	48 hours	Embryo development	EC10	35.1	measured	static	4.2 m	5.7	15	8.0	29.6	Filtered seawater	Arnold et al. 2010a Q1

Chemical form	Species	Taxo-nomic group	Age and/ or size of test organism	Test duration	Effect	End-point	Value (µg/l)	Chemical Analysis	Exposure	Cu back-ground (µg l^{-1})	DOC	Temp (°C)	pH	Salinity (g/l)	Test water	Reference and data quality code
Cu in 2%HNO_3	*Dendraster excentricus*	ECH	Embryo	72 hours	Embryo development	EC10	17.3	measured	static	0.2 m	1.2	15	7.8	32	Filtered seawater	Arnold et al. 2010a Q1
Cu in 2%HNO_3	*Dendraster excentricus*	ECH	Embryo	72 hours	Embryo development	EC10	30.4	measured	static	1.2 m	2.1	15	7.8	32	Filtered seawater	Arnold et al. 2010a Q1
Cu in 2%HNO_3	*Dendraster excentricus*	ECH	Embryo	72 hours	Embryo development	EC10	41.3	measured	static	2.3 m	3.5	15	7.9	32	Filtered seawater	Arnold et al. 2010a Q1
Cu in 2%HNO_3	*Dendraster excentricus*	ECH	Embryo	72 hours	Embryo development	EC10	61.7	measured	static	3.2 m	5.0	15	7.9	32	Filtered seawater	Arnold et al. 2010a Q1
Cu in 2%HNO_3	*Dendraster excentricus*	ECH	Embryo	72 hours	Embryo development	EC10	23.5	measured	static	0.4 m	1.3	15	8.0	33.1	Filtered seawater	Arnold et al. 2010a Q1
Cu in 2%HNO_3	*Dendraster excentricus*	ECH	Embryo	72 hours	Embryo development	EC10	24.1	measured	static	2.3 m	1.8	15	8.1	34.3	Filtered seawater	Arnold et al. 2010a Q1
Cu in 2%HNO_3	*Strongylo-centrotus purpuratus*	ECH	Embryo	72 hours	Embryo development	EC10	12.3	measured	static	0.2 m	1.2	15	7.8	32	Filtered seawater	Arnold et al. 2010a Q1
Cu in 2%HNO_3	*Strongylo-centrotus purpuratus*	ECH	Embryo	72 hours	Embryo development	EC10	22.1	measured	static	1.2 m	2.1	15	7.8	32	Filtered seawater	Arnold et al. 2010a Q1
Cu in 2%HNO_3	*Strongylo-centrotus purpuratus*	ECH	Embryo	72 hours	Embryo development	EC10	22.7	measured	static	2.3 m	3.5	15	7.9	32	Filtered seawater	Arnold et al. 2010a Q1
Cu in 2%HNO_3	*Strongylo-centrotus purpuratus*	ECH	Embryo	72 hours	Embryo development	EC10	35.6	measured	static	3.2 m	5.0	15	7.9	32	Filtered seawater	Arnold et al. 2010a Q1
Cu in 2%HNO_3	*Strongylo-centrotus purpuratus*	ECH	Embryo	72 hours	Embryo development	EC10	14.2	measured	static	0.4 m	1.3	15	8.0	33.1	Filtered seawater	Arnold et al. 2010a Q1
Cu in 2%HNO_3	*Strongylo-centrotus*	ECH	Embryo	72 hours	Embryo development	EC10	17.7	measured	static	2.3 m	1.8	15	8.1	34.3	Filtered seawater	Arnold et al. 2010a Q1

Chemical form	Species	Taxo-nomic group	Age and/ or size of test organism	Test duration	Effect	End-point	Value (µg/l)	Chemical Analysis	Exposure	Cu back-ground (µg l^{-1})	DOC	Temp (°C)	pH	Salinity (g/l)	Test water	Reference and data quality code
	purpuratus															
Copper sulphate	*Strongylocentrotus purpuratus*	ECH	Embryo	96 hours	Embryo development	NOEC	9.1	measured	Static	0.9 m	2.47	15.8	8.09	34.2	filtered seawater	Rosen et al. 2008 Q1
Copper sulphate	*Strongylocentrotus purpuratus*	ECH	Embryo	96 hours	Embryo development	NOEC	14.1	measured	Static	2.5 m	3.43	16.0	8.2	35.4	filtered seawater	Rosen et al. 2008 Q1
Cu in 2%HNO_3	*Mytilus edulis*	MOL	Embryo	48 hours	Embryo development	EC10	2.03	measured	static	0.28 m	0.6	15	7.8	30.5	Filtered seawater	Arnold et al 2009 Q1
Cu in 2%HNO_3	*Mytilus edulis*	MOL	Embryo	48 hours	Embryo development	EC10	6.69	measured	static	1.5 m	2.5	15	7.9	30.4	Filtered seawater	Arnold et al 2009 Q1
Cu in 2%HNO_3	*Mytilus edulis*	MOL	Embryo	48 hours	Embryo development	EC10	12.7	measured	static	2.13 m	4.1	15	8.0	30.5	Filtered seawater	Arnold et al 2009 Q1
Cu in 2%HNO_3	*Mytilus edulis*	MOL	Embryo	48 hours	Embryo development	EC10	20.7	measured	static	3.72 m	5.9	15	7.9	30.2	Filtered seawater	Arnold et al 2009 Q1
Cu in 2%HNO_3	*Mytilus edulis*	MOL	Embryo	48 hours	Embryo development	EC10	3.89	measured	static	0.28 m	0.6	15	7.8	30.5	Filtered seawater	Arnold et al 2009 Q1
Cu in 2%HNO_3	*Mytilus edulis*	MOL	Embryo	48 hours	Embryo development	EC10	8.11	measured	static	1.5 m	2.5	15	7.9	30.4	Filtered seawater	Arnold et al 2009 Q1
Cu in 2%HNO_3	*Mytilus edulis*	MOL	Embryo	48 hours	Embryo development	EC10	15.4	measured	static	2.13 m	4.1	15	8.0	30.5	Filtered seawater	Arnold et al 2009 Q1
Cu in 2%HNO_3	*Mytilus edulis*	MOL	Embryo	48 hours	Embryo development	EC10	22.2	measured	static	3.72 m	5.9	15	7.9	30.2	Filtered seawater	Arnold et al 2009 Q1

3 Copper bioavailability in the marine environment

A bioavailability correction for Cu in the marine environment has been proposed (ECI 2008). This bioavailability correction is based on the complexation of Cu by DOC in seawater, and has been assessed against short term toxicity tests for six species (Arnold et al. 2010a). These tests are considered to be valid for PNEC derivation, despite their relatively short duration, as they are based on embryo development and are amongst some of the more sensitive endpoints in the chronic effects database. Studies on the toxicity of Cu to different developmental stages of the blue mussel (*Mytilus trossulus*) have identified embryos as more sensitive than either sperm or eggs (Fitzpatrick et al 2008).

A comparative study of metal toxicity to oyster and mussel embryos and crab larvae (Martin et al. 1981) found the 48 hour embryo development tests to be much more sensitive to copper than the 96 hour mortality test on the crab larvae. Another comparative study (Nacci et al. 1986) compared early embryo growth and sperm cell toxicity tests on the sea urchin *Arbacia punctulata* against Microtox and conventional acute toxicity tests on *Menidia menidia* (Atlantic silverside) and *Mysidopsis bahia* (opossum shrimp). The early embryo growth and sperm cell toxicity tests were of 4 and 1 hours in duration respectively, and were found to be approximately 10 times more sensitive to copper toxicity than acute tests on fish (96 hours) and invertebrates (48 hours), confirming the high sensitivity of these types of tests to copper. Microtox was of slightly greater sensitivity than the standard tests. The majority of tests undertaken to assess the effect of DOC on Cu toxicity in seawater have used terrestrially derived DOC added to seawater, rather than DOC extracted from seawater. A significant component of marine DOC is, however, believed to be derived from terrestrial sources due to riverine inputs, although some studies (e.g. Nadella et al. 2009) have indicated that high concentrations of terrestrially derived DOC may be inhibitory, although the levels considered (12 and 20 mg l^{-1}) are likely to be above those typically found in the marine environment.

3.1 Background to a correction for the effect of DOC

In order to derive a DOC bioavailability correction factor relevant to chronic data the European Copper Institute were asked to provide the data used in the previous correction, including data from Arnold et al (2010a). Pacific EcoRisk was contracted to compile and summarise analytical chemistry and toxicity data previously developed in support of these various studies of the toxicity of copper to marine species (Pacific EcoRisk 2010). A summary of the Arnold (2005) publication is also provided as this data forms a large part of the dataset used for deriving the DOC correction.

A protective effect of DOC on the marine macroalga *Fucus vesiculosus* has also been observed (Brooks et al. 2007) in a 14 day germling growth test. The authors reported a protective effect of DOC on the EC50 values from the tests, but also suggested that some of the DOC bound Cu may also be bioavailable. The study measured labile Cu concentrations by Differential Pulse Anodic Stripping Voltammetry (DPASV). Techniques such as DPASV also include some of the more labile complexed metals in addition to the free metal ion (Van Leeuwen et al. 2005). Given that it is either the free metal ion activity, or the sum of several inorganic species, that is assumed to be

related to toxicity in freshwaters (Di Toro et al. 2001a,2001b, Santore 2001) the interpretation of a possible toxic contribution from the operationally defined “ASV labile” fraction should be treated with caution. The no-effect concentration (NEC) values presented by Brooks et al. (2007) indicate a significant positive relationship between $\log_e$(total Cu, µg l^{-1}) and DOC concentrations at the NEC, as was observed for the EC50 values. The slopes from the two regression relationships were 0.63 (se = 0.054) for the regression based on NEC values, and 0.49 (se = 0.066) for the regression based on EC50 values. The slopes are not significantly different from one another at the 95% confidence level, suggesting that the bioavailability modifying effect of DOC is expected to be of a similar magnitude to that identified from EC50 values (e.g. Arnold et al. 2009).

When the data of Brooks et al. (2007) are compared to the slopes derived for bioavailability corrections due to DOC by Arnold et al. (2010) they appear to be higher, indicating either a greater bioavailability reducing effect of the terrestrially derived commercial humic acid used in the experiments by Brooks et al. (2007), or a lower copper availability to the macroalga tested. The slope function derived on the basis of *Fucus vesiculosus* NEC values was significantly different from the slope functions derived from EC50 values by Arnold et al. (2010), although the slope function derived from the EC50 data of Brooks et al. (2007) was not significantly different from the slope functions derived by Arnold et al. (2010). This suggests a relatively similar effect of DOC on all of these organisms.

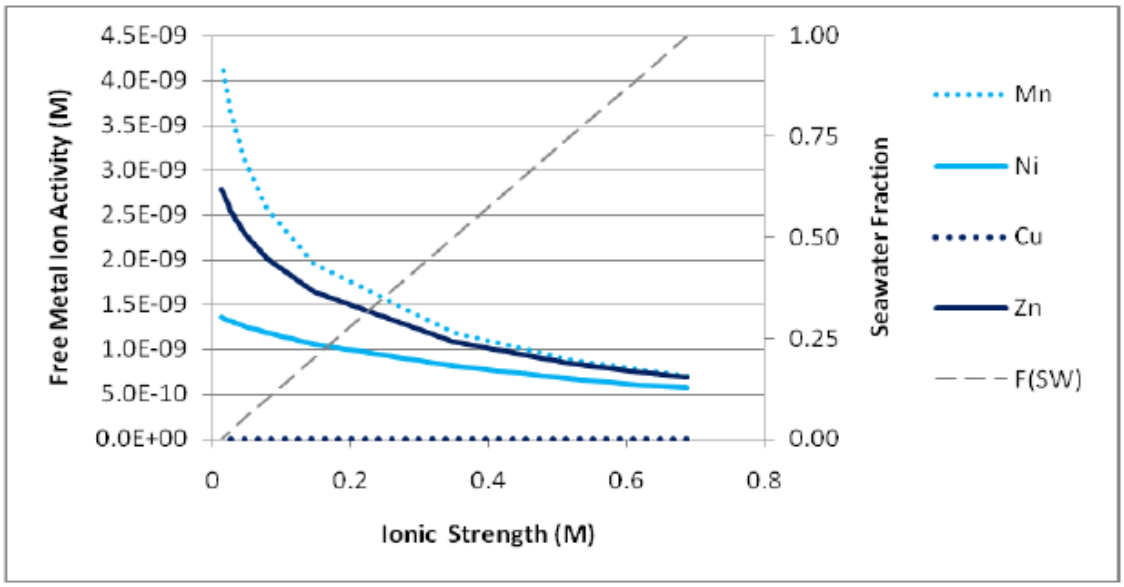

Figure 3.1 Changes in the free metal ion activity during estuarine mixing for Mn, Ni, Cu, and Zn, as predicted by WHAM.

These findings are consistent with expectations resulting from chemical speciation calculations for copper in seawater, which indicate that Cu in seawater is likely to be extensively bound to organic colloids as it is in freshwaters. Figure 3.1 shows predictions of the free ion activity of Cu, Mn, Ni, and Zn as a function of ionic strength for a range of water chemistries representative of estuarine mixing, and Figure 3.2 shows the fraction of each metal which is bound to colloidal organic matter (see Table 3.1 for details of the waters). The conditions of the seawater used for the calculations are shown in Table 2.2. Chemical speciation calculations were made using WHAM 6.0.13 (NERC 2001), this is a chemical speciation model which is able to predict the behaviour of metals in natural waters, and their interactions with natural organic matter.

Marine waters have very high ionic strength, which affects the activity coefficients which relate the chemical activity of a species to its concentration. The procedures used for the calculation of activity coefficients in freshwaters cannot be applied to the high ionic strength conditions found in seawater. Activity coefficients were therefore calculated using the Davies equation, as this is able to provide better estimates at high ionic strength. The free cupric ion activity ranges from 1 x 10^{-12} M in freshwater to 6 x 10^{-13} M in seawater, whereas the free metal ion activities of Mn, Ni, and Zn are all reduced during the transition to seawater. This is due to the formation of other metal complexes. The reductions in free metal ion activities are likely to result from the increased ionic strength of seawater compared to freshwater.

Table 3.1 Composition of key parameters in waters used for WHAM calculations

Parameter	Seawater	Freshwater
pH	8.2	7.8
Na (M)	0.47	0.005
Ca (M)	0.01	0.0017
Fulvic acid (mg l^{-1})	2	2
Ionic strength (M)	0.7	0.013
Zn (M)	10^{-8}	10^{-8}
Cu (M)	10^{-8}	10^{-8}

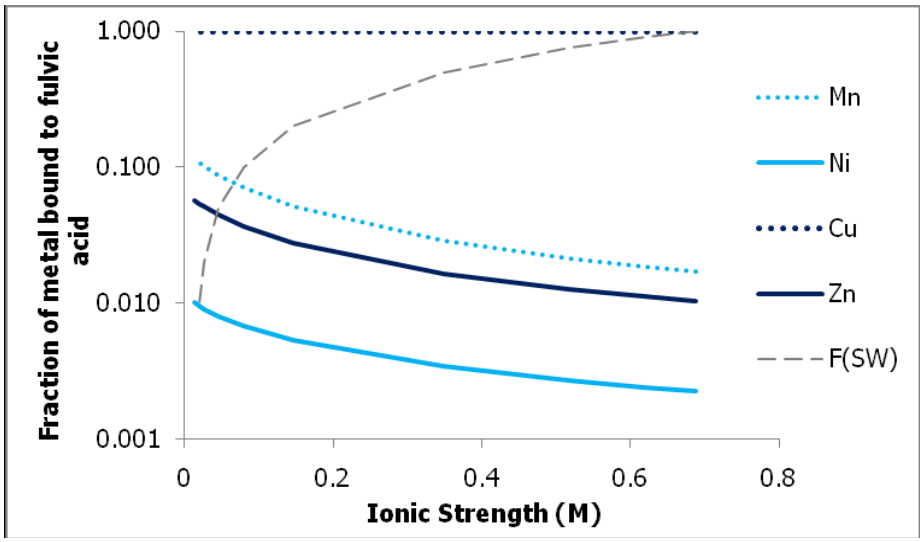

Figure 3.2 Changes in the fraction of metal bound to fulvic acid during estuarine mixing for Mn, Ni, Cu, and Zn, as predicted by WHAM.

The binding of these metals to fulvic acid in seawater is shown in Figure 3.3 as the fraction of the total metal bound to the DOC. A concentration of 10 nM was assumed for each of the trace metals Cu, Mn, Ni and Zn, and activity coefficients were calculated according to the Davies equation due to the high ionic strength. Whilst the fraction of Ca bound to fulvic acid is small, the total quantity of metal bound to the fulvic acid is over 100 times higher than for Cu, due to the much higher concentration of Ca in seawater than the concentrations of trace metals. Other trace metals such as Mn, Ni,

and Zn are all predicted to be much less extensively bound to fulvic acid, with around one percent, or less, of the total dissolved metal concentrations being associated with the fulvic acid.

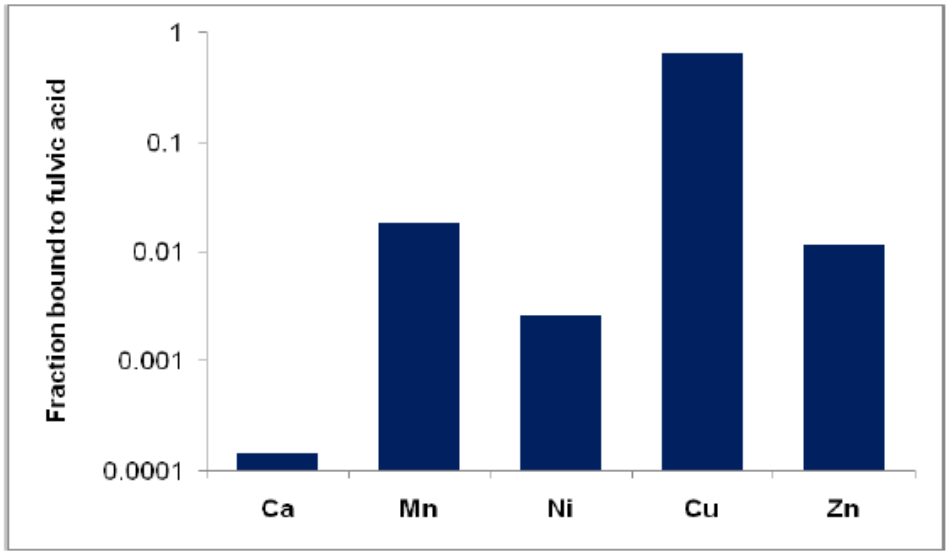

Figure 3.3 Predicted fractions of 5 metals bound to fulvic acid in seawater.

Whilst only a small fraction of the total Ca is bound to the fulvic acid, this represents a considerable loading of the total number of binding sites available. Whilst the majority of the Cu is predicted to be bound to the fulvic acid this element occupies only a relatively small fraction of the available binding sites. Only around one percent of Zn is predicted to be bound to the fulvic acid. This is due to the importance of Ca competition in Zn binding to fulvic acid, whereas Ca has a much weaker competitive effect against Cu binding. The free metal ion activity of Cu is reduced considerably, relative to that of the other trace metals considered in the predictions (Figure 3.4), due to the complexation of Cu by fulvic acid. The free metal ion activities of the other trace metals, including Zn, are relatively high, confirming the more limited interaction with the organic ligands.

