COMMENTS ON THE UK TAG MARINE EQS

We thank the UK TAG for allowing us to provide input into the assessment. We are pleased to note that

- the RA report has been used
- the new information of single species EC10 values and the marine mesocosm was integrated into the report.
- A bio-availability correction was integrated in the marine EQS

However, in response to the document "Science Report – Marine Environmental Quality Standard for Copper" prepared on behalf of the UK Environment Agency, The European Copper Institute (ECI) and the EU Antifouling Copper Task Force (EUACTF) have prepared this document to highlight observations which they believe should be considered.

Background - ECI Approach

Defining DOC correlation

The ECI approach was based upon utilising all data for which there were DOC vs. toxicity data (Mytilus, *Fucus*, *Dendraster* etc. databases). An overall correlation was defined as;

$$EC50 \ \alpha \ aDOC^{0.6136}$$

Q1 data from the entire toxicity database were normalised using this correlation to 2.0 mg/l DOC, 1.0 mg/l active DOC, and the HC5-50 from the Species Sensitivity Distribution was derived. From this value, a site specific PNEC could be calculated as;

$$PNEC_{site\_specific} = [PNEC \text{ (at 2.0 mg/l DOC)}] \times [\text{active DOC}]^{0.6136}$$

HC5-50 = 5.2 µg/l at 2.0 mg/l DOC
HC5-50 = 3.4 µg/l at 1.0 mg/l DOC

Background – UK EA Approach

The toxicity database was extended to include new data. This is highly appreciated

Defining DOC correlation

The approach proposed in the document "Science Report – Marine Environmental Quality Standard for Copper" applied a linear model to describe the effect of DOC on EC10 values derived from the *Mytilus galloprovincialis* database. The linear model was considered to be preferable to the power model used in the VRAR, on the basis that the adjusted r^2 value for the power model was 0.57, compared to a value of 0.69 for the linear model when applied to this particular EC10 database. The mechanism by which DOC affects

toxicity in both fresh and marine waters was 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.

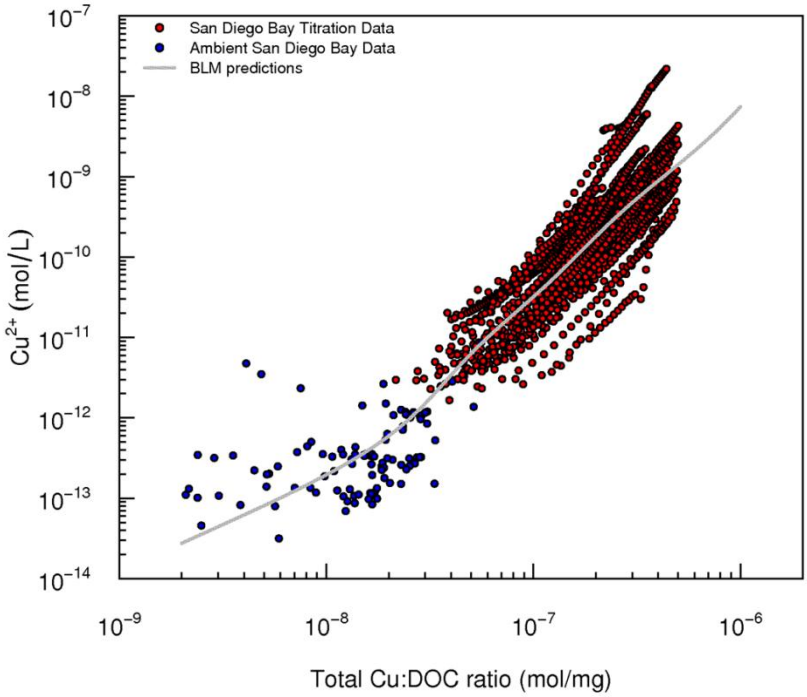
Below we will therefore discuss (1) linear versus non linear DOC normalization; (2) the use of EC10 versus EC50 data for assessing DOC normalization equation; (3) Predictive capacity of the linear versus the non-linear power function and (4) a revised assessment of the HC5 and marine EQS for copper

FUNDAMENTAL PRINCIPLE – LINEAR OR NON-LINEAR?

The approach described in the document "Science Report – Marine Environmental Quality Standard for Copper" assumes that the mechanism by which DOC affects toxicity in both fresh and marine waters is same, i.e. the copper which is bound to DOC is not available for biological uptake and does not, therefore, contribute to toxicity. If a linear correlation between DOC and toxicity for a particular species is expected, then an underlying assumption is that the DOC has similar effective binding along the DOC concentration gradient. This has been demonstrated for the freshwater environment, wherein freshwater DOC is a complex mixture of allochthonous carbon (derived from terrestrial input), consisting primarily of humic and fulvic material and autochthonous carbon (generated in the water column through microbial and algal activity), highly proteinaceous in nature and is characterized by amino acid structural groups.

This assumption is not proven for the marine environment, wherein dilution of the terrestrial DOC might lead to a change in the ratio of allochthonous to autochthonous carbon.

If the assumption of linearity is correct, the control of the free cupric ion would be expected to be constant across the range of Cu(total) vs. DOC scenarios. Titration data (Santore, 2009) suggests that this is not the case, and that at lower ratios, higher than expected cupric ion concentrations are observed.



