Comparison of nickel toxicity to cladocerans in soft versus hard surface waters

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Abstract

The aims of the present study were to investigate (1) whether cladocerans living in soft water (operationally defined hardness < 10 mg CaCO₃/L) are intrinsically more sensitive to Ni than cladocerans living in hard water (operationally defined hardness > 25 mg CaCO₃/L) and (2) whether a single bioavailability model can be used to predict the protective effect of water hardness on the toxicity of Ni to cladocerans in both soft and hard water. To address these research questions, acute and chronic bioassays were conducted with 10 different cladoceran species collected in soft and hard water lakes in Sweden. Soft water organisms were tested in a ‘soft’ and a ‘moderately hard’ test water (nominal hardness = 6.25 and 16.3 mg CaCO₃/L, respectively). Hard water organisms were tested in a ‘moderately hard’ and a ‘hard’ test water (nominal hardness = 16.3 and 43.4 mg CaCO₃/L, respectively). The results of the toxicity tests in the ‘moderately hard’ test water revealed no significant differences between the intrinsic sensitivity of soft versus hard water organisms. Modeling exercises indicated that water hardness significantly reduced Ni toxicity to both the soft and the hard water organisms tested. Although predictions of chronic toxicity were sufficiently accurate using the same log $K_{\text{CaBL}}$ and log $K_{\text{MgBL}}$ (i.e. the model parameters describing the effect of hardness) for all organisms under consideration, predictions of acute toxicity were significantly more accurate when separate log $K_{\text{CaBL}}$ and log $K_{\text{MgBL}}$ values were derived for the soft and the hard water organisms tested. This is due to the fact that the relative decrease of acute Ni toxicity to soft water organisms in ‘moderately hard’ compared to ‘soft’ test water was significantly higher than for hard water organisms in ‘hard’ compared to ‘moderately hard’ test water.

Keywords: Nickel; Water fleas; Cladocerans; Field-collected organisms; Water hardness; Bioavailability; Metal risk assessment; Modeling; Biotic ligand model

1. Introduction

The importance of considering water hardness in the risk assessment of metals in freshwater is obvious as it protects freshwater biota against the toxicity of cationic metals (De Schamphelaere and Janssen, 2002; Heijerick et al., 2002; Niyogi and Wood, 2004), including Ni (Chapman et al., 1980; Meyer et al., 1999; Pyle et al., 2002; Hoang et al., 2004; Keithly et al., 2004). This protective effect can be considered the result of competition between Ca²⁺ and/or Mg²⁺ and the free metal ion (Me²⁺) for binding to transport sites and/or sites of toxic action (commonly termed ‘biotic ligand’ (BL), Di Toro et al., 2001) at the organism–water interface. Stability constants representing the strength of binding of each of these cations to the BL (log $K_{\text{CaBL}}$, log $K_{\text{MgBL}}$, and log $K_{\text{MeBL}}$) are used in biotic ligand models (BLMs) to predict the relationship between water hardness and toxicity of metals (e.g., Di Toro et al., 2001; De Schamphelaere and Janssen, 2002), including Ni (Wu et al., 2003, 2007a,b; Keithly et al., 2004, 2007a,b). For crustaceans (Ceriodaphnia dubia and Daphnia magna), investigated hardness levels ranged from 42 to 476 mg CaCO₃/L (Chapman et al., 1980; Keithly et al., 2004; De Schamphelaere et al., 2006; Deleebeeck et al., 2007a,b). For fish (Pimephales promelas and Oncorhynchus mykiss), relations between hardness and Ni toxicity have been established within an overall hardness range of 20–305 mg CaCO₃/L (Meyer et al., 1999; Pyle et al., 2002; Hoang et al., 2004; Deleebeeck et al., 2007a). For crustaceans (Ceriodaphnia dubia and Daphnia magna), investigated hardness levels ranged from 42 to 476 mg CaCO₃/L (Chapman et al., 1980; Keithly et al., 2004; De Schamphelaere et al., 2006; Deleebeeck et al., 2007b). All crustacean Ni bioavailability models developed so far (Wu et al., 2003, 2007a,b; Keithly et al., 2004; De Schamphelaere et al., 2006, 2007a,b) are based on experiments with D. magna and C. dubia. These models have a lower hard-
ness boundary between 42 and 50 mg CaCO₃/L. Cladocerans are known to be one of the organism groups that are most sensitive to metals (Brix et al., 2001; Von Der Ohe and Liess, 2004), including Ni (Keithly et al., 2004). An important question therefore is whether or not bioavailability models based on hardness–toxicity relationships for hardness levels ≥ 42 mg CaCO₃/L can be used to predict the effect of hardness on Ni toxicity at lower hardness levels. Based on acute Cu toxicity experiments with fathead minnows, Van Genderen et al. (2005) demonstrated that an extrapolation of the acute Cu BLM of Santore et al. (2001) below hardness levels of 50 mg CaCO₃/L may result in underestimation of Cu toxicity. For Ni however, the applicability of the existing bioavailability models below their lower hardness boundary has not been evaluated so far.

In Europe, Ni is considered a priority substance in the water framework directive (European Commission, 2000), implying that environmental quality standards are required for the whole European Union. About 30% of the European waters have hardness < 42 mg CaCO₃/L and several surface waters in geographic areas such as Scandinavia, Scotland and Northern Portugal are characterized by low hardness (Salminen et al., 2005). Hence, in order to correctly evaluate the risk of Ni in such waters and such areas, information is needed on how low hardness affects Ni toxicity.

Ni is known to be an ionoregulatory toxicant to D. magna, by impairing unidirectional Mg²⁺ uptake, which results in a net decrease of whole body Mg²⁺ (Pane et al., 2003). Several studies have suggested that the interaction between Ni²⁺ and Mg²⁺ is the result of competition for uptake at Mg²⁺ transport channels (Snayel et al., 1991; Pane et al., 2006a,b). The existence of a shared uptake pathway could explain why Mg protects against Ni toxicity in freshwater organisms. The protective effect of Ca is expected to be primarily related to its stabilizing effect on membrane permeability (McWilliams, 1983; Hunn, 1985; Evans, 1987).

Reviewing mechanisms of acute Cu and Ag toxicity, two ionoregulatory toxicants disrupting Na homeostasis, Grosell et al. (2002) argued that a species’ sensitivity towards exposure to Cu and Ag is determined by its Na uptake rate (in the absence of toxicants) and by its sensitivity to loss of Na from its body fluids. Similiarly, the Ni sensitivity of an organism could be dependent on its Mg uptake rate, the affinity of its transport channels for Mg or its sensitivity to loss of Mg. Since water hardness has already been demonstrated to affect the affinity of organisms not only for uptake of Mg (Snayel et al., 1991, Salmonella typhimurium) but also for Ca (Neufeld and Cameron, 1993, crustaceans), it might be expected that organisms living in waters with different hardness levels have different intrinsic Ni sensitivities and/or do not experience a similar protective effect of hardness.

