EURO-ECOLE
Assessment of the Bioavailability and Potential Ecological Effects of Copper in European Surface Waters

Subproject 4:
Evaluation and improvement of the ecological relevance of laboratory generated toxicity data.

Final report prepared for the European Copper Institute

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EXECUTIVE SUMMARY

§1. The EURO-ECOLE project aimed at providing data and tools which would allow a more scientifically-based risk assessment of metals in general, and copper in particular. The project tried to adress various important science gaps: effect of copper acclimation on the sensitivity of different laboratory species, copper toxicity and acclimation in a mesocosm study, copper toxicity and bioavailability in different natural surface waters using both laboratory and resident species and an extensive literature study on the effect of copper background concentrations on field algal assemblages.
The specific aims of EURO-ECOLE subproject 4 were to collect surface waters from 10 sites, representing potentially different European regions, and to evaluate the variation in acute and chronic toxicity of copper to different species exposed in these waters. Toxicity tests were performed with the green freshwater algae *Raphidocelis subcapitata* Printz (formerly known as *Selenastrum capricornutum*), the freshwater flea *Daphnia magna* Straus and, where possible, with resident crustacean species.

§2. For *D. magna*, the acute 48h-EC_{50} in surface waters ranged from 8.9 to 648 µg/L Cu. With the exception of site 4, the 48h-EC_{50}s obtained with the surface waters were always higher than those derived with standard laboratory medium (EEG-medium). The largest Water Effect Ratio (WER) was found in site 09 (Ankeveense Plassen), where the 48h-EC_{50} was 20 times higher than tahat obtained in standard test medium.
Chronic toxicity ranged between 23 and 367 µg/L Cu. The difference between 21d-EC_{50} and 21d-NOEC was always less than a factor of 1.8, with the NOEC being the more sensitive endpoint. The acute to chronic ratios (ACRs) for *D. magna* (48h-EC_{50} divided by the 21d-NOEC) varied between 1.1 and 3.9. It was noted that during chronic *D. magna* toxicity tests that the Cu-concentration range in which a daphnid does not reproduce anymore but still survives, is very narrow, resulting in small ACRs.
For 86% of the datapoints the difference between observed and BLM-predicted 48h-EC_{50} was less than a factor 1.5, suggesting that the observed differences in acute Cu-toxicity can be related to the physico-chemical characteristics of the test medium.

§3. For the algae *R. subcapitata* 72h-EC_{50}s (biomass as endpoint) ranged from 32 to 212 µg/L Cu. The Water Effect Ratio's varied from 1.2 to 15.4. A significant linear relationship (R^2 = 0.73, $p<0.05$) was found between the DOC concentration and the 72h-EC_{50}, but no relationship was found between the effect concentration and hardness or pH. Detailed laboratory experiments revealed that hardness and pH affect copper toxicity to algae. However, in the toxicity test performed in EURO-ECOLE 4 project the effect of these factors was masked by the fact that the toxicity enhancing-effect of one parameter was compensated by the toxicity reduction of another. Indeed, because of the co-variance of pH and hardness in the final site selection, the effect of DOC on copper toxicity becomes more pronounced and was the only factor for which a significant relationship with Cu-toxicity was observed.

§4. Depending on the test species and origin, the local species were more or less sensitive than the laboratiy strains. In all cases, the factor of difference was always less than 3, with the observed 48h-EC_{50}s of resident species varying between 14.1 and 289 µg/L Cu.

§5. Conclusions

The effect of varying physico-chemistry on Cu-toxicity to *D. magna* can be summarized as follows:

- an increase of DOC leads to an increase of the 48h-EC_{50} (decrease of Cu-toxicity): complexation of Cu^{2+};
- an increase of water hardness leads to an increase of the 48h-EC_{50} (decrease of Cu-toxicity): competition between Cu^{2+} and Ca^{2+}/Mg^{2+} for binding (and uptake);
- an increase of the pH leads to an increase of the 48h-EC_{50} (decrease of Cu-toxicity): effect of speciation > the (possible) competition between H^{+} and Cu^{2+} for binding to the Biotic Ligand;
- the observed relationship between acute and chronic toxicity indicates that the effects of varying physico-chemistry on chronic Cu-toxicity are similar to those affecting acute toxicity.

The effect of the varying physico-chemistry on Cu-toxicity to *R. subcapitata* can be summarized as follows:

- an increase of DOC leads to an increase of the 72h-EbC_{50} (decrease of Cu-toxicity): complexation of Cu^{2+};
- an increase of water hardness leads to an increase of the 72h-EbC_{50} (decrease of Cu-toxicity): competition between Cu^{2+} and Ca^{2+}/Mg^{2+} for binding (and uptake);
- an increase of the pH leads to a decrease of the 72h-EbC_{50} (increase of Cu-toxicity): effect of (possible) competition between H^{+} and Cu^{2+} for binding to the Biotic Ligand > speciation effect (+ additional bioavailability and toxicity of copper hydroxydes and copper carbonates?).

In the natural waters with low pH (<7) and low hardness (< 50 mg/L as $CaCO_3$), *D. magna* was the more sensitive species. However, in the waters with high pH (>7) and high hardness (>100 mg/L as $CaCO_3$), algae appeared to be the more sensitive species. This difference can be explained by the different effect of pH on Cu-toxicity to both species.

§6. Dissemination of project results (to date)

Presentations

- ***Heijerick DG, Bossuyt BT and Janssen CR.***
  Copper toxicity for *Daphnia magna*, *Raphidocelis subcapitata* and local crustaceans in six European surface waters.
  11th Annual Meeting of SETAC Europe "From basic science to decision-making: the Environmental Odyssey" – 6-10 May 2001, Madrid, Spain.

- ***Janssen CR, Heijerick DG, De Schamphelaere KAC and Bossuyt B.***
  Copper toxicity in European surface waters: observed vs. predicted toxicity as a function of water characteristics.
  6th International Conference on the Biogeochemistry of Trace Elements (ICOBTE). July 29 – August 2, 2001, Guelph, Ontario, Canada.

Publications (in preparation)

Heijerick et al. (to be decided)
Effect of varying physicochemistry of European surface waters on the copper toxicity to the green algae *Raphidocelis subcapitata*.

Heijerick et al. (to be decided)
Copper toxicity to *Daphnia magna* as a function of key water characteristics.

1 Introduction

Like all materials, metals may present risks to man and the environment. These risks are being managed through the establishment of environmental quality criteria and standards. Recently it has been recognized by both regulators, industry and academic scientists that standard procedures for deriving environmental quality criteria for metals are inadequate to accurately assess the metal's potential impact on the ecological quality of ecosystems. The main shortcomings of present risk assessment procedures used for the aquatic environment can be summarized as follows (Janssen et al. 2000):

- Metals are naturally occuring elements of which the (natural) background concentration may vary considerably depending on the geographic area and season;
- Methods for accounting for metal bioavailability are not incorporated in most regulatory systems: the bioavailability and toxicity of a metal varies as a result of speciation, complexation and competition processes;
- Background concentrations of essential metals like copper may affect the sensitivity of resident communities, which is not be reflected in toxicity tests with laboratory strains.

Some regulatory authorities like the US-EPA have recognised the importance of considering toxicity-modifying water characteristics in risk assessments of metals in natural waters and have developed procedures for setting site-specific WQC: the water effect ratio (WER) procedure and the recalculation method (US-EPA, 1994). There are, however, a number of disadvantages to these procedures. Firstly, they do not take the metal complexation capacity of dissolved organic carbon into account, which has been shown to decrease metal toxicity (Erickson et al., 1996; Kim et al., 1999; Ma et al., 1999). Secondly, the change of metal speciation (and thus bioavailability/toxicity) as a function of pH is neglected. Finally, the toxicity reducing potential of magnesium and sodium is not considered in a similar way as that of calcium in the applied adjustment procedures (US-EPA, 1994).
Additionally, in literature there is evidence that the sensitivity of organisms and communities towards metals is affected by the background metal concentration of their natural environment (Muyssen and Janssen, 2001a, 2001b; Muyssen et al., 2001). As the Cu background concentration can vary considerably in different surface waters it may be hypothesized that the sensitivity of resident species and communities may vary accordingly. Organisms used in standard aquatic ecotoxicity tests recommended by various international regulatory organisations, are cultured in media which usually have a constant and very low copper background concentration which make these organisms extremely sensitive to elevated Cu concentrations (Janssen and Heijerick, 2001; Muyssen and Janssen, 2001a). Consequently, standard toxicity tests for regulatory purposes (using these organisms) may produce very low toxicity values which are not representative for the natural tolerance of these organisms.

The specific aims of this subproject are:

- to collect surface waters and resident organisms at 10 selected reference sites. These sites are selected on the basis on differences in Cu-background concentrations and factors affecting copper bioavailability (pH, hardness, DOC) and reflect the geographic variability of copper background concentrations across Europe
- to perform toxicity tests, evaulating copper with standard test species, using the 10 collected surface waters and a standard laboratory test medium
- to perform acute toxicity tests, evaluating copper toxicity, with organisms collected at the 10 reference sites.

2 Materials and Methods

2.1 Toxicity testing

Two international recognised test species were selected for the evaluation of copper toxicity in natural waters: the freshwater flea *Daphnia magna* Straus and the freshwater green alga *Raphidocelis subcapitata* Printz (formerly known as *Selenastrum capricornutum*).
The different toxicity tests were performed in accordance with the OECD Guidelines 201 and 202. A common test protocol was followed by each of the four participating laboratories (IUCT, IRSA, LISEC and GU). The applied test procedures are given in Annex A.

To ensure compatibility of the test results, round robin testing was performed with the different participating laboratories. The results of these tests are presented in section 3.1 to 3.3.

2.2 Data treatment

Both the 48h-EC_{50} and 21d-EC_{50} for *D. magna* were calculated using the probit method (Finney, 1971). Immobility was the endpoint in the acute toxicity test, whereas net reproductive rate (Ro) was considered in the chronic exposure. Ro was calculated using the the following formulae (Southwood, 1976):

$$Ro = \sum_{x=0}^{21} l_x . m_x$$

with l_x the age-specific survival and m_x the reproduction rate.

For the determination of the effect concentration (72h-EbC_{50}) for the algae *R. subcapitata*, the growth inhibition was plotted against the Cu-concentration and a (sigmoidal) concentration-response relationship was fitted using Sigmaplot. Growth inhibition percentages were calculated using the 'area under the growth curve' methodology (OECD, 1984).

The raw data of all toxicity tests that were performed in this study are given in Annex B (lab species) and C (local species).

2.3 Chemical analysis.

Full chemical characterisation of the surface waters was performed by the Water Research Center (WRc). These data are presented in the EURO-ECOLE Subproject 2 – Report.
The following physico-chemical parameters were also determined in the participating institutes: pH (Consort pH meter, P407), hardness (Aquamerck® 1.11104.0001 total hardness titration test), oxygen concentration (WTW, Oxi330 oximeter) and dissolved Cu-concentrations in each test medium were analysed using a flame or a graphite furnace atomic absorption spectrophotometer. Calibration standards (Sigma-Aldrich, Steinheim, Germany) and a reagent blank were analysed with every ten samples.

2.4 Site selection

Based on an extensive study of literature data and existing surface water monitoring database (SWAD, Surface WAter Database, Heijerick and Janssen, 2000), 10 sites representing different types of European surface water were selected. An overview of the different sites and the selection criteria are presented in Table 1.

Table 1: Final site selection EURO-ECOLE.