A comparison of metal binding to fulvic acid in freshwater and seawater is shown in Figure 3.5. The compositions of the key parameters in the freshwater are shown in Table 3.2. The amount of Ca bound to fulvic acid in freshwater is slightly higher than that in seawater, despite the much lower concentration of Ca in the freshwater. Cu binding to fulvic acid is relatively unaffected by the changing ionic strength and Ca concentrations between freshwater and seawater, reflecting the high affinity of Cu for organic ligands, and the limited competition for binding by Ca. Conversely, Mn, Ni, and Zn are all predicted to show reduced binding to fulvic acid in seawater due to the increased competition for binding by Ca.

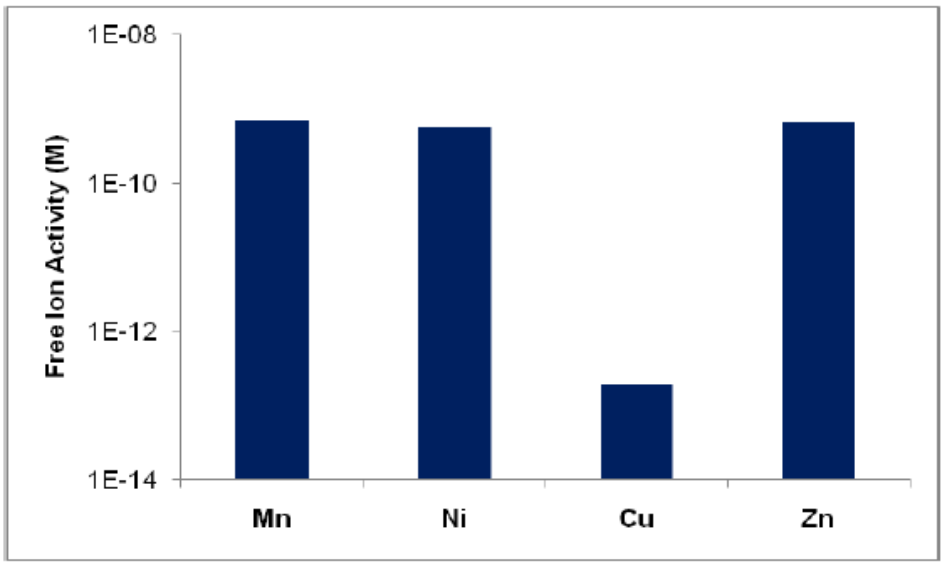

Figure 3.4 Predicted free ion activities of 4 metals in seawater in the presence of fulvic acid.

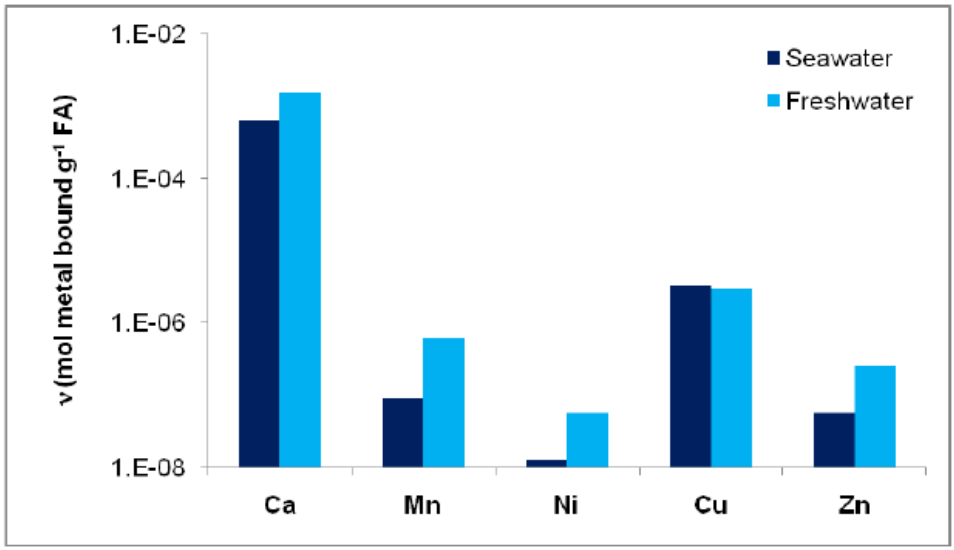

Figure 3.5 Comparison of the predicted amount of 5 metals bound to fulvic acid in seawater and a freshwater.

The predicted binding of Cu by fulvic acid is consistent with the results of several laboratory studies which have observed a reduction in the toxicity of Cu with increasing DOC concentrations. This may be related to reductions in Cu free metal ion activities with increasing complexation by organic ligands in a similar manner to that which results in the reduced bioavailability of Cu in freshwaters.

3.2 Development of a DOC correction

A correction for the effect of DOC on the toxicity of copper to marine organisms is derived in the VRAR for copper. A similar correction is derived here for application to

the quality standard, but its derivation and implementation differ slightly. The correction proposed in the VRAR establishes a power relationship between the DOC concentration and the EC10 or PNEC of the form shown in Equation 1.

$$EC10 = a \,.\, DOC^{\,b} \qquad \text{Eq. 1}$$

Where a and b are fitted constants that respectively define the intercept and slope of the regression line when displayed on a log-log graph.

This relationship indicates that there is a non-linear relationship between the DOC concentration and the EC10 or PNEC. The situation usually encountered is for a linear relationship to be observed between the concentration of DOC and the EC10 or PNEC. A linear relationship between these parameters has been observed in the case of all freshwater biotic ligand models, and is also considered to be more appropriate in the case of copper bioavailability in marine systems. Over the range of DOC concentrations encountered in typical marine systems the two different approaches are expected to produce similar results, although under the more extreme conditions of high DOC some differences may be anticipated.

Both linear and power models were assessed for the effect of DOC on copper toxicity to the five species for which test results over a range of DOC concentrations were available. The power model was compared to the linear model by fitting a linear model to log transformed data for both the dependent and independent variables since this allowed a more direct comparison of the goodness of fit to be performed. For *Crassostrea virginica* and *Strongylocentrotus purpuratus* the power model provided a very slightly improved fit, whereas for the other three species an improvement in model fit was observed for the linear model. The greatest difference in model fits was observed for the *Mytilus galloprovincialis* dataset, which was considerably more extensive than the other datasets available.

The linear models for *C. virginica*, *Dendraster excentricus*, *Mytilus edulis,* and *S. purpuratus* are shown in Figure 3.6, and the power models are shown in Figure 3.7. Given that there is little difference in the predictive capability of the two model expressions for these four species, and that the linear model appears to offer an improved fit to the *Mytilus galloprovincialis* dataset the linear model is used for the bioavailability correction. This is also consistent with observations from freshwater bioavailability corrections which indicate that the effect of DOC complexation on metal toxicity is linear.

There is very little difference in the ability of the linear or power models to fit any of the individual datasets. The fit of the linear model to the largest (*Mytilus galloprovincialis*) dataset provides a slightly higher adjusted r^2 value (0.69) than is obtained for the power model (0.57). However, AIC (Akaike Information Criterion) values for both models are almost identical which indicates that neither model is able to provide an improved description of the dataset.

The theory of trace metal bioavailability suggests that organisms respond to an “available” fraction of the dissolved metal concentration. This “available fraction may correspond to the free metal ion activity, or to a number of different inorganic species, or may in some cases include a fraction of the colloid (DOC) bound species. It is typically assumed that the sensitivity to the “available” fraction of the metal is constant for a species, and that it is changes in the relative “availability” of the dissolved metal concentration that cause effects to be observed at different dissolved concentrations according to the water chemistry (i.e. variation in DOC concentration). It is unlikely that the sensitivity of species to the “available” concentration of a metal changes with changes in water chemistry conditions, such as the DOC concentration. This suggests

that a linear model for relating changes in bioavailability to DOC may be preferable.

Although the majority of the studies investigating the bioavailability of copper in marine systems have focused on very short term embryo development tests there is one additional 14 day germling growth study on the marine macroalga *Fucus vesiculosus* (Brooks et al. 2007) which has also included testing at different DOC concentrations. This is the only long term study demonstrating a bioavailability effect, although the protective effect of DOC was found to be very similar to that observed for the embryo development tests. This supports the use of these short term tests to derive a bioavailability correction for Cu in marine systems.

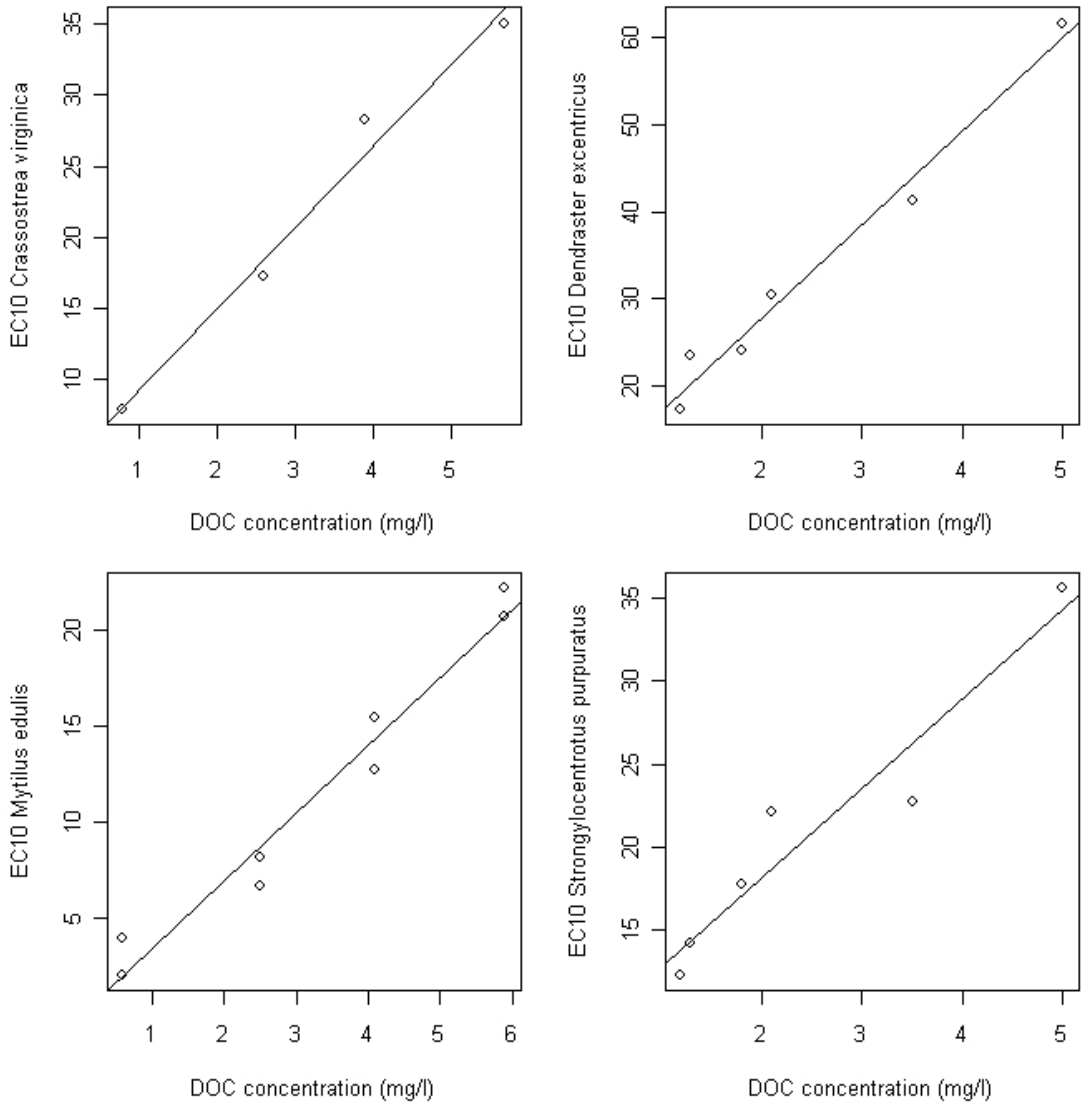

Figure 3.6 Linear models for the effect of DOC on copper toxicity to *Crassostrea virginica*, *Dendraster excentricus*, *Mytilus edulis,* and *Strongylocentrotus purpuratus*

Table 3.2 Parameters of linear models for the effect of DOC on copper toxicity

Species	Slope	r2	p
Crassostrea virginica	5.77	0.980	0.00996
Dendraster excentricus	10.80	0.979	0.000175
Mytilus edulis	3.54	0.965	1.39E-05
Strongylocentrotus purpuratus	5.40	0.918	0.00257
Mytilus galloprovincialis	3.60	0.689	<2E-16

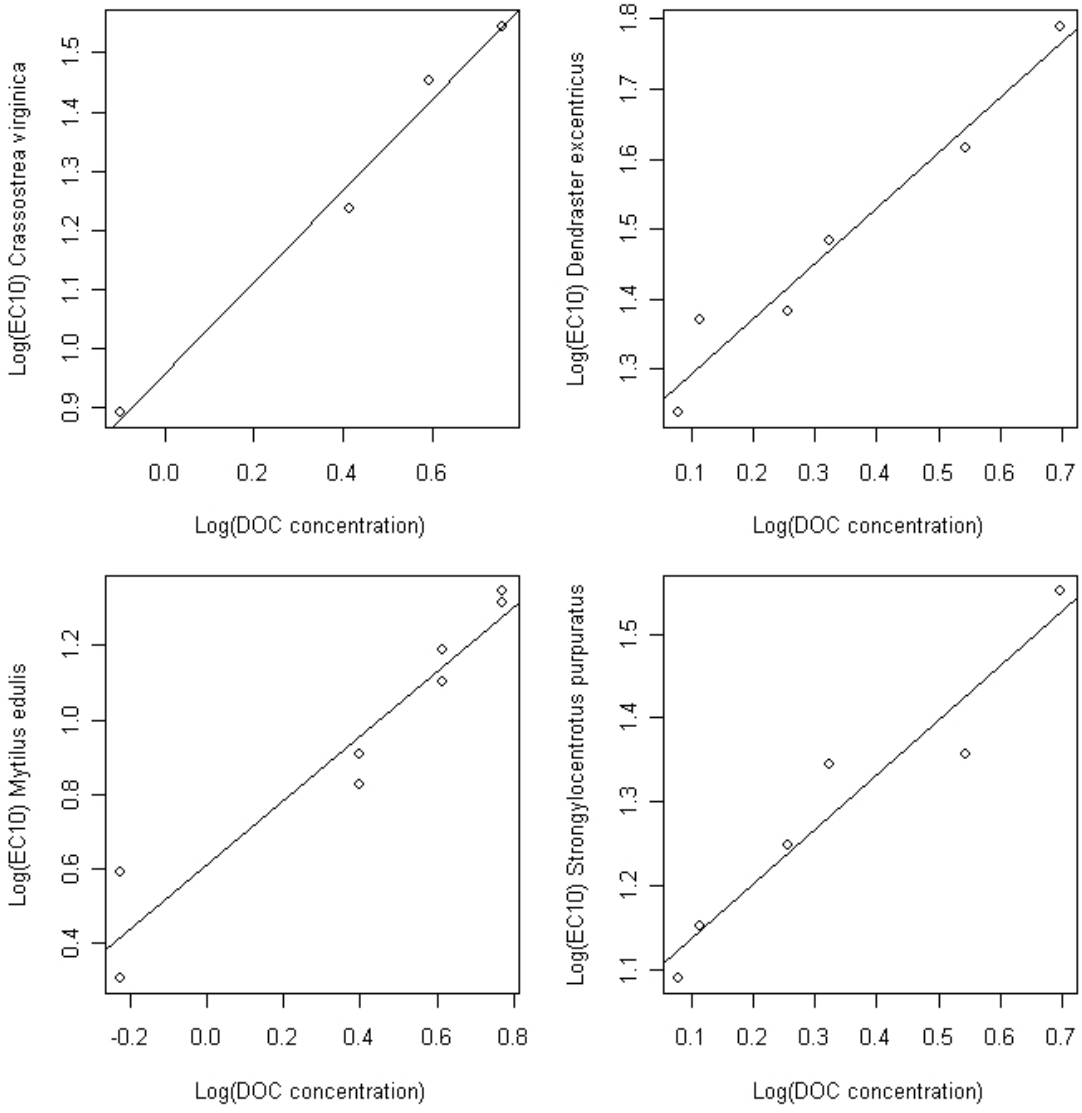

Figure 3.7 Power models for the effect of DOC on copper toxicity to *Crassostrea virginica*, *Dendraster excentricus*, *Mytilus edulis,* and *Strongylocentrotus purpuratus*

Ninety-five test results are available for the effect of Cu on *M. galloprovincialis* at different DOC concentrations, which indicate a protective effect of DOC on Cu toxicity to this species (Arnold 2005 (54), Arnold et al. 2006 (21), Arnold et al. 2007 (3), Arnold et al. 2009 (7) and Arnold et al. 2010 (10)), these data are shown in Figure 3.8.

The slopes of the regressions in Figure 3.8 based on total and added Cu are 3.6 and 3.3 (µg l^{-1} per mg l^{-1} DOC) respectively. More limited studies have also been performed on four additional species (*S. purpuratus*, *M. edulis*, *D. excentricus*, and *C.virginica*) (Arnold et al. 2010). For these species the slopes of the responses in the EC10 to increases in DOC concentration were higher than that for *Mytilus galloprovincialis* with the exception of that observed for *M. edulis* where the observed slope was very similar to that observed for *M. galloprovincialis*. It is therefore considered to be appropriate to apply a correction based on the response of *M. galloprovincialis* to increases in DOC to correct the generic PNEC for Cu according to local DOC conditions, since this species demonstrates the least DOC-related reduction in Cu toxicity, so any derived PNEC should be protective of other species also.

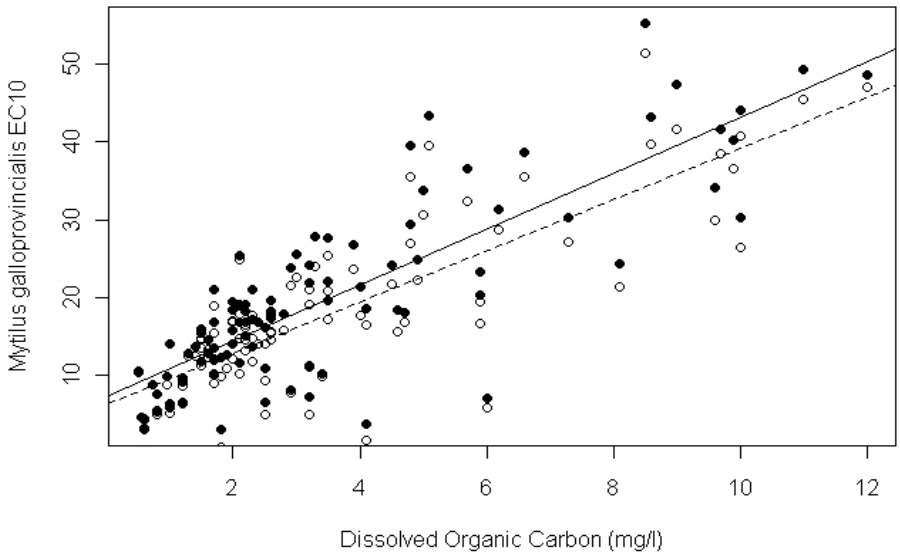

Figure 3.8 Effect of DOC on Cu toxicity to *Mytilus galloprovincialis*, filled circles represent total Cu concentrations (ug/l), and open circles represent added Cu concentrations 9ug/l). The solid line represents the regression based on total Cu, and the dashed line represents the regression based on added Cu.