Therefore, the two major research questions of this study were (1) whether organisms living in soft water are intrinsically more sensitive to Ni than organisms living in hard water and (2) whether a single bioavailability model can be used to predict the protective effect of water hardness on acute and chronic toxicity of Ni to organisms in both soft and hard water. Soft water was operationally defined as water having hardness below the 5th percentile of the hardness distribution in European surface waters (i.e. <10 mg CaCO₃/L, Salminen et al., 2005). Waters having hardness >25 mg CaCO₃/L were operationally defined as hard waters throughout this study. To address both research questions simultaneously, we determined the acute and chronic Ni toxicity to (1) cladocerans collected in soft surface waters and tested in a ‘soft’ and a ‘moderately hard’ test solution and (2) cladocerans collected in hard surface waters and tested in a ‘moderately hard’ and a ‘hard’ test solution. Nominal hardness of these test solutions was 6.25, 16.3 and 43.4 mg CaCO₃/L, respectively.

2. Materials and methods

2.1. Sampling of water and organisms

First, the geographical distribution of water hardness throughout Sweden (data source: Swedish Agricultural University, http://info1.ma.slu.se) was visualized using a Geographic Information System (GIS, Arcview 3.7a, ESRI Inc., 1996). Next, regions with hardness <10 mg CaCO₃/L (arbitrarily termed soft water regions) and >25 mg CaCO₃/L (arbitrarily termed hard water regions) were delineated. Regions within a 10-mile radius (~16km) around cities and other urban zones were rejected to avoid confounding effects of anthropogenic activities. A digital land use map including urban zones and cities was therefore downloaded from the GIS data depot (http://data.geocomm.com). Finally, one hard water region and one adjoining soft water region were selected for sampling in an area approximately 200–300 km northwest of Stockholm (geographic area 60°04′49″–61°59′49″N and 15°46′17″–17°14′08″E).

During an exploratory sampling campaign, 13 soft and 13 hard water lakes were investigated. Live zooplankton samples were collected using a plankton sampling net with mesh size 100 μm. Cladoceran species present in the zooplankton samples were identified using the key of Scourfield and Harding (1966). Based on the results of the exploratory sampling campaign, five soft and four hard water lakes were selected for in-depth investigation. Main selection criteria were hardness, pH (no extremes), species diversity and accessibility of the sites. Anthropogenic influence was very low as evidenced by extremely low trace metal concentrations and NO₃⁻, NH₄⁺ and PO₄³⁻ concentrations (measured in the field using test kits of Merck, Darmstadt, Germany) being <10, <0.2 and <0.25 mg/L, respectively. Water hardness in the selected soft and hard water lakes varied between 4.68 and 7.16 and between 33.9 and 52.9 mg CaCO₃/L, respectively; pH varied between 6.20 and 6.91 in the soft water lakes and between 7.63 and 8.07 in the hard water lakes. Live zooplankton samples of these nine lakes were transported to the laboratory within 72 h after collection. At the time of sampling, water temperature was between 19.9 and 26.8°C. Overall, 19 cladoceran species were successfully transported to the laboratory. They belonged to 5 families: Sidae (1 species), Daphniidae (7), Bosminidae (1), Chydoridae (8) and Macrothricidae (2). For culturing the field-collected species, water was filtered (0.45 μm) at each site and transported to the laboratory. The following physico-chemical parameters were measured:
analyzed: pH (pH-meter P407, Consort, Turnhout, Belgium), dissolved organic and inorganic carbon (DOC and IC) (TOC-5000, Shimadzu, Duisburg, Germany), Ca, Mg, Na, K, Fe, Al, Mn, Ni, Cu, Zn, Pb, Cr, As and Cd (ICP-OES, Perkin Elmer 3300 DV) and Cl, NO₃ and SO₄ (Ion Chromatography, Dionex QIC analyzer, IONPAC AS4A). An overview of the geographic coordinates and the main physico-chemical characteristics of the selected lakes is given in Table 1.

2.2. Culturing of cladoceran populations

We established cultures of all species that were successfully transported to the laboratory. All organisms were cultured in filtered water (0.45 μm) from their lake of origin and were kept at 20°C and under a light cycle of 12L:12D. The animals were fed ad libitum with an algal mix of *Pseudokirchneriella subcapitata* and *Chlamydomonas reinhardtii* in a 3:1 ratio (on a cell number basis). Culture medium was renewed once a week.

2.3. Test design

Organisms originating from soft water lakes were tested in a ‘soft’ and a ‘moderately hard’ test medium and organisms originating from hard water lakes were tested in a ‘moderately hard’ and a ‘hard’ test medium. The physico-chemical composition of the ‘soft’ and the ‘hard’ test medium reflected the geometric mean of major anion (with the exception of SO₄) and cation (with the exception of Na, see further) concentrations in the five selected soft and the four selected hard water lakes, respectively. Physico-chemical composition of the ‘moderately hard’ test medium was determined as the geometric mean of the ‘soft’ and ‘hard’ test medium. To avoid complications related to a combined modification of pH and hardness, pH was maintained around 7 in all test waters. This was achieved by adding the same amount of NaHCO₃ to each test medium. Water hardness of the ‘soft’, the ‘moderately’ hard and the ‘hard’ test medium was 6.25, 16.3 and 43.4 mg CaCO₃/L, respectively. Nominal composition of the test media is given in detail in Table 2.

2.4. Toxicity testing

All tested species are presented in Table 3. Test organisms were only taken from cultures in which organisms had been growing and reproducing well for several months. All tests were performed at 20°C under a light cycle of 12L:12D.

Acute toxicity tests were performed according to OECD guideline 202 (OECD, 1996) with the exception that juveniles of <48 h old (instead of <24 h old) were used to initiate tests. Each experiment consisted of a control and 5–7 Ni (added as NiCl₂) treatments. Each treatment was performed with three replicates (polyethylene cups containing 50 mL of test medium) using 5 to 10 organisms per replicate. The number of ‘immobilized’ juveniles in each cup was recorded after 24 and 48 h. Test results were accepted and reported only when mortality in the controls did not exceed 10%.

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**Table 1**

Geographic coordinates and main physico-chemical parameters of the four hard and five soft water lakes of which live zooplankton samples were transported to the laboratory.