Site	pH	Hardness mg/L as $CacO_3$	DOC mg/L	Cu µg/L
Bihain – Belgium (river)	5.9	10.1	8.89	0.4-0.9
Clywydog – Wales (lake)	6.3	10.0	2.72	1.5-2.8
Skårsjön – Sweden (lake)	5.5	7.9	12.0	<0.4
Somerain – Belgium (river)	6.1	27.8	1.45	<0.4
Monate – Italy (lake)	8.2	42	2.52	0.5
Marken – The Netherlands (lake)	8.3	220	6.42	1.8-3.2
Rhine – Germany (river)	8.1	191	1.99	2.2
Mole – England (river)	7.6	132	6.13	2.5
Ankeveen – The Netherlands (river)	7.4	166	17.8	1.7-2.7
Segrino – Italy (lake)	8.2	169	1.70	<0.4
Mesocosm/IUCT	7.6	47.3	1.03	<0.4

3. Results

3.1 Round robin tests

Three different standard ecotoxicity tests were performed with *Daphnia magna* and *Raphidocelis subcapitata*, using $CuSO_4.7H_2O$ as toxicant. The tests, performed by each of the four participating laboratories in Subproject 4, were conducted according to the test procedures described in the test protocols (Annex A). The four participating laboratories were:

- Fraunhofer Institute – Schmallenberg (IUCT)
- Ghent University – Laboratory of Environmental Toxicology (GU)
- Iistituto di Ricercaz Sulle Acque (IRSA)
- LISEC

Tests were conducted in standard artificial test medium: EEG-medium for tests with *D. magna*, ISO-medium for the test with *R. subcapitata*. The composition of the standard test media is given in Table 2. Originally, each laboratory used their own strain of *D. magna* and *R. subcapitata*. Initial experiments using EEG-medium revealed major differences (up to a factor of 10) between the different *D. magna* strains. Therefore the UG-strain was send to the different laboratories involved in Subproject 4; Toxicity tests with Cu and surface waters collected in April-June 2001 were performed with these *D. magna* strains.

The following ecotoxicity tests were performed:

- acute 48h-immobility test with *D. magna*
- chronic 21d-reproduction test with *D. magna*
- 72h-growth inhibition test with the green algae *R. subcapitata*

Table 2: Composition of the standard test media used in the round robin tests.

EEG-medium (OECD Guideline 202, 1984)	
$CaCl_2.2H_2O$	294 mg/L
$MgSO_4.7H_2O$	123 mg/L
$NaHCO_3$	64.8 mg/L
KCl	5.75 mg/L
ISO-medium (1989)	
NH_4Cl	15 mg/L
$MgCl_2.6H_2O$	12 mg/L
$CaCl_2.2H_2O$	18 mg/L
$MgSO_4.7H_2O$	15 mg/L
KH_2PO_4	1.6 mg/L
$FeCl_3.6H_2O$	80 µg/L
$Na_2EDTA.2H_2O$	100 µg/L
H_3BO_3	185 µg/L
$MnCl_2.4H_2O$	415 µg/L
$ZnCl_2$	3 µg/L
$CoCl_2.2H_2O$	1.5 µg/L
$CuCl_2.2H_2O$	10 ng/L
$Na_2MoO_4.2H_2O$	7 µg/L
$NaHCO_3$	50 mg/L

3.2 Round robin results for *D. magna*

Tables 3 presents the results of the round robin ecotoxicity tests with $CuSO_4.7H_2O$, using *D. magna* as test spieces. All test were – unless mentioned otherwise – performed in reconsituted standard EEG-medium. All reported effect concentrations are based on AAS-measured copper concentrations.

Table 3: Results of the round-robin exercise with *D.* magna exposed to Cu (in µg/L).

	GU	LISEC	IRSA	IUCT[c]
48h-EC50 with lab-strain (95% CL)	27.4 (23.4 – 32.1)	18.8 (17.0 – 22.0)	119[b]	15.0 (13.1 – 17.2) 13.4 (11.0 – 14.6)
48h-EC50 with the GU-strain (95% CL)		---[a]	19.2 (16.7 – 22.1)	65.5 (57.2 – 74.7)
21d-EC50 with the lab-strain (95% CL)	16.6 (13.6 – 21.4)	3.4[b,d]	---[a]	7.37 (4.8 – 9.9)

a: not performed
b: no confidence limits could be calculated
c: in reconstituted tap water, IUCT
d: not valid; control reproduction < 60 juveniles/daphnid

Ghent University and LISEC found similar 48h-EC_{50}s for their *D. magna* strain (18.8 to 27.4), tested in EEG-medium. The strain used by IRSA appeared to be less sensitive by one order of magnitude (119 µg/L). Therefore, the *D. magna* strain used by Ghent University was send to IRSA and was used for the round robin test and for the ecotoxicological evaluation of the three sites for which this institute was responsible. When using the GU-strain, an 48h-EC_{50} of 19.2 µg/L in EEG-medium was found. Since the *D.magna* strain originating from Ghent University showed a comparable sensitivity when tested by GU or by IRSA, this strain was used in the acute and chronic toxicity tests performed by IRSA.
IUCT initially performed the acute *D. magna* test with their strain in reconsituted tap water and found 48h-EC_{50}s, comparable to the values found by the other laboratories (13.4 and 15 µg/L). The GU-strain appeared to be less sensitive in this test medium (65 µg/L) than the strain normally used by IUCT. These findings demonstrate the importance of culture conditions on the sensitivity of test organisms.
Raw data of the round robin acute toxicity tests with *D. magna* are reported in Annex B.

Both LISEC and IRSA encountered various problems during the chronic testing of their allocated test sites (no valid test results were obtained due to low reproduction rates of the daphnids). Only the tests performed bu Ghent University were wildheld in this study. This institute found chronic 21d-EC_{50}s of 21 µg/L in EEG-medium. Raw data of the (valid) round robin chronic toxicity tests with *D. magna* are reported in Annex B.

3.3 Round robin results for *R. subcapitata*

Table 4 summarizes the results of the round robin tests with the green algae *R. subcapitata*. No significant differences in Cu-sensitivity of the algae used in the different laboratories were observed. The 72h-EbC_{50}s ranged from 13.4 to 22.0 µg/L, which is in accordance with earlier reported Cu-toxicity levels for this algal species tested in ISO-medium.

Table 4: Results of the round-robin exercise with *R. subcapitata* exposed to Cu (in µg/L)

	GU	**LISEC**	**IRSA**	**IUCT**
72h-EbC_{50} for *R. subcapitata* (95% CL)	22.0 (18.0 – 27.0)	14.1 (7.5 – 22.3)	13.4 (11.7 – 15.2)	---[a]

a : not performed

3.4 Ecotoxicological evaluation of the Cu-toxicity in 11 natural surface waters

In this section, the main physico-chemical characteristics (determined by WRc) of the 11 testsites are presented, together with the observed Cu-effect concentrations for *D. magna* (acute and chronic), *R. subcapitata* and, where available, for local species. All reported effect concentrations are based on measured copper concentrations unless mentioned otherwise. Raw data are reported in Annex B (*D. magna*, *R. subcapitata*) and Annex C (local species).

3.4.1 Location 1 : Bilhain, Belgium

Physico-chemistry
Table 5 presents the main physico-chemical characteristics of the surface water sampled at Bilhain in Autumn 2000. Measurements were done by WRc.

Table 5: Physico-chemical characterisation of surface water collected at Bihain, Belgium

	Autumn 2000
Alkalinity (as mg/L $CaCO_3$)	< 5.0
pH	5.69
Hardness (mg/L $CaCO_3$)	10.1
DOC (mg/L)	8.89
Calcium (mg/L)	2.5
Magnesium (mg/L)	0.95
Conductivity (µs/cm)	46.2
Suspended Solids (mg/L)	3.0
$Copper_{tot.}$ (µg/L)	< 0.4
$Copper_{diss.}$ (µg/L)	0.62

Ecotoxicity results

Table 6 presents the results of the acute (48h-EC_{50}) and chronic (21d-EC_{50}, 21d-NOEC) toxicity test with *D. magna*, the 72h-EbC_{50} for the algae *R. subcapitata* and the 48h-EC_{50} for a local crustacean (not determined).

Table 6: Ecotoxicity of Cu tested in surface water collected at Bihain. Effect concentrations in µg/L Cu (+ 95%CL).

Endpoint	**Sampling period Autumn 2000**
48h-EC_{50} for *D. magna* LISEC-strain	59.9 (52.5 – 69.1) (nominal)
21d-EC_{50} for *D. magna* LISEC-strain	---[a]
21d-NOEC for *D. magna* LISEC-strain	---[a]
72h-EbC_{50} for *R. subcapitata*	152 (108 - 216)[b]
24h-EC_{50} for a local crustacean tested in EEG-medium	10 (0–31) (nominal)

a: not valid; control reproduction < 60 juveniles/daphnid
b: test performed at pH 7.5 (± the pH of the ISO-medium)

3.4.2 Location 2: Lake Clywydog, Wales

Physico-chemistry

Table 7 presents the main physico-chemical characteristics of the surface water sampled at Lake Clywydog for both sampling periods. Measurements were done by WRc.

Table 7: Physico-chemical characterisation of surface water collected at Clywydog, Wales.

	Sampling period	
	Autumn 2000	**Spring 2001**
Alkalinity (as mg/L $CaCO_3$)	7.1	41.8
pH	6.74	7.00
Hardness (mg/L $CaCO_3$)	10.0	12.4
DOC (mg/L)	2.72	2.34
Calcium (mg/L)	2.2	3.1
Magnesium (mg/L)	1.11	1.15
Conductivity (µs/cm)	49.0	50.7
Suspended Solids (mg/L)	1.3	< 1
$Copper_{tot.}$ (µg/L)	1.5	2.8
$Copper_{diss.}$ (µg/L)	1.6	1.39

Ecotoxicity results

Table 8 presents the results of the acute (48h-EC_{50}) and chronic (21d-EC_{50}, 21d-NOEC) toxicity test with *D. magna*, the 72h-EbC_{50} for the algae *R. subcapitata* and the 48h-EC_{50} for a cyclopoid copepod.

Table 8: Ecotoxicity of Cu tested in surface water collected at Lake Clywydog. Effect concentrations in µg/L Cu (+ 95%CL).

Endpoint	**Autumn 2000**	**Spring 2001**
48h-EC_{50} for *D. magna*	37.9 (33.2 – 43.3)	33.8 (31.4 – 36.6)
21d-EC_{50} for *D. magna*	34.4 (31.2 – 38.6)	26.2 (23.2 – 30.3)
21d-NOEC for *D. magna*	28	21.5
72h-EbC_{50} for *R. subcapitata*	97.4[a]	84 (61 – 128)
48h-EC_{50} for a cyclopoid copepod in EEG-medium	---[b]	3647 (1883 – 7062) 2329 (1487 – 3615)

a: no confidence limits could be calculated
b: not performed

3.4.3 Location 3: Skårsjön, Sweden

Physico-chemistry

Table 9 presents the main physico-chemical characteristics of the surface water sampled at Skårsjön. Measurements were done by WRc.

Table 9: Physico-chemical characterisation of surface water collected at Skårsjön, Sweden.

	Sampling period Autumn 2000
Alkalinity (as mg/L $CaCO_3$)	< 5.0
pH	6.14
Hardness (mg/L $CaCO_3$)	7.9
DOC (mg/L)	12.0
Calcium (mg/L)	2.4
Magnesium (mg/L)	0.48
Conductivity (µs/cm)	26.4
Suspended Solids (mg/L)	< 1
$Copper_{tot.}$ (µg/L)	<0.4
$Copper_{diss.}$ (µg/L)	0.25

Ecotoxicity results

Table 10 presents the results of the acute (48h-EC_{50}) and chronic (21d-EC_{50}, 21d-NOEC) toxicity test with *D. magna*, the 72h-EbC_{50} for the algae *R. subcapitata* and the 48h-EC_{50} for a local species (*Scapholeberis* sp.). Tests with surface water from Location 3 were performed by LISEC and GU.

Table 10: Ecotoxicity of Cu tested in surface water collected at Skårsjön. Effect concentrations in µg/L Cu (+ 95%CL).