The slopes of the relationships established between DOC and the dissolved Cu concentration at the EC10 for the 5 tested species were compared in order to assess whether there were any significant differences between them. Significant differences were found between several of the smaller datasets (the slopes of the responses of *Crassostrea virginica* and *Strongylocentrotus purpuratus* were both found to be significantly different from those of *Mytilus edulis* and *Dendraster excentricus*. However, only the slope of the *Dendraster excentricus* model was found to be significantly different to that of *Mytilus galloprovincialis*. This is considered to be due to the fact that the *Mytilus galloprovincialis* dataset is the most extensive and covers the broadest range of conditions, it is also most likely to reflect any variation that may occur due to other parameters which are not considered by the bioavailability model. This suggests that there are differences in the bioavailability responses of different species.

A linear model is used here to describe the effect of DOC on Cu toxicity to these organisms because this model was considered to be preferable to the power model used in the VRAR, at least in terms of consistency with other bioavailability based approaches (Figure 3.9). The adjusted r^2 value for the power model was 0.57, compared to a value of 0.69 for the linear model. A linear relationship between increases in DOC concentrations and increases in toxicity endpoints is observed for freshwater bioavailability corrections (ECI 2008) and a linear correction is therefore also considered to be preferable in the case of a bioavailability correction for the marine environment. The mechanism by which DOC affects toxicity in both fresh and marine waters is considered to be the same, i.e. the copper which is bound to DOC is not available for biological uptake and does not, therefore, contribute to toxicity.

The active DOC concentration is assumed to be 50% of the total DOC concentration in natural seawater samples, and the relationships established can be expressed in terms of the active DOC concentration. This assumes that a fraction of the measured DOC is inactive with respect to copper binding, and the approach taken here is consistent with the approach taken for freshwaters (ECI 2008).

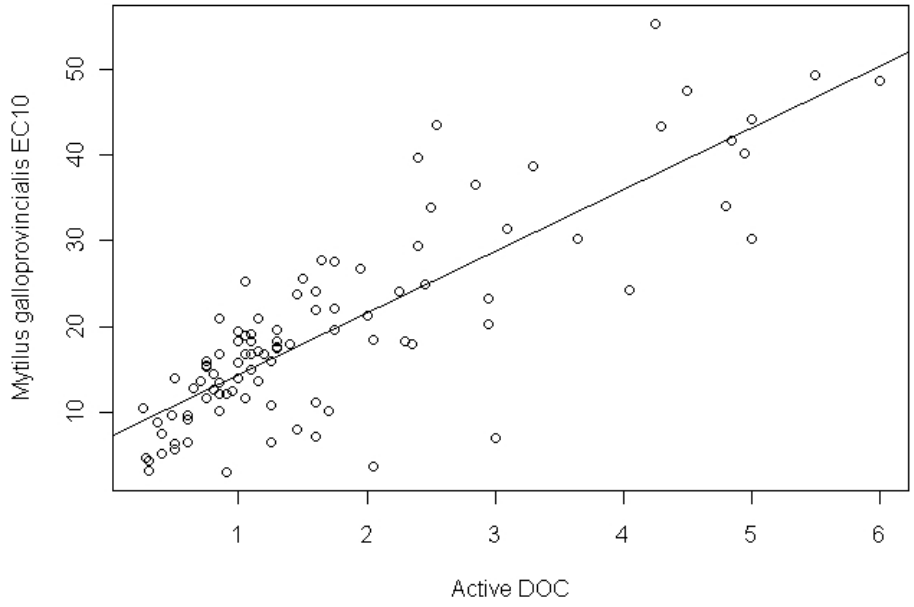

Figure 3.9 Effect of active DOC on Cu toxicity to *Mytilus galloprovincialis.* The solid line indicates the regression model describing the effect of DOC on toxicity.

The prediction of *Mytilus galloprovincialis* EC10 based on total Cu concentrations is:

EC10 = 3.6 x DOC + 7.1 Eq 2

The correction factor is:

Site PNEC = 3.6 x (DOC – DOCref) +PNEC(ref) Eq 3

Where DOCref is the DOC concentration relating to the reference conditions (which would usually be high bioavailability conditions)

If the active DOC concentration is considered to be 50% of the total DOC concentration then the prediction of *Mytilus galloprovincialis* EC10 based on total Cu concentrations is:

$$EC10 = 7.2 \times DOC_{act} + 7.1 \quad \text{Eq 4}$$

The correction factor is:

$$\text{Site PNEC} = 7.2 \times (DOC_{act} - DOC_{act}(ref)) + PNEC(ref) \quad \text{Eq 5}$$

Where DOC_{act} is the active DOC concentration (assumed to be 50% of the total DOC concentration in natural seawater) and $DOC_{act}(ref)$ is the active DOC concentration relating to the reference conditions (which would usually be high bioavailability conditions). The DOC concentration for the reference condition is set to 1.0 mg l^{-1}, and the active DOC concentration at the reference conditions is therefore assumed to be 0.5 mg l^{-1}.

4 PNEC for marine water bodies

Using the DOC correction described in the previous section each individual NOEC/L(E)C10 value was normalised to a predefined DOC concentration of 0.5 mg l^{-1} active DOC (equivalent to 1 mg l^{-1} measured DOC in natural seawater) and used to construct the species sensitivity distribution.

Based on the 29 species NOECs (after normalisation and using geometric means where applicable) presented in Table 4.1 and use of the program ETX 2.0 (Van Vlaardingen et al., 2004) for deriving an SSD (Figure 4.1), the median (i.e. 50 per cent confidence) 5th percentile cut-off value of 2.64 µg l^{-1}Cu is calculated with a lower 90 per cent CI of 1.6 µg l^{-1} and an upper 90 per cent CI of 3.9 µg l^{-1}. The assumption that the input data are normally distributed is accepted at the highest level (P = 0.01) using the Anderson–Darling Goodness-of-Fit (AD statistic 0.33308), the Kolmogorov–Smirnov test and the Cramer van Mises tests for normality.

Table 4.1 Normalised "species mean" NOECs/L(E)C10s used for the derivation of the species sensitivity distribution calculations

Species	Taxa	ECx ref(µg $l^{-1)}$
*Phaeodactylum tricornutum**	Alga (unicellular)	2.03
Skeletonema costatum	Alga (unicellular)	3.22
Nitzschia thermalis	Alga (unicellular)	35.02
Macrocystis pyrifera	Alga (multicellular)	6.60
*Fucus vesiculosis**	Alga (multicellular)	15.67
*Neanthes arenaceodentata**	Annelid	9.17
Ciona intestinaslis	Ascidian	19.02
Goniastrea aspera	Cnidarian	10.60
Acropora tenuis	Cnidarian	13.70
Lobophytum compactum	Cnidarian	32.40
Pandalus danae	Crustacean	6.30
Cancer anthonyi	Crustacean	8.10
Artemia franciscana	Crustacean	8.47
Tisbe battagliai	Crustacean	14.40
Tisbe furcata	Crustacean	15.50
Penaeus mergulensis	Crustacean	29.40
Penaeus monodon	Crustacean	141.40
*Paracentrotus lividus**	Echinoderm	8.66
*Strongylocentrotus purpuratus**	Echinoderm	11.07
*Dendraster excentricus**	Echinoderm	26.13
*Atherinopsis affinis**	Fish	55.35
Cyprinodon variegatus	Fish	57.12
*Mytilus edulis**	Mollusc	3.01
*Crassostreas gigas**	Mollusc	4.69
Placopecten magellanicus	Mollusc	6.40
*Mytilus galloprovincialis**	Mollusc	7.10
Mercenaria mercenaria	Mollusc	8.80
*Crassostrea virginica**	Mollusc	13.34
Prototheca staminea	Mollusc	14.40

*Calculated from the geometric mean of multiple results from the same species.

According to the ECHA Guidance (2008a), an assessment factor of 1–5 should be applied in order to derive the PNEC from the 5th percentile of the SSD. The size of this assessment factor needs to be justified by taking into account aspects such as:

- data comprehensiveness and quality;
- fit to the assumed distribution;
- the occurrence of NOEC values below the 5th percentile of the SSD;
- the results of field tests (if available);

There are several reasons in the case of a saltwater copper PNEC to use an assessment factor smaller than 5.

All goodness of fit tests for a normal distribution, of the log transformed data, (Anderson–Darling, Kolmogorow–Smirnov, and Cramer van Mises) are accepted at the highest significance level (99 per cent, p = 0.01). There is a relatively large database, resulting in high reliability of the 5th percentile value. This is also shown by the small difference between the 50 per cent CI and the 90 per cent CI (less than a factor of 2). This would support an AF smaller than 5. Goodness of fit to other distributions is summarised in Table 4.2.

Table 4.2 Goodness of fit statistics for various theoretical data distributions

Distribution	AIC	BIC
lognormal	228.0	230.7
exponential	234.5	235.8
gamma	236.0	238.7
weibull	236.5	239.2
cauchy	240.0	242.7
logistic	261.4	264.2
normal	276.9	279.6

Akaike (AIC) and Bayesian (BIC) information criteria were used to compare alternative distribution models. The fit of the data distribution was tested against several theoretical distributions, and the goodness of fit is described by the AIC (Akaike Information Criterion) (Burnham and Anderson 2002). This statistic can be used to select which of several models provides the best description, and the lowest value indicates the best model. Also included is the BIC (Bayesian Information Criterion) which is very similar and is treated in the same way (the lowest value indicates the best fitting model) (Burnham and Anderson 2002).It can therefore be concluded that the distribution of the species sensitivity data fits the lognormal distribution better than it fits any of the other tested models.

The number of chronic NOEC values (*n*=29 'species mean' NOEC values) meets the general requirement for the number of input data (minimum requirement: 10 NOEC values, preferably more than 15 NOEC values, for different species covering at least eight taxonomic groups). Chronic NOEC values are available for three unicellular algal species, two multicellular algal species, one annelid species, three cnidarian species,

seven crustacean species, three echinoderm species, two fish species and seven mollusc species. The input database does not include all groups recommended in ECHA (2008a) for setting up an SSD, but this guidance is specifically for the freshwater compartment. Higher plants and insects are not represented in the dataset, but these taxonomic groups are considered to be of marginal relevance for the assessment of saltwater. Lepper (2005) suggests that the same assessment factor on the result of the SSD (the 5% cut-off value) that is considered appropriate for inland waters can be applied for salt waters if the data set used to establish the SSD comprises long-term NOECs for at least two additional marine taxonomic groups other than fish, crustaceans and algae (e.g. echinoderms, molluscs, or cnidarians), showing that these additional marine groups are not more sensitive than other taxa. Representatives of all three of these taxonomic groups are available. The data are derived from high quality studies, report ecologically relevant endpoints and the tests cover complete life cycles or sensitive life stages.

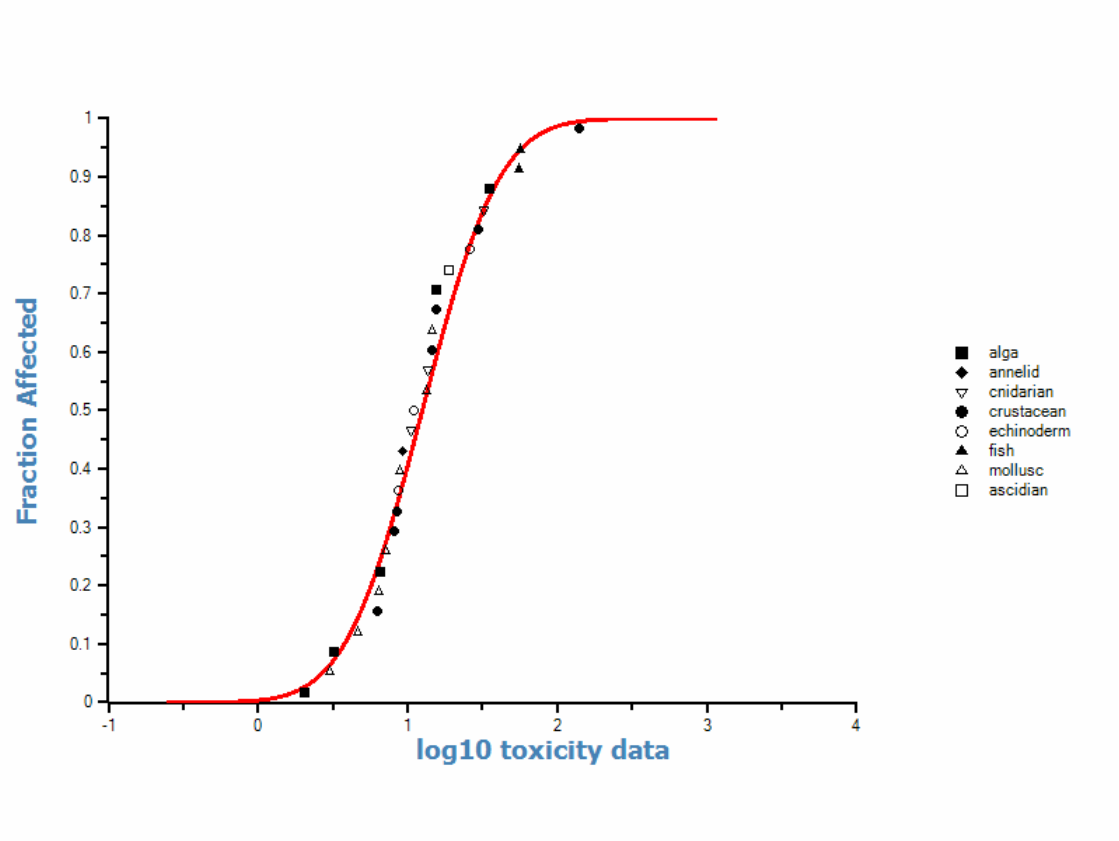

Figure 4.1 Species sensitivity distribution of selected chronic Cu endpoints.

The lowest species-specific mean NOEC (2.03 µg Cu l^{-1} for *Phaeodactylum tricornutum*) is only slightly below (factor 1.3) the HC5 value of 2.64µg Cu l^{-1}. NOECs below the HC5 do not generally occur when the sample size is below 10-20, but the availability of more data points increases the probability of NOECs below the value of the HC5. The occurrence of one NOEC below the HC5 (n = 29) is possibly a statistical phenomenon. Additional data from the mesocosm study (see section 4.1) are available for *P. tricornutum*. Foekema et al. (2010) reported seeing a bloom of the diatom *P. tricornutum* on day 26 in the 9.9 µg Cu l^{-1} mesocosms and to a lesser extent in the lower treatments and the controls suggesting that this species is less sensitive in the field compared to that shown in laboratory testing.

The Cu VRAR proposes a procedure in which all of the individual ecotoxicity data for the SSD are recalculated to the DOC concentration at a specific site for calculating the PNEC at each site. In a case where a single linear normalisation method is used to perform this recalculation of the ecotoxicity data it will result in a similar change to the EC10 or NOEC value for each individual data point. This will affect the mean, but not the standard deviation, of the SSD and is therefore not considered to provide any practical benefit over application of the correction to the HC5 value derived for the reference conditions. Correction of the HC5 value is considered to offer a simpler, and hence more practical, approach towards adjusting the PNEC value to suit site specific DOC conditions. This approach may differ slightly in the results under different normalisation conditions due to the different model expressions used to describe the effect of DOC on copper toxicity. Given that there is little to discriminate between the models used in this report and the VRAR, the variability in the results is expected to be small.

4.1 Field and mesocosm studies

An outdoor marine mesocosm study (Foekema et al. 2010) was performed over 83 days to investigate the effects on a range of organisms of continuous exposure to a concentration series from 1 (untreated controls) to 31 µg l^{-1}dissolved copper. Eighteen mesocosms, three per concentration, were provided with a layer of natural sediment below a layer of natural seawater containing a natural plankton community. Various macro-invertebrate species were added to each mesocosm in known numbers. Clear adverse direct effects were observed on zooplankton, bivalves and sponges and, to a lesser extent, on the periphyton development and the shell growth of gastropods in the two highest treatments. The phytoplankton concentration and related primary production increased as a response to the reduced grazing pressure. Further details on the experimental design are given in the study summary in Appendix 2.

A summary of the results is provided in Table 4.3. No effects were observed in the two lowest treatments, 2.9 and 5.7µg l^{-1}, respectively. Some effects on periphyton development and the zooplankton community were observed in the intermediate treatment (9.9µg l^{-1}) but these were of short-term duration. The most sensitive endpoint was a reduction in reproduction success of the bivalve cockle (*Cerastoderma edule*) which was observed at concentrations ≥ 9.9µg l^{-1}. Twenty-five cockles were introduced into each mesocosm. At the end of the study approximately 50% survival was observed in concentrations up to and including 9.9µg l^{-1}. No cockles survived in the highest treatment and there was significant mortality in the 16µg l^{-1} treatment ($p < 0.01$). Growth, shell length and average ash-free dry weight were similar in all treatments with a sufficient number of survivors. In the control mesocosms and in the two lowest treatments the introduced cockles reproduced successfully resulting in approximately 50 juvenile cockles per m^2 at the end of the study. In the higher treatments the number of juveniles was significantly reduced, or completely absent (31µg l^{-1}). The juveniles in the 9.9µg l^{-1} treatment were also significantly smaller than those found in the lower treatment and control mesocosms. Juvenile cockles were observed in one replicate 16µg l^{-1} mesocosm during the final sampling. These juveniles were very small with an average length of 1.8 mm compared to the length (7 to 8 mm) of juveniles sampled in control mesocosms.