<table>
<thead>
<tr>
<th>Site number</th>
<th>Geographic coordinates</th>
<th>Name of lake</th>
<th>pH</th>
<th>Ca (mg/L)</th>
<th>Mg (mg/L)</th>
<th>Hardness (mg CaCO₃/L)</th>
<th>Na (mg/L)</th>
<th>K (mg/L)</th>
<th>Cl (mg/L)</th>
<th>SO₄ (mg/L)</th>
<th>Alkalinity b (mg CaCO₃/L)</th>
<th>DOC (mg C/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H 10</td>
<td>60°05’45’’N 16°18’05’’E</td>
<td></td>
<td>8.07</td>
<td>15.1</td>
<td>3.65</td>
<td>52.9</td>
<td>3.51</td>
<td>3.02</td>
<td>6.95</td>
<td>6.99</td>
<td>35.4</td>
<td>16.8</td>
</tr>
<tr>
<td>H 11</td>
<td>60°05’32’’N 15°59’13’’E</td>
<td></td>
<td>7.91</td>
<td>16.9</td>
<td>1.44</td>
<td>48.2</td>
<td>13.4</td>
<td>9.25</td>
<td>8.29</td>
<td>3.91</td>
<td>72.1</td>
<td>10.3</td>
</tr>
<tr>
<td>H 12</td>
<td>60°04’49’’N 16°00’37’’E</td>
<td>Målsjön</td>
<td>7.71</td>
<td>12.9</td>
<td>2.66</td>
<td>43.2</td>
<td>7.45</td>
<td>2.35</td>
<td>12.1</td>
<td>18.3</td>
<td>24.0</td>
<td>11.6</td>
</tr>
<tr>
<td>H 13</td>
<td>60°33’44’’N 15°53’30’’E</td>
<td>Lintjärnen</td>
<td>7.63</td>
<td>11.3</td>
<td>1.39</td>
<td>33.9</td>
<td>18.4</td>
<td>0.389</td>
<td>27.4</td>
<td>5.40</td>
<td>25.2</td>
<td>7.75</td>
</tr>
<tr>
<td>S 14</td>
<td>61°40’25’’N 15°52’51’’E</td>
<td></td>
<td>6.41</td>
<td>1.98</td>
<td>0.538</td>
<td>7.16</td>
<td>1.58</td>
<td>0.167</td>
<td>1.57</td>
<td>1.79</td>
<td>6.86</td>
<td>5.57</td>
</tr>
<tr>
<td>S 17</td>
<td>61°42’25’’N 15°59’01’’E</td>
<td>Abborrtjärnen</td>
<td>6.20</td>
<td>1.94</td>
<td>0.404</td>
<td>6.50</td>
<td>1.81</td>
<td>0.207</td>
<td>2.92</td>
<td>1.40</td>
<td>2.74</td>
<td>12.2</td>
</tr>
<tr>
<td>S 18</td>
<td>61°40’18’’N 15°52’51’’E</td>
<td>Oktjärn</td>
<td>6.91</td>
<td>1.71</td>
<td>0.480</td>
<td>6.24</td>
<td>1.43</td>
<td>0.241</td>
<td>1.20</td>
<td>1.94</td>
<td>6.99</td>
<td>5.15</td>
</tr>
<tr>
<td>S 19</td>
<td>61°39’40’’N 15°46’17’’E</td>
<td></td>
<td>6.44</td>
<td>1.26</td>
<td>0.371</td>
<td>4.68</td>
<td>1.19</td>
<td>0.234</td>
<td>1.14</td>
<td>1.10</td>
<td>3.26</td>
<td>6.80</td>
</tr>
<tr>
<td>S 21</td>
<td>61°56’54’’N 16°11’42’’E</td>
<td>Lilla Svartsjön</td>
<td>6.69</td>
<td>1.96</td>
<td>0.535</td>
<td>7.09</td>
<td>1.42</td>
<td>0.078</td>
<td>1.95</td>
<td>1.45</td>
<td>6.02</td>
<td>7.79</td>
</tr>
</tbody>
</table>

*Dissolved Ni concentration was below the method detection level (MDL) of the ICP-OES (i.e. <1 μg/L) in all lakes. NO₃, NH₄ and PO₄ concentrations were all <10, <0.2 and <0.25 mg/L, respectively. Trace metal concentrations were all very low and can be obtained from the authors on request.

a Name of lake not indicated on topographic map or in situ.
b Alkalinity was calculated from measured inorganic carbon (IC) and pH, using thermodynamic stability constants taken from Stumm and Morgan (1996).*
Chronic tests were initiated with juveniles of <48 h old. For each bioassay, a control and 5 Ni concentrations (added as NiCl₂) were prepared. At the start of testing, a single juvenile was transferred to each of the 10 replicates per concentration (polyethylene cups containing 50 mL of test medium). Animals were fed daily with an algal mix of *P. subcapitata* and *C. reinhardtii* in a 3:1 ratio (on a cell number basis). Food quantities were dependent on the species tested and varied between 3 × 10⁶ and 12 × 10⁶ cells per individual per day. Every other day, the test medium was renewed, parent mortality noted, and the number of produced juveniles counted. Tests were continued until control organisms had released a third brood. This is in accordance with the survival and reproduction test method of the US EPA for *C. dubia* (US EPA, 2002). Depending on the species, this occurred between 16 and 21 days after test initiation (indicated in Table 3). Test results were accepted and reported only when mortality in the controls did not exceed 20%.

### 2.5. Chemical analyses

Water temperature, oxygen saturation and pH were measured at the beginning and at the end of testing for acute tests and at each medium renewal for chronic tests. The glass electrode for pH measurements was calibrated with pH 4, pH 7 and pH 10 buffers (Merck, Darmstadt, Germany). Samples for measurement of total Ca and Mg and dissolved Ni concentrations (filtration through a 0.45 µm filter, Gelman Sciences, Ann Arbor, MI, USA) were taken at the end of testing for acute tests and once a week for chronic tests. They were acidified (1%, v/v) with 0.14 N HNO₃ (NormatomTM ultrapure, Ann Arbor, MI, USA) were taken at the end of testing for acute tests and once a week for chronic tests. They were acidified (1%, v/v) with 0.14 N HNO₃ (NormatomTM ultrapure, Ann Arbor, MI, USA) were taken at the end of testing for acute tests and once a week for chronic tests. They were acidified (1%, v/v) with 0.14 N HNO₃ (NormatomTM ultrapure, Ann Arbor, MI, USA) were taken at the end of testing for acute tests and once a week for chronic tests. They were acidified (1%, v/v) with 0.14 N HNO₃ (NormatomTM ultrapure, Ann Arbor, MI, USA) were taken at the end of testing for acute tests and once a week for chronic tests. They were acidified (1%, v/v) with 0.14 N HNO₃ (NormatomTM ultrapure, Ann Arbor, MI, USA) were taken at the end of testing for acute tests and once a week for chronic tests. They were acidified (1%, v/v) with 0.14 N HNO₃ (NormatomTM ultrapure, Ann Arbor, MI, USA) were taken at the end of testing for acute tests and once a week for chronic tests. They were acidified (1%, v/v) with 0.14 N HNO₃ (NormatomTM ultrapure, Ann Arbor, MI, USA) were taken at the end of testing for acute tests and once a week for chronic tests. They were acidified (1%, v/v) with 0.14 N HNO₃ (NormatomTM ultrapure, Ann Arbor, MI, USA) were taken at the end of testing for acute tests and once a week for chronic tests.

#### 2.6. Calculation and statistical comparison of effect concentrations

Acute 48-h EC50 values, chronic LC50 values and their respective 95% confidence intervals were calculated using the trimmed Spearman–Karber method (Hamilton, 1977). Observed ‘immobility’ (acute tests) or mortality (chronic tests) at each measured Ni concentration was used as input for the calculations. Chronic EC10 and EC50 values (exposure concentrations resulting in 10 and 50% decrease in reproduction, respectively) and their 95% confidence intervals were calculated using a logistic model described by De Schamphelaere and Janssen (2004) and the Levenberg–Marquardt method (Levenberg, 1944; Marquardt, 1963), respectively. In this paper all EC10, EC50 and LC50 values are based on dissolved Ni measurements. NOEC and LOEC values can be obtained from the authors on request.

Acute EC50s, chronic EC50s and chronic LC50s for soft water organisms in ‘moderately hard’ test medium were statistically compared to those for hard water organisms in the same test medium using the Mann–Whitney *U* test (*p* < 0.05).