Endpoint	**LISEC** (LISEC-strains)	**Ghent University** (GU-strains)
48h-EC_{50} for *D. magna*	13.4 (12.4 – 14.4)[a] (nominal)	35.2 (30.6 – 40.6)
21d-EC_{50} for *D. magna*	--[b]	23.0 (18.4 – 29.2)
21d-NOEC for *D. magna*	--[b]	20
72h-EbC_{50} for *R. subcapitata*	212 (163-273)[c]	194 (106 – 322)
24h-EC_{50} for local cladocera (*Scapholeberis* sp.)	> 59	---
48h-EC_{50} for local cladocera (*Scapholeberis* sp.)	14.1 (8.51 – 25.1)	---

a: observed mortality at lower conc. > mortality at higher conc. after 24h exposure period
b: no valid test performed
c: test performed at pH 7.6 (± the pH of the ISO-medium)

3.4.4 Location 4 : Somerain, Belgium

Physico-chemistry

Table 11 presents the main physico-chemical characteristics of the surface water sampled at Somerain. Measurements were done by WRc.

Table 11: Physico-chemical characterisation of surface water collected at Somerain, Belgium.

	Sampling period Autumn 2000
Alkalinity (as mg/L $CaCO_3$)	13.5
pH	7.10
Hardness (mg/L $CaCO_3$)	27.8
DOC (mg/L)	1.45
Calcium (mg/L)	6.7
Magnesium (mg/L)	2.65
Conductivity (µs/cm)	85.2
Suspended Solids (mg/L)	< 1
$Copper_{tot.}$ (µg/L)	< 0.4
$Copper_{diss.}$ (µg/L)	0.14

Ecotoxicity results

Table 12 presents the results of the acute (48h-EC_{50}) and chronic (21d-EC_{50}, 21d-NOEC) toxicity test with *D. magna*, the 72h-EbC_{50} for the algae *R. subcapitata* and the 48h-EC_{50} for a local caldoceran (*Eurycerus lamellatus*).

Table 12: Ecotoxicity of Cu tested in using surface water collected at Somerain. Effect concentrations in µg/L Cu (+ 95%CL).

Endpoint	**Autumn 2000**
48h-EC_{50} for *D. magna* LISEC-strain	---[a]
21d-EC_{50} for *D. magna* LISEC-strain	---[a]
21d-NOEC for *D. magna* LISEC-strain	---[a]
72h-EbC_{50} for *R. subcapitata*	32 (23 – 42)[b]
24h-EC_{50} for local cladocera (*Eurycerus lamellatus*)	18.0 (15.9 –20.5)
48h-EC_{50} for local cladocera (*Eurycerus lamellatus*)	15.1 (13.3 – 17.1)

a: no valid test performed
b: test performed at pH 7.4 (± the pH of the ISO-medium)

3.4.5 Location 5 : Lake Monate, Italy

Physico-chemistry

Table 13 presents the main physico-chemical characteristics of the surface water sampled at Lake Monate. Due to bad weather conditions in Autumn 2000, this site was only sampled in Spring 2001. Measurements were done by WRc.

Table 13: Physico-chemical characterisation of surface water collected at lake Monate, Italy.

	Sampling period Spring 2001
Alkalinity (as mg/L $CaCO_3$)	50.6
pH	7.66
Hardness (mg/L $CaCO_3$)	--
DOC (mg/L)	2.52
Calcium (mg/L)	13.6
Magnesium (mg/L)	3.5
Conductivity (µs/cm)	104
Suspended Solids (mg/L)	0.6
$Copper_{tot}$. (µg/L)	0.5
$Copper_{diss}$. (µg/L)	0.78 (< 0.3)

Ecotoxicity results

Table 14 presents the results of the acute (48h-EC_{50}) and chronic (21d-EC_{50}, 21d-NOEC) toxicity test with *D. magna* and the 72h-EbC_{50} for the algae *R. subcapitata*. The used *D. magna strain* originated from Ghent University. No valid chronic toxicity test with *D. magna* was performed with this sample.

Table 14: Ecotoxicity of Cu tested in surface water collected at lake Monate. Effect concentrations in µg/L Cu (+ 95%CL).

Endpoint	**Spring 2001**
48h-EC_{50} for *D. magna* GU-strain	39.9 (34.9 – 46.0)
21d-EC_{50} for *D. magna* GU-strain	---[a]
21d-NOEC for *D. magna* GU-strain	---[a]
72h-EbC_{50} for *R. subcapitata*	52.7 (48.8 – 57.5)

a: not performed

3.4.6 Location 6 : Marken, The Netherlands

Physico-chemistry

Table 15 presents the main physico-chemical characteristics of the surface water sampled at Marken (Maarkermeer) for both sampling periods. Measurements were done by WRc.

Table 15: Physico-chemical characterisation surface water collected at Marken, The Netherlands.

	Sampling period	
	Autumn 2000	**Spring 2001**
Alkalinity (as mg/L $CaCO_3$)	106	120
pH	8.00	7.84
Hardness (mg/L $CaCO_3$)	220	238
DOC (mg/L)	6.42	8.24
Calcium (mg/L)	61.0	66.7
Magnesium (mg/L)	16.3	17.07
Conductivity (µs/cm)	793	787
Suspended Solids (mg/L)	12.1	29.8
$Copper_{tot}$. (µg/L)	1.8	3.2
$Copper_{diss}$. (µg/L)	0.51	1.33

Ecotoxicity results

Table 16 presents the results of the acute (48h-EC_{50}) and chronic (21d-EC_{50}, 21d-NOEC) toxicity test with *D. magna*, the 72h-EbC_{50} for the algae *R. subcapitata* and the 48h-EC_{50} for a cyclopoid copepod and for the juvenile perch (*Perca fluviatilis*) .

Table 16: Ecotoxicity of Cu tested in surface water collected at Marken (Maarkermeer). Effect concentrations in µg/L Cu (+ 95%CL).

Endpoint	**Autumn 2000**	**Spring 2001**
48h-EC_{50} for *D. magna* GU-strain	188 (165 – 211)	257.8 (231 – 286)
21d-EC_{50} for *D. magna* GU-strain	130 (117 - 143) (nominal)	118 (98.2 – 136)
21d-NOEC for *D. magna* GU-strain	110 (nominal)	71.4
72h-EbC_{50} for *R. subcapitata*	108.0[a]	65.4 (51 – 80)
96h-EC_{50} for the juvenile perch *P. fluviatilis* tested in local medium	---[b]	688.5 (566 – 838) 566.3 (460 – 697)
96h-EC_{50} for the juvenile perch *P. fluviatilis* tested in EEG-medium	---[b]	227.9 (149 – 349) 169.2 (129 – 222)
48h-EC_{50} for a cyclopoid copepod, tested in EEG-medium	---[b]	6917 (5425 – 8818) 8468 (5124 – 14000)

a: no confidence limits could be calculated
b: not performed

3. 4.7 Location 7 : River Rhine (Koblenz), Germany

Physico-chemistry

Table 17 presents the main physico-chemical characteristics of the surface water sampled at the Rhine. Due to bad weather conditions in Autumn 2000, this site was only sampled in Spring 2001. Measurements were done by WRc.

Table 17: Physico-chemical characterisation of surface water collected at Koblenz, Germany.

	Sampling period Spring 2001
Alkalinity (as mg/L $CaCO_3$)	160
pH	7.93
Hardness (mg/L $CaCO_3$)	191
DOC (mg/L)	1.99
Calcium (mg/L)	60.7
Magnesium (mg/L)	9.48
Conductivity (µs/cm)	450
Suspended Solids (mg/L)	15.8
$Copper_{tot}$. (µg/L)	2.2
$Copper_{diss}$. (µg/L)	(1.7)

Ecotoxicity results

Table 18 presents the results of the acute (48h-EC_{50}) and chronic (21d-EC_{50}, 21d-NOEC) toxicity test with *D. magna* and the 72h-EbC_{50} for the algae *R. subcapitata*. Tests with surface water from Location 7 were performed by IRSA and GU. In both laboratories the *D. magna* strain from Ghent University was used.

Table 18: Ecotoxicity of Cu tested in surface water from the River Rhine. Effect concentrations in µg/L Cu (+ 95%CL).

Endpoint	**IRSA**	**Ghent University**
48h-EC_{50} for *D. magna* GU-strain	70.3 (55.7 – 82.4)	119 (106 – 133)
21d-EC_{50} for *D. magna* GU-strain	---[a]	75.6 (66.6 – 88.8)
21d-NOEC for *D. magna* GU-strain	---[a]	68.8
72h-EbC_{50} for *R. subcapitata*	48.0 (38.8 – 59.9)	33.9 (30 – 44)

a: effect concentration > 32 µg/L (nominal)

3.4.8 Location 8 : River Mole, South-England

Physico-chemistry

Table 19 presents the main physico-chemical characteristics of the surface water sampled at the river Mole. Due to bad weather conditions in Autumn 2000, this site was only sampled in Spring 2001. Measurements were done by WRc.

Table 19: Physico-chemical characterisation surface water collected at the river Mole, England.

	Sampling period Spring 2001
Alkalinity (as mg/L $CaCO_3$)	98.1
pH	7.69
Hardness (mg/L $CaCO_3$)	132
DOC (mg/L)	6.13
Calcium (mg/L)	42.6
Magnesium (mg/L)	6.21
Conductivity (µs/cm)	364
Suspended Solids (mg/L)	6.08
$Copper_{tot}$. (µg/L)	2.5
$Copper_{diss}$. (µg/L)	2.2

Ecotoxicity results

Table 20 presents the results of the acute (48h-EC_{50}) and chronic (21d-EC_{50}, 21d-NOEC) toxicity test with *D. magna*, the 72h-EbC_{50} for the algae *R. subcapitata* and the 48h-EC_{50} for a local species. The used *D. magna strain* originated from Ghent University.

Table 20: Ecotoxicity of Cu tested in surface water colected at the river Mole. Effect concentrations in µg/L Cu (+ 95%CL).

Endpoint	**Spring 2001**
48h-EC_{50} for *D. magna* GU-strain	175 (165 – 185)
21d-EC_{50} for *D. magna* GU-strain	133 (117 – 147)
21d-NOEC for *D. magna* GU-strain	106
72h-EbC_{50} for *R. subcapitata*	113 (63 – 167)

3.4.9 Location 9 : Ankeveense plassen, The Netherlands

Physico-chemistry

Table 21 presents the main physico-chemical characteristics of the surface water sampled at the Ankeveense Plassen for both sampling periods. Measurements were done by WRc.

Table 21: Physico-chemical characterisation of surface water collected at the Ankeveense Plassen, The Netherlands.

	Sampling period	
	Autumn 2000	**Spring 2001**
Alkalinity (as mg/L $CaCO_3$)	56.6	29.7
pH	7.84	7.35
Hardness (mg/L $CaCO_3$)	166	134
DOC (mg/L)	17.8	20.4
Calcium (mg/L)	52.3	43.4
Magnesium (mg/L)	8.58	6.15
Conductivity (µs/cm)	377	269
Suspended Solids (mg/L)	2.6	21.2
$Copper_{tot}$. (µg/L)	1.7	2.7
$Copper_{diss}$. (µg/L)	1.70	3.26

Ecotoxicity results

Table 22 presents the results of the acute (48h-EC_{50}) and chronic (21d-EC_{50}, 21d-NOEC) toxicity test with *D. magna*, the 72h-EbC_{50} for the algae *R. subcapitata* and the 48h-EC_{50} for local *D. magna*, *D. longispina* and a cyclopoid copepod.

Table 22: Ecotoxicological evaluation using surface water from the Ankeveense Plassen. Effect concentrations in µg/L Cu.

Endpoint	**Autumn 2000**	**Spring 2001**
48h-EC_{50} for *D. magna* GU-strain	686 (667 – 706)	648 (612 – 689)
21d-EC_{50} for *D. magna* GU-strain	367 (337 – 397)	265 (227 – 310)
21d-NOEC for *D. magna* GU-strain	300 (nominal)	181
72h-EbC_{50} for *R. subcapitata*	245 (180 – 304)	163 (117 – 213)
48h-EC_{50} for local *D. magna*	289 (242 – 344)	---[a]
48h-EC_{50} for local *D. longispina*	215 (179 – 259)	---[a]
48h-EC_{50} for a local species (copepod) in EEG-medium	---[a]	714 (218 – 1811) 1258 (901 – 1756)

a: not performed

3.4.10 Location 10 : Lake Segrino

Physico-chemistry

Table 23 presents the main physico-chemical characteristics of the surface water sampled at Lake Segrino. Due to bad weather conditions in Autumn 2000, this site was only sampled in Spring 2001. Measurements were done by WRc.