Table 4.3 NOEC and LOEC concentrations derived from the mesocosm study after continuous exposure to dissolved copper for 83 days (Foekema et al. 2010)

Group	NOEC µg l^{-1}	LOEC µg l^{-1}	Effect
Phytoplankton			
Chlorophyll-a	9.9	16	Increased concentration
Primary production	9.9	16	Increase
Community structure	9.9	16	Change
Nitzschia closterium	9.9	16	Increased density
Chroococcus turgidus	9.9	16	Increased density
Perinidium sp.	16	31	Increased density
Flagellates	16	31	Increased density
Periphyton			
Chlorophyll-a	5.7	9.9	Temporarily decreased development
Zooplankton			
Acartia clausi	5.7	9.9	Temporarily reduced density
Copepod nauplii	5.7	9.9	Temporarily Increased density
Centrophagus hamatipes	9.9	16	Reduced density
Temora longicornis	9.9	16	Reduced density
Copepod copepodites	16	31	Reduced density
Euterpina sp.	31	>31	No effect observed
Sponges			
Halichondria panicea	9.9	16	Biomass decrease, mortality
Molluscs			
Cerastoderma edule	5.7	9.9	Reduced reproduction success
Littorina littorea	9.9	16	Reduced shell growth
Polychaetes			
Arenicola marina	31	>31	No effects observed
Amphipods			
Corophium volutator	31	>31	No effects observed

An EC10 value of 6.2 µg l^{-1} (95% confidence interval 3.1 to 12.4 µg l^{-1}) dissolved copper can be derived for the number of juvenile cockles per m^2 at the end of the experiment (see Figure 4.2). The EC50 for the reduction in the number of juvenile

cockles at the end of the experiment was 8.4 μg l^{-1} (95% confidence interval 6.1 to 11.7 μg l^{-1}) dissolved Cu. This suggests a very steep dose-response for this endpoint

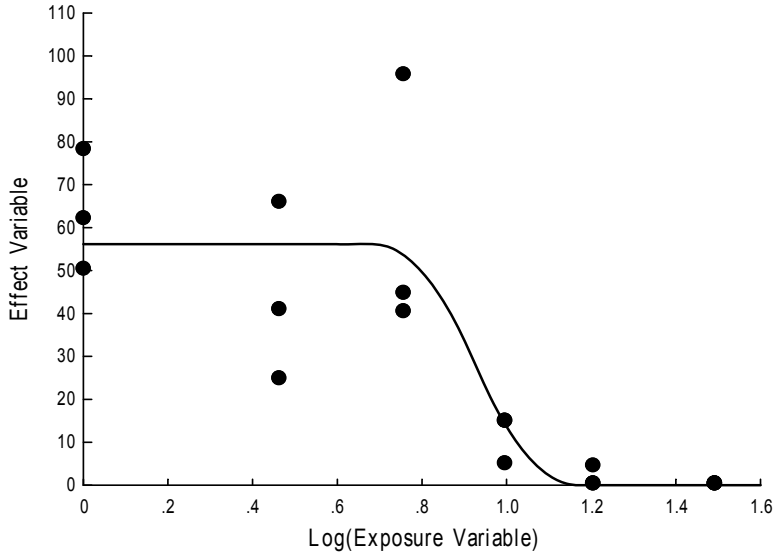

Figure 4.2 Dose response for the number of juvenile cockles m^{-2} at the end of the mesocosm study.

Samples for the analysis of DOC concentrations were taken on days -9, -2, 5 and weekly thereafter. DOC concentrations in untreated mesocosms increased from 2.8 (day -9) to 4.2 mgl^{-1} during the course of the study, and DOC concentrations at the lower copper treatment levels were comparable up to the 9.9 μg l^{-1} dissolved Cu treatment. DOC concentrations in the two higher treatment levels (16 and 31 μg l^{-1}dissolved Cu) increased to higher levels than were observed at the lower treatment levels.

In order enable a more consistent comparison of the results of the mesocosm study with the PNEC derived for Cu in marine systems, a comparison against the PNEC derived in this report has been performed. The site specific PNEC values which are appropriate to the DOC concentrations observed in the study, and the risk characterisation ratios (the ratio of the exposure concentration to the bioavailability adjusted EQS) based on the Cu exposure in the mesocosms, are shown in Table 4.4.

Table 4.4 PNEC and RCR values calculated for lower level Cu treatments from mesocosm study for lower and upper range of observed DOC concentrations.

Cu	DOC	PNEC	RCR
1	2.8	5.05	0.20
2.9	2.8	5.05	0.57
5.7	2.8	5.05	**1.13**
9.9	2.8	5.05	**1.96**
1	4.2	6.92	0.14
2.9	4.2	6.92	0.42
5.7	4.2	6.92	0.82
9.9	4.2	6.92	**1.43**

The Risk Characterization Ratios indicate that the DOC normalization used for the final EQS proposal (see Section 4.3) is likely to be protective when compared to the outcome of the mesocosm study. A slight risk is indicated for the 5.7 µg l^{-1} Cu treatment at low DOC concentrations, and this treatment level was the lowest NOEC recorded in the study. Risk characterisation ratios of between 1.4 and 2.0 are predicted for the 9.9 µg l^{-1} Cu treatment level, although the level of effect observed at this treatment level for *Cerastoderma edule* was relatively high (greater than 50 percent). Apart from the reduction in reproduction success for the mollusc, *C. edule*, the other effects observed at this concentration were transitory. In light of the information from the mesocosm study, compliance with the proposed EQS is likely to ensure the protection of cockle communities.

4.2 Recommendations from the Peer Review Meeting

The suggested PNEC/EQS is generic, i.e. it refers to a reference scenario of environmental conditions that determine copper bioavailability and thus toxicity. For marine waters this reference scenario is for 0.5 mg l^{-1} active DOC. For marine waters with different DOC the monitored environmental Cu concentrations should be normalised to the reference condition in order to render them comparable with the generic PNEC. The site specific PNEC is calculated from the site specific DOC concentration according to Equation 4 (Section 3.2).

The reference PNEC is derived by the application of an assessment factor to the HC5 from the reference SSD. From the above it can be seen that the copper marine effects database contains a large number of high quality NOEC values (58 for 28 species, in addition to the comprehensive data available for *M. galloprovincialis*). The correlation between DOC and the consequent reduction in toxicity, at the EC10 level, was assessed for five species and found to result in a greater reduction in copper toxicity for most of the other species for which the effect of DOC on copper toxicity was assessed. Two related mussel species (*Mytilus galloprovincialis* and *Mytilus edulis*) both showed a more limited effect of DOC on reducing copper toxicity, and the relationship derived for application in normalising the PNEC to site specific conditions is based on the relationship for *Mytilus galloprovincialis* in order to ensure that it is protective of other marine organisms. All species-specific copper NOECs/EC10s were therefore normalised assuming a DOC value of 0.5 mg l^{-1} active DOC. This is considered to be equivalent to a measured DOC concentration of 1 mg l^{-1} in field samples due to the assumption that only 50 percent of DOC is active in terms of copper binding. The DOC correction is therefore only applied to the PNEC in cases where the DOC concentration exceeds 1.0 mg l^{-1}, in other cases (where the DOC concentration is less than 1.0 mg l^{-1}) the reference PNEC is applied unadjusted. The resultant SSD met all goodness of fit tests for a lognormal distribution and this distribution was shown to be the most appropriate. In addition to the species covered in the single species studies, additional species and taxonomic groups were evaluated in the mesocosm study, e.g. periphyton communities.

Given the above an assessment factor of 1 is considered to be appropriate in this case.

AF = 1 Reference PNEC = 2.64 / 1 = **2.64µg l^{-1} dissolved Cu**

The site specific PNEC, in µg l^{-1} dissolved copper is therefore calculated according to Equation 6 if an assessment factor of 1 is to be applied in deriving the reference PNEC.

AF = 1 Site PNEC = 2.64 + (7.2 x ((0.5 x DOC) – 0.5)) Eq 6

A question arose as to whether the PNEC derived using an assessment factor of 1 was sufficiently protective of the effects observed on the reproduction of cockles in the

mesocosm study or whether a larger assessment factor of 3 should be applied. As the DOC correction applied at the DOC concentrations found in the mesocosm study resulted in a relatively high site specific PNEC and the reference PNEC has relatively little influence on the site specific EQS, which is controlled predominantly by the bioavailability correction it was proposed to retain the assessment factor of 1.

4.3 Final PNEC for Cu Marine

The recommendation resulting from the EQS Peer Review Meeting was to apply an assessment factor of 3 to the reference PNEC used to derive the EQS, but not to adjust the DOC correction factor. The result of this is that the DOC correction applied effectively controls the site specific PNEC where DOC concentrations are slightly elevated. The proposed EQS may also not be adequately protective of the most sensitive endpoint in the mesocosm study, which was for the commercially relevant species the cockle. A reduction in the abundance of juvenile cockles by more than 50% was observed at the LOEC in the study (9.9 $\mu g\ l^{-1}$ dissolved Cu).

As has been discussed previously, both linear and power models can be fitted to the datasets for individual species. The linear model implies that there is a consistent absolute increase in the EC10 value for a given increase in the DOC concentration. This applies to the different species, but the slope of the regression varies between species. The power model implies that there is a consistent proportional increase in the EC10 for a given proportional increase in the DOC concentration. The slopes of these models are consistent between species, which indicates that the relative effect of DOC is consistent between species, i.e. a doubling of the DOC concentration will result in an x-fold increase in the EC10. The model used for derivation of the EQS should ideally be consistent with both of these issues.

It therefore follows that the correction which is applied to the linear model in order to correct for the intrinsic toxicity (the intercept constant) must also be applied to the DOC correction (the slope constant). As the *Mytilus galloprovincialis* data set is used to derive the bioavailability correction it is the constants from this model which need to be adjusted to account for the difference in sensitivity. The correction factor is the reference toxicity value (the HC5 from the SSD, 2.64 $\mu g\ l^{-1}$) divided by the specific toxicity value derived from the species specific bioavailability data (y-axis intercept of the linear regression model, 7.1 $\mu g\ l^{-1}$). The correction factor is therefore 0.372, and is applied to both constants from the linear equation. The resulting DOC corrected PNEC is therefore calculated according to Equation 7 (which simplifies to Equation 8), and is applied in cases where the DOC concentration exceeds 1 mg l^{-1}.

$$PNEC_{Site\ Specific} = \left(7.2 * \frac{2.64}{7.1}\right) * \left(\frac{DOC}{2} - 0.5\right) + \left(7.1 * \frac{2.64}{7.1}\right) \quad \text{Eq. 7}$$

$$PNEC_{Site\ Specific} = 2.677 * \left(\frac{DOC}{2} - 0.5\right) + 2.64 \quad \text{Eq. 8}$$

The published power model was used for the ESR voluntary assessment, and has also been proposed under REACH. Whilst the linear model has not been published it is generally consistent in its approach with that taken for the derivation of a bioavailability correction for Pb in freshwaters under the WFD. A comparison of the models is given in Table 4.5.

Table 4.5 Comparison of Bioavailability Modelling Approaches

Model Approach	Linear EC10	Power EC50
Endpoint for model	EC10	EC50
Previous use	none	ESR, REACH, published
Bioavailability theory	yes	no link to freshwater BLMs
Mytilus galloprovincialis r^2	0.69	0.71

Quoted r^2 values for the fit to the *Mytilus galloprovincialis* data are not for identical datasets.

When fitted to the same dataset both approaches provide approximately equivalent descriptions of the data. Both the revised linear modelling approach and the power modelling approach maintain a consistent relative difference in the predicted EC10 value for a given DOC concentration, when compared to the EC10 for the test species at that DOC concentration. The linear modelling approach maintains a linear relationship between the DOC concentration and the predicted EC10 or EQS value.

Remaining Uncertainties

Cockles were found to be the most sensitive species in a mesocosm study (NOEC 5.7 μg l^{-1}, LOEC 9.9 μg l^{-1}, at an average DOC concentration of approximately 3.5 mg l^{-1}). The study was conducted over 83 days, and the most sensitive endpoint for this species was for the density of juvenile cockles at the end of the test. A relatively high level of effect (>50%) was observed at the LOEC for this endpoint.

Derivation of the EQS

The reference EQS is derived as the HC5 from an SSD containing data for 29 marine species under low DOC conditions. An assessment factor of 1 is considered to be appropriate for derivation of the EQS from the HC5. The EQS can be adjusted for bioavailability according to either a linear (Equation 4) or a power model (Equation 3) to describe the effect of DOC on copper toxicity to marine organisms. The power model is consistent with that proposed under REACH, whilst the linear model is most consistent with the approach recently proposed for lead in freshwaters under the WFD.

Site Specific PNEC Calculation

Under REACH the $PNEC_{Site\ Specific}$ is derived according to Equation 9:

$$PNEC_{Site\ Specific} = 5.2 \times \left(\frac{DOC}{2}\right)^{0.6189} \qquad \text{Eq. 9}$$

The $PNEC_{Site\ Specific}$ using the linear model is derived as follows:

For DOC concentrations of less than, or equal to, 1 mg l^{-1} the reference PNEC is applied, and for DOC concentrations of greater than 1 mg l^{-1} the $PNEC_{Site\ Specific}$ is calculated according to Equation 8.

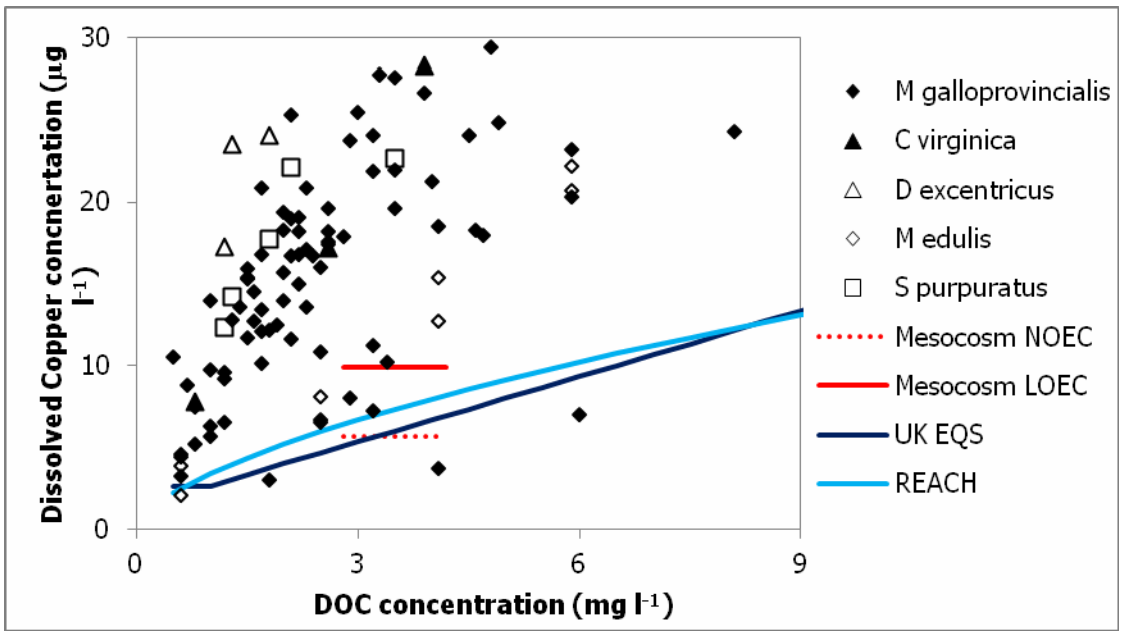

Figure 1 Comparison of PNECs derived using linear and power models with ecotoxicity data

There is little practical difference between the two approaches in terms of the site specific PNEC or EQS values predicted. Both approaches result in site specific standards which are close to the NOEC for the most sensitive endpoint in the mesocosm study over the relevant range of DOC concentrations. The linear model is slightly more conservative than the power model over the range of DOC concentrations observed in the mesocosm study. The linear model will, however, result in higher PNEC values at extreme DOC concentrations than the power model proposed under REACH.

Whilst it was considered appropriate to apply an assessment factor to derive the PNEC Following the Peer Review meeting, this was due to the fact that at slightly elevated DOC concentrations applying the DOC correction dominated the site specific PNEC, and the contribution form the reference PNEC was relatively small. Revising the bioavailability model to be consistent with the interspecies similarities identified through the fitting of power models makes the effects of the DOC correction less important in terms of the site specific EQS value. A greater level of protection is therefore ensured at the typical DOC concentrations likely to be encountered in UK coastal waters, and the EQS is not excessively conservative at low DOC concentrations.

5 Indicative compliance assessment for copper in the marine environment for England and Wales

In this section we use the PNECs developed in the previous section to assess compliance for Cu using selected marine monitoring data from England and Wales. The process is described below, firstly considering the monitoring dataset used, then estimating ambient background concentrations before going onto to assess compliance against the PNECs and considering the influence of DOC. The compliance assessment is, as far as is possible, undertaken in accordance with recent Water Framework Directive guidance (EC 2010).

5.1 The monitoring dataset

Marine monitoring data for all coastal regions of England and Wales were collated by the Environment Agency from the years 2000 – 2008 inclusive. The primary search requirement was for sites for which dissolved Cu data were measured. A summary of the physico-chemical data collated for estuarine and marine sites are shown in Tables 5.1 and 5.2. The designation and classification of sites as either estuarine (or marine) has been undertaken by the Environment Agency. This classification is based on the WFD Common Implementation Strategy guidance document No. 2"[4]

Table 5.1 Mean (SD) salinity, pH and DOC values for estuaries in England and Wales (monitoring data 2000 – 2008)

Region	Salinity (Parts per thousand)	Number of samples	pH	Number of samples	DOC (mgl^{-1})	Number of samples
Wales	27.97(7.40)	948	8.01(0.25)	765	-	0
Thames	16.34(9.61)	1412	7.67(0.30)	2271	3.36(1.46)	1047
South West	25.57 (8.54)	4402	7.87(0.34)	4259	2.62(1.51)	55
Southern	25.99(9.55)	1181	7.88(0.31)	2448	-	0
North West	22.42 (8.42)	368	7.86(0.32)	1397	2.48(4.32)	4

[4]The guidance document can be found at http://circa.europa.eu/Public/irc/env/wfd/library?l=/framework_directive/guidance_documents/guidancesnos2sidentifica/_EN_1.0_&a=d

Region	Salinity (Parts per thousand)	Number of samples	pH	Number of samples	DOC (mgl^{-1})	Number of samples
North East	25.59 (8.06)	1125	7.82(0.32)	1549	1.83(7.38)	3
Anglian	25.02 (8.63)	2203	8.00(0.26)	4553	5.70(3.73)	191

Table 5.2 Mean (SD) salinity, pH and DOC values for seawater England and Wales (monitoring data 2000 – 2008)

Region	Salinity (Parts per thousand)	Number of samples	pH	Number of samples	DOC (mgl^{-1})	Number of samples
Wales	30.89(3.55)	995	8.03(0.18)	835	1.65(1.07)	13
Thames	N/A	N/A	7.63(0.32)	17	-	-
South West	29.13 (6.98)	787	8.04(0.16)	443	-	-
Southern	33.16(2.21)	2382	8.07(0.16)	2595	5.94	1
North West	31.18 (2.4)	513	8.02(0.24)	2096	2.78(2.77)	47
North East	33.87 (2.81)	337	7.93(0.31)	510		
Anglian	30.18 (7.60)	290	8.05(0.27)	341	2.01(1.67)	38

As with most historical Environment Agency monitoring data there are considerable regional differences. Some regions have measured DOC in estuaries (Thames), but most have very little measured DOC data. The considerable variability in coverage of the data highlights the historical autonomous differences between regions. Nevertheless, for some parameters, salinity and pH, most regions have at least some data. The estuarine data, not surprisingly, shows lower values and a greater variability (as seen in the standard deviations) in salinity as compared to the marine data.