#### 2.7. Modeling and predicting the effect of hardness on Ni toxicity

One way to quantitatively evaluate and compare the effects of water hardness on Ni toxicity observed for the different populations is by assuming that the observed effects can be explained by an underlying model, such as the BLM. The choice of a model may result in a biased interpretation, and the potential implications of choosing a particular model will therefore be discussed in the discussion section. According to the BLM concept, E/LC50s based on Ni²⁺ activity can be predicted for population *i* in test water *j* using the following equation, assuming that the protective effects of Ca and Mg against Ni toxicity can be represented by competitive unidentate binding to a single BL site,
Table 3
Toxicity of Ni to all field populations tested

<table>
<thead>
<tr>
<th>Site number</th>
<th>Species</th>
<th>Chronic test duration (days)</th>
<th>Hard</th>
<th>Moderately hard</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Acute</td>
<td>chron. EC50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>EC10</td>
<td>EC50</td>
</tr>
<tr>
<td>H1</td>
<td>Ceriodaphnia quadrangula</td>
<td>17</td>
<td>517.0 (252.0–1061)</td>
<td>33.1 (–)</td>
</tr>
<tr>
<td></td>
<td>Daphnia longispina</td>
<td>21</td>
<td>510.6 (290.3–898.3)</td>
<td>113 (–)</td>
</tr>
<tr>
<td></td>
<td>Alona affinis</td>
<td>16</td>
<td>5540 (3827–8019)</td>
<td>–a</td>
</tr>
<tr>
<td></td>
<td>Camptocer cus lilljeborgi</td>
<td>1085 (624.2–1886)</td>
<td>–a</td>
<td>–a</td>
</tr>
<tr>
<td>H1</td>
<td>Ceriodaphnia pulchella</td>
<td>17</td>
<td>981.0 (708.8–1358)</td>
<td>27.6 (3.51–217)</td>
</tr>
<tr>
<td></td>
<td>Chydrorus ovalis</td>
<td>17</td>
<td>4256 (3205–5653)</td>
<td>–a</td>
</tr>
<tr>
<td>H1</td>
<td>Simocephalus v etul tus</td>
<td>21</td>
<td>1485 (963.1–2291)</td>
<td>23.3 (–)</td>
</tr>
<tr>
<td>S1</td>
<td>Ceriodaphnia quadrangula</td>
<td>17</td>
<td>97.31 (66.71–142.0)</td>
<td>2.95 (2.08–4.19)</td>
</tr>
<tr>
<td></td>
<td>Peracantha truncata</td>
<td>17</td>
<td>2200 (1782–2716)</td>
<td>4.92 (1.68–14.4)</td>
</tr>
<tr>
<td>S1</td>
<td>Simocephalus serrulatus</td>
<td>17</td>
<td>640.7 (505.4–812.2)</td>
<td>6.86 (–)</td>
</tr>
<tr>
<td>S1</td>
<td>Bosmina coregoni</td>
<td>165.3 (135.9–201.1)</td>
<td>–a</td>
<td>–a</td>
</tr>
</tbody>
</table>

Effect concentrations (48-h EC50s, chronic LC50s, chronic EC10s and EC50s for the endpoint reproduction) are reported as μg dissolved Ni/L. Test duration of chronic tests was dependent on the time needed for control animals to release three broods. Numbers between brackets represent 95% confidence intervals—if not reported, no reliable confidence intervals could be calculated due to steep concentration response curves.

- a No reproduction observed.
- b No LC50 could be calculated—70% survival at highest exposure concentration of 118 μg/L.
as described by De Schamphelaere and Janssen (2002):

\[
E/\text{LC50}_{\text{Ni}^{2+},i,j,\text{predicted}} = E/\text{LC50}_{0,i,j} + K_{\text{CaBL}}(\text{Ca}^{2+})_j + K_{\text{MgBL}}(\text{Mg}^{2+})_j
\]

where \(E/\text{LC50}_{0,i,j}\) is the sensitivity parameter (may be interpreted as the \(E/\text{LC50}_{0,i,j}\) for population \(i\) in the hypothetical case that no competing cations are present), \((\text{Ca}^{2+})_j\) and \((\text{Mg}^{2+})_j\) are the chemical activities of the competing cations in test water \(j\), and \(K_{\text{CaBL},i}\) and \(K_{\text{MgBL},i}\) are the stability constants for binding of \(\text{Ca}^{2+}\) and \(\text{Mg}^{2+}\) to the BL of population \(i\). Chemical speciation of Ni and other ions was calculated using the WHAM VI software (Tipping, 1998; NERC, 2001). Stability constants for inorganic complexes were taken from Smith et al. (2004) and used to replace the constants in the default thermodynamic database of the software. The model presented in Eq. (1) requires the estimation of three model parameters. The sensitivity parameter \(E/\text{LC50}_{0,i,j}\) can be estimated for each single population \(i\). The parameters describing the protective effect of hardness, i.e. \(K_{\text{CaBL}}\) and \(K_{\text{MgBL}}\), may also vary among species/populations. However, our limited dataset (only two hardness levels tested per population) does not allow estimation of these constants for each individual species/population. Furthermore, since the Ca:Mg ratio was similar in all test solutions, it is not possible to optimize \(K_{\text{CaBL}}\) and \(K_{\text{MgBL}}\) independently of each other. We dealt with these two limitations by estimating \(K_{\text{CaBL}}\) and \(K_{\text{MgBL}}\) for groups of tested populations instead of for individual populations (see further) and by assuming a fixed ratio between \(K_{\text{CaBL}}\) and \(K_{\text{MgBL}}\) equal to the ratio of these two constants obtained for \(D.\) magna (acute toxicity: Deleebeeck et al., 2007b; chronic toxicity: De Schamphelaere et al., 2006). Maximum likelihood estimation (MLE) was followed to obtain best-fit values for all model parameters. For large sample sizes, minimizing the sum of squared errors (SSE) is identical to maximizing the likelihood (Kutner et al., 2005):

\[
\text{SSE} = \sum_{i,j} \left( \log \left( \frac{E/\text{LC50}_{0,i,j,\text{predicted}}}{E/\text{LC50}_{0,i,j,\text{observed}}} \right) - 1 \right)^2
\]

A first modeling exercise (model 1) assumed \(K_{\text{CaBL}} = K_{\text{MgBL}} = 0\). In other words, it was assumed that effects of hardness on Ni toxicity are not significant. Fitting of Eq. (1) was thus reduced to estimating the sensitivity parameter \(E/\text{LC50}_{0,i,j}\) for all populations \(i\). A second modeling exercise (model 2) consisted of optimizing \(K_{\text{CaBL}}\) and \(K_{\text{MgBL}}\) based on the combined toxicity dataset of the soft and the hard water organisms tested. A third modeling exercise (model 3) differed from the second in that optimal values for \(K_{\text{CaBL}}\) and \(K_{\text{MgBL}}\) were derived for the soft and the hard water organisms separately.