Table 23: Physico-chemical characterisation of surface water collected at lake Segrino, Italy.

	Sampling period Spring 2001
Alkalinity (as mg/L $CaCO_3$)	146
pH	8.16
Hardness (mg/L $CaCO_3$)	169
DOC (mg/L)	1.7
Calcium (mg/L)	58.5
Magnesium (mg/L)	5.6
Conductivity (µs/cm)	277
Suspended Solids (mg/L)	1.5
$Copper_{tot.}$ (µg/L)	< 0.4
$Copper_{diss.}$ (µg/L)	0.4

Ecotoxicity results

Table 24 presents the results of the acute (48h-EC_{50}) and chronic (21d-EC_{50}, 21d-NOEC) toxicity test with *D. magna* and the 72h-EbC_{50} for the algae *R. subcapitata*. The used *D. magna strain* originated from Ghent University.

Table 24: Ecotoxicological evaluation using surface water from Lake Segrino. Effect concentrations in µg/L Cu (+ 95%CL).

Endpoint	**Spring 2001**
48h-EC_{50} for *D. magna* GU-strain	56.2 (45.5 – 93.5)
21d-EC_{50} for *D. magna* GU-strain	---[a]
21d-NOEC for *D. magna* GU-strain	---[a]
72h-EbC_{50} for *R. subcapitata*	36.9 (34.4 – 39.8)

a: effect concentration > 32 µg/L (nominal)

3.4.11 Location 11 : Mesocosm water

Physico-chemistry

Table 25 presents the main physico-chemical characteristics of the surface water of the mesocosm-water. Measurements were done by WRc.

Table 25: Physico-chemical characterisation of mesocosm water collected at IUCT.

	Autumn 2000
Alkalinity (as mg/L $CaCO_3$)	32.7
pH	7.29
Hardness (mg/L $CaCO_3$)	47.3
Calcium (mg/L)	14.0
Magnesium (mg/L)	2.94
Conductivity (µs/cm)	113
Suspended Solids (mg/L)	1.6
$Copper_{tot}$. (µg/L)	<0.4
$Copper_{diss}$. (µg/L)	0.1

Ecotoxicity results

Table 26 presents the results of the acute (48h-EC_{50}) and chronic (21d-EC_{50}, 21d-NOEC) toxicity test with *D. magna*, the 72h-EbC_{50} for the algae *R. subcapitata* and the 48h-EC_{50} for local *D. longispina*.

Table 26: Ecotoxicity of Cu tested in mesocosm water. Effect concentrations in µg/L Cu (+ 95%CL).

Endpoint	**Autumn 2000**	**Spring 2001**
48h-EC_{50} for *D. magna*	19.5 (17.8 – 21.5)	19.7 (17.9 – 21.8)
IUCT-strain		64.2 (55.3 – 75.5)[a]
21d-EC_{50} for *D. magna* IUCT-strain	---[b]	32.8 (25.4 – 46.9)[a]
21d-NOEC for *D. magna* IUCT-strain	---[b]	29[a]
48h-EC_{50} for a local species (*D. longispina*) in reconst. tap water	---[b]	13.9 (12.8 – 15.3)
48h-EC_{50} for a local species (*D. longispina*) in mesocosm-water	---b	30.6 (27.2 – 34.5)

a: *D. magna* strain Ghent University
b: not performed

4.5.12 Overview of all toxicity data

Table 27 presents the toxicity data with local species. Table 28 gives an overview of all the effect concentrations and NOECs, respectively, obtained for *D. magna* and *R. subcapitata*.

Table 27: Overview of all toxicity tests performed with local species.

Site	Test Laboratory	Test species	Endpoint	Effect Concentration
01 – autumn	LISEC	cladoceran	24h-EC_{50} in EEG-medium	10 (0–31) (nominal)
02 – spring	GU	copepod	48h-EC_{50} in EEG-medium	3647 (1883–7062) 2329 (1487–3615)
03 – autumn	LISEC	*Scapholeberis* sp	24h-EC_{50} in local medium	> 59
03 – autumn	LISEC	*Scapholeberis* sp	48h-EC_{50} in local medium	14.1 (8.51 – 25.1)
04 – autumn	LISEC	*Eurycerus lamellatus*	24h-EC_{50} in local medium	18.0 (15.9 –20.5)
04 – autumn	LISEC	*Eurycerus lamellatus*	48h-EC_{50} in local medium	15.1 (13.3 – 17.1)
06 – spring	GU	*Perca fluviatilis* (fish)	96h-EC_{50} in local medium	689 (566 – 838) 566 (460 – 697
06 – spring	GU	*Perca fluviatilis* (fish)	96h-EC_{50} in EEG medium	228 (149 – 349) 169 (129 – 222)
06 – spring	GU	copepod	48h-EC_{50} in EEG-medium	6917 (5425 – 8818) 8468 (5124 – 14000)
09 – autumn	GU	*D. magna*	48h-EC_{50} in local medium	288.7 (242.3 – 343.9)
09 – autumn	GU	*D. longispina*	48h-EC_{50} in local medium	215.1 (178.7 – 259.0)
09 – spring	GU	copepod	48h-EC_{50} in EEG-medium	714 (218 – 1811) 1258 (901 – 1756)
11 – spring	IUCT	*D. longispina*	48h-EC_{50} in reconst. water	13.9 (12.8 – 15.3)
11 – spring	IUCT	*D. longispina*	48h-EC_{50} in local medium	30.6 (27.2 – 34.5)

Table 28: Overview of all toxicity data (EC50s, NOECs) generated with *D. magna* and *R. subcapitata*

		D. magna			***R. subcapitata***	
		48h-EC_{50}	**21d-EC50**	**21d-NOEC**	**72h-EbC_{50}**	**72h-NOEC**
01 – autumn	LISEC	59.9 (52.5 – 69.1)[a]	---[b]	---[b]	152 (108 - 216)	46.5
02 – autumn	GU	37.9 (33.2 – 43.3)	34.4 (31.2 – 38.6)	28	---[c]	52.9
02 – spring	GU	33.8 (31.4 – 36.6)	26.2 (23.2 – 30.3)	21.5	84 (61 – 128)	61.8
03 – autumn	LISEC	13.4 (12.4 – 14.4)[a]	---[d]	---[d]	212 (163-273)	50.5
03 – autumn	GU	35.2 (30.6 – 40.6)	23.0 (18.4 – 29.2)	20	194 (106 – 322)	94.7
04 – autumn	LISEC	---[b]	---[b]	---[b]	32 (23 – 42)	---[c]
05 – spring	IRSA	42.4 (36.5 – 49.4)	---[d]	---[d]	52.7 (48.8 – 57.5)	17.9
06 – autumn	GU	188 (165 – 211)	130 (117 – 143)	110[a]	108 (68 – NR)	49
06 – spring	GU	257 (231 – 286)	118 (98.2 – 136)	71.4	65.4 (51 – 80)	35.4
07 – spring	IRSA	70.3 (55.7 – 82.4)	---[e]	---[e]	48.0 (38.8 – 59.9)	23.1
07 – spring	GU	119 (106 – 133)	75.6 (66.6 – 88.8)	68.8	33.9 (30 – 44)	19.3
08 – spring	GU	175 (165 – 185)	133 (117 – 147)	106	112.6 (63 – 167)	56.4
09 – autumn	GU	686 (667 – 706)	367 (337 – 397)[a]	300[a]	245.3 (180 – 304)	164
09 – spring	GU	648 (612 – 689)	265 (227 – 310)	181	163.1 (117 – 213)	65.5
10 – spring	IRSA	56.2 (45.5 – 93.5)	---[e]	---[e]	36.9 (34.4 – 39.8)	15.7
11 – autumn	IUCT	19.5 (17.8 – 21.5)	---[d]	---[d]	---[d]	---[d]
11 – spring	IUCT	19.7 (17.9 – 21.8)	---[d]	---[d]	---[d]	---[d]
11 – spring[f]	IUCT	64.2 (55.3 – 75.5)	32.8 (25.4 – 46.9)	29	---[d]	---[d]

a: nominal values
b: no valid test performed (control reproduction < 60)
c: observed concentration-response relationship does not allow the calculation of a reliable value
d: not performed
e: effect concentration > 32 µg/L (nominal)
f: Ghent University strain

4 Discussion

4.1 Acute toxicity of Cu to *D. magna*

General

Figure 1 gives an overview of the different acute (48h) effect concentrations found for *D. magna*. For those sites where two sampling periods were performed (autumn, spring), the data obtained with medium collected in the spring are included in the figure (no significant difference with the autumn results were observed). The lowest EC_{50}s are noted for sites 01 to 05 and for site 11. These six sites exhibited a low water hardness (< 50 mg/L as $CaCO_3$). For site 04, no valid acute toxicity test was performed, but the observed concentration-response relationship suggests an 48h-EC_{50} ranging between 13.5 and 19.0 µg/L Cu. This datapoint, however, has not been included in the discussion of the test results.
Figure 2 presents the same data, expressed as Water Effect Ratio's, i.e. the observed 48h-EC_{50} in local medium divided by the 48h-EC_{50} of the round-robin test (performed in ISO-medium with a hardness of ± 250 mg/L As $CaCO_3$). The used round-robin values were 31.7 µg/L, 18.8 µg/L, 19.2 µg/L and 14.2 µg/L for GU, LISEC, IRSA and IUCT, respectively.

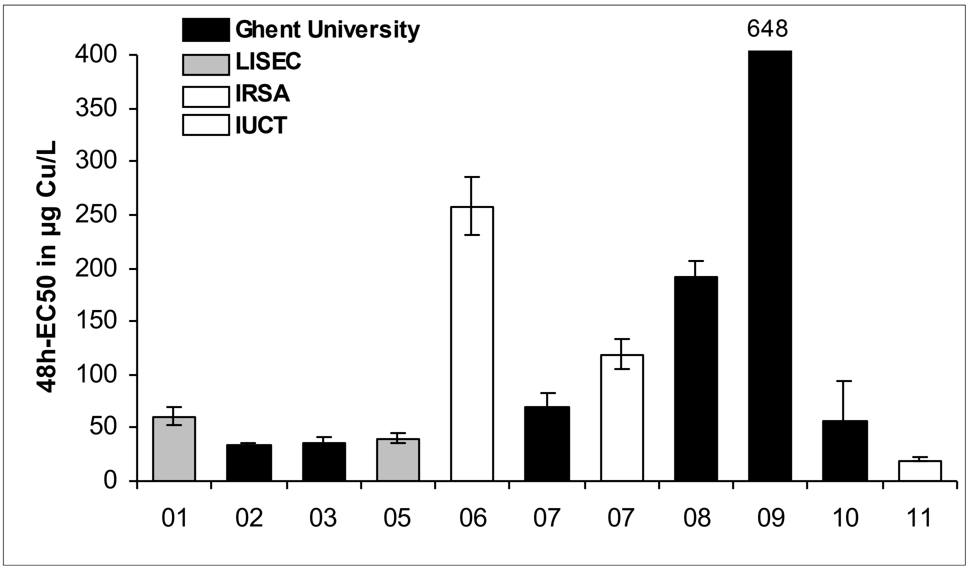

Figure 1: 48h-EC_{50} for *D. magna* in 10 surface waters spiked with $CuSO_4$.

The acute 48h-EC_{50} ranged from 33.8 to 648 µg/L Cu. For all sites, *D. magna* was less sensitive to Cu when tested in surface water compared to standard laboratory medium (EEG-medium). The largest WER was found in site 09 (Ankeveense Plassen), were the 48h-EC_{50} was 20 times higher compared to that obtained in EEG-medium.

The differences in toxicity values obtained using surface waters collected in different seasons (autumn – spring) are presented in Figure 3. It can be concluded that no major differences were noted between the effect concentrations found in autumn 2000 and spring 2001. This is is accordance with the results of 1) other tests performed with these waters (see below) and 2) the physico-chemical data of the surface waters which revealed only slight differences between the two samples.