This means that at low total Cu/DOC, the free Cu ion concentration is low and thus, the binding strength high. At high total Cu/DOC, the free ion concentration is relatively higher and thus, the binding strength lower.

The causes of the non-linearity may include differences between terrestrial and marine NOM, chemical changes in NOM in saline environments, inadequacy of ion activity corrections for saline environment, and proton interactions with DOM.

Conclusion : the speciation data indicate a non-linear relationship. Although not fully explained mechanistically, the observed non-linearity should not be ignored

EC10 VS EC50 ESTIMATION

The original report by Pacific EcoRisk on which the EC10 correlation is based reported all the NOEC, LOEC, EC10, EC20 and EC50 data for the several species on which correlation testing was performed. Based upon the raw data, ECx data were calculated using several approaches;

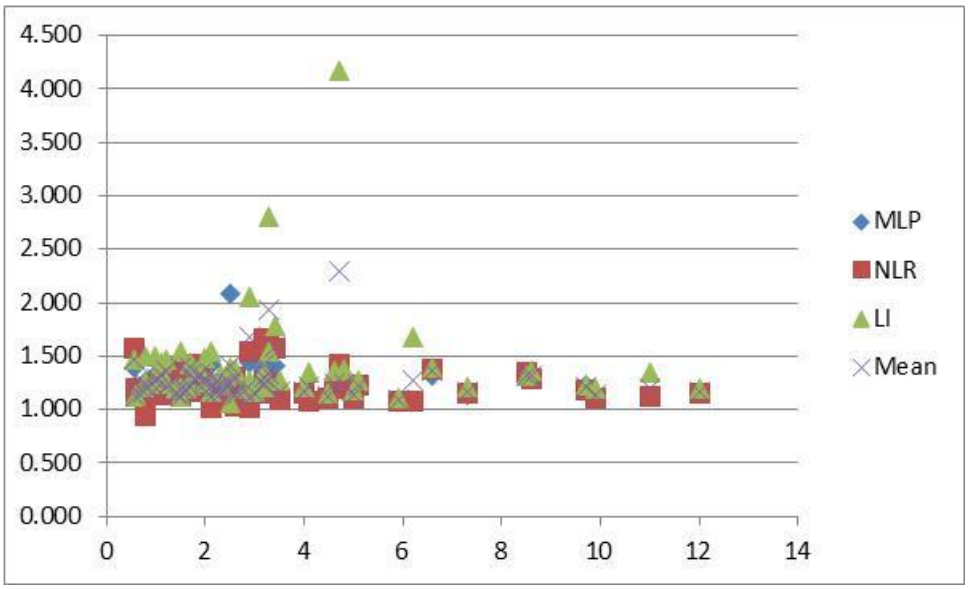
- Trimmed Spearman-Karber (TSK) [EC50 only]
- Maximum Likelihood Probit (MLP)
- Non-Linear Regression (NLR)
- Linear Interpolation (LI)

Comparing the methods, the variability in EC10 estimate was consistently worse than EC50 values, typically by a factor (%RSD) of 2-3 times. The

conclusion that a linear approach better fits less predictive data should not be the basis for defining a correlation.

As could be expected, an assessment of the Acute to Chronic Ratio (ACR) from the *Mytilus* database shows that the ACR is relatively constant for this species, regardless of either calculation method or DOC concentration.

Mean EC50:EC10 ratio; 1.27
10th percentile: 1.14
90th percentile: 1.40

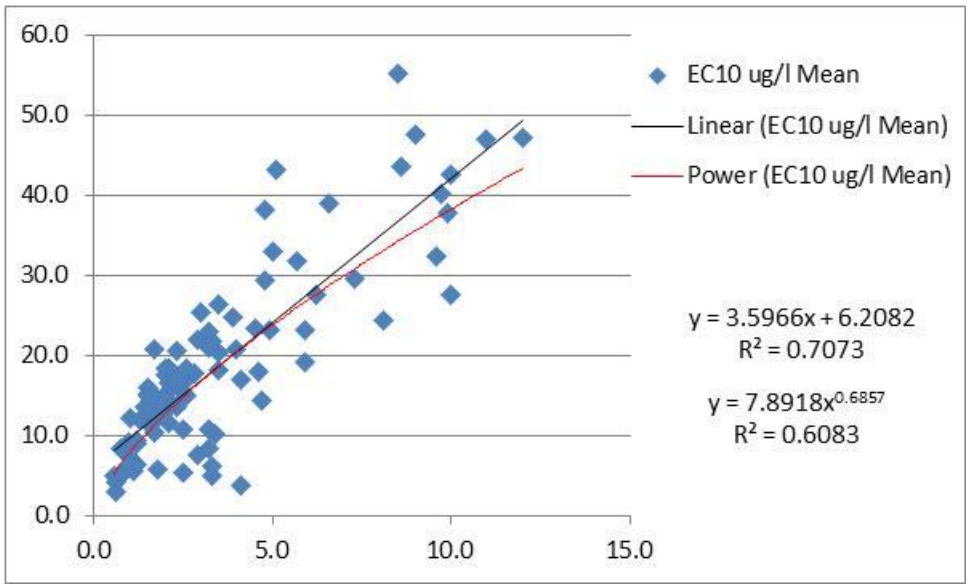


Combining the lower reliability of the EC10 calculations with the supported conclusion that EC50 data are equally valid for derivation of a relationship between DOC and toxicity, it may be concluded that the use of the EC50 data would provide a more reliable description of the correlation.