In this sequence of modeling exercises, model 3 can be considered a ‘nested’ extension (sensu Kutner et al., 2005) of model 2, which in turn is a ‘nested’ extension of model 1. Obviously, the SSE for model 3 will be lower than for model 2, since 1 more parameter is estimated. Similarly, the SSE for model 2 will be lower than for model 1. The performance of model 3 was compared to that of model 2 and the performance of model 2 was compared to that of model 1 using the likelihood ratio test (Kutner et al., 2005). The statistic for the likelihood ratio test, denoted by \(G^2\) (Kutner et al., 2005; Jonker et al., 2005) is calculated using the following equation:

\[
G^2_{1-2} = N \times \frac{\text{SSE}_{\text{Model 1}}}{\text{SSE}_{\text{Model 2}}}
\]

\[
G^2_{2-3} = N \times \frac{\text{SSE}_{\text{Model 2}}}{\text{SSE}_{\text{Model 3}}}
\]

\(N\) is the number of LC50 or EC50 data that were used to fit the model. Large-sample theory states that, when \(N\) is large, \(G^2_{1-2}\) (or \(G^2_{2-3}\)) is approximately distributed as \(\chi^2\) with 1 degree of freedom since one more parameter is estimated in model 2 versus model 1 (or model 3 versus model 2). The \(\chi^2\) cumulative probability distribution function delivers the probability \(\alpha\) for \(G^2\), where \(\alpha\) is the probability of validity of the null-hypothesis, i.e. that model 1 and model 2 (or model 2 and model 3) have the same predictive capacity. When \(\alpha < p = 0.05\), model 2 was considered significantly better than model 1 (or model 3 was considered significantly better than model 2). A significantly better model 2 means that the protective effect of hardness should be incorporated into the model. A significantly better model 3 means that Ni toxicity to the soft and the hard water populations tested should be modeled using separate \(K_{\text{CaBL}}\) and \(K_{\text{MgBL}}\) values. A final modeling exercise (model 4) was conducted to evaluate the predictive capacity of a model that uses the \(K_{\text{CaBL}}\) and \(K_{\text{MgBL}}\) values derived for the standard test organism \(D.\) magna (acute toxicity: Deleebeeck et al., 2007b; chronic toxicity: De Schamphelaere et al., 2006). The sensitivity parameter \(E/\text{LC50}_{0,i,j}\) was optimized for each population \(i\). For all four modeling exercises, model parameter values and values for SSE, \(G^2\) and \(\alpha\) are reported in Table 4.

3. Results

3.1. Acute toxicity test results

Acutely, seven hard and four soft water populations were tested successfully. Three cladoceran families were represented among the tested species: Daphniidae (Ceriodaphnia pulchella, Ceriodaphnia quadrangula, Daphnia longispina, Simocephalus serrulatus and Simocephalus vetulus), Bosminidae (Bosmina coregoni) and Chydoridae (Alona affinis, Camptocercus lilljeborgi, Chydorus ovalis and Peracantha truncata). For \(C.\) quadrangula both a soft and a hard water population was tested. An overview of all tested populations and their 48-h EC50s is given in Table 3. Overall, the 48-h EC50s of the soft water populations varied between 97.31 and 2200 \(\mu\)g/L in ‘soft’ test water and between 140.6 and 2726 \(\mu\)g/L in ‘moderately hard’ test water. The 48-h EC50s of the hard water populations varied between 400.6 and 3335 \(\mu\)g/L in ‘moderately hard’ test water and between 510.6 and 5540 \(\mu\)g/L in ‘hard’ test water. Thus, there is an upward shift of the EC50 ranges with increasing hardness. Also, for all individual populations, increasing hardness resulted in decreased toxicity. For the soft water populations,
Table 4
Model parameters (log $K_{\text{CABL}}$, log $K_{\text{MABL}}$ and $E/\text{LC50}_{\text{Ni}^{2+},i}$, i.e. the population-specific sensitivity parameter), average prediction errors and prediction error ranges for four different modeling exercises based on acute 48-h EC50s and chronic E/LC50s (see Table 3 for chronic test duration for each tested population)

<table>
<thead>
<tr>
<th>Model</th>
<th>Acute 48-h EC50</th>
<th>Chronic LC50</th>
<th>Chronic EC50</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>log $K_{\text{CABL}}$</td>
<td>–</td>
<td>3.2</td>
<td>3.2/2.7</td>
</tr>
<tr>
<td>log $K_{\text{MABL}}$</td>
<td>–</td>
<td>2.6</td>
<td>2.6/3.6</td>
</tr>
<tr>
<td>SSE</td>
<td>0.365</td>
<td>0.235</td>
<td>0.144</td>
</tr>
<tr>
<td>$G^{2b}$</td>
<td>–</td>
<td>9.71</td>
<td>10.7a</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>–</td>
<td>0.00183d</td>
<td>0.00103d</td>
</tr>
<tr>
<td>Average prediction error</td>
<td>1.3</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Prediction error range</td>
<td>1.0–1.8</td>
<td>1.0–1.7</td>
<td>1.1–1.4</td>
</tr>
</tbody>
</table>

Species/population | Population-specific sensitivity parameter $E/\text{LC50}_{\text{Ni}^{2+},i}$ (µM)

Hard
- Ceriodaphnia quadrangula | 6.2 | 6.7 | 4.7 | 4.9 | 0.29 | 0.060 | 0.099 | 0.14 | 0.27 | 0.024 | 0.062 | 0.14 |
- Daphnia longispina | 6.6 | 4.8 | 4.8 | 5.1 | 1.0 | 0.20 | 0.34 | 0.49 | 1.2 | 0.095 | 0.25 | 0.57 |
- Alona affinis | 55 | 41 | 41 | 43 | 0.24 | 0.049 | 0.081 | 0.12 | – | – | – | – |
- Camptocercus lilljeborgi | 10 | 7.4 | 7.4 | 7.8 | – | – | – | – | – | – | – | – |
- Ceriodaphnia pulchella | 12 | 9.3 | 9.3 | 9.8 | 0.38 | 0.080 | 0.13 | 0.19 | 0.30 | 0.027 | 0.070 | 0.16 |
- Chydorus ovalis | 51 | 36 | 36 | 39 | – | – | – | – | – | – | – | – |
- Simocephalus vetulus | 12 | 8.4 | 8.4 | 8.9 | 0.31 | 0.068 | 0.11 | 0.16 | 0.24 | 0.022 | 0.058 | 0.13 |

Soft
- Ceriodaphnia quadrangula | 1.7 | 1.5 | 1.1 | 1.5 | 0.11 | 0.043 | 0.024 | 0.077 | 0.15 | 0.029 | 0.024 | 0.11 |
- Peracantha truncata | 36 | 32 | 23 | 33 | 0.36 | 0.15 | 0.080 | 0.26 | 0.39 | 0.076 | 0.063 | 0.28 |
- Simocephalus serrulatus | 14 | 12 | 8.9 | 13 | 0.30 | 0.13 | 0.072 | 0.23 | 0.29 | 0.062 | 0.052 | 0.22 |
- Bosmina coregoni | 4.4 | 4.0 | 2.9 | 4.1 | – | – | – | – | – | – | – | – |

Model 1 = no hardness effect assumed; model 2 = log $K$ values determined for soft and hard water populations combined; model 3 = log $K$ values determined for soft and hard water populations separately; model 4 = Daphnia magna model as described by Deleebeeck et al. (2007b, acute) and De Schamphelaere et al. (2006, chronic). SSE = Sum of squared errors (Eq. (2)); $G^{2} = \text{test statistic for likelihood ratio test (Eq. (3))}$; $\alpha = \text{probability of validity of null-hypothesis (if } \alpha < p = 0.05, \text{null-hypothesis is rejected, i.e. model performance is significantly better than previous model)}$. log $K_{\text{CABL}}$, log $K_{\text{MABL}}$, and prediction errors of retained models are printed in bold.

a log $K_{\text{CABL}}$ and log $K_{\text{MABL}}$ values for hard and soft water populations are given before and after the slash, respectively.
b $N = 22$ acute 48-h EC50s, 16 chronic LC50s, 14 chronic LC50s (Eq. (3)).
c Values for statistical comparison of model 2 with model 1.
d Values for statistical comparison of model 3 with model 2.