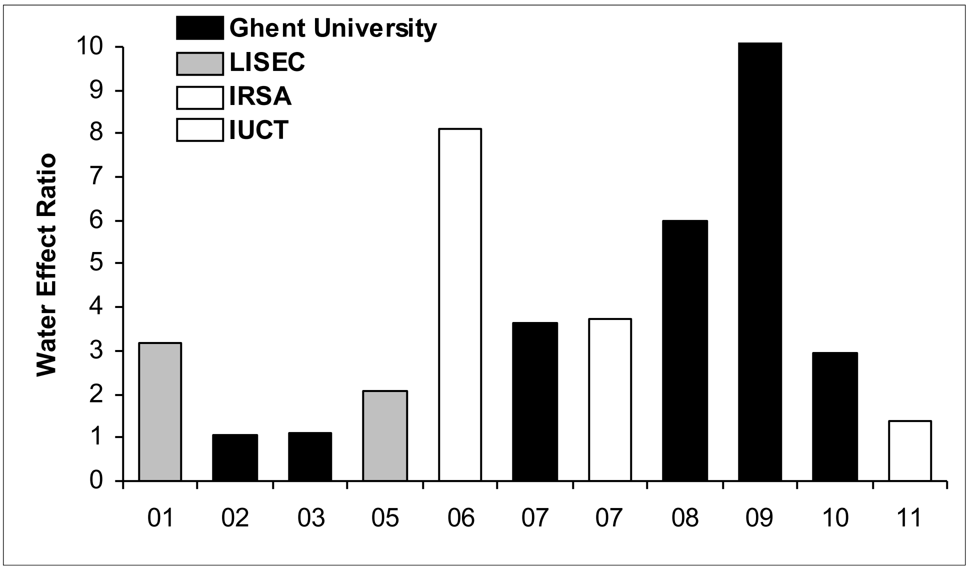

Figure 2: Water Effect Ratio for *D. magna* (acute) in 10 surface waters spiked with $CuSO_4$.

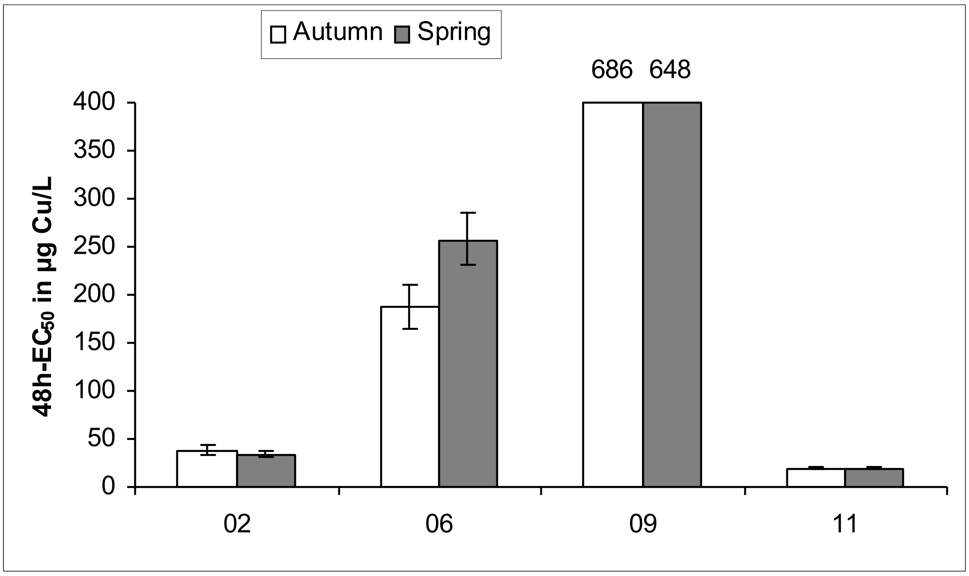

Figure 3: Seasonal differences on observed 48h-EC_{50} for *D. magna* in 4 surface waters.

Relationship observed toxicity – physicochemistry

A possible relationship between the observed Cu-toxicity in the different waters and the physicochemistry of these waters was examined using the Biotic Ligand Model-approach.

- The BLM used in this study is based on the model developed by Di Toro et al. (2001) and Paquin et al. (2001), as modified by De Schamphelaere and Janssen (2002) and De Schamphelaere et al. (2002). The latter model takes into account: 1) Mg-competition and 2) the bioavailability (and hence the toxicity) of $Cu(OH)^+$ and $CuCO_3$.

Figure 4 presents the observed acute Cu-toxicity against the BLM-predicted Cu-toxicity for *D. magna* for the different tested surface waters.

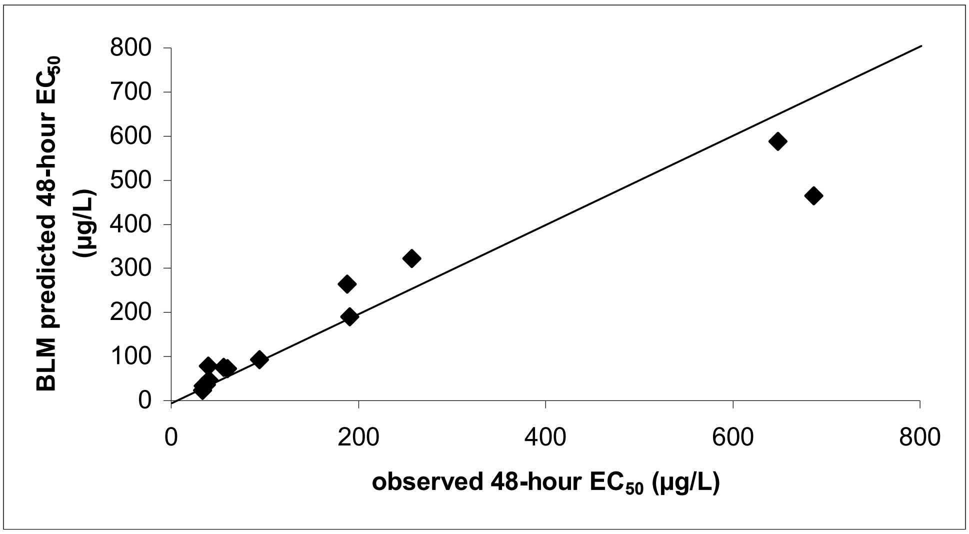

Figure 4: Observed vs. BLM-predicted 48h-EC_{50} for *D. magna* in natural surface waters spiked with copper.

For 11 of the 13 toxicity values (85%) the difference between observed and predicted 48h-EC_{50} was less than a factor 1.5. These data and model predictions clearly indiciate that the observed differences in acute Cu-toxicity can be related to the physico-chemical characteristics of the testmedium. The observed toxicity data also demonstrate that, depending on the type of surface water, Cu-toxicity to *D. magna* can vary more than one order of magnitude, i.e. the difference between the lowest and highest observed 48h-EC_{50} was a factor of 19.2.

4.2 Chronic toxicity of Cu to *D. magna*

<u>General</u>

Valid chronic toxicity tests with *D. magna* were obtained for only 6 sites. All test were performed using the same strain of *D. magna*. Figure 5 presents the 21d-EC_{50} and 21d-NOEC for Cu tested in these surface waters.

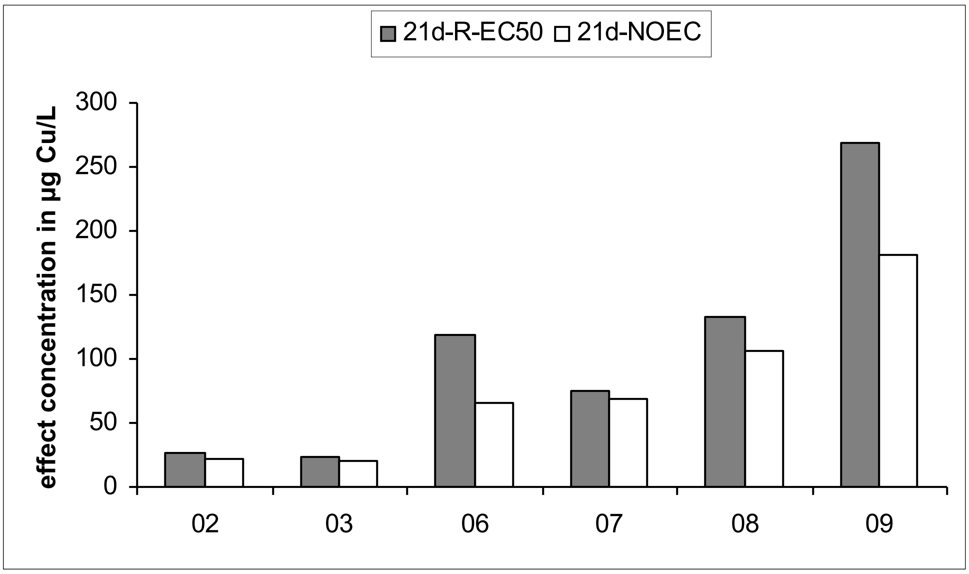

Figure 5: Chronic toxicity of Cu to *D. magna* in 6 different surface waters.

The observed 21d-EC_{50}s ranged between 23.0 and 367 µg/L Cu, i.e. a factor of 15. Although this difference is considerally less than that observed for the acute toxicity (77), it should be noted that there is no chronic value available for site 04, which had an acute toxicity of 8.9 µg/L. This is approximately 3 times lower than lowest chronic value which was found for site 03.
The difference between 21d-EC_{50} and 21d-NOEC was always less than a factor of 1.8 (with the NOEC being the more sensitive endpoint). The relatively small difference between these two endpoints is a result of the steep concentration-response slope: the concentration range of copper in which reproduction is partially inhibited (between 0 and 100%) is rarely larger than a factor of 3. This can also explain the low acute to chronic ratios for *D. magna* (48h-EC_{50} divided by the 21d-NOEC), which vary between 1.1 and 3.9. Indeed, observations during chronic toxicity tests with copper indicate that the Cu-concentration range in which a daphnid does not reproduce anymore but still survives is very narrow.

The chronic WERs for these six surface waters are given in Figure 6. An 21d-EC_{50} in EEG medium of 16.6 µg/L Cu was used for the determination of the WERs.

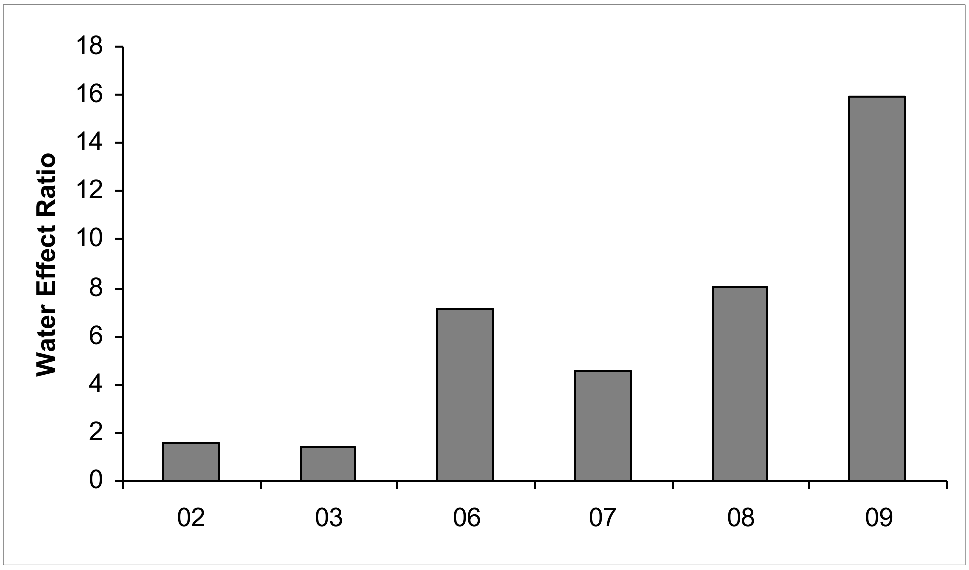

Figure 6: Chronic WERs for *D. magna* exposed to six surface waters spiked with Cu..