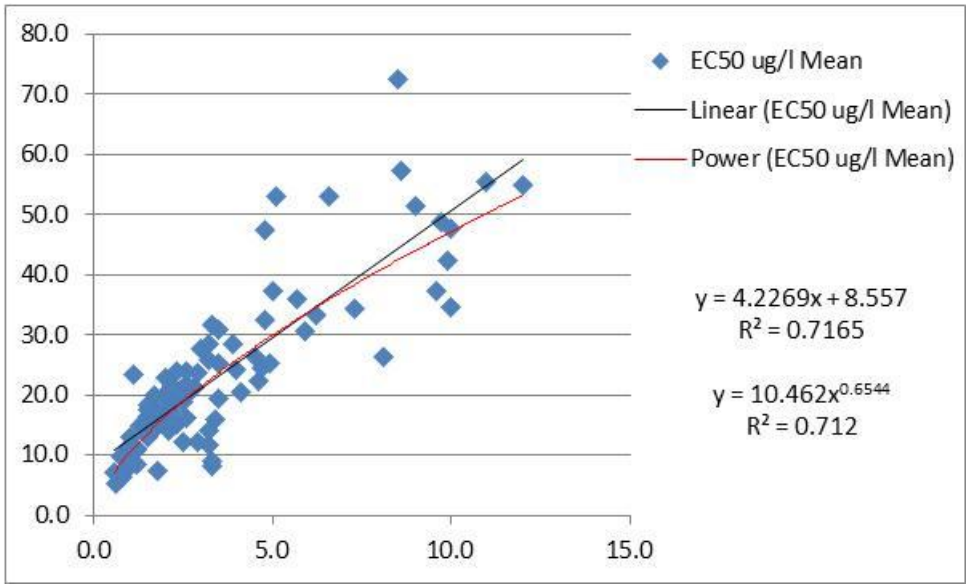
Conclusion : Considering that the EC10/EC50 ratio is rather constant, but EC50 data are more robust.

EC10 VS EC50 CORRELATIONS WITH DOC

Analysis of the correlation between DOC and EC10 does show that, regardless of calculation method, a linear fit better describes the available dataset (Plots of mean EC10 values presented – all plots reported in Appendix 1);



However, when assessing the different EC50 data, which are more consistent and an appropriate surrogate for the EC10 data in respect of describing the relationship between DOC concentration and toxicity, the two methods appear to be equally appropriate;



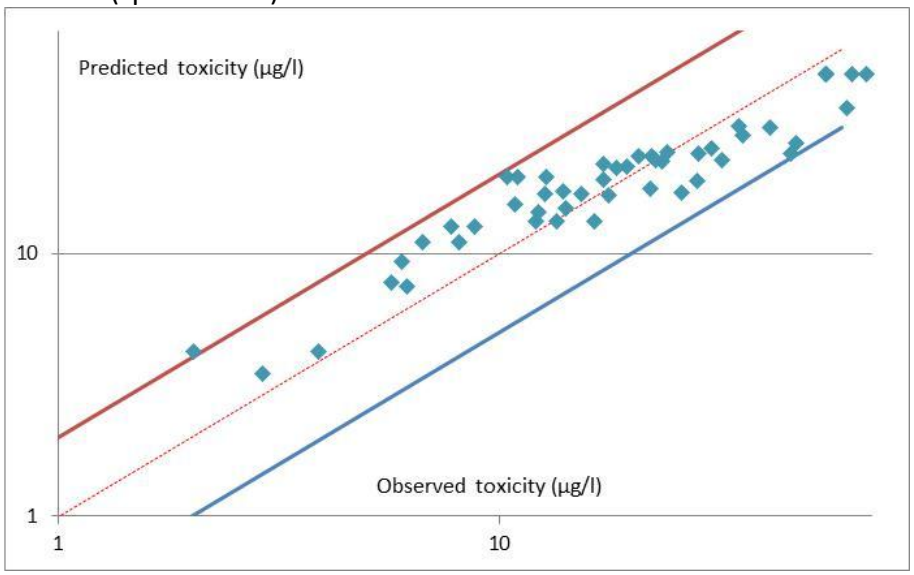
Conclusion : The EC50 –DOC relation are considered as more robust