3.2. Chronic toxicity test results

The same populations were used in chronic assays, with the exception of C. lilljeborgi, C. ovalis and B. coregoni. Test duration, LC50s, EC10s and EC50s are reported in Table 3. Overall, the LC50s of the soft water populations varied between 3.85 and 21.0 µg/L in ‘soft’ test water and between 13.9 and 47.3 µg/L in ‘moderately hard’ test water. For the hard water populations, LC50s varied between 9.85 and 48.3 µg/L in ‘moderately hard’ test water and between 25.1 and >118 µg/L in ‘hard’ test water. The EC50s of soft water populations varied between 4.41 and 15.3 µg/L in ‘soft’ test water and between 23.4 and 54.2 µg/L in ‘moderately hard’ test water. For the hard water populations, EC50s varied between 11.2 and 58.6 µg/L in ‘moderately hard’ test water and between 28.9 and 125 µg/L in ‘hard’ test water. For the soft water populations, an increase of (nominal) hardness from 6.25 to 16.3 mg CaCO3/L resulted in a 1.2- to 3.4-fold increase of the 48-h EC50s. For the hard water populations, 48-h EC50s increased by a factor 1.1–3.3 with an increase of (nominal) hardness from 6.25 to 16.3 mg CaCO3/L. Considering all 48-h EC50s obtained in the ‘moderately hard’ test water, no significant differences were observed between those obtained with soft and obtained with hard water organisms (Mann–Whitney $U$ test, $p < 0.05$). However, for C. quadrangula, the 48-h EC50 for the soft water population was a factor 2.8 lower than the 48-h EC50 for the hard water population in the same test medium. The observed effects of increasing hardness are visualized in Fig. 1.
ness clearly protected all tested species against chronic exposure to Ni. Overall, the LC50s and EC50s obtained with the soft water organisms in the ‘moderately hard’ test water did not significantly differ from those obtained with the hard water organisms in the same test water (Mann–Whitney U test, \( p < 0.05 \)). Moreover, the soft water population of *C. quadrangula* was observed to be equally sensitive as the hard water population when tested in the same test medium. The observed effects of increasing hardness are visualized in Fig. 1.

3.3. Biotic ligand model application

The first modeling exercise (model 1) assumed \( K_{\text{CaBL}} = K_{\text{MgBL}} = 0 \). Fitting of Eq. (1) was thus reduced to estimating the sensitivity parameter \( E/LC50_{0.5} \) for each population \( i \). Average prediction errors were 1.3 (1.0–1.8), 1.6 (1.1–2.2) and 1.8 (1.3–2.6) for the models based on acute 48-h EC50s, chronic LC50s and chronic EC50s, respectively (Table 4). In the second modeling exercise (model 2), \( K_{\text{CaBL}} \) and \( K_{\text{MgBL}} \) were optimized based on the combined toxicity dataset for soft and hard water populations. The acute model (\( \log K_{\text{CaBL}} = 3.2, \log K_{\text{MgBL}} = 2.6 \)) predicted toxicity with an average error of factor 1.2 (1.0–1.7). Optimized \( \log K \) values for chronic LC50s (\( \log K_{\text{CaBL}} = 4.2, \log K_{\text{MgBL}} = 3.9 \)) and EC50s (\( \log K_{\text{CaBL}} = \log K_{\text{MgBL}} = 4.6 \)) were more than 1 log-unit higher than in the acute model. Chronic LC50s and EC50s were predicted with an average error of factor 1.3 (1.0–1.7) and 1.3 (1.0–1.8), respectively (Table 4). Prediction errors were clearly reduced compared to the scenario where no hardness effect was taken into account (model 1). In all cases, the likelihood ratio test indicated a very significant improvement of predictions using model 2 compared to model 1 (\( \alpha < p = 0.05 \), acutely; \( \alpha < p = 0.001 \), chronically; Table 4). This demonstrates the need to incorporate the protective effect of hardness into the Ni bioavailability model. In the third modeling exercise (model 3), optimal values for \( \log K_{\text{CaBL}} \) and \( \log K_{\text{MgBL}} \) were determined for soft and hard water populations separately. \( \log K \) values for the acute model were \( \log K_{\text{CaBL}} = 4.2 \) and \( \log K_{\text{MgBL}} = 3.6 \) for soft water populations and \( \log K_{\text{CaBL}} = 3.2 \) and \( \log K_{\text{MgBL}} = 2.6 \) for hard water populations. This model predicted acute Ni toxicity with an average error of factor 1.2 (1.1–1.4). Optimal \( \log K \) values for chronic LC50s were \( \log K_{\text{CaBL}} = 4.6 \) and \( \log K_{\text{MgBL}} = 4.2 \) for soft water populations and \( \log K_{\text{CaBL}} = 3.9 \) and \( \log K_{\text{MgBL}} = 3.5 \) for hard water populations. Chronic EC50s were predicted in an optimal manner using \( \log K_{\text{CaBL}} = \log K_{\text{MgBL}} = 4.7 \) and 4.1 for soft and hard water populations, respectively. These models predicted chronic LC50s and EC50s with an average error of factor 1.2 (1.1–1.6) and 1.3 (1.0–1.8), respectively (Table 4). For both endpoints the optimized \( \log K_{\text{CaBL}} \) and \( \log K_{\text{MgBL}} \) values were higher for the soft water populations than for the hard water populations. Average prediction errors and/or prediction error ranges obtained using separate \( \log K \) values for soft and hard water populations (model 3) were slightly (acute 48-h LC50s and chronic LC50s) or not (chronic EC50s) lower than those obtained using a single set of \( \log K \) values for soft and hard water populations (model 2). The likelihood ratio test indicated that acutely, predictions with model 3 were significantly better than those obtained with model 2 (\( \alpha < p = 0.05 \)). Chronically however, no significant differences were observed (\( \alpha > p = 0.05 \)). The predictive capacity of the retained models (acute: model 3; chronic: model 2) is visualized in Fig. 2.

4. Discussion

In this study, we investigated the effect of water hardness on the acute and chronic toxicity of Ni to field-collected cladocerans within a (nominal) hardness range of 6.25–16.3 mg CaCO₃/L for cladocerans collected in soft water and 16.3–43.4 mg CaCO₃/L for cladocerans collected in hard water. The objectives were to determine (1) whether organisms living in soft water are intrinsically more sensitive to Ni than organisms living in hard water, and (2) whether a single bioavailability model can be used to predict the protective effect of water hardness on acute and chronic toxicity of Ni to organisms in both soft and hard water. We
focused on the effect of water hardness since it has been demonstrated that it is one of the most important factors affecting Ni toxicity. Although several other factors have been demonstrated not to affect Ni toxicity to cladocerans in waters with hardness ≥ 42 mg CaCO₃/L (e.g., Na and K, Deleebeeck et al., 2007b), it must be kept in mind that these findings are not yet confirmed in softer waters such as the test waters used in this study.