5.2 Ambient background concentrations (ABC) for Cu in marine waters in England and Wales

All of the measured monitoring data for Cu was as dissolved (< 0.45 µm) Cu. These data were collated by site and by region and the frequency distribution of these data are shown in Tables 5.3 and 5.4. In the estimation of an ambient background concentration for Cu at marine and estuarine sites around England and Wales we have taken a pragmatic approach by using a low percentile (e.g. 5^{th} or 10^{th}) of the distribution

of monitoring data for a dissolved metal (cf. EC 2010). This approach is relatively precautionary, but is reasonable when used either in a suitable tiered regulatory framework or as context in assessing whether an EQS is implementable, given that the monitoring data will likely include contributions from natural and anthropogenic (point and diffuse) metals sources.

A tiered approach towards compliance assessment has been proposed for metals in which biotic ligand models may be applied (Environment Agency 2008c). Such an approach may also be considered for metals where *no* correction for bioavailability is possible. In such cases, failure of the quality standard would potentially trigger an investigation of the background concentrations relevant to the assessment site.

Employing the estimated ABCs within a tiered risk assessment approach will allow any further efforts to refine ABCs, either at the level of the whole hydrometric area or individual water bodies, to be targeted according to risk. If a location for which an uncertain ABC has been derived is not considered to be at risk then it would not be necessary for further consideration of the reliability of the ABC, provided that there is confidence that the uncertainty is not too large (which could result in an unprotective risk characterisation).

We consider that the ABCs derived within this report are appropriate for application within a tiered approach towards compliance assessment. The derived ABCs are relatively conservative values. This means that they are unlikely to overestimate the relevant ABC for any individual water body. However, it also means that in some localised areas higher background concentrations may be applicable.

ABCs would be fixed for the six coastal English regions and Wales and the results are shown at the 5th percentile and would be considered after the influence of DOC has been accounted for. The reason for this is the relative uncertainty associated with the ABCs compared to the DOC correction, which is based on ecotoxicological evidence.

Table 5.3 Percentiles of dissolved Cu concentrations (µg l^{-1}) for estuaries in Regions of England and Wales (monitoring data 2000 – 2008)

	Region (and number of samples)						
Percentile	**Anglian** (1167)	**North East** (58)	**North West** (80)	**Southern** (351)	**South West** (1724)	**Thames** (149)	**Wales** (816)
5th	0.84	0.46	0.76	0.88	0.60	1.99	0.45
10th	1.01	0.50	0.98	1.14	0.60	2.42	0.53
15th	1.09	0.52	1.12	1.38	0.70	2.70	0.61
25th	1.25	0.58	1.31	1.74	0.80	3.15	0.69
50th	1.52	0.74	1.95	2.49	1.20	3.95	0.94
75th	2.02	0.98	2.81	3.90	1.80	4.69	1.46
90th	2.67	1.40	4.33	6.02	2.60	6.59	2.33
95th	3.39	1.78	5.73	7.74	3.60	7.95	3.17

From Table 5.3 the greatest estimated ABC set at the 5th percentile is for Thames region at 1.99 µg Cu l^{-1} and the lowest for Wales of 0.45 µg Cu l^{-1}. For the marine waters (Table 5.4) the range between maxima and minima is much less than for estuarine waters, but there are few measured Cu data for Thames region.

Table 5.4 Percentiles of dissolved Cu concentrations (µg l^{-1}) for seawater for coastal regions of England and Wales (monitoring data 2000 – 2008)

	Region (and number of samples)						
Percentile	**Anglian** (729)	**North East** (351)	**North West** (2257)	**Southern** (2790)	**South West** (479)	**Thames** (2)	**Wales** (891)
5th	0.65	0.34	0.64	0.59	0.47		0.54
10th	0.75	0.41	0.72	0.67	0.50		0.63
15th	0.81	0.44	0.78	0.70	0.56		0.69
25th	0.96	0.51	0.85	0.80	0.60		0.80
50th	1.26	0.67	1.05	1.10	0.80	8.77	1.09
75th	1.70	1.02	1.43	1.59	1.30		1.49
90th	2.16	1.78	2.30	2.30	2.60		2.13
95th	2.68	2.91	3.27	2.91	3.89		2.68

5.3 Indicative compliance assessment for Cu

Only sites for which both measured dissolved Cu data and measured DOC were available were used in the indicative compliance assessment. There were relatively few sites for which these matched data were available (n = 36). This indicative compliance assessment was performed assuming an AF of either 1 or 3 applied to the reference EQS. For comparison the compliance assessment has also been performed following the PNEC derivation approach proposed under REACH, and also following an approach which applies an assessment factor to the DOC correction of the EQS. Monitoring data reported by Jones and Bolam (2007) for a further 27 marine sites with both dissolved copper and DOC concentrations were also included in this assessment.

Site specific PNEC values for the 36 sites range from 2.7 to 17.2 µg l^{-1} dissolved. Following the approach proposed under REACH results in PNEC values between 3.3 and 15.5 µg l^{-1}. Average (mean) DOC concentrations for the sites ranged from 0.9 to 11.9 mg l^{-1}, and resulted from between 1 and 62 samples per site. The PNEC values calculated for the sites reported by Jones and Bolam (2007) cover a much narrower range because the average DOC concentrations at the sites were all within the range of 1 to 2 mg l^{-1}.

An indicative compliance assessment has been performed for these sites on the basis of mean measured DOC concentrations (mg l^{-1}) and mean measured dissolved copper concentrations (µg l^{-1}). Six out of 63 sites (9.5%) have risk characterisation ratios of greater than 1 when assessed against the final EQS proposal, although the “failures” are relatively marginal with risk characterisation ratios of between 1.12 and 1.46. There

are slightly fewer failures (4) when assessed against the PNEC proposal recommended under REACH. The risk characterisation ratios for the 63 sites with matched dissolved copper and DOC monitoring data are summarised in Figure 5.1, for assessments the final EQS proposal and the approach proposed under REACH.

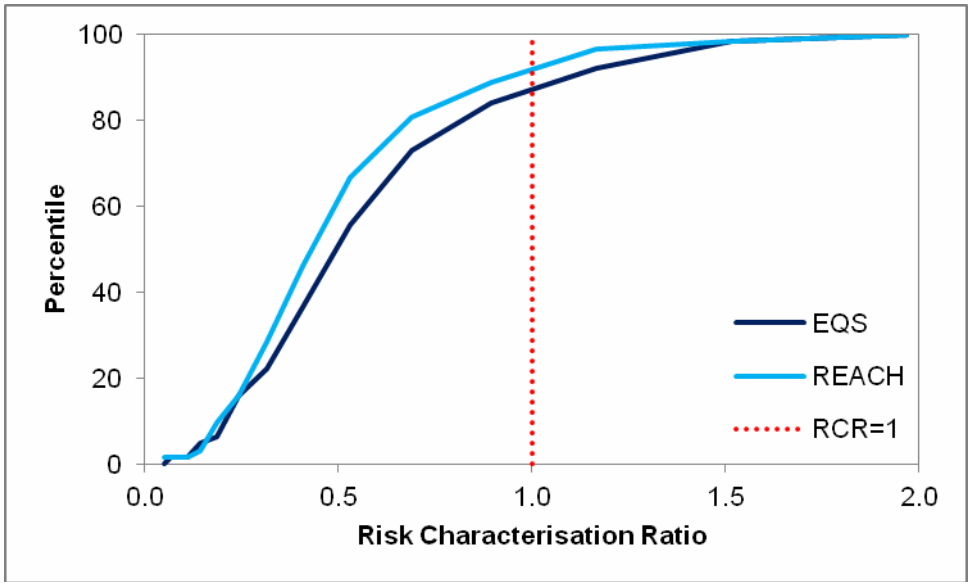

Figure 5.1 Cumulative frequency distribution of Risk Characterisation Ratios for 63 sites with matched dissolved copper and DOC monitoring data, for the EQS proposal (dark blue), the proposal under REACH (pale blue.

The approach taken to derive the site specific PNEC values has relatively little influence on the proportion of these sites which are expected to pass or fail a proposed quality standard. Limited quantities of sites with monitoring data for both dissolved copper and DOC concentrations mean that the true compliance situation could differ considerably from that indicated by the limited data available here.

6. Conclusions and recommendations

6.1 Availability of data

The number of chronic NOEC values (*n*=29 'species mean' NOEC values) meets the general requirement for the number of input data for a SSD approach to PNEC derivation. Chronic NOEC values are available for three unicellular algal species, two multicellular algal species, one annelid species, three cnidarian species, seven crustacean species, three echinoderm species, two fish species and seven mollusc species. The data are derived from high quality studies, report ecologically relevant endpoints and the tests cover complete or sensitive life stages. All goodness of fit tests for a normal distribution, of the log transformed data, are accepted at the highest significance level. In addition to the species covered in the single species studies, additional species and taxonomic groups were evaluated in the mesocosm study, e.g. periphyton communities.

6.2 Suitability of DOC correction

Both linear and power models were assessed for the effect of DOC on copper toxicity to five species for which test results over a range of DOC concentrations were available. Given that there was little difference in the predictive capability of the two model expressions for four out of the five species, and that the linear model appeared to offer an improved fit to the *Mytilus galloprovincialis* dataset the linear model was used for the bioavailability correction. Two related mussel species (*M. galloprovincialis* and *M.edulis*) both showed a more limited effect of DOC on reducing copper toxicity, and the relationship derived for application in normalising the PNEC to site specific conditions is based on the relationship for *Mytilus galloprovincialis* and is corrected to take account of differences in organism sensitivity in order to ensure that it is protective of other marine organisms. All species-specific copper NOECs/EC10s were therefore normalised assuming a DOC value of 0.5 mg l^{-1} active DOC. This is considered to be equivalent to a measured DOC concentration of 1 mg l^{-1} in field samples due to the assumption that only 50 percent of DOC is active in terms of copper binding.

6.3 PNEC for marine water bodies

Based on the 29 species NOECs (after normalisation and using geometric means where applicable) and use of the program ETX 2.0 for deriving an SSD, the median (i.e. 50 per cent confidence) 5^{th} percentile cut-off value of 2.64 µg l^{-1}Cu is calculated with a lower 90 per cent CI of 1.6 µg l^{-1} and an upper 90 per cent CI of 3.9 µg l^{-1}. The assumption that the input data are normally distributed is accepted at the highest level (P = 0.01) using the Anderson–Darling Goodness-of-Fit, the Kolmogorov–Smirnov test and the Cramer van Mises tests for normality.

The reference PNEC is derived by the application of an assessment factor to the HC5 from the reference SSD. Given the taxonomic breadth and relevant end points available from a high quality dataset and the availability of a DOC correction factor an assessment factor of 1 is recommended.

Reference PNEC = 2.64 / 1 = 2.64 µg l^{-1} dissolved Cu

The site specific PNEC, in μg l^{-1} dissolved copper is calculated as follows:

Site PNEC = 2.64 + (2.677 x ((0.5 x DOC) – 0.5))

Correction of the HC5 value is considered to offer a simple and more practical, approach towards adjusting the PNEC value to suit site specific DOC conditions where DOC concentrations exceed 1 mg l^{-1}.

The proposed EQS is shown in Figure 6.1 along with relevant ecotoxicity data and the most sensitive endpoint from the mesocosm study. Also shown for comparison are site specific PNEC values calculated according to both the REACH proposal and the previous EQS proposal.

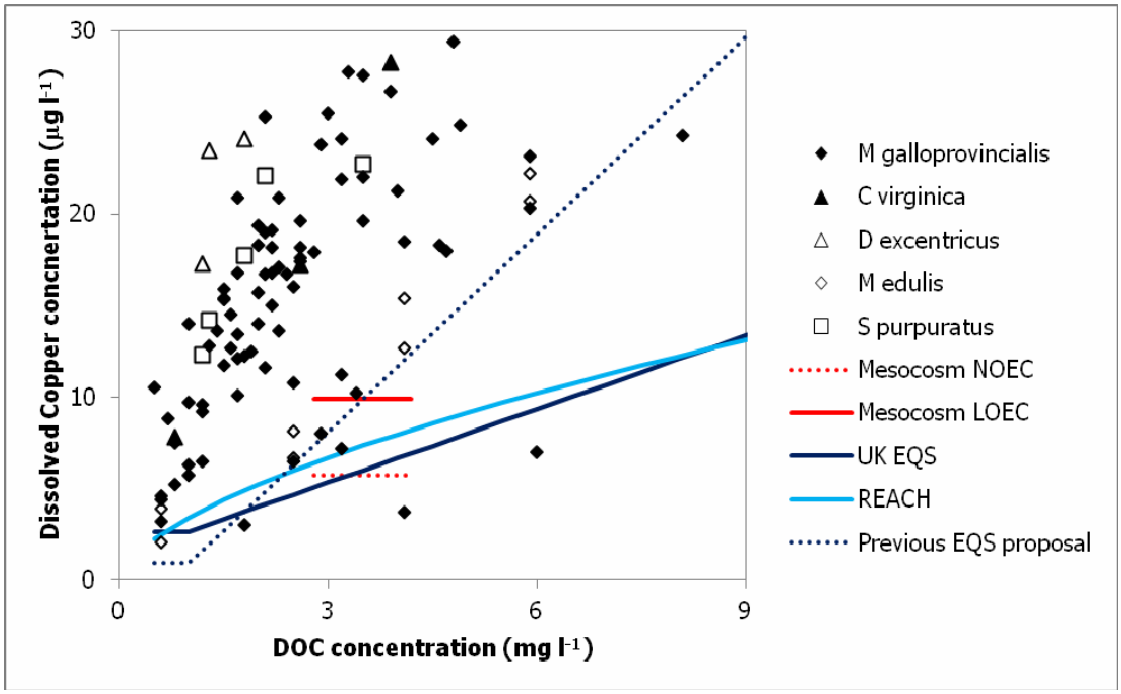

Figure 6.1 Proposed EQS for Cu in marine waters as a function of DOC concentration and ecotoxicity data.

The lowest EC10 in the ecotoxicity database is 2.03 μg l^{-1} for the algae *Phaeodactylum tricornutum*. Applying an assessment factor of 10 to this value to derive a PNEC according to the deterministic approach would result in a PNEC of 0.2 μg l^{-1} dissolved copper. This value is below the 5th percentile of coastal monitoring data for all of the Environment Agency regions with data available, and as such is unlikely to represent a practical basis for the derivation of an EQS due to being within the range of typical background concentrations in estuarine and coastal waters.

6.4 Indicative compliance assessment for copper

A reference EQS of 2.64 μg Cu l^{-1} is greater than the estimated ambient background concentrations set at the 5th percentile of the frequency distribution of measured Cu concentrations for all regions in England Wales for estuaries and coastal waters.

An indicative compliance assessment was performed for 36 Environment Agency monitoring sites, and a further 27 sites identified from the literature, on the basis of mean measured DOC concentrations and mean measured dissolved copper concentrations over the period 2000-08. Site specific PNEC values range from 2.68 to

17.2 μg l^{-1} dissolved copper, and were calculated according to the mean DOC concentration at the site. Six out of 63 sites (9.5%) have risk characterisation ratios of greater than 1 when assessed against the final EQS proposal, although the “failures” are relatively marginal with risk characterisation ratios of between 1.12 and 1.46.

6.5 Recommendations

An assessment factor of 1 is recommended for the derivation of the PNEC from the ecotoxicity data. An assessment factor of 3 was considered for application to the reference PNEC, although this did not result in adequate protection of the most sensitive species in ecotoxicity studies (cockles). The DOC correction has been revised to be consistent with both the similarities and differences between the response to DOC concentrations between different species. This results in a smaller effect of DOC on the site specific PNEC, and ensures that a greater degree of protection is assured for conditions with slightly elevated DOC concentrations.

The Reference PNEC is 2.64 mg l^{-1} dissolved Cu, and site specific PNEC values are calculated according to the following equation where DOC concentrations are greater than 1 mg l^{-1}:

$$PNEC_{Site\ Specific} = 2.677 * \left(\frac{DOC}{2} - 0.5\right) + 2.64$$

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Appendix 1. Saltwater toxicity data

Table A1-1: Overview of the NOEC values and physico-chemical parameters for saltwater algae and higher plants

Chemical form	Species	Age and/or size of test organism	Test duration	Effect	End-point	Value (µg/l)	Chemical Analysis	Exposure	Temp. (°C)	Cu back-ground (µg/l)	DOC	pH	Salinity (g/l)	Test water	Reference and data quality code
Copper	*Macrocystis pyrifera,* motile zoospore	Zoo-spores	19 days	sporophyte growth	NOEC	10.2	measured	static renewal	13-15	<0.6m	2.0e	7.8-8.3	35-37	artificial filtered seawater	Anderson et al., 1990 Q1
Copper	*Macrocystis pyrifera,* motile zoospore	Zoo-spores	19 days	Germ-ination	NOEC	(50.1)	measured	static renewal	13-15	<0.6m	2.0e	7.8-8.3	35-37	artificial filtered seawater	Anderson et al., 1990 Q1
Copper	*Macrocystis pyrifera,* motile zoospore	Zoo-spores	19 days	germ tube growth	NOEC	10.2	measured	static renewal	13-15	<0.6m	2.0e	7.8-8.3	35-37	artificial filtered seawater	Anderson et al., 1990 Q1
Copper chloride	*Fucus vesiculosus*	Zoo-spore	14 days	Growth	NOEC	11	measured	flow through	21	4.2m	1.67m	8.1	30.9	natural filtered seawater	Brooks 2006d* Q1
Copper chloride	*Fucus vesiculosus*	Zoo-spore	14 days	Growth	NOEC	14	measured	flow through	21	2.5m	1.05	8.1	31.1	natural filtered seawater + 0.09 mg DOC l^{-1} added as humic acids	Brooks 2006d* Q1
Copper chloride	*Fucus vesiculosus*	Zoo-spore	14 days	Growth	NOEC	18.5	measured	flow through	21	2.3m	2.11m	8.1	31.0	natural filtered	Brooks 2006d*

Chemical form	Species	Age and/or size of test organism	Test duration	Effect	End-point	Value (µg/l)	Chemical Analysis	Exposure	Temp. (°C)	Cu back-ground (µg/l)	DOC	pH	Salinity (g/l)	Test water	Reference and data quality code
														seawater+ 0.56 mg DOC l^{-1} added as humic acids	Q1
Copper chloride	*Fucus vesiculosus*	Zoo-spore	14 days	Growth	NOEC	32	measured	flow through	21	2.9m	2.56m	8.1	31.4	natural filtered seawater + 1.65 mg DOC l^{-1} added as humic acids	Brooks 2006d* Q1
Copper chloride	*Fucus vesiculosus*	Zoo-spore	14 days	Growth	NOEC	46	measured	flow through	21	2.8m	2.88m	8.1	30.9	natural filtered seawater + 2.03 mg DOC l^{-1} added as humic acids	Brooks 2006d* Q1
Copper sulphate	*Phaeo-dactylum tricornutum*	10^3 cellsml^{-1}	72 hours	Growth rate	EC10	2.9	measured	static	20	NR	1.0m	8.2-8.3	31.0	natural filtered seawater	Simpson 2003 Q1
Copper chloride	*Skeletonema costatum*	10^4 cells ml^{-1}	72 hours	Growth rate	NOEC	7.5	measured	static	20	<0.4m	2.19m	8.2-8.6	31.0	natural filtered seawater	Smyth 2006a Q1
Copper chloride	*Phaeo-dactylum tricornutum*	10^4 cellsml^{-1}	72 hours	Growth rate	NOEC	5.7	measured	static	20	<0.4m	2.19m	8.2-8.3	31.0	natural filtered seawater	Smyth 2006b Q1
Copper sulphate	*Nitzschia thermalis*	NR	NR	Growth	NOEC	32	nominal	static	0.5e	0.3e	16	NR	NR	Artificial seawater	Metaxas and Lewis 1991

Chemical form	Species	Age and/or size of test organism	Test duration	Effect	End-point	Value (µg/l)	Chemical Analysis	Exposure	Temp. (°C)	Cu back-ground (µg/l)	DOC	pH	Salinity (g/l)	Test water	Reference and data quality code
														(Aquil) excl EDTA	Q2
Copper sulphate	*Skeletonema costatum*	NR	NR	Growth	NOEC	25	nominal	static	0.5e	0.3e	16	NR	NR	Artificial seawater (Aquil) excl EDTA	Metaxasand Lewis 1991 Q2
Not reported	*Proro-centrum minimum*	NR	96 hours	Growth	NOEC	632	nominal	static	0.5e	2.0e	19	8.2	NR	Nutrient deficient filtered natural seawater	Miaoet al. 2005 Q2
Not reported	*Dunaliella tertiolecta*	NR	96 hours	Growth	NOEC	3160	nominal	static	0.5e	2.0e	19	8.2	NR	Nutrient deficient filtered natural seawater	Miaoet al. 2005 Q2
Not reported	*Synecho-coccus sp.*	NR	96 hours	Growth	NOEC	8.7	nominal	static	0.5e	2.0e	19	8.2	NR	Nutrient deficient filtered natural seawater	Miaoet al. 2005 Q2
Not reported	*Thallasiosira weisflogii*	NR	96 hours	Growth	NOEC	318	nominal	static	0.5e	2.0e	19	8.2	NR	Nutrient deficient filtered natural seawater	Miaoet al. 2005 Q2

E = value was estimated
M = value was measured and reported
NR = Not reported (For background concentration results are based on measured concentrations, therefore this does not affect the validity of the final result)

NOEC values in parentheses are not included in the derivation of a species mean NOEC, because they are not the most sensitive biological endpoint for the species
* Manuscript since published: Brooks et al., Ecotoxicology and Environmental Safety, 70, 88-98.