PREDICTED VS. OBSERVED TOXICITY

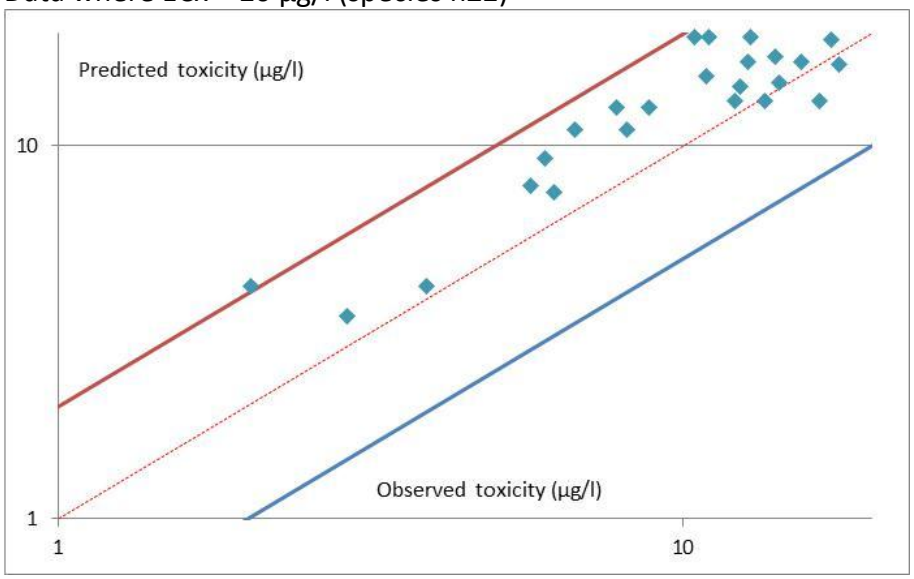
The comparison of the predictive capacity of the linear model vs the power function shows increased predictive capacity of the power model vs the linear model.. Applying the derived models, adapted to organism sensitivity where appropriate for species where $n \geq 2$, the following plots clearly demonstrate the better predictive power of the power model at lower DOC/ECx values are observed.

Linear model predictions (logarithmic scale; red line = 1:1, ± factor 2 limits shown)

All data (species n≥2)

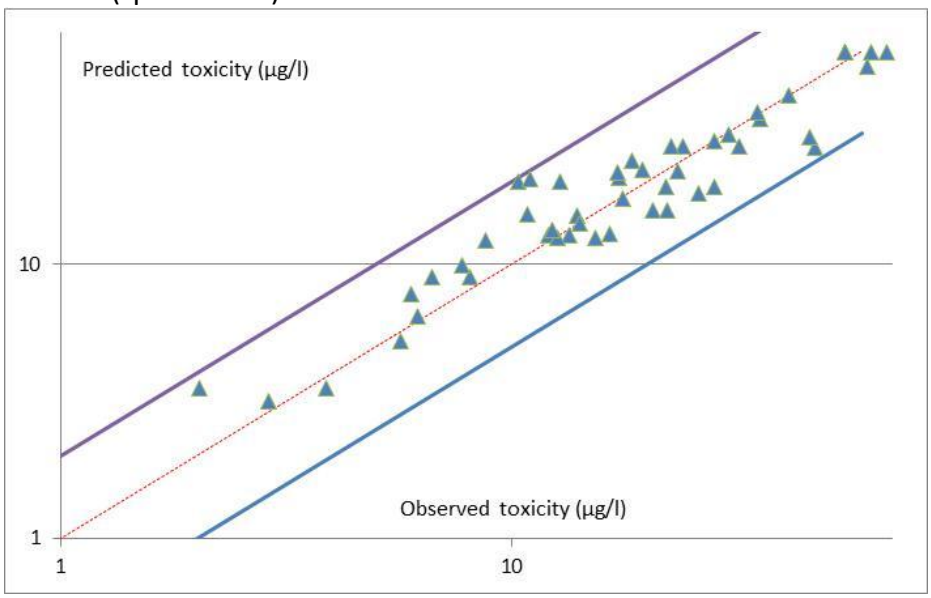


Data where ECx < 20 µg/l (species n≥2)

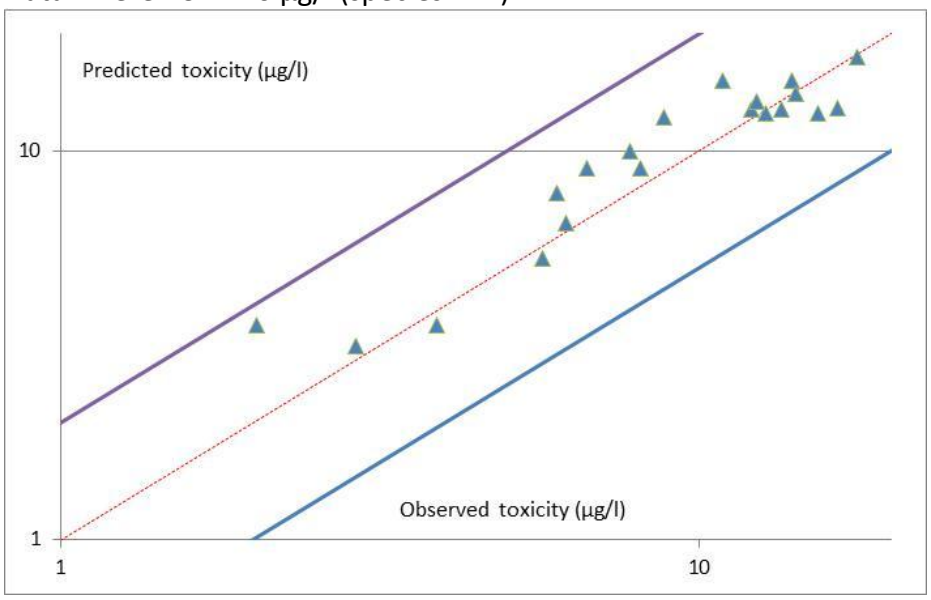


Power model predictions (logarithmic scale; red line = 1:1, ± factor 2 limits shown)

All data (species n≥2)



Data where ECx < 20 µg/l (species n≥2)



CONCLUSIONS

- Given the lower reliability of the EC10 data, the reason that the linear model best fits the data could be explained by the increased uncertainty relating to the endpoint, rather than any chemical or biological mechanism. The differences in chemical interactions within freshwater and marine water, supported by the titration data from marine water, suggest that the linear model, appropriate for the freshwater environment, may not be the best conceptual model for the marine environment.