The calculated WERs range from 1.4 (site 03) to 16 (site 9), which is comparable with the range that was determined for the acute Cu-toxicity.

Relationship observed toxicity – physicochemistry
although efforts are ongoing, no chronic BLM for Cu-toxicity prediction has been developed yet. Therefore, the chronic and acute effect concentrations for *D. magna* are compared (Figure 7) in order to examine wether the variation of the chronic toxicity for the different sites is similar to the observed variation in acute toxicity, the latter being related to the physico-chemistry of the test medium as demonstrated in the previous section through the use of the acute Cu-BLM.
The correlation coefficient (R^2) of 0.951 indicates that the same factors causing the variability in acute toxicity are most probably also responsible for the observed chronic variability.

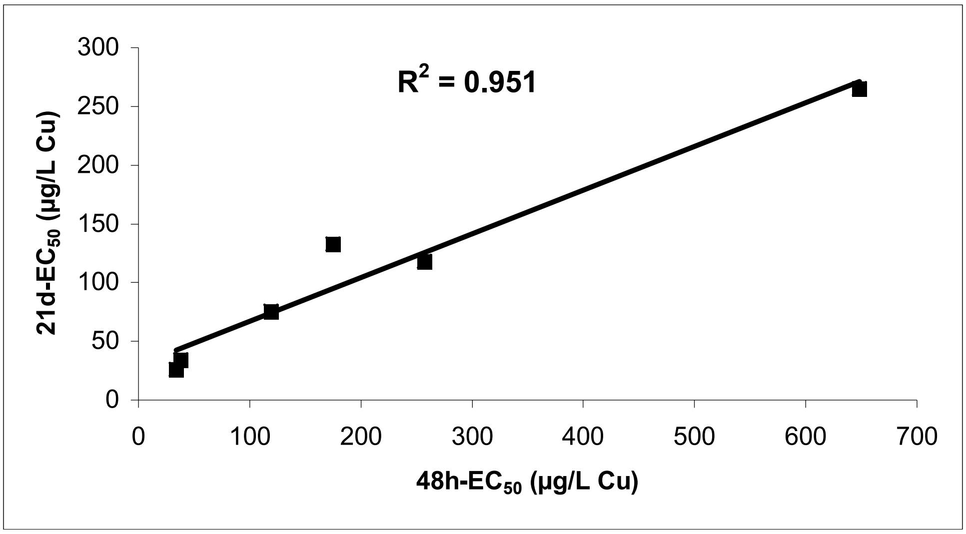

Figure 7: Correlation between the observed acute and chronic Cu-toxicity *to D. magna* in six surface waters.

4.3 Toxicity of Cu to *R. subcapitata*

<u>General</u>

The 72h-EbC_{50}s with the different surface waters spiked with Cu are presented in Figure 8. For those sites where two sampling periods were performed (autumn, spring), the data obtained with medium collected in the spring are presented in the figure (no significant difference with the autumn results were observed). The 72h-EC_{50}s range from 32 to 212 µg/L Cu, with the highest values obtained in surface water from Site 02 and the lowest effect concentration with water from Site 04. No statistical differences were noted between the results obtained for Site 03 and 07 which were tested in two laboratories.

Compared with the results for *D. magna* (acute), the variation of Cu-toxicity in the different test media appears to be smaller (factor difference of 6.6 for *R. subcapitata* vs. 77 and 10 for the acute and chronic *D. magna*, respectively).

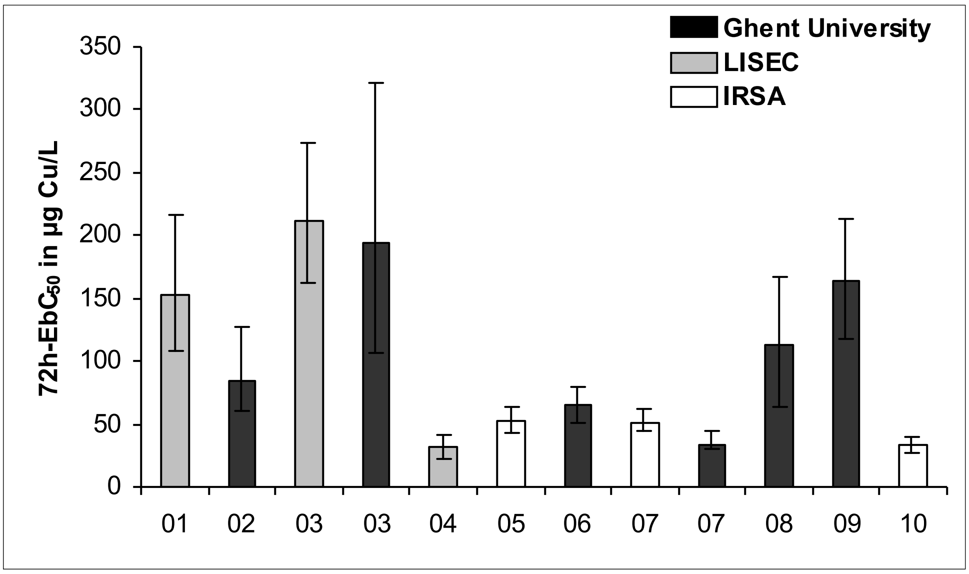

Figure 8: 72h-EC_{50} for *R. subcapitata* in 11 surface waters spiked with $CuSO_4$.

Figure 9 presents the same data, this time expressed as Water Effect Ratios. The used reference 72h-EbC_{50}s in standard (ISO) test medium are 28 µg/L Cu, 13.7 µg/L Cu and 13.4 µg/L Cu for GU, LISEC and IRSA, respectively. The Water Effect Ratio's for *R. subcapitata* vary from 1.2 (Site 07) to 15.4 (Site 3).

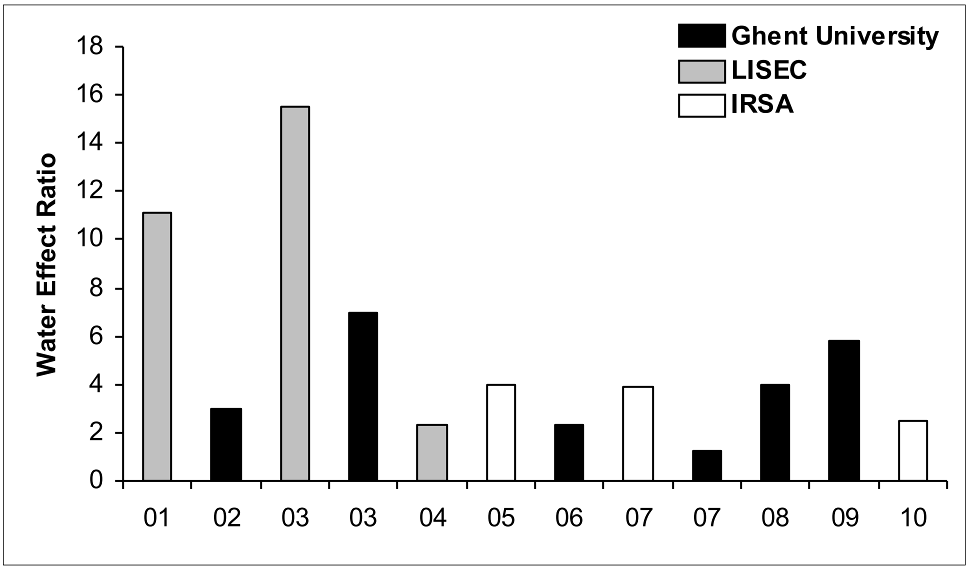

Figure 9: Water Effect Ratio for *R. subcapitata* in 10 surface waters spiked with $CuSO_4$

The observed differences between different seasons (autumn – spring) are presented in Figure 10. It can be concluded that no major differences were noted between the EC_{50}s found in autumn 2000 and spring 2001. This is in accordance with (1) the results obtained with *D. magna* and (2) the data from the physico-chemical characterisation for both samples which revealed only slight differences between the two samples.

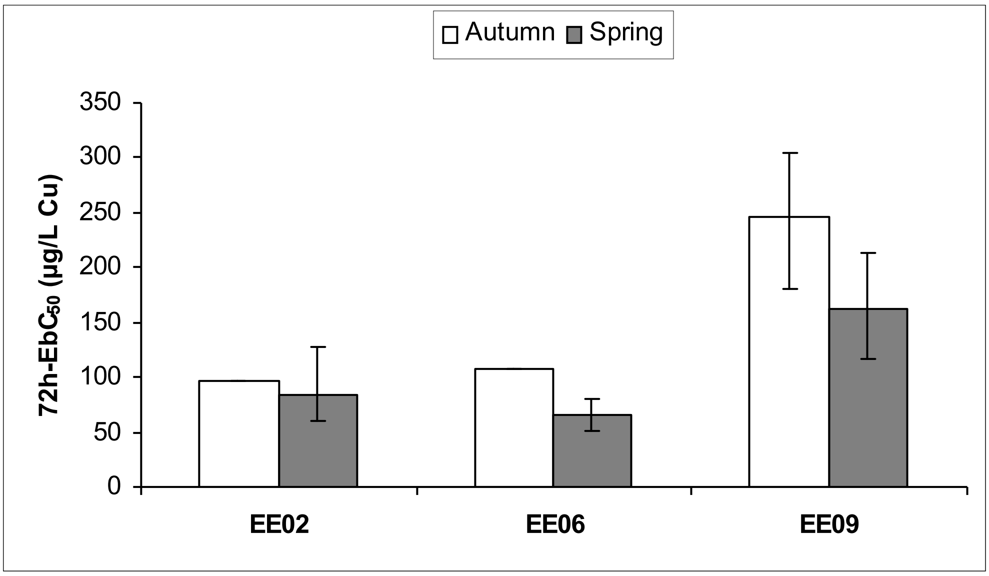

Figure 10: Seasonal differences on observed 72h-EcC_{50} for *R. subcapitata* in 3 surface waters.

Relationship observed toxicity – physicochemistry

Although research efforts are ongoing, no BLM for predicting Cu-toxicity to algae has been developed. A similar analysis as the one that has been performed with the chronic Cu-toxicity data for *D. magna* (see section 4.6.2) was conducted by plotting the acute Cu-toxicty data for *D. magna* against the 72h-EbC$_{50}$ for *R. subcapitata* (Figure 11). However, no relationship between both datasets was found, indicating that the physico-chemistry of the test media affects Cu-toxicity to *R. subcapitata* differently than to *D. magna*.

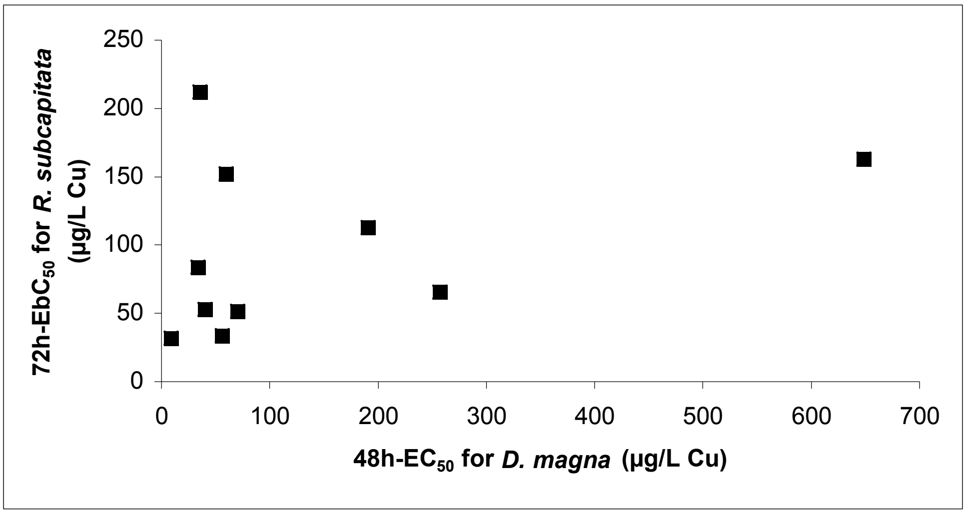

Figure 11: 48h-EC$_{50}$ for *D. magna* vs. 72h-EbC$_{50}$ for *R. subcapitata* in 10 surface waters spiked with $CuSO_4$.