4.1. Modeling the effect of hardness

Statistical comparison of the predictive capacity of model 2 (hardness effect for soft and hard water populations described

using a single set of log $K_{\text{CaBL}}$ and log $K_{\text{MgBL}}$ values) with the predictive capacity of model 1 (no hardness effect included) revealed that in all cases, model 2 performed significantly better (acutely, $p < 0.05$; chronically, $p < 0.001$). This demonstrates that the protective effect of hardness on Ni toxicity at low hardness levels is sufficiently important to be included in Ni bioavailability models and risk assessment exercises. The explanation for the protective effect of Mg can be found in the mechanism of Ni toxicity. For D. magna, it was demonstrated that both acute and chronic exposure to Ni resulted in a decrease of the unidirectional Mg²⁺ uptake rate and the whole body Mg²⁺ concentration (Pane et al., 2003). This most likely originates from the fact that Ni²⁺ and Mg²⁺, having similar dehydrated ionic radii (0.066 and 0.069 nm, respectively, Weast, 1973), compete for uptake at the same Mg²⁺ transport systems (Snavely et al., 1991; Pane et al., 2006a,b). Although less effective than Mg²⁺, Ca²⁺ is also a competitive inhibitor of Ni²⁺ uptake at Mg²⁺ transporters (Snavely et al., 1991). However, the protective effect of Ca is expected to be primarily due to its stabilizing effect on membrane permeability (McWilliams, 1983; Hunn, 1985; Evans, 1987), hereby offering protection against Ni-induced loss of Mg²⁺ from the haemolymph. Further research is needed to elucidate the underlying physiological mechanisms of the protective effect of Ca against Ni toxicity in crustaceans.

To determine whether a single bioavailability model can be used to predict the protective effect of water hardness on acute and chronic toxicity of Ni to organisms in both soft and hard water, the predictive capacity of model 3 (log $K_{\text{CaBL}}$ and log $K_{\text{MgBL}}$ determined for soft and hard water populations separately) was statistically compared to the predictive capacity of model 2 (log $K_{\text{CaBL}}$ and log $K_{\text{MgBL}}$ determined for soft and hard water populations combined). Only acute Ni toxicity was significantly better predicted using model 3 ($p < 0.05$). The average prediction error and the prediction error range of the acute model 2 were only slightly lower and smaller than those obtained when no hardness effect was assumed (model 1). Closer inspection of the data indicated that this was because prediction errors remained high mainly for soft water populations (e.g., factor 1.7 deviation for B. coregoni with both models). Model performance significantly improved when log $K_{\text{CaBL}}$ and log $K_{\text{MgBL}}$ values were derived for soft and hard water populations separately (model 3). In model 3, the log $K_{\text{CaBL}}$ and log $K_{\text{MgBL}}$ values for soft water organisms are about 1 log-unit higher than those for hard water organisms. This is due to the fact that the relative decrease of acute Ni toxicity to soft water organisms in ‘moderately hard’ compared to ‘soft’ test water was significantly higher than for hard water organisms in ‘hard’ compared to ‘moderately hard’ test water. A possible explanation for this could be that soft water organisms have higher affinities for Mg²⁺ and/or Ca²⁺ uptake and thus experience a stronger competitive effect between Mg²⁺ and/or Ca²⁺ and Ni²⁺ at increasing hardness compared to hard water organisms. Snavely et al. (1991) reported the existence of three different types of Mg transporters in the prokaryote S. typhimurium. CorA is a low-affinity channel, of which the expression and functioning is not affected by the prevailing external Ca²⁺ or Mg²⁺ concentrations. MgtA and MgtB are high-affinity transport systems, with affinities for Mg²⁺ and
Ni$^{2+}$ being two orders of magnitude higher than those of CorA. The expression of MgtA and MgtB is increased at very low Ca$^{2+}$ and/or Mg$^{2+}$ concentrations. On the other hand, Mg$^{2+}$ influx via MgtA and MgtB is decreased at increasing Mg$^{2+}$ concentrations. Both Mg$^{2+}$ and Ca$^{2+}$ inhibited Ni$^{2+}$ uptake via each of these three Mg transport systems. Obviously, if similar low- and high-affinity transport systems for Mg$^{2+}$ (and Ni$^{2+}$) exist in cladocerans, this could explain why the relative decrease in acute Ni toxicity at increasing hardness was significantly higher for soft water cladocerans compared to hard water cladocerans. For Ca, it has already been demonstrated that crustaceans living in soft water have higher affinities for Ca$^{2+}$ uptake than crustaceans living in harder water (Neufeld and Cameron, 1993).

Since Ca$^{2+}$ is reported to be a less effective competitive inhibitor for Mg$^{2+}$ uptake via Mg$^{2+}$ transport systems, a higher affinity of more than one transport system. This could explain the higher stability constants of both chronic models. Physiological research with cladocerans is needed to confirm this hypothesis. It should be noticed that the possible existence of multiple binding sites for Ni$^{2+}$, Mg$^{2+}$ and/or Ca$^{2+}$ cannot be taken into account in a one-binding-site modeling approach. Fitting log $K_{CaBL}$ and log $K_{MgBL}$ assuming only one binding site would mean that we are estimating some kind of ‘average’ affinity of more than one transport system. This could explain the higher estimates of log $K_{CaBL}$ and log $K_{MgBL}$ for the soft water populations. Borgmann et al. (2005) have demonstrated the usefulness of considering two biotic ligand sites with different affinities for competing cations in explaining bioavailability of Cu to Hyalella azteca.

Our finding that increasing hardness does not affect the acute toxicity of Ni to soft and hard water organisms to a similar extent seems to contradict two other studies. First, Erickson et al. (1997) observed that acclimation hardness did not affect the acute toxicity of Cu to fathead minnow at two different hardness levels. However, fish were only acclimated to high hardness levels (45.8 and 210 mg CaCO$_3$/L), which is an important difference with our study. Furthermore, one could argue that long-term acclimation and/or adaptation of organisms to the hardness of their natural environment is more likely to result in differences than short-term acclimation in the laboratory. Second, De Schamphelaere et al. (2007) found no relation between the protective effect of Na on the acute toxicity of Cu to field cladocerans and the Na concentration or hardness of their waters of origin. However, too little data were available to infer general theories of how a different physico-chemical environment may result in differing effects of competing cations on the acute toxicity of Cu to cladocerans.

Chronically, the predictive capacity of model 3 was not significantly different from the predictive capacity of model 2. As opposed to what was observed for acute Ni toxicity, the difference between the soft and the hard water populations with regard to the effect of increasing hardness on chronic Ni toxicity was too small to require separate model parameters. This is not due to the fact that fewer populations were tested chronically. A model fitting exercise using only the acute toxicity data for populations that were also tested chronically revealed that the performance of model 3 was still significantly better than the performance of model 2 ($p < 0.05$). Perhaps the explanation for the difference between the acute and chronic toxicity observations lies in the two main differences between acute and chronic test conditions, i.e. longer exposure duration and presence of food in chronic exposures. First, it has been demonstrated that feeding can protect against metal-induced ionoregulatory disturbance (e.g., Baldisserotto et al., 2004). It has been suggested that this may result in shifts in protective effects of cations on metal toxicity (e.g., De Schamphelaere et al., 2004). Second, physiological acclimation is more likely to occur during longer exposures. Hogstrand et al. (1995) demonstrated that the affinity of Ca$^{2+}$ transport systems (shared by Zn$^{2+}$) in rainbow trout gills was modified during long-term exposure to Zn. Further research is needed to investigate how feeding and exposure duration influence the effect of increasing hardness on chronic Ni toxicity to soft and hard water organisms.