- A constant ACR across the range of DOCs suggests that the more reliable EC50 data can be used to define the relationship between toxicity and DOC concentration.

- While the linear model better fits the overall EC10 dataset, at lower DOC concentrations (i.e. more sensitive environments), the power model is better able to predict toxicity.

CALCULATION OF HC5 VALUES (TO BE RE-DONE)

The following assessment is therefore proposed:

1. The EC10 values used in the document "Science Report – Marine Environmental Quality Standard for Copper" are the primarily sourced from the Non Linear Regression analysis of the data.

2. Use the Power function for DOC normalization
    a. Refine the equation used in the risk assessment, including the new data
    b. Demonstrate the predictive power of the model from predicted-Observed curves on the full ecotox database

3. Calculate the HC5 values using the E_TX model, which assumes a Normal distribution. For completeness, also assess the distribution of log transformed data was also analysed with BestFit, which makes no assumption on distribution.

UNCERTAINTY ANALYSIS (IN PREPARATION)

Normalize the PNEC to the mean DOC concentration of 3.6 mg C/L from the relevant mesocosms, by using the revised power function formula (eg VRAR:

$PNEC_{3.6\ mgC/L} = PNEC_{2\ mgC/L} * (3.6/2)^{0.6136})$

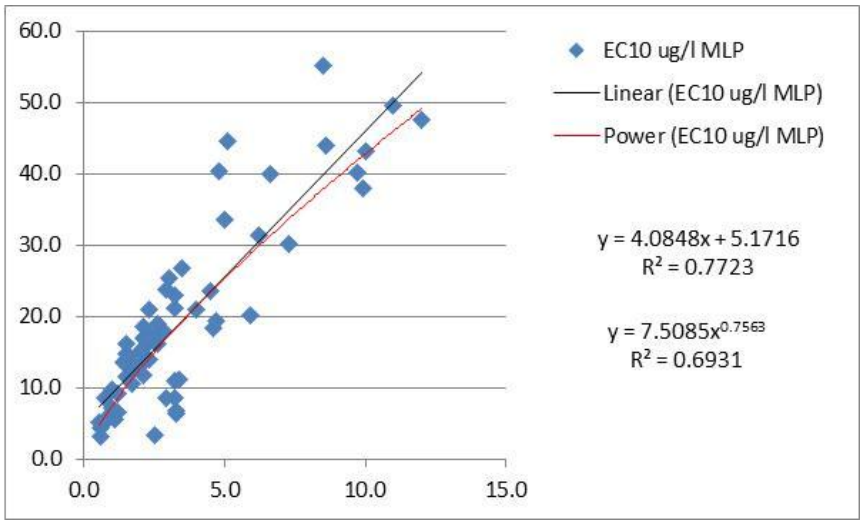
Use the "chronic value" for the most sensitive species from the mesocosm as reference point. The Maximum allowable toxicant concentration (MATC) is the maximum concentration at which a chemical can be present and not be toxic to the test organism(s), and is synonymous with the representative term "Chronic Value" (ChV), defined as the geometric mean of the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC). This can be mathematically represented as: ChV = 10^([log (LOEC x NOEC)]/2).

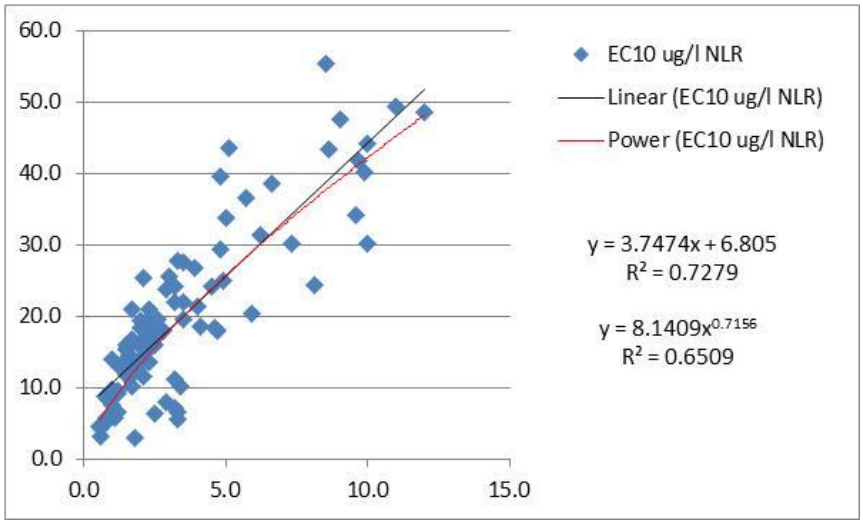
In the mesocosm test, analysis of the NOEC (5.7 µg/l) and LOEC (9.9 µg/l) returns a calculated ChV of 7.5 µg/l, an intermediate concentration between