Table A1-2: Overview of the NOEC values and physico-chemical parameters for saltwater invertebrates

Chemical form	Species	Taxo-nomic group	Age and/ or size of test organism	Test duration	Effect	End-point	Value (µg/l)	Chemical Analysis	Exposure	Cu back-ground (µg l^{-1})	DOC	Temp (°C)	pH	Salinity (g/l)	Test water	Reference and data quality code
Copper	*Penaeus mergulensis*	CRU	Juvenile	14 days	growth	NOEC	33	measured	Flow through	<1m	2.0e	27	NR	20	Diluted natural seawater	Ahsanullah, M. et al 1995 Q1
Copper	*Penaeus monodon*	CRU	Juvenile	14 days	growth	NOEC	145	measured	Flow through	<1m	2.0e	27	NR	20	Diluted natural seawater	Ahsanullah, M. et al 1995 Q1
Copper sulphate	*Tisbe furcata*	CRU	Life cycle	100 days max	Survival and reproduction	NOEC	19.1	measured	Static renewal	NR	2.0e	15	8	34	Natural seawater	Bechmann R.K., 1994 Q1
Copper Chloride	*Artemia franciscana*	CRU	Cysts	48 hours	Hatching success	NOEC	6.6	measured	Static	0.2m	0.48m	25	7.8 – 8.1	NR	Artificial seawater	Brix, 2006 Q1
Copper chloride	*Mytilus edulis*	MOL	Embryo	48 hours	Development	NOEC	6.2	measured	Flow through	1.8m	1.51m	13	8.3	32	natural seawater	Brooks, S. 2006 Q1
Copper chloride	*Crassostreas gigas*	MOL	Embryo	24 hour	Development	NOEC	10.89	measured	Flow through	2.8m	2.19m	21 ± 1	8.0 - 8.2	31.1 - 34.2	natural seawater + 0.1 mg DOC l^{-1} added as humic acids	Brooks, S. 2006 Q1
Copper chloride	*Crassostreas gigas*	MOL	Embryo	24 hour	Development	NOEC	10.42	measured	Flow through	2.5m	3.36m	21 ± 1	8.0 - 8.2	31.1 - 34.2	natural seawater + 0.81 mg	Brooks, S. 2006 Q1

Chemical form	Species	Taxo-nomic group	Age and/ or size of test organism	Test duration	Effect	End-point	Value (µg/l)	Chemical Analysis	Exposure	Cu back-ground (µg l^{-1})	DOC	Temp (°C)	pH	Salinity (g/l)	Test water	Reference and data quality code
															$DOCl^{-1}$ added as humic acids	
Copper chloride	*Crassostreas gigas*	MOL	Embryo	24 hour	Development	NOEC	12.83	measured	Flow through	3.0m	3.36m	21 ± 1	8.0 - 8.2	31.1 - 34.2	natural seawater + 1.02 mg DOC l^{-1} added as humic acids	Brooks 2006 Q1
Copper chloride	*Crassostreas gigas*	MOL	Embryo	24 hour	Development	NOEC	19.53	measured	Flow through	3.6m	3.88m	21 ± 1	8.0 - 8.2	31.1 - 34.2	natural seawater + 1.85 mg DOC l^{-1} added as humic acids	Brooks 2006 Q1
Copper chloride	*Crassostreas gigas*	MOL	Embryo	24 hour	Development	NOEC	28.19	measured	Flow through	1.1m	4.66m	21 ± 1	8.0 - 8.2	31.1 - 34.2	natural seawater + 2.77 mg DOC l^{-1} added as humic acids	Brooks 2006 Q1
Copper chloride	*Crassostreas gigas*	MOL	Embryo	24 hour	Development	NOEC	47.13	measured	Flow through	3.2m	5.19m	21 ± 1	8.0 - 8.2	31.1 - 34.2	natural seawater + 3.13 mg DOC l^{-1} added as humic	Brooks 2006 Q1

Chemical form	Species	Taxo-nomic group	Age and/ or size of test organism	Test duration	Effect	End-point	Value (µg/l)	Chemical Analysis	Exposure	Cu back-ground (µg l^{-1})	DOC	Temp (°C)	pH	Salinity (g/l)	Test water	Reference and data quality code
															acids	
Copper sulphate	*Placopecten magellanicus*	MOL	Adult	8 weeks	gonad development	NOEC	10.0	measured	Flow-through	2.5-3.4m	2.0e	6.6	Not repo rted	25	Natural seawater	Gould, 1988 Q1
Copper chloride	*Eurytemora affinis*	CRU	<24 hrs	8 days	Mortality, fecundity and maturation	NOEC	51.1	measured	Semi-static	<3m	2.0e	25±2	7.9 - 8.8	14 - 17	natural estuarin e water	Hall, L., 1997 Q1
Copper chloride	*Paracentrotus lividus*	ECH	Embryo	48 hours	Development	NOEC	8.8	measured	Static	<0.4m	1.83m	18	8.2 – 8.3	34.4	natural seawater	Hurd, K. 2006a Q1
Copper nitrate	*Mercenaria mercenaria*	MOL	Larvae	96 hours	Development	NOEC	7.0	measured	Static	1m	0.5e	24	8.0 – 8.5	26.5	Artificial seawater	LaBreche, 2002 Q1
Copper chloride	*Paracentrotus lividus*	ECH	Embryo	48 hours	Development	NOEC	16.5	measured	Static	0.32 – 1.45m	2.0e	20	8.1	35	natural seawater	Lorenzo, J. 2006 Q1
Copper chloride	*Neanthes arenaceodentata*	Polycha ete	3-4 week larva	28 days	growth	NOEC	13.5	measured	Flow through	2±1m	2.0m	NR	NR	32	filtered natural seawater	Pesch et al., 1986 Q1
Copper chloride	*Neanthes arenaceodentata*	Polycha ete	3-4 week larva	28 days	growth	NOEC	12.1	measured	Flow through	2±1m	2.0m	NR	NR	32	filtered natural seawater	Pesch et al., 1986 Q1
Copper Chloride	*Mytilus edulis*	MOL	1.0-1.5 cm individuals	10 days	growth rate	NOEC	6.0	measured	daily renewal of solutions	2.0 – 2.4m	2.0m	NR	NR	NR	Filtered seawater	Redpath 1985 Q1
Copper chloride	*Goniastrea aspera*	CNI	Larvae	72 hours	Motility	NOEC	14.2	measured	Static	1.2m	2.0e	NR	NR	NR	Natural seawater	Reichelt-Brushett, 2004 Q1
Copper chloride	*Acropora tenuis*	CNI	Larvae	48 hours	Settlement success	NOEC	17.3	measured	Static	0.63m	2.0e	NR	NR	NR	Natural seawater	Reichelt-Brushett, 2000 Q1
Copper chloride	*Lobophytum compactum*	CNI	Eggs/sperm	5 hours	Fertilisation success	NOEC	36.0	measured	Static	NR	2.0e	NR	NR	NR	Natural seawater	Reichelt-Brushett,

Chemical form	Species	Taxo-nomic group	Age and/ or size of test organism	Test duration	Effect	End-point	Value (µg/l)	Chemical Analysis	Exposure	Cu back-ground (µg l^{-1})	DOC	Temp (°C)	pH	Salinity (g/l)	Test water	Reference and data quality code
																2005 Q1
Copper chloride	*Protothaca staminea*	MOL	5.2 to 5.8 cm total length	30 days	Mortality	NOEC	18	measured	Flow-through	0.35m	2.0e	12.3	8.1	32	natural seawater	Roesijida, G. 1980 Q1
Copper sulphate	*Mytilus galloprovincialis*	MOL	Embryo	48 hours	Development	NOEC	5.9	measured	Static	0.6m	0.9m	15	NR	NR	Filtered seawater	Rosen, 2005 Q1
Copper sulphate	*Mytilus galloprovincialis*	MOL	Embryo	48 hours	Development	NOEC	7.5	measured	Static	1.5m	0.9m	15	NR	NR	Filtered seawater	Rosen, 2005 Q1
Copper sulphate	*Mytilus galloprovincialis*	MOL	Embryo	48 hours	Development	NOEC	9.2	measured	Static	0.7m	1.5m	15	NR	NR	Filtered seawater	Rosen, 2005 Q1
Copper sulphate	*Mytilus galloprovincialis*	MOL	Embryo	48 hours	Development	NOEC	9.7	measured	Static	1.0m	0.9m	15	NR	NR	Filtered seawater	Rosen, 2005 Q1
Copper chloride	*Tisbe battagliai*	CRU	<24 hrs	21 days	Survival	NOEC	18	measured	Semi-Static	2.0m	2.79m	20 ± 1	8.1-8.4	35	natural seawater	Williams, T. 2006 Q1
Copper chloride	*Tisbe battagliai*	CRU	<24 hrs	21 days	Development	NOEC	18	measured	Semi-Static	2.0m	2.79m	20 ± 1	8.1-8.4	35	natural seawater	Williams, T. 2006 Q1
Copper chloride	*Tisbe battagliai*	CRU	<24 hrs	21 days	Reproduction	NOEC	18	measured	Semi-Static	2.0m	2.79m	20 ± 1	8.1-8.4	35	natural seawater	Williams, T. 2006 Q1
Copper sulphate	*Pandalus danae*	CRU	Larvae	>42 days	Mortality	NOEC	9.9	measured	Flow through	0.47m	2.0e	8.7-10.3	7.9-9.7	29.8-30.6	natural seawater	Young et al., 1979 Q1
Copper sulphate	*Pandalus danae*	CRU	Larvae	>42 days	Development	NOEC	9.9	measured	Flow through	0.47m	2.0e	8.7-10.3	7.9-9.7	29.8-30.6	natural seawater	Young et al., 1979 Q1
Copper chloride	*Ciona intestinalis*	Chordata	Fertilised embryos	20 hours	Embryonic development	NOEC	16	Nominal	Static	0.5e	0.3e	18–23	7.4–8.8	34	Artifical seawater	Bellas et al 2004 Q2
Copper chloride	*Ciona intestinalis*	CHOR	Fertilised embryos	20 hours	Larval attachment	NOEC	32	Nominal	Static	0.5e	0.3e	18–23	7.4–8.8	34	Artifical seawater	Bellas et al 2004 Q2

Chemical form	Species	Taxo-nomic group	Age and/ or size of test organism	Test duration	Effect	End-point	Value (µg/l)	Chemical Analysis	Exposure	Cu back-ground (µg l^{-1})	DOC	Temp (°C)	pH	Salinity (g/l)	Test water	Reference and data quality code
Copper chloride	*Mytilus edulis*	MOL	2 months, 4.5 mm	21 months	Growth	NOEC	7.9	Measured	Flow-through	3.0m	2.0e	2.6 to 24	NR	25	Natural seawater	Calabrese, A., et al 1984 Q2
Copper Chloride	*Crassostrea virginica*	MOL	Larvae	14 days	mortality	LC10	12.6	nominal	renewal every 24 hours	13.4m	2.0e	25	NR	24	filtered natural seawater	Calabrese, A. et al. 1977 Q2
Copper Chloride	*Mercenaria mercenaria*	MOL	Larvae	8-10 days	mortality	LC10	6.2	nominal	renewal every 24 hours	13.4m	2.0e	24	NR	24	filtered natural seawater	Calabrese, A. et al. 1977 Q2
Copper Chloride	*Crassostrea virginica*	MOL	Larvae	14 days	growth	EC10	14.8	nominal	renewal every 24 hours	13.4m	2.0e	24	NR	24	filtered natural seawater	Calabrese, A. et al. 1977 Q2
Copper Chloride	*Mercenaria mercenaria*	MOL	Larvae	8-10 days	growth	EC10	5.5	nominal	renewal every 24 hours	13.4m	2.0e	24	NR	24	filtered natural seawater	Calabrese, A. et al. 1977 Q2
Copper sulphate	*Aiptasia sp*	COE	Polyps	8 weeks	population growth	NOEC	70	measured	Static replenish ment	1.1e	2.0e	24	8.3	34	Natural seawater	Kaiser et al 2003 Q2
Copper Chloride	*Mysidopsis bahia*	CRU	Larvae	35 days	mortality	NOEC	(77)	measured	Flow through	2.9m	2.0e	20-25	NR	30	natural sea water	Lussier et al., 1985 Q2
Copper Chloride	*Mysidopsis bahia*	CRU	Larvae	35 days	reproduction	NOEC	38	measured	Flow through	2.9m	2.0e	20-25	NR	30	natural sea water	Lussier et al., 1985 Q2
Copper chloride	*Cancer anthonyi*	CRU	embryo	7 days	mortality	NOEC	10	nominal	renewal every working day	1.7m	2.0e	NR	7.8	34	filtered natural seawater	MacDonald et al., 1988 Q2
Copper chloride	*Cancer anthonyi*	CRU	embryo	11 days	mortality	NOEC	10	nominal	renewal every working	1.7m	2.0e	NR	7.8	34	filtered natural seawater	MacDonald et al., 1988 Q2

Chemical form	Species	Taxo-nomic group	Age and/ or size of test organism	Test duration	Effect	End-point	Value (µg/l)	Chemical Analysis	Exposure	Cu back-ground (µg l^{-1})	DOC	Temp (°C)	pH	Salinity (g/l)	Test water	Reference and data quality code
									day							
Not reported	*Echinogammarus perlotti*	CRU	Juvenile	21 days	Mortality	NOEC	100	Nominal	renewal twice weekly	6.0m	0.3e	10	NR	33	Artifical seawater	Rainbow et al 1989 Q2
Not reported	*Elminius modestus*	CRU	Juvenile	28 days	Mortality	NOEC	6	Nominal	renewal twice weekly	6.0m	0.3e	10	NR	33	Artifical seawater	Rainbow et al 1989 Q2
Not reported	*Palaemon elegans*	CRU	Juvenile	21 days	Mortality	NOEC	316	Nominal	renewal twice weekly	6.0m	0.3e	10	NR	33	Artifical seawater	Rainbow et al 1989 Q2
Not reported	*Carcinus maenass*	CRU	Juvenile	21 days	Mortality	NOEC	1000	Nominal	renewal twice weekly	6.0m	0.3e	10	NR	33	Artifical seawater	Rainbow et al 1985 Q2
Not reported	*Elminius modestus*	CRU	Juvenile	21 days	Mortality	NOEC	316	Nominal	renewal days 3, 7, 11, 16	6.0m	0.3e	10	NR	33	Artifical seawater	Rainbow et al 1985 Q2
Not reported	*Palaemon elegans*	CRU	35-50 mm	21 days	Survival	NOEC	316	Nominal	Static renewal	6.0m	0.3e	NR	NR	NR	Artifical seawater (Tropic Marin Neu)	White, & Rainbow, 1982 Q2
Copper chloride	*Eudistylia vancouveri*	ANN	Larvae	35 days	Growth	NOEC	6.1	Nominal	Flow-through	0.3m	2.0e	8.2	7.8	30.4	Natural seawater	Young, et al, 1979 Q2
Copper chloride	*Argopecten irradians*	MOL	Adult	56 days	Spawning	NOEC	>10.2	Measured	Flow-through	1.8m	2.0e	14 (8-18)	NR	29-32	Natural seawater	Zaroogian, & Johnston, 1983 Q2

E = Value was estimated
M = Value was measured and reported
NR = Not reported (For background concentration results are based on measured concentrations, therefore this does not affect the validity of the final result)
ANN = annelid, CHOR = chordate, COE = coelenterata, CRU = crustacean, MOL = mollusca

Table A1-3: Overview of the NOEC values and physico-chemical parameters for saltwater fish