A second important observation with regard to chronic exposure to Ni is that overall, the protective effect of water hardness appears to be more important than in acute exposures. As a result, the log $K_{CaBL}$ and log $K_{MgBL}$ values of the chronic models are higher than those of the acute model (Table 4). This may be related to the high acute to chronic ratios (48 h EC50/chronic LC50 = 62.4 (4.35–304); 48 h EC50/chronic EC50 = 41.4 (4.10–144); note that both averages and maxima are substantially higher due to extremely high ratios for the chydorids tested). Since chronic Ni toxicity generally occurs at much lower Ni concentrations, high-affinity sites (as discussed above) might be more involved in chronic than in acute toxicity. This could explain the higher stability constants of both chronic models.

For risk assessment purposes, it was considered interesting to evaluate the predictive capacity of the Ni bioavailability models developed for the standard test organism D. magna (De Schamphelaere et al., 2006; Deleebeeck et al., 2007b) when used for predicting Ni toxicity to the field-collected cladocerans tested in this study (modeling exercise 4). Although the majority of the predictions that had to be made were for hardness levels below the lower hardness boundary of both models (i.e. 46 mg CaCO$_3$/L for the acute model; 42 mg CaCO$_3$/L for the chronic models), acute and chronic Ni toxicity to the investigated field-cladocerans were fairly well predicted (Table 4). However, it must be acknowledged that the D. magna-based models were still less accurate than the models specifically developed for the tested field-cladocerans. Further research would be needed to explain why log $K_{CaBL}$ and log $K_{MgBL}$ values for D. magna (Table 4, model 4) were lower than those derived for the field-cladocerans (Table 4, model 2 and 3). Perhaps this has to do with the fact that D. magna is maintained in the laboratory at a hardness of ~200 mg CaCO$_3$/L, which is substantially higher than the water hardness of the lakes of origin of the soft and hard water populations we investigated (i.e. 4.68–52.9 mg CaCO$_3$/L).

### 4.2. Intrinsic sensitivity

In order to compare the sensitivity of soft and hard water cladocerans to Ni, both soft and hard water populations were...
tested in a similar ‘moderately hard’ test medium (nominal hardness = 16.3 mg CaCO₃/L). Acutely or chronically, significant differences were observed between the E/LC50s for the soft and the hard water populations tested. However, for C. quadran­gula – the only species of which both a soft and a hard water population were tested – the acute sensitivity of the soft water population was significantly higher than the acute sen­sitivity of the hard water population. Chronically, no significant sensitivity difference was observed. The observed factor 2.8 difference in acute sensitivity between both populations of C. quadran­gula is within the range of inter-population sensitivity differences observed by Muyssen et al. (2005) for Zn (factor 1.2–4.6) using a range of field-collected populations of cladoceran species. Among field populations of D. magna, Barata et al. (1998) observed a difference in Cu sensitivity of up to factor 7. For S. vetulus, Bossuyt and Janssen (2005) even observed intra-population sensitivity differences of up to factor 2.9 for Cu. The lower acute Ni sensitivity of the soft water population of C. quadran­gula is hence not necessarily related to the low water hardness of its lake of origin.

Comparison of the E/LC50s for species belonging to differ­ent cladoceran families clearly reveals the markedly lower acute sensitivity to Ni of most Chydroridae (A. affinis, C. ovalis and P. truncata) compared to the other tested families (Daphniidae and Bosminidae). Overall, species of the Chydroridae that were tested by Bossuyt and Janssen (2005) and Muyssen et al. (2005) also exhibited a relatively low sensitivity to Cu and Zn in acute exposures. Why chy­drids are – at least acutely – less sensitive to metals than daphnids is still an unresolved research ques­tion that certainly deserves further attention. Comparison of the toxicity data for the hard water organisms in ‘hard’ test water (nominal hardness = 43.4 mg CaCO₃/L) with toxicity data for C. dubia (Keithly et al., 2004) in water with comparable hardness (50 mg CaCO₃/L) reveals that both acutely and chronically, all tested field species were less sensitive to Ni than the standard test organism C. dubia. D. magna exhibits intermediate sensitivity compared to the tested field species, as demonstrated by toxicity data at hardness levels of 42–50 mg CaCO₃/L (Chapman et al., 1980; De Schamphelaere et al., 2006; Deleebeeck et al., 2007b).

Since the sensitivity parameter of bioavailability models (in this study: E/LC50 Ni^{2+},0,i) has been previously suggested to represent the intrinsic sensitivity of a species/population (De Schamphelaere et al., 2007), one may expect that Ni sensitiv­ity differences as observed through comparison of effect data obtained in the ‘moderately hard’ test water would be reflected in the E/LC50 Ni^{2+},0,i values. This is clearly the case for individual species/populations. As a result, based on the E/LC50 Ni^{2+},0,i values for the retained models (acute: model 3; chronic: model 2), no significant sensitivity difference was revealed between the soft and the hard water populations either. However, here also, the implications of using a single-site bioavailability model should not be overlooked. A single-site model ignores the possi­ble existence of multiple binding sites with different affinities for both Ni^{2+} and competing cations (Mg^{2+} and Ca^{2+}), as discussed above. If water hardness determines the relative presence of low­and high-affinity binding sites for Ni^{2+} and Mg^{2+} and/or Ca^{2+}, the Ni sensitivity of an organism is expected to be dependent on the water hardness of its environment. If the soft and/or the hard water cladocerans used in this study experienced a sensitivity shift by exposing them to Ni in the ‘moderately hard’ test water, to which they were not acclimated before testing, the sensitivity parameter E/LC50 Ni^{2+},0,i would represent the average Ni sen­sitivity of population i at different hardness levels. This could have masked sensitivity differences between soft and hard water cladocerans.

5. Conclusion

It can be concluded from this study that cladocerans living in soft water (hardness < 10 mg CaCO₃/L) are not intrinsically more sensitive to Ni than cladocerans living in hard water (hard­ness > 25 mg CaCO₃/L). The protective effect of water hardness between 6.25 and 43.4 mg CaCO₃/L is significant and should therefore be incorporated in Ni bioavailability models and risk assessment exercises. Bioavailability models predicting acute Ni toxicity performed significantly better when the distinction was made between organisms in soft and in hard water. This is due to the fact that the relative decrease of acute Ni toxicity to soft water organisms in ‘moderately hard’ compared to ‘soft’ test water was significantly higher than for hard water organisms in ‘hard’ compared to ‘moderately hard’ test water. For chronic Ni toxicity, the difference between soft and hard water organisms was not important enough to require separate modeling. Fur­ther research is needed to mechanistically explain the protective effect of water hardness on Ni toxicity to soft and hard water organisms.

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