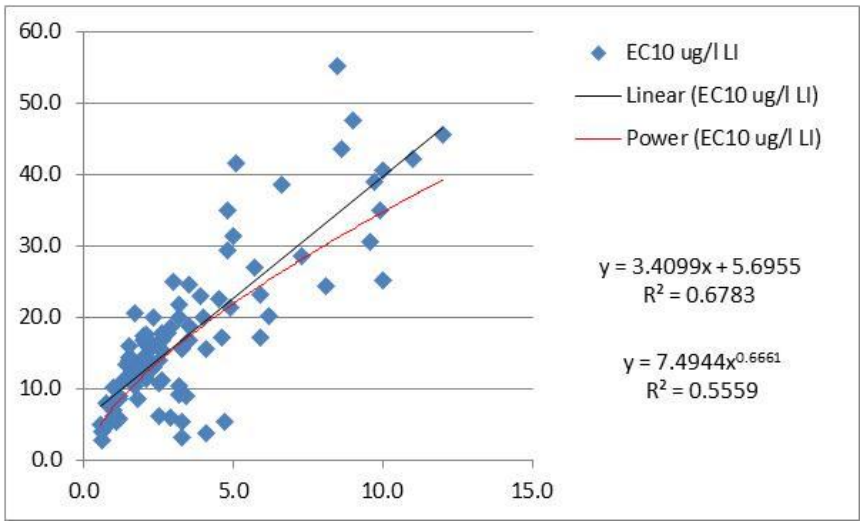
the NOEC and the LOEC treatment in the mesocosm study, equal to the PNEC calculated according to the VRAR PNEC derivation.

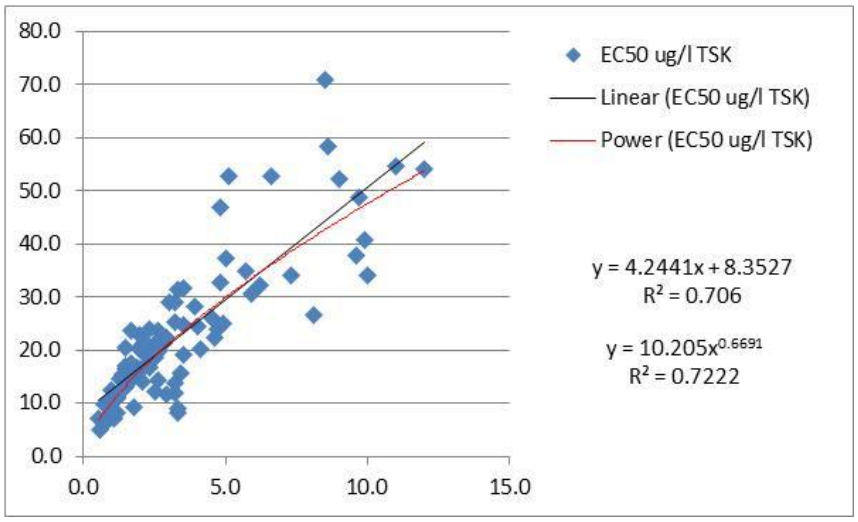
Compare the predictive power of the PNEC for the mesocosm results.