Chemical form	Species	Taxo-nomic group	Age and/ or size of test organism	Test duration	Effect parameter	End-point	Value (µg/l)	Chemical Analysis	Exposure	Cu back-ground (µg l^{-1})	DOC	Temp (°C)	pH	Salinity (g/l)	Test water	Reference and data quality code
Copper chloride	*Atherinops affinis*	Fish	early blastula embryo	12 days	embryo abnormalities	NOEC	(123)	measured	static	<3m	2.0e	21	7.1-7.7	33	filtered natural seawater	Anderson et al., 1991
Copper chloride	*Atherinops affinis*	Fish	early blastula embryo	12 days	hatchability	NOEC	(123)	measured	static	<3m	2.0e	21	7.1-7.7	33	filtered natural seawater	Anderson et al., 1991
Copper chloride	*Atherinops affinis*	Fish	early blastula embryo	12 days	young abnormalities	NOEC	63	measured	static	<3m	2.0e	21	7.1-7.7	33	filtered natural seawater	Anderson et al., 1991 Q1
Copper chloride	*Atherinops affinis*	Fish	early blastula embryo	12 days	embryo abnormalities	NOEC	(115)	measured	static	<3m	2.0e	21	7.1-7.7	33	filtered natural seawater	Anderson et al., 1991
Copper chloride	*Atherinops affinis*	Fish	early blastula embryo	12 days	hatchability	NOEC	(115)	measured	static	<3m	2.0e	21	7.1-7.7	33	filtered natural seawater	Anderson et al., 1991
Copper chloride	*Atherinops affinis*	Fish	early blastula embryo	12 days	young abnormalities	NOEC	68	measured	static	<3m	2.0e	21	7.1-7.7	33	filtered natural seawater	Anderson et al., 1991 Q1
Copper chloride	*Atherinops affinis*	Fish	early blastula embryo	12 days	embryo abnormalities	NOEC	55	measured	static	<3m	2.0e	21	7.1-7.7	33	filtered natural seawater	Anderson et al., 1991 Q1
Copper chloride	*Atherinops affinis*	Fish	early blastula embryo	12 days	hatchability	NOEC	55	measured	static	<3m	2.0e	21	7.1-7.7	33	natural seawater	Anderson et al., 1991 Q1
Copper chloride	*Atherinops affinis*	Fish	early blastula embryo	12 days	young abnormalities	NOEC	55	measured	static	<3m	2.0e	21	7.1-7.7	33	natural seawater	Anderson et al., 1991 Q1

Chemical form	Species	Taxo-nomic group	Age and/ or size of test organism	Test duration	Effect parameter	End-point	Value (µg/l)	Chemical Analysis	Exposure	Cu back-ground (µg l^{-1})	DOC	Temp (°C)	pH	Salinity (g/l)	Test water	Reference and data quality code
Copper chloride	*Cyprinodon variegates*	Fish	Egg	7 days	hatchability	NOEC	(109)	measured	Flow through	<0.4m	1.19m	25	8.0-8.3	23.5-27	natural seawater	Hurd 2006b
Copper chloride	*Cyprinodon variegates*	Fish	Embryo-larval stages	32 days	Survival	NOEC	(109)	measured	Flow through	<0.4m	1.19m	25	8.0-8.3	23.5-27	natural seawater	Hurd 2006b
Copper chloride	*Cyprinodon variegates*	Fish	Embryo-larval stages	32 days	Embryo development (weight)	NOEC	57.8	measured	Flow through	<0.4m	1.19m	25	8.0-8.3	23.5-27	natural seawater	Hurd 2006b Q1

Appendix 2. Study summaries

Reference	Arnold 2005

Information on the test species	
Test species used	*Mytilus galloprovincialis*
Source of the test organisms	Carlsbad Aquafarms, Carlsbad, CA, USA
Holding conditions prior to test	Not stated. All tests conducted by the same laboratory, Pacific EcoRisk, Martinez, CA, USA.
Life stage of the test species used	embryos

Information on the test design	
Methodology used	USEPA1995. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to west coast marine and estuarine organisms. Washington DC: USEPA. EPA/600/R-95/136.
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Natural field collected seawaters from 13 sites in San Francisco Bay north of the Dumbarton Bridge. Samples were collected 4 times: Sept 2000, Feb 2001, April 2001 and June 2001. Clean-water reference site in the Pacific Ocean outside San Francisco Bay. Further samples were collected in Feb 2003 from Puget Sound, Galveston Bay and Narragansett Bay.
Test concentrations used	Concentrations spanning the EC50 of *Mytilus* sp. together with a positive, negative and sea salt control.
Number of replicates per concentration	Stated as per guideline
Number of organisms per replicate	Stated as per guideline
Nature of test system (static, semi-static or flow-through, duration, feeding)	Stated as per guideline
Measurement of exposure concentrations	Yes.
Measurement of water quality parameters	Tests are reported as acceptable according to guidelines. Salinity of all samples was adjusted upward using reagent grade GP2 artificial sea salts or downward using reverse osmosis deionized water to a constant 30‰ to meet guideline. DOC ranged from 0.3 – 10 mg C l^{-1}.
Test validity criteria satisfied	Tests are reported as acceptable according to guidelines
Water quality criteria satisfied	Tests are reported as acceptable according to guidelines

Study conducted to GLP	Not stated
Comments	Comprehensive unpublished supporting data made available to wca. 54 data sets of acceptable quality were taken forward and used in the DOC bioavailability correction factor analysis

Reliability of study	**Reliable without restriction**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	Arnold et al. 2006

Information on the test species	
Test species used	*Mytilus galloprovincialis*
Source of the test organisms	Carlsbad Aquafarms, Carlsbad, CA, USA
Holding conditions prior to test	Not stated. All tests conducted by the same laboratory, Pacific EcoRisk, Martinez, CA, USA.
Life stage of the test species used	embryos

Information on the test design	
Methodology used	USEPA1995. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to west coast marine and estuarine organisms. Washington DC: USEPA. EPA/600/R-95/136.
Form of the test substance	Not stated
Source of the test substance	Not stated
Type and source of the exposure medium	Natural field collected seawaters from Mugu Lagoon and lower sections of Callegaus Creek, Ventura County, California, USA. Samples were collected 3 times: Aug 2003, Jan 2004, and Mar 2004. Reference site water collected from Pacific Ocean at Granite Canyon marine Laboratory, Carmel, CA.
Test concentrations used	Concentrations spanning the EC50 of *Mytilus* sp. together with a positive, negative and sea salt control.
Number of replicates per concentration	Stated as per guideline
Number of organisms per replicate	Stated as per guideline
Nature of test system (static, semi-static or flow-through, duration, feeding)	Stated as per guideline
Measurement of exposure concentrations	Yes.
Measurement of water quality parameters	Tests are reported as acceptable according to guidelines. Salinity of all samples was adjusted upward using reagent grade GP2 artificial sea salts or downward using reverse osmosis deionized water to a constant 30 ± 2‰ to meet guideline. DOC ranged from 1 – 12 mg C l^{-1}.
Test validity criteria satisfied	Tests are reported as acceptable according to guidelines
Water quality criteria satisfied	Tests are reported as acceptable according to guidelines
Study conducted to GLP	Not stated
Comments	Comprehensive unpublished supporting data made available to wca. 21 data sets of acceptable quality were taken forward and

	used in the DOC bioavailability correction factor analysis

Reliability of study	**Reliable without restriction**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	Arnold et al. 2007

Information on the test species	
Test species used	*Mytilus galloprovincialis*
Source of the test organisms	Carlsbad Aquafarms, Carlsbad, CA, USA
Holding conditions prior to test	Not stated. All tests conducted by the same laboratory, Pacific EcoRisk, Martinez, CA, USA.
Life stage of the test species used	embryos

Information on the test design	
Methodology used	USEPA1995. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to west coast marine and estuarine organisms. Washington DC: USEPA. EPA/600/R-95/136.
Form of the test substance	$CuNO_3$
Source of the test substance	Inorganic Ventures, Lakewood, NJ, USA
Type and source of the exposure medium	3 separate solutions made using Crystal Sea marinemix, HW Marinemix or GP2 synthetic sea salts dissolved in reverse osmosis, deionized water at 30 ± 2‰. Control water was 0.45 µm filtered natural seawater collected from University of California at Davis Bodega Bay Marine Laboratory and adjusted to 30 ± 2‰ salinity with reverse osmosis, deionized water.
Test concentrations used	Not stated
Number of replicates per concentration	Stated as per guideline
Number of organisms per replicate	Stated as per guideline
Nature of test system (static, semi-static or flow-through, duration, feeding)	Stated as per guideline
Measurement of exposure concentrations	Yes.
Measurement of water quality parameters	Crystal Sea: DOC 6 mg l^{-1}; pH 8.6; hardness 240 mg $CaCO_3$ l^{-1}; HW Marinemix: DOC 0.8 mg l^{-1}; pH 7.95; hardness 244 mg $CaCO_3$ l^{-1}; GP2: DOC 1 mg l^{-1}; pH 8.14; hardness 267 mg $CaCO_3$ l^{-1}.
Test validity criteria satisfied	Concurrent reference toxicity tests were performed and results were within the established mean ± 2 standard deviations of the point estimate generated by the 20 most recent reference toxicity tests.
Water quality criteria satisfied	Tests are reported as acceptable according to guidelines
Study conducted to GLP	Not stated

Comments	Comprehensive unpublished supporting data made available to wca. 3 data sets of acceptable quality were taken forward and used in the DOC bioavailability correction factor analysis

Reliability of study	**Reliable without restriction**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	Arnold et al. 2009

Information on the test species	
Test species used	*Mytilus galloprovincialis* *Mytilus edulis*
Source of the test organisms	*M. galloprovincialis* – commercial culture, Carlsbad Aquafarms, Carlsbad, CA, USA *M. edulis* – wild population, Aquatic Research Organisms, Hampton, NH, collected from Seabrook, NH, USA.
Holding conditions prior to test	Upon receipt and prior to spawning, adult bivalves held in filtered seawater at 15°C. To induce spawning gravid adults placed into clean 0.45 µm-filtered seawater at 20°C. Individuals moved to separate container at start of release of sperm or eggs for isolation and collection of gametes. Gametes examined for viability. Best quality gametes used to prepare freshly-fertilized embryos with resulting embryos examined ~ 1 h post fertilization to ensure viability.
Life stage of the test species used	embryos

Information on the test design	
Methodology used	*M. galloprovincialis* - USEPA1995. Short-term methodsfor estimating the chronic toxicity of effluents and receiving waters to westcoast marine and estuarine organisms. Washington DC: USEPA. EPA/600/R-95/136. *M. edulis* – ASTM 1993. Guide for conducting static acute toxicity tests starting with embryos of four species of saltwater bivalve molluscs. Standard E724-98.
Form of the test substance	Commercial Cu standard (Cu in 2% HNO_3)
Source of the test substance	Inorganic Ventures, Lakewood, NJ, USA
Type and source of the exposure medium	Test samples with 4 DOC concentrations made from 2 ambient samples having different natural DOC concentrations. Low DOC: Pacific Ocean at University of California at Davis Granite Canyon Marine Laboratory, Carmel, CA, USA. High DOC: South San Francisco Bay. Low DOC water salinity adjusted to 30 ± 2‰ using laboratory reverse osmosis-deionized water. High DOC water salinity increased to 30 ± 2‰ using GP2 synthetic sea salt.
Test concentrations used	Low DOC: 0, 4, 6, 8, 12, 17, 24 and 34 µg l^{-1} nominal. Intermediate low, intermediate high and high

	DOC: 0, 6, 8, 12, 17, 24, 34, 49, 70 and 100 µg l^{-1} nominal. Concurrent reference toxicity tests performed.
Number of replicates per concentration	5
Number of organisms per replicate	~ 150 – 300 embryos
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	Yes.
Measurement of water quality parameters	Temp 15°C; photoperiod 16:8 light/dark
Test validity criteria satisfied	Yes: > 90% of the surviving control embryos achieved normal development of the “D-hinge” stage.
Water quality criteria satisfied	pH 7.82 – 7.98; hardness 5150 – 5280 mg $CaCO_3$ l^{-1} (low to high DOC)
Study conducted to GLP	Not stated
Comments	8 data sets of acceptable quality for each species were taken forward and used in the DOC bioavailability correction factor analysis.

Reliability of study	**Reliable without restriction**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	Arnold et al. 2010a

Information on the test species	
Test species used	*Mytilus galloprovincialis* *Crassostrea virginica* *Dendraster excentricus* *Strongylocentrotus purpuratus*
Source of the test organisms	*M. galloprovincialis* – commercial culture, Carlsbad Aquafarms, Carlsbad, CA, USA *C. virginica* – wild population, Aquatic Research Organisms, Hampton, NH, USA. *D. excentricus* and *S. purpuratus*wild populations obtained from a commercial supplier (Dave Gutoff).
Holding conditions prior to test	Upon receipt and prior to spawning, adult bivalves held in filtered seawater at 15°C. To induce spawning gravid adults placed into clean 0.45 µm-filtered seawater at 20°C (*M. galloprovincialis*) and 30°C (*C. virginica*). Individuals moved to separate container at start of release of sperm or eggs for isolation and collection of gametes. Gametes examined for viability. Best quality gametes used to prepare freshly-fertilized embryos with resulting embryos examined ~ 1 h post fertilization to ensure viability. Upon receipt adult echinoderms were rinsed with filtered seawater and injected with 0.5 ml of a 0.5M KCl solution to induce spawning, after which indicviduals were inverted over separate glass beakers for isolation and collection. Gametes examined for viability. Best quality gametes used to prepare freshly-fertilized embryos with resulting embryos examined ~ 1 h post fertilization to ensure viability.
Life stage of the test species used	Embryos ~ 1 h post fertilization

Information on the test design	
Methodology used	*M. galloprovincialis* - USEPA1995. Short-term methodsfor estimating the chronic toxicity of effluents and receiving waters to westcoast marine and estuarine organisms. Washington DC: USEPA. EPA/600/R-95/136.

	C. virginica – ASTM 1993. Guide for conducting static acute toxicity tests starting with embryos of four species of saltwater bivalve molluscs. Standard E724-98. *D. excentricus* and *S. purpuratus* US EPA guidelines, specific reference not cited.
Form of the test substance	Commercial Cu standard (Cu in 2%HNO_3)
Source of the test substance	Inorganic Ventures, Lakewood, NJ, USA
Type and source of the exposure medium	Test samples with 4 DOC concentrations made from 2 ambient samples having different natural DOC concentrations. Low DOC: Pacific Ocean at University of California at Davis Granite Canyon Marine Laboratory, Carmel, CA, USA. High DOC: South San Francisco Bay. Low DOC water salinity adjusted to 30 ± 2‰ using laboratory reverse osmosis-deionized water. High DOC water salinity increased to 30 ± 2‰ using GP2 synthetic sea salt. Salinity adjustments 32 ± 2‰ for *D. excentricus* and *S. purpuratus*. Two additional waters, north end and south end of San Diego Bay used for *D. excentricus* and *S. purpuratus*.
Test concentrations used	8 nominal concentrations for low DOC exposures and 10 nominal concentrations for all other DOC exposures. Sea-salt control also included. Concurrent reference toxicity tests performed.
Number of replicates per concentration	5
Number of organisms per replicate	~ 150 – 300 embryos
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	Yes.
Measurement of water quality parameters	Temp 15°C; photoperiod 16:8 light/dark
Test validity criteria satisfied	Yes: > 90% (*M. galloprovincialis*) or > 70% (*C. virginica*) of the surviving control embryos achieved normal development of the “D-hinge” stage. After 72 h ≥ 80% surviving control *D. excentricus* and *S. purpuratus* embryos achieved normal development of pluteus larvae.
Water quality criteria satisfied	Full water chemistry details provided to wca. Representative values as follows: pH 7.84 – 8.09; hardness 5980 – 7270 mg $CaCO_3$ l^{-1}
Study conducted to GLP	Not stated
Comments	6 data sets of acceptable quality for *D. excentricus* and *S. Purpuratus*, 4 data sets for *C. Virginica* and 10 for*M. galloprovincialis* were taken forward and used in the DOC bioavailability correction factor analysis.
Reliability of study	**Reliable without restriction**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	Baumann et al. 2009

Information on the test species	
Test species used	Brown algae - *Ascophyllum nodosum, Fucus vesiculosus,* Green algae - *Ulva intestinalis, Cladophora rupestris,* Red algae - *Chondrus crispus, Palmaria palmate, Polysiphonia lanosa*
Source of the test organisms	Irish Atlantic coastal site – near Spiddal, Co. Galway (53° 13'N, 9° 17'W)
Holding conditions prior to test	Plants acclimatised under laboratory conditions for 48 h.
Life stage of the test species used	Whole plants of all species used except for *A. nodosum*, where only apical plant parts were collected. All plants were sized between 40-60 mm, except *U. intestinalis* which were up to 100 mm in length to ensure sufficient biomass.

Information on the test design	
Methodology used	No standard guideline methodology cited. Chlorophyll fluorescence of photosystemII was measured (yield) on day 0, 4, 7 and 14. Fluorescence yield as the overall quantum yield of photochemistry of photosystemII was measured using a PAM-2000 fluorometer. 3 individual measurements were taken on each plant.
Form of the test substance	Copper chloride
Source of the test substance	Puriss grade, Sigma-Aldrich
Type and source of the exposure medium	Sterile, unenriched seawater control (Cu concentration 0.01 µmol l^{-1} [0.06 µg l^{-1}]).
Test concentrations used	0 (control), 0.1, 1 and 10 µmol l^{-1} (nominal concentrations).
Number of replicates per concentration	1
Number of organisms per replicate	3 plants of each species
Nature of test system (static, semi-static or flow-through, duration, feeding)	Semi-static. Plants transferred to fresh culture medium on day 4 and 7.
Measurement of exposure concentrations	No. Cu concentration in exposure media measured after experimental procedure on day 14.
Measurement of water quality parameters	Temperature 10 ± 1°C; light 20µmol m^{-2} s^{-1} provided by fluorescence tubes.
Test validity criteria satisfied	None set.
Water quality criteria satisfied	Only temperature recorded

Study conducted to GLP	Not stated
Comments	No significant effect of different Cu treatments on the yield of *A. nodosum*, *F. vesiculosus*, *C. rupestris*, *U. intestinalis* and *P. lanosa* over the 14-day period. The 10µmol l^{-1} (63.5 µg l^{-1}) treatment significantly reduced yield in *C. crispus* by day 7 and post day 7 for *P. palmata*.

Reliability of study	**Unreliable** (the difference factor between tested concentrations was high (0, 0.1, 1 and 10 µmol l^{-1}) and only one replicate per concentration containing 3 plants)
Relevance of study	**Relevant**
Klimisch Code	**3**

Reference	Debelius et al. 2009

Information on the test species	
Test species used	*Nannochloropsis gaditana, Isochrysis galbana* (T-iso), *Chaetoceros* sp., *Rhodomonas salina, Tetraselmis chuii*
Source of the test organisms	Marine Microalgal Culture Collection, Instituto de Ciencias marinas de Andalucia.
Holding conditions prior to test	Filtered, sterilized seawater from Bay of Cadiz. Enriched with NO_3^- (124 µM), PO_4^{3-} (4 µM) and for Chaetoceros sp., SiO_2 (50 µM). Background Cu < 1 µg l^{-1}. Cultures maintained at 20 ± 1°C, under continuous white light (30 µmol m^{-2} s^{-2}) and aseptically transferred to fresh media weekly to maintain cells in logarithmic growth phase.
Life stage of the test species used	Exponentially growing populations

Information on the test design	
Methodology used	72 h toxicity test. No standard methodology cited. Data obtained by flow cytometry.
Form of the test substance	Not stated
Source of the test substance	Merck
Type and source of the exposure medium	Same as culture medium, described above.
Test concentrations used	0, 5, 10, 20, 40, 80, 120, 200, 300 and 600 µg l^{-1} (nominal).
Number of replicates per concentration	Test performed in triplicate
Number of organisms per replicate	Initial cell density (cells ml^{-1}): *N. gaditana* – 42 x 10^4; *Isochrysis galbana* (T-iso) – 6.6 x 10^4; *Chaetoceros* sp. – 4.2 x 10^4; *Rhodomonas salina* – 1.5 x 10^4; *Tetraselmis chuii* – 1 x 10^4.
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	Yes, 5 out of the 10 exposure concentrations. 5.2, 16.7, 87.2, 186, 590 µg l^{-1}.
Measurement of water quality parameters	20 ± 1°C; pH 8.
Test validity criteria satisfied	Yes - cell density in controls increased by a factor of 8 within 72 h.
Water quality criteria satisfied	Yes
Study conducted to GLP	Not stated
Comments	No reliable NOEC/EC10 data could be calculated. NOEC values were estimated graphically and assigned as Q3 data.