To further explore possible relationships, the observed 72h-EbC$_{50}$s were plotted against the DOC-concentration (Figure 12), the hardness (Figure 13) and pH (Figure 14) of the surface waters. A significant linear relationship ($R^2 = 0.73$, $p<0.05$) was found between the DOC concentration and the 72h-EbC$_{50}$, but no relationship was found between the effect concentration and one of the two other physicochemical parameters. These findings, however, should not lead to the conclusion that only DOC is important for assessing Cu-toxicity to *R. subcapitata* in natural waters.

Recent studies by Heijerick *et al*. (in prep.) and De Schamphelaere *et al*. (in prep.) have evaluated the effects of individual changes of calcium, magnesium and pH on zinc and copper toxicity, respectively. These studies revealed that an increase of calcium or magnesium from 0.25 to 2 mM (covering the range of hardness found in natural surface waters) reduced copper toxicity (expressed as Copper$_{dissolved}$) by a factor of approxicmately 4. A pH-increase from 6 to 8.5, on the other hand (a pH-range covering the majority of the natural variability in European surface waters) lead to an increase of copper toxicity with a similar factor of 4.

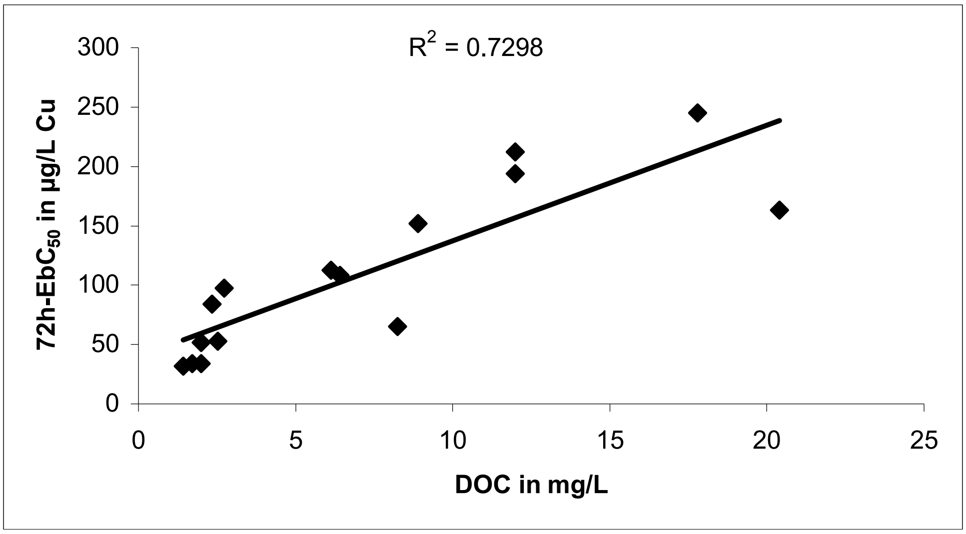

Figure 12: Copper toxicity to the alga *R. subcapitata* as a function of the DOC-concentration of the surface water.

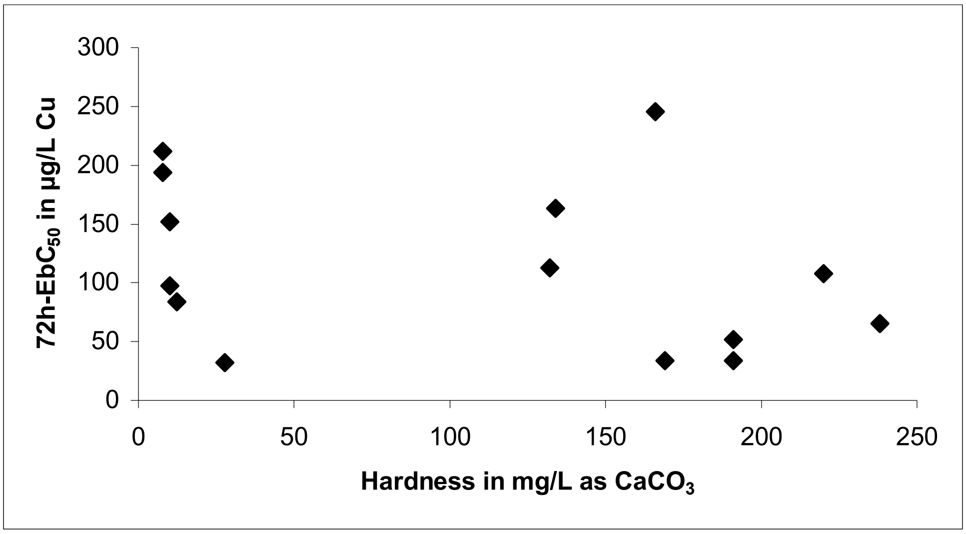

Figure 13: Copper toxicity to the alga *R. subcapitata* as a function of the hardness of the surface water.

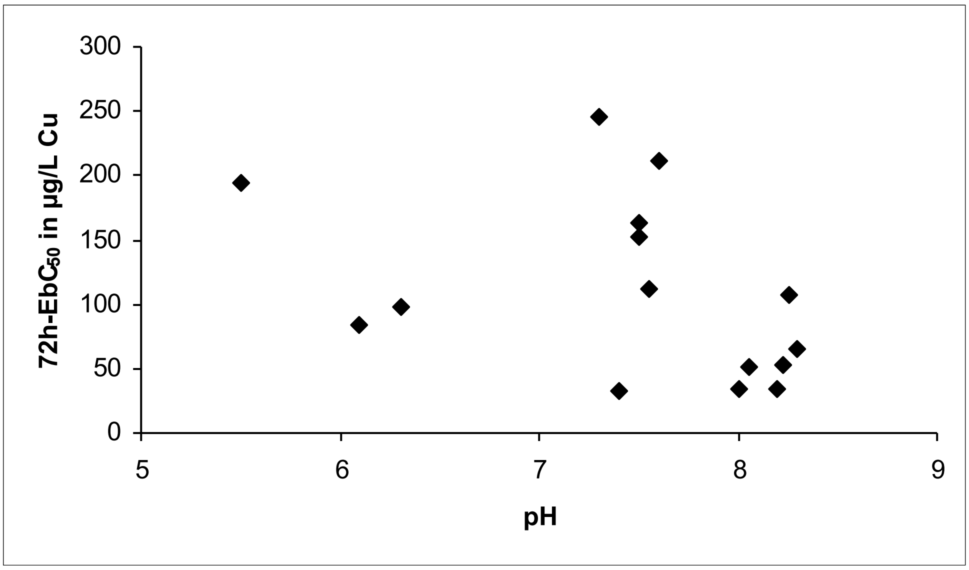

Figure 14: Copper toxicity to the alga *R. subcapitata* as a function of the pH of the surface water during the test period.

So, both parameters clearly affect copper toxicity to algae when they are varied while all other parameters remain constant. The absence of any relation between these parameters and copper toxicity (Figures 13 and 14), however, can be (partially) explained when the correlation between hardness and pH in the surface waters is considered. Analysis of the Surface Water Database (Heijerick and Janssen, 2000), a database containing monitoring data of surface waters from different European countries, revealed a positive relationship between pH and hardness. This co-variance between both parameters is reflected in the final selection of the sampling sites in this project: site 01 to 04 have a low pH and low hardness there where site 06 to 10 have a relatively high hardness and pH.

This co-variance has its consequences on the observed algal Cu-toxicity in the used surface waters. Whenever low pH has a protective effect for algae to Cu-toxicity, reduced protection is available by the presence of low calcium and/or magnesium concentrations. On the other hand, high concentrations of hardness-ions offer a higher protection against copper toxicity (by a factor of 4), but this is accompanied by a higher pH which increases Cu-toxicity with a similar factor of 4. Consequently, the effect of a variation of one of these parameters on copper toxicity is "neutralised" by the opposite effect of the other parameter which varies concurrently. The effect of DOC on copper toxicity becomes thus more pronounced and the observed relationship could well be used as an indicative tool for predicting copper toxicity to *R. subcapitata* in European surface waters. This relationship remains valid as long as there are no changes in the correlation between hardness and pH. In Europe, few surface waters are found with low hardness and high pH, or vice versa. However, in Northern America these types of surface waters are more common and estimations of copper toxicity based on the DOC-concentrations, as suggested by these data, may not be valid.

Taking the individual effects of pH and hardness on copper toxicity to *R. subcapitata* into account, combined with the co-variance of pH and hardness, it is possible to explain the variability in observed Cu-toxicity to algae using the physicochemistry (i.e. DOC) of the tested surface waters in this study.

NOEC versus the 72h-EbC50

In Figure 15 the NOEC and the 72h-EbC_{50} are presented for the algal tests performed by the different laboratories.

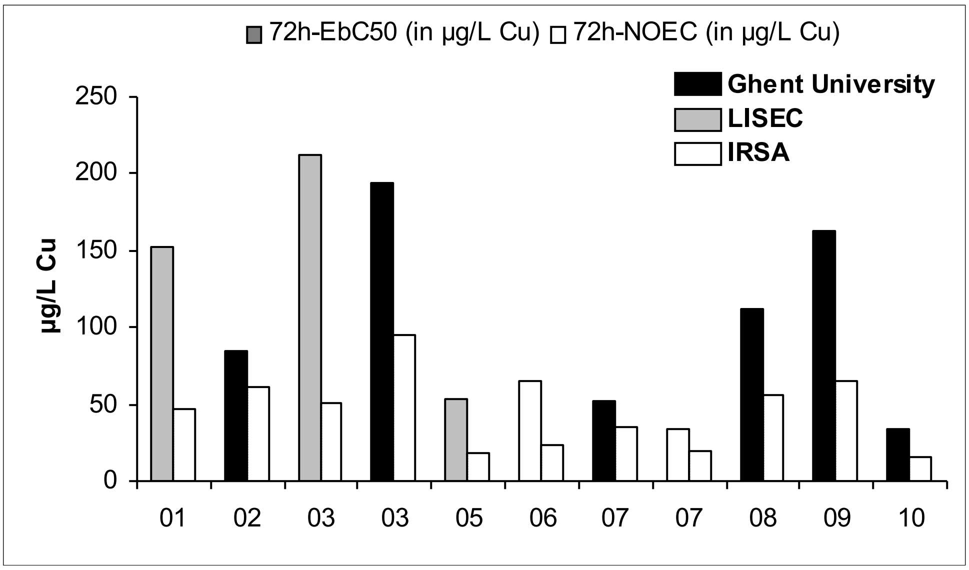

Figure 15: Copper toxicity (72h-EbC_{50}s and 72h-NOECs) to the alga *R. subcapitata* in 10 surface waters

Generally, the 72h-NOEC is a factor 1.4 to 3.3 lower than the 72h-EbC_{50}. For site 04 (not included in Figure 15) a factor difference of 7.6 was observed. This large difference was due to an outlier in the test results, resulting in an overestimation of the NOEC (4.2 µg/L Cu). The concentration-response relationship for this test suggests that the 'true' NOEC for this medium is situated around 15µg/L, which would correspond with a factor difference of ± 2.

4.4 Species-dependent variability of the observed effect concentrations

As mentioned in section 4.6.3, an increase of pH is associated with an increase of copper toxicity to the algae *R. subcapitata*. This effect, however, is not the same for all organisms: De Schamphelaere and Janssen (2001, accepted) demonstrated that for *D. magna* an increase of the pH resulted in lower acute copper toxicity. The effect of increasing hardness and/or DOC on acute copper toxicity to daphnids and algae are similar: in both cases copper toxicity is reduced. Based on these findings, some major differences are expected when the toxicity data for both species are compared. Figure 16 presents the 48h-EC_{50} for *D. magna* and the 72h-EbC_{50} for *R. subcapitata* for the 10 sites.