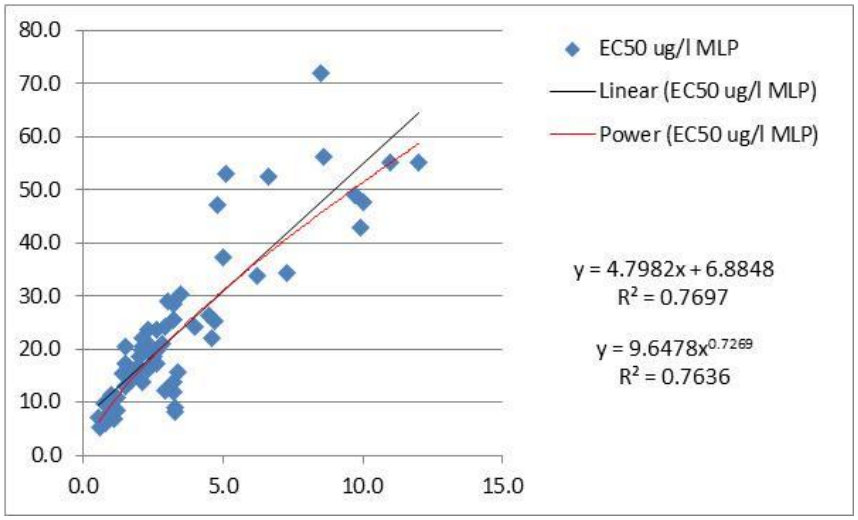
APPENDIX 1 – Individual ECx plots

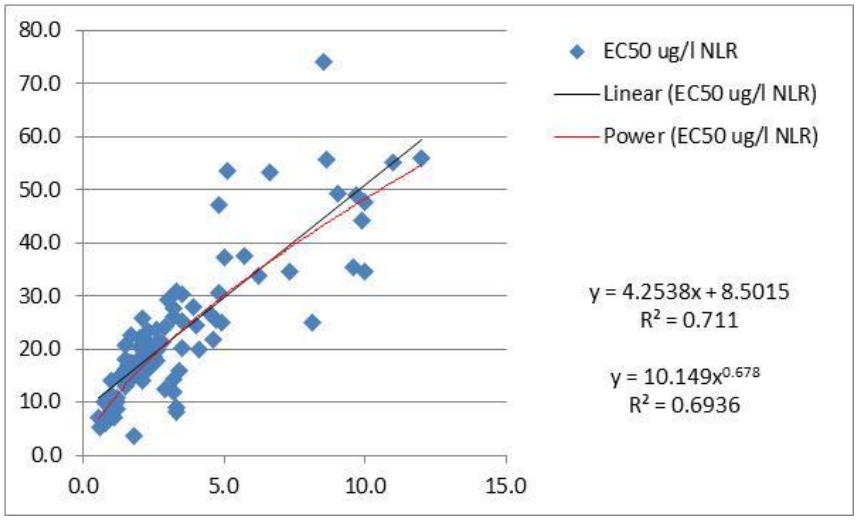


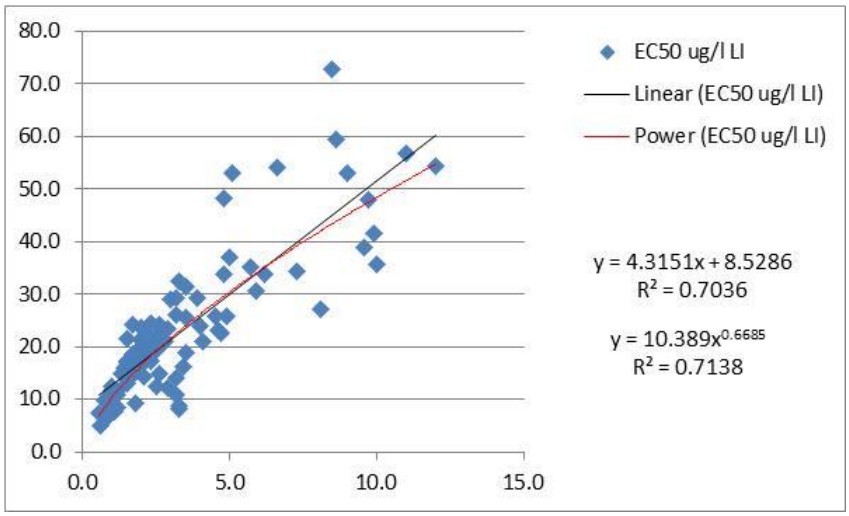





80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0.0
5.0
10.0
15.0
EC50 ug/l TSK
Linear (EC50 ug/l TSK)
Power (EC50 ug/l TSK)
y = 4.2441x + 8.3527
R² = 0.706
y = 10.205x^0.6691
R² = 0.7222


80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0.0
5.0
10.0
15.0
EC50 ug/l MLP
Linear (EC50 ug/l MLP)
Power (EC50 ug/l MLP)
y = 4.7982x + 6.8848
R² = 0.7697
y = 9.6478x^0.7269
R² = 0.7636

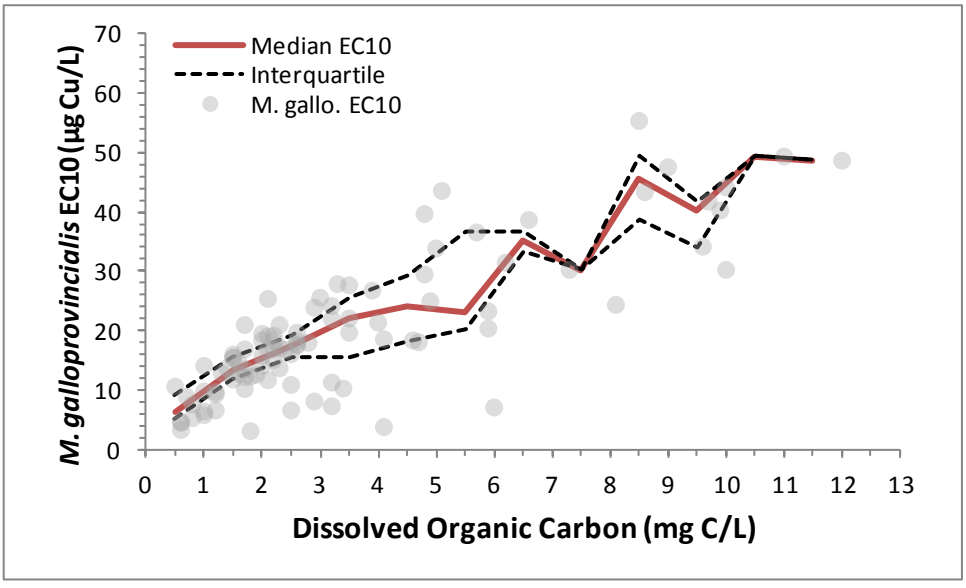

80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0.0
5.0
10.0
15.0
EC50 ug/l NLR
Linear (EC50 ug/l NLR)
Power (EC50 ug/l NLR)
y = 4.2538x + 8.5015
R² = 0.711
y = 10.149x^0.678
R² = 0.6936