Reliability of study	**Unreliable** (NOEC values based on graphical interpretation)
Relevance of study	**Relevant**
Klimisch Code	**3**

Reference	Fitzpatrick et al. 2008

Information on the test species	
Test species used	*Mytilus trossulus*
Source of the test organisms	Collected from natural intertidal populations in pristine area, Broken Island Group, near Bamfield, British Columbia (48.45N, 125.10W)
Holding conditions prior to test	Adults cleaned, separated and transferred to aerated, flowing seawater baths (11 - 13°C) for up to 24 h prior to testing. Adults (15-20) then placed in 10 l seawater bath (22 - 25°C) to induce spawning. Immediately gamete release observed, mussels were placed in isolated 250 ml beakers, on ice, containing filtered seawater where gametes were released into the water column. Adults were only used if they continued to release gametes after transfer to the beaker.
Life stage of the test species used	Sperm, eggs, embryos

Information on the test design	
Methodology used	(1) *Effects on sperm motility and fertilization success*. Sperm motility was assessed from 11 males at ~ 3.2, 32 and 100 minutes, subsequent to the Cu spike, for each of 7 concentrations. To control for variability between and among males and females, fertilization trials were conducted mixing sperm from a single male (from each of 7 treatments) with eggs from a single female. The experiment was repeated 7 times with different individuals at each concentration. (2) *Effects of Cu on egg viability.* Females (n = 8) allowed to release eggs for ~ 15 min. A homogenized egg/water solution was spiked with appropriate Cu solution and held on ice for 100 min prior to fertilization success trials. As before, sperm from a single male used to fertilize eggs from a single female. The experiment was repeated 8 times with different individuals at each concentration. (3) *Effects of Cu on embryo development.* Standard methodology – Standard operating procedure 21.1 blue mussel embryo (*Mytilus galloprovincialis*) National Institute of Water and Atmospheric Research Ltd, Hamilton, New Zealand. 2005. 48 h exposure, 5 replicates.
Form of the test substance	Copper chloride
Source of the test substance	Sigma-Aldrich
Type and source of the exposure medium	Filtered seawater
Test concentrations used	0, 0.32, 1.0, 3.2, 10, 32 and 100 µg l^{-1} (nominal)
Number of replicates per concentration	See above - methodology used

Number of organisms per replicate	See above - methodology used
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	Yes. 1.47 (control/background), 1.15, 2.37, 4.63, 11.67, 27.4, 71.0 µg l^{-1} (mean measured).
Measurement of water quality parameters	Only temperature reported
Test validity criteria satisfied	Not stated
Water quality criteria satisfied	Yes for temperature
Study conducted to GLP	Not stated
Comments	20 ± 3.5% abnormal embryos in controls. US EPA guidelines require ≤ 10% abnormalities in surviving controls. Similar effect seen in lowest 3 test concentrations.

Reliability of study	**Unreliable** (embryo test - control response too high)
Relevance of study	**Relevant**
Klimisch Code	**3**

Reference	Foekema et al. 2010

Information on the test species	
Test species used	Plankton and small invertebrates were introduced with the water and/or sediment during the installation of the mesocosms. Other introduced species: *Cerastoderma edule* (cockle) *Littorina littorea* (periwinkle) *Corophium volutator* (mud shrimp) *Arenicola marina* (lugworm) *Halichandria panicea* (bread-crumb sponge) Macroalgae were introduced as juvenile plants on substrates – *Ulva intestinalis* (sea lettuce)
Source of the test organisms	All field collected
Holding conditions prior to test	Introduced into mesocosms prior to dosing: *Cerastoderma edule* day -29 *Littorina littorea* – day -10 *Corophium volutator* – day -27 *Arenicola marina* – day -26 *Halichandria panicea* – day -1 *Ulva intestinalis* – day + 69
Life stage of the test species used	*Cerastoderma edule* 25.1 ± 1.5 mm *Littorina littorea* – 16.3 ± 1.2 mm *Arenicola marina*– 6.0 g *Halichandria panicea* – 63 ± 15 g

Information on the test design	
Methodology used	No standard guideline available for marine mesocosm tests. Recommendations from the 'HARAP' and 'CLASSIC' workshops for freshwater mesocosms taken into consideration where applicable.
Form of the test substance	Copper (II) sulphate pentahydrate – $CuSO_4 \cdot 5H_2O$
Source of the test substance	Sigma-Aldrich
Type and source of the exposure medium	Seawater collected from Oosterschelde, a relatively pristine tidal bay. Mesocosms allowed to establish for 33 d prior to dosing with Cu.
Test concentrations used	1.0 (control, natural background), 2.6, 5.2, 9.0, 15 and 27 µg l^{-1} nominal. 1.0, 2.9, 5.7, 9.9, 16.4 and 31.1 µg l^{-1}mean measured. Co-efficient of variance 7 – 14 % in treated mesocosms over the study period.
Number of replicates per concentration	3
Number of organisms per replicate	*Cerastoderma edule* - 25 *Littorina littorea* - 40 *Corophium volutator* - 300 *Arenicola marina* - 20 *Halichandria panicea* - 2 *Ulva intestinalis* – 'seedlings' on 2 glass slides
Nature of test system (static, semi-static or	Outdoor, partially buried, circular glass-fibre

flow-through, duration, feeding)	tanks (180 cm height x 190 cm diameter (top) and 175 cm (bottom). ~ 20 cm depth natural sediment and water column ~ 140 cm. Sediment was collected from the coastal North Sea (90% grain size between 250 and 500 µm and ~ 0.5% organic matter). Dosing solution was applied continuously to compensate for dissipation of the dissolved Cu due to sorption to sediment, tank wall etc. Mesocosms aerated and covered with transparent lid. Evaporation losses were replenished with demineralised water after 45 d to reduce the increased salinity level.
Measurement of exposure concentrations	Yes, samples analysed 3 times per week
Measurement of water quality parameters	Yes, water temp, salinity and pH twice weekly. Nutrient levelshardness every other week. DOC weekly. Wks 1 – 3 water temp ~ 15°C, days 34 to 47 temperatures up to 20°C. Highest temperatures recorded after day 58 with a maximum of 24°C on day 65. DO ~ 100% saturation until day 70. After this date saturation reached 140% in some individual mesocosms with no clear relationship to treatment. pH 7.8 – 8.1. No significant differences between treatments prior to day 26. Significant differences observed in highest treatment mesocosms compared to controls from day 26 onwards and in the 16 µg l^{-1} treatment from day 44. No significant differences in salinity between mesocosms ~ 32.8‰. DOC in water of untreated mesocosms increased from 2.8 to 4.2 mg l^{-1} during the study. A comparable development was observed in all mesocosms up to the 9.9 µg l^{-1} treatments. Statistically significant increase in DOC in highest 2 treatments from day 19 and 43 onwards for the 31 and16 µg l^{-1} treatments, respectively.
Test validity criteria satisfied	No validity criteria stated
Water quality criteria satisfied	Yes
Study conducted to GLP	N/A
Comments	Statistical analysis for single species and endpoints: significance of differences between the controls and the treated mesocosms was tested using a one-way ANOVA with a Dunnet's multiple comparison test as post test for single data sets. In case of time series a two-way ANOVA with Bonferoni post-test for time series was applied. Functional groups:

	Principal Response Curve (PRC) analyses were performed to evaluate response in time of phytoplankton and zooplankton community and primary production to treatment. Mesocosm LOEC 9.9 µg l^{-1} Mesocosm NOEC 5.7 µg l^{-1} Mesocosm NOEAEC (No Observed Ecological Adverse Effect Concentration) 5.7 µg l^{-1}

Reliability of study	**Reliable without restriction**
Relevance of study	**Relevant**
Klimisch Code	**1**

Reference	Hall et al. 2008

Information on the test species	
Test species used	*Eurytemora affinis*
Source of the test organisms	Chesapeake Biological Laboratory (Solomons, MD)
Holding conditions prior to test	Acclimated to required salinity at ~ 2 ppt per day change (starting from 10 ppt). Culture water autoclaved and filtered (1 µm) estuarine water, Choptank River at the University of Maryland, Horn Point Laboratory (Cambridge, MD). Salinity was adjusted with Kent Marine sea salts and reverse osmosis water. Copepod cultures fed daily with equal volumes *Thalassiosira weissflogii* and *Isochrysis galbana*.
Life stage of the test species used	~ 1 d old

Information on the test design	
Methodology used	96 h toxicity test.
Form of the test substance	Copper (II) chloride dihydrate
Source of the test substance	Sigma-Aldrich
Type and source of the exposure medium	As culture water.
Test concentrations used	0, 16, 28, 50, 90, 160, and 284 µg l^{-1} (nominal for 96-h 2.5 ppt salinity tests). 0, 28, 46, 72, 114, 180 and 284 µg l^{-1} (nominal for 96-h 5, 15 and 25 ppt salinity tests). 0, 28, 42, 66, 100, 152 and 232 µg l^{-1} (nominal for 2 mg l^{-1} DOC test). 0, 28, 46, 72, 114, 180 and 284 µg l^{-1} (nominal for 4 mg l^{-1} DOC test). 0, 42, 66, 100, 152, 232 and 354 µg l^{-1} (nominal for 6 and 8 mg l^{-1} DOC test).
Number of replicates per concentration	4
Number of organisms per replicate	12 - 16
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static, 16:8 light/dark photoperiod; daily feeding, no aeration unless DO < 40% saturation. 25 ± 1°C
Measurement of exposure concentrations	Yes. The percent loss of Cu ranged 0.4 to 35% among all experiments. The % difference between the mean measured concentration and nominal concentrations ranged from 1.8 to 27%.
Measurement of water quality parameters	Yes – test start and end in control, low, middle and high test concentrations
Test validity criteria satisfied	Control survival > 80%.
Water quality criteria satisfied	Yes
Study conducted to GLP	Not stated
Comments	Experimental variable – salinity and DOC

Reliability of study	**Reliable with restrictions** (some minor omissions in reporting)

Relevance of study	**Not Relevant** (estuarine species)
Klimisch Code	**2**

Reference	Kwok et al. 2008

Information on the test species	
Test species used	*Tigriopus japonicus*
Source of the test organisms	Field collected at Cape d'Aguilar marine Reserve, Hong Kong in warm-wet season (May to Aug) and in cold-dry season (Oct – Nov).
Holding conditions prior to test	Cultures acclimated in laboratory at 22 - 25°C, salinity 30 - 35‰ and photoperiod 16:8 light:dark cycle. Fed finely ground green algae *Enteromorpha* spp and commercial phytoplankton concentrate (Kent marine Phytoplex, US)
Life stage of the test species used	< 24 h

Information on the test design	
Methodology used	Modified ASTM protocol: ASTM E2317-04
Form of the test substance	Copper chloride ($CuCl_2$)
Source of the test substance	BDH Ltd
Type and source of the exposure medium	Artificial seawater (Sea salt: Tropic Marine, Germany)
Test concentrations used	0 and 10 µg l^{-1} nominal
Number of replicates per concentration	8
Number of organisms per replicate	12. When the copepods reached copepodid stage 5, male and female copepods were paired up and mated within each replicated for each treatment. Life cycle testing was terminated after release of the 2nd brood of nauplii from each mating pair.
Nature of test system (static, semi-static or flow-through, duration, feeding)	Test period 20 – 30d with static renewal every 96 h; 12:12 light/dark photoperiod; 25 ± 1°C; salinity 30 ± 0.5‰; pH 7.9 – 8.0. Fed 2 µl of algal solution containing 10^7 *Skeletonema costatum* cells ml^{-1} every 96h.
Measurement of exposure concentrations	No
Measurement of water quality parameters	Yes
Test validity criteria satisfied	Validity criteria not stated. 10.4 ± 4% control mortality.
Water quality criteria satisfied	Yes
Study conducted to GLP	No
Comments	Test repeated 10 times.

Reliability of study	**Unreliable** (No reliable NOEC data can be derived from this study as only a single copper concentration was tested)
Relevance of study	**Relevant**
Klimisch Code	**3**

Reference	Manyin and Rowe 2010

Information on the test species	
Test species used	*Palaemonetes pugio*
Source of the test organisms	Larval *P pugio* derived form laboratory-bred stocks of wild caught adults collected from Patuxent River, MD, USA.
Holding conditions prior to test	
Life stage of the test species used	0 or 1 d post hatch

Information on the test design	
Methodology used	Life cycle test – no standard guideline cited but well described.
Form of the test substance	$CuCl_2$
Source of the test substance	Not stated
Type and source of the exposure medium	Instant Ocean (Aquarium Systems, Mentor, OH) sea salts mixed with filtered (reverse osmosis) tap water. pH adjusted to 7.8 or 7.9 with 1 M NaOH.
Test concentrations used	Control, 9 and 26 µg Cu^{2+} l^{-1}. Concentration of free Cu ions (Cu^{2+}) was buffered by adding nitrilotriacetic acid (NTA), at 5 x 10^{-5} M
Number of replicates per concentration	4
Number of organisms per replicate	110 embryos. Only middle 80% of individuals to metamorphose were retained for juvenile exposure, Juvenile exposure was initiated on day 28 by transferring 55 individuals into replicate tanks. Gravid females were removed daily to individual jars.
Nature of test system (static, semi-static or flow-through, duration, feeding)	Semi-static, renewal every 96 h for larval exposures and 7 to 8 days juvenile and adult exposures. Fed *Artemia* nauplii daily; diet ofr juveniles and adults supplemented with coarsely ground dry food.
Measurement of exposure concentrations	Yes: due to the addition of NTA to the exposure water, the total Cu concentrations were similar for the 2 treatments (3200 and 3270 µg $l^{-1}$and as a result of variation in samples and limits of analytical resolution, concentrations in the 2 treatments could not be reliably distinguished using either ICP-MS or AA. Therefore, only the minimal free ion concentrations were reported.
Measurement of water quality parameters	Salinity 10 ppt. Temp 25°C.
Test validity criteria satisfied	Survival average > 95% for each life stage
Water quality criteria satisfied	Yes
Study conducted to GLP	Not stated
Comments	

Reliability of study	**Unreliable** (NTA present in test medium)
Relevance of study	**Not Relevant**(Salinity below the cut-off level)
Klimisch Code	**3**

Reference	Nadella et al. 2009

Information on the test species	
Test species used	*Mytilus trossolus*
Source of the test organisms	Adults collected from natural intertidal populations in the Broken Island Group, near Bamfield BC, Canada.
Holding conditions prior to test	Animals cleaned and transferred to aerated flowing seawater baths maintained at 11 -13°C and allowed to acclimate for 24 h. Spawning was induced using thermal shock (22-25°C)
Life stage of the test species used	embryos

Information on the test design	
Methodology used	NIWA, 2005. Standard operating procedure 21.1 Blue mussel embryo (*Mytilus galloprovincialis*) acute toxicity protocol.
Form of the test substance	$CuCl_2 \cdot 2H_20$ (analytical grade)
Source of the test substance	Sigma
Type and source of the exposure medium	Filtered seawater (0.2 µm) DOC from 3 sources – Luther Marsh, Ontario Canada. Nordic Reservoir and Suwannee River, purchased as freeze-dried powders, International Humic Substances Society, St. Paul, MN, USA) and reconstituted in filtered seawater.
Test concentrations used	0, 3.2, 10, 20, 32 and 100 µg l^{-1} nominal
Number of replicates per concentration	5
Number of organisms per replicate	600 – 1000 individuals
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static
Measurement of exposure concentrations	Yes
Measurement of water quality parameters	Yes: range of values for representative test solutions – pH 7.5 -7.96; DO > 5 mg l^{-1}; temp 21 ± 1°C.
Test validity criteria satisfied	Yes > 80% control embryos develop into normal D-shaped larvae.
Water quality criteria satisfied	Yes
Study conducted to GLP	Not stated
Comments	EC50 and EC20 data presented in the paper with insufficient detail to calculate a EC10 or NOEC.

Reliability of study	**Reliable with restrictions**
Relevance of study	**Relevant**
Klimisch Code	**2**

Reference	Rosen et al. 2008

Information on the test species	
Test species used	*Mytilus galloprovincialis* *Strongylocentrotus purpuratus*
Source of the test organisms	*M. galloprovincialis* – commercial culture, Carlsbad Aquafarms, Carlsbad, CA, USA *S. purpuratus*– field collected subtidally from local field populations by Marinus Scientific, Garden Grove, Calfornia, USA.
Holding conditions prior to test	Mussels induced to spawn by thermal shock by raising water temperature ~ 10°C. Sea urchins induced by injection of 0.5 M KCl into the peristomal membrane.
Life stage of the test species used	Embryos within 4 h of fertilization

Information on the test design	
Methodology used	USEPA1995. Short-term methodsfor estimating the chronic toxicity of effluents and receiving waters to westcoast marine and estuarine organisms. Washington DC: USEPA. EPA/600/R-95/136. These guidelines were modified as follows: to obtain sufficient biomass for weight measurements and tissue analysis experiments were conducted at higher initial embryo concentrations (60 and 40 embryos ml^{-1}, for mussels and sea urchins, respectively)
Form of the test substance	$CuSO_4 \cdot 5H_20$
Source of the test substance	Not stated
Type and source of the exposure medium	Filtered seawater (0.45 µm in the field) collected from San Diego Bay (north and south)
Test concentrations used	7 concentrations bracketing the expected LC50
Number of replicates per concentration	3
Number of organisms per replicate	60 and 40 embryos ml^{-1}, for mussels and sea urchins, respectively
Nature of test system (static, semi-static or flow-through, duration, feeding)	Static. No feeding required. Temp controlled light chambers - 15°C; photoperiod 16:8 light/dark.
Measurement of exposure concentrations	Yes.
Measurement of water quality parameters	pH, DO, temp and salinity measured daily in surrogate beakers that also included embryos.
Test validity criteria satisfied	Yes: > 90% of the surviving control embryos achieved normal development of the “D-hinge”

	stage.
Water quality criteria satisfied	Mean value range given for 5 different exposure scenarios. pH 7.85 – 8.2; DO 7.28 – 8.65 mg l^{-1}; Temp 14.8 - 16°C; DOC 1.3 – 3.43 mg l^{-1}.
Study conducted to GLP	Not stated
Comments	Data for *M. galloprovincialis* not used as value derived for the bioavailability correction factor used in the SSD.

Reliability of study	**Reliable without restriction**
Relevance of study	**Relevant**
Klimisch Code	**1**