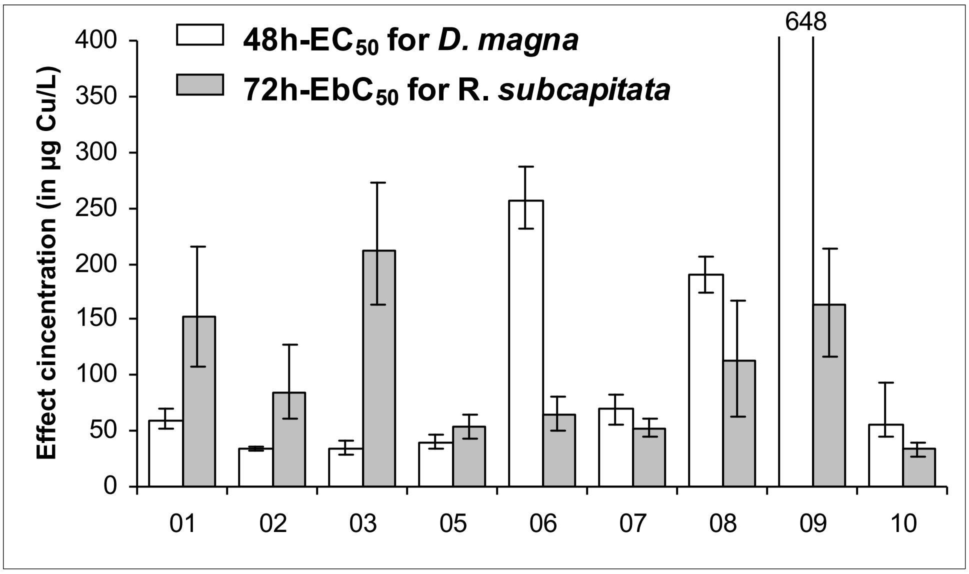

Figure 16: Cu-toxicity to *D. magna* and *R. subcapitata* in 9 European surface waters.

For site 01 to 05 – the sites with relatively low pH and low hardness – *D. magna* is the more sensitive species. Indeed, a low pH and a low hardness maximize Cu-bioavailability and toxicity for *D. magna*, while a low pH has a protective effect for *R. subcapitata*. As a result, *D.magna* is up to 6.2 times more sensitive than *R. subcapitata*.
An opposite effect is noted for the sites with high pH and hardness (sites 06 to 10). Here, all three physico-chemical parameters reduce acute copper toxicity to *D. magna*. The high pH, however, enhances copper toxicity for the algae. Consequently, higher copper toxicity for algae is noted in these waters compared to daphnids (up to a factor of 3.9 and 4.0 for sites 06 and 09, respectively). In tests with surface water from site 05, a site with relatively low hardness (± 50 mg/L as $CaCO_3$) and moderate pH (7.66), both species showed a comparable sensitivity.

These findings indicate that the physicochemistry of the test medium not only determines the toxicity of copper, but also determines which species is more sensitive.

4.5 Copper toxicity for local species

The toxicity of copper for local organisms, and more specific, resident cladocerans was investigated for 5 of the studied sites (Figure 17).

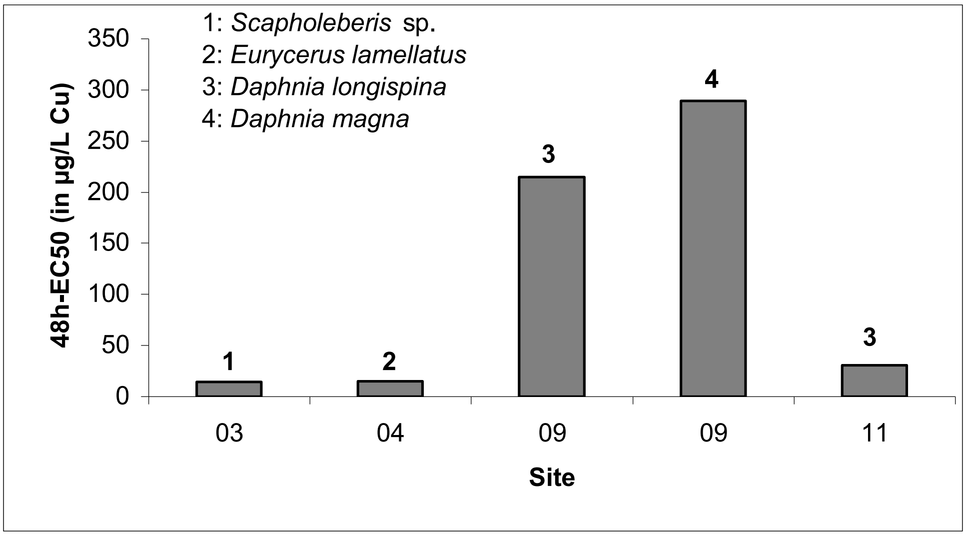

Figure 17: 48h-EC_{50} of copper (in µg/L Cu) for resident organisms tested in the local surface water.

The observed 48h-EC_{50}s varies between 14.1 and 289 µg/L Cu (factor difference of 20.5). The lowest observed copper toxicty is observed in surface water originating from site 9. This is in accordance with the results obtained with daphnids cultured under laboratory conditions (see Figure 1). The relevance of using laboratory cultured daphnids for predicting effects 'in the field' was evaluated through calculation of the ratio between the *D. magna* 48h-EC_{50} and the local species 48h-EC_{50}, both tested in natural (local) surface water (Figure 18).

Depending on the test species and origin, the local species were more or less sensitive than the lab strain (both tested in local test medium). For instance, *D. longispina* originating from site 09 was more sensitive to copper than the lab species, but the *D. longispina* strain from location 11 (mesocosm study) proved to be less sensitive. In all cases, the factor of difference was always less than 3.

Other tested local species were copepods (site 02, 06 and 09) and the perch *P. fluviatilis* (site 06). However, these organisms proved to be far less sensitive to copper compared to daphnids and algae. Copepod effect concentrations in surface water varied from 714 to 8468 µg/L. For the juvenile perch, an 96h-EC_{50} of 689 µg/L was found in surface water.

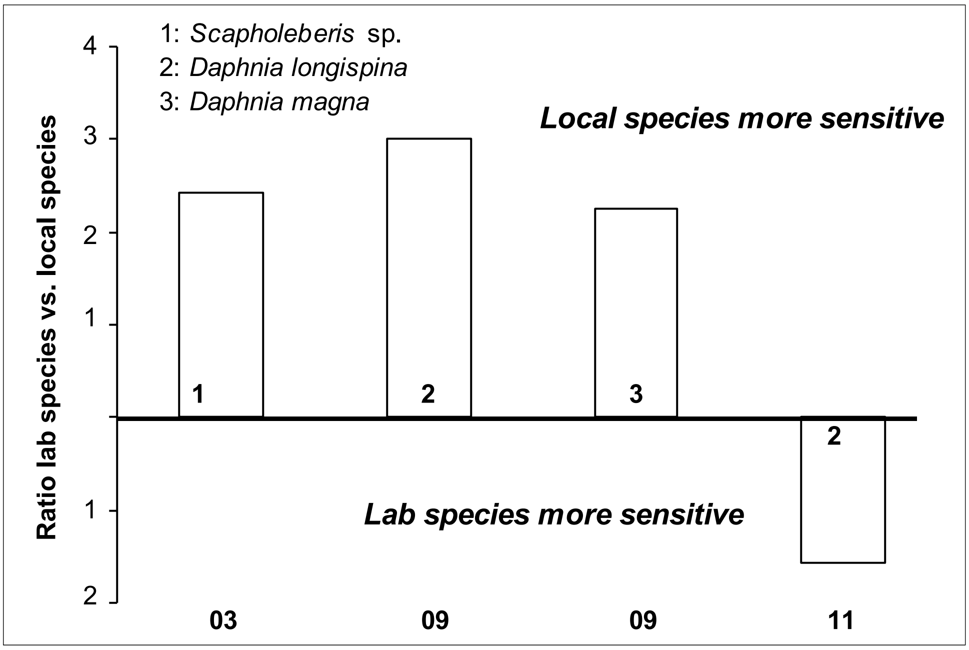

Figure 18: Ratio between the *D. magna* 48h-EC_{50} and the local species 48h-EC_{50} for 4 different surface waters.

4.6 Conclusions

The 48h-EC_{50} for *Daphnia magna* ranged between 33.8 and 648 µg/L $Cu_{diss.}$, a difference of almost 1.5 orders of magnitude (factor difference of 19.2). The observed acute Cu-toxicity could well be predicted (within a factor of 1.5) using the Biotic Ligand Model – approach (De Schamphelaere and Janssen, 2001). The effect of varying physico-chemistry on Cu-toxicity to *D. magna* can be summarized as follows:

- an increase of DOC leads to an increase of the 48h-EC_{50} (decrease of Cu-toxicity): complexation of Cu^{2+};
- an increase of water hardness leads to an increase of the 48h-EC_{50} (decrease of Cu-toxicity): competition between Cu^{2+} and Ca^{2+}/Mg^{2+} for binding (and uptake);
- an increase of the pH leads to an increase of the 48h-EC_{50} (decrease of Cu-toxicity): effect of speciation > the (possible) competition between H^+ and Cu^{2+} for binding to the Biotic Ligand.

Chronic toxicity (21d-EC_{50}, Ro as endpoint) of Cu to *D. magna* was determined for 6 sites and varied between 23 and 367 µg/L (± one order of magnitude). The observed relationship between acute and chronic toxicity indicates that the effects of varying physico-chemistry on chronic Cu-toxicity are similar to those affecting acute toxicity. No chronic BLM for *D. magna* is presently available.
The difference between the 21d-EC_{50} and the NOEC was always less than a factor of 1.8, with the latter being the more sensitive endpoint. The relatively small difference between these two endpoints is a result of the steep slope of the concentration-response curve.

The 72h-EC_{50}s for *Raphidocelis subcapitata* varied between 32 to 212 µg/L Cu_{diss}. Compared to the results for *D. magna* (acute), the variation of Cu-toxicity in the different surface waters appears to be much smaller (factor difference of 6.6 for *R. subcapitata* vs. 19.2 for *D. magna*). DOC appeared to be the most important factor affecting copper toxicity in natural waters: the effects of the varying physico-chemistry on Cu-toxicity to *R. subcapitata* can be summarized as follows:

- an increase of DOC leads to an increase of the 72h-EbC_{50} (decrease of Cu-toxicity): complexation of Cu^{2+};
- an increase of water hardness leads to an increase of the 72h-EbC_{50} (decrease of Cu-toxicity): competition between Cu^{2+} and Ca^{2+}/Mg^{2+} for binding (and uptake);
- an increase of the pH leads to a decrease of the 72h-EbC_{50} (increase of Cu-toxicity): effect of (possible) competition between H^+ and Cu^{2+} for binding to the Biotic Ligand > speciation effect (+ additional bioavailability and toxicity of copper hydroxydes and copper carbonates?).

The observed correlation between hardness and pH in European surface waters (high pH and hardness or low pH and hardness) was taken into account when test sites were selected. Therefore, both parameters always had an opposite effect on copper toxicity, resulting in the observed relation between 72h-EbC_{50} and DOC.
No BLM for predicting Cu-toxicity to algae has been published yet.

The acute to chronic ratio for *D. magna* (48h-EC_{50} divided by the 21d-NOEC) varied between 1.1 and 3.9. This small difference between acute and chronic toxicity is a result of the typical concentration-response of Cu. Observations during chronic toxicity tests with copper indicate that the Cu-concentration range in which a daphnid survives but does not reproduce anymore is very small.

In the natural waters with low pH (<7) and low hardness (< 50 mg/L as $CaCO_3$), *D. magna* was the more sensitive species. However, in the waters with high pH (>7) and high hardness (>100 mg/L as $CaCO_3$), algae appeared to be the more sensitive species. This difference can be explained by the different effect of pH on Cu-toxicity to both species.

The sensitivity of local cladocerans was comparable (less than a factor of 3 difference) to the sensitivity of the laboratory species (*D. magna*). Depending on the origin of the resident cladocerans, the laboratory species were more or less sensitive to copper.

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