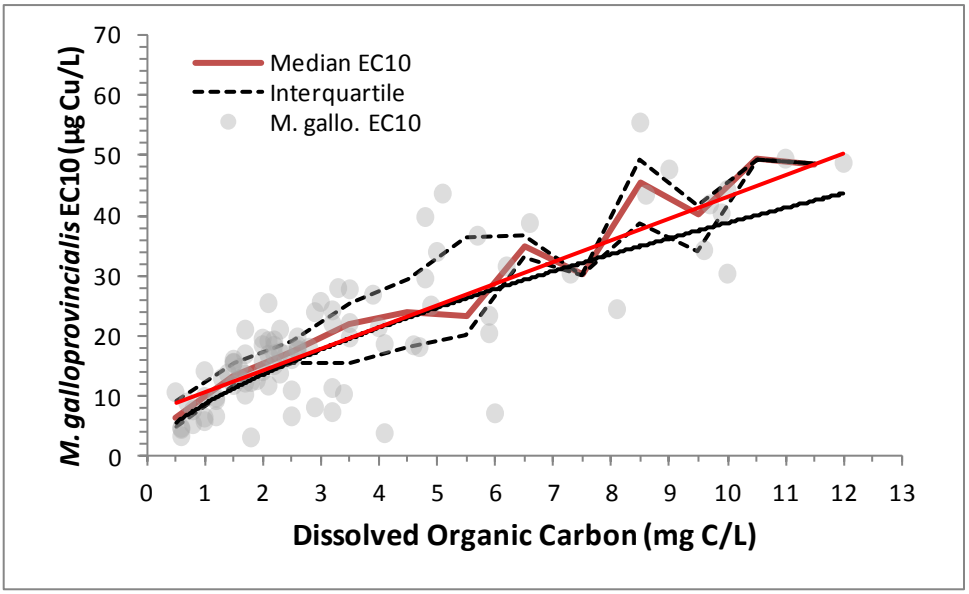
APPENDIX 2: MEDIAN EC10 ANALYSIS

The following plots of Median EC10 and interquartiles with *M. galoprovincialis* EC10 data have been prepared. These plots clearly show that, while a linear model may provide a better fit for estimating the Median EC10 over the overall dataset, in more sensitive waters with a lower DOC content, the power model is better able to predict toxicity, and would provide a more protective basis for setting a PNEC.

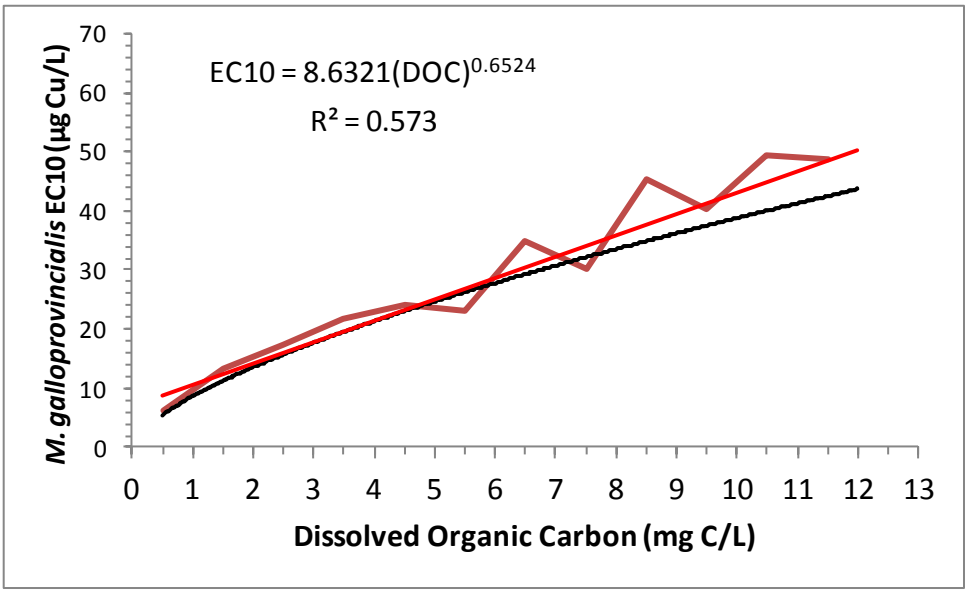
Plot of Median EC10 and interquartiles with *M. galoprovincialis* EC10 data (gray dots).



Plot of Median EC10, interquartiles, *M. galoprovincialis* EC10 data, simple linear regression model (red line), and power regression model (black line).


Median EC10
Interquartile
M. gallo. EC10
M. galloprovincialis EC10 (µg Cu/L)
Dissolved Organic Carbon (mg C/L)
0
10
20
30
40
50
60
70
0
1
2
3
4
5
6
7
8
9
10
11
12
13

Plot of Median EC10 (brown line), simple linear regression model (red line), and power regression model (black line).



Plot of Median EC10 (brown line), simple linear regression model (red line), and power regression model (black line) focusing on dissolved organic carbon range less than 4 mg C/L.