DEVELOPMENT OF MODELS FOR THE PREDICTION OF SHORT-TERM AND LONG-TERM TOXICITY TO *HYALELLA AZTECA* FROM SEPARATE EXPOSURES TO NICKEL AND CADMIUM

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I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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ABSTRACT

The free metal ion is the form most associated with toxicity to aquatic organisms. Mathematical models have been developed to predict acute toxicity of metals to aquatic organisms that incorporate information regarding the concentration of the metal in solution, its complexation by various organic and inorganic ligands as well as its competition with other cations for sites of uptake on organisms. If the site of uptake on an organism (the biotic ligand) is considered as one of many other anionic ligands in solution, principles of thermodynamics may be applied to predict uptake and toxicity. This study investigates whether principles of the Biotic Ligand Model (BLM), originally developed to predict acute metal toxicity to fish, may be applied to metal accumulation and toxicity in short-term and long-term exposures of *Hyalella azteca*, an epibenthic invertebrate. The two metals selected for study were cadmium and nickel, which are elevated in Ontario freshwaters in the vicinity of mining and smelting activities.

Models were developed to predict the uptake and toxicity of nickel and cadmium, by carrying out the following steps:

1) Short-term bioaccumulation tests were performed with adult *Hyalella* to identify which of the common cations present in freshwater compete with nickel or cadmium for sites of uptake on the organism and to determine whether steady state conditions had been achieved within the test exposure period.

2) To identify which of the cations investigated in step 1 influence short-term and long-term toxicity of nickel or cadmium, seven-day and 28-day toxicity tests were performed using young *Hyalella.*

3) Cations shown to influence uptake and toxicity of each metal were included in mathematical expressions to predict bioaccumulation and toxicity. The models were then evaluated by comparison to observed data.

In tests with nickel, toxicity corresponded to increasing whole-body nickel concentrations and with increasing concentrations of nickel in solution. The whole-body concentration of nickel associated with 50% mortality of the exposed organisms (LA50) was less variable than the corresponding water concentration (LC50), supporting BLM theory, which assumes toxicity is a function of accumulation above a critical threshold. Whole-body nickel concentrations reached steady state within 48 hours of exposure, providing supportive evidence that equilibrium existed between nickel in solution and nickel bound to the ligand.

In both short-term and long-term exposures, hydrogen and calcium were the only cations found to significantly influence nickel accumulation and toxicity, although some influence by magnesium was suggested in solutions of low calcium and alkalinity. Analysis of short-term (seven-day) bioaccumulation and long-term (28-day) toxicity data established three potential models to explain the observed accumulation and/or toxicity. One of the models included calcium and hydrogen as competitors to nickel (consistent with BLM theory), while the other two models incorporated the non-competitive effect of calcium on the biotic ligand in addition to, or instead of, the competitive action of calcium (not currently considered by BLM theory). Short-term accumulation observed in the tests with adults was best predicted by the competitive (BLM-type) model. However, long-term accumulation and toxicity were predicted equally well by both competitive and non-competitive models.

Tests with cadmium showed that cadmium toxicity also increased with increasing whole-body concentrations and with increasing cadmium in solution. Similarly, the LA50 for cadmium was much less variable than the range in LC50s from the same exposures. Calcium and hydrogen appeared to influence accumulation and toxicity in short-term exposures; however, only calcium clearly influenced cadmium uptake and toxicity in long-term exposures. Modeling of the accumulation data over time indicated that whole-body steady state for cadmium would be reached within approximately seven days in dechlorinated tap water and within 28 days in low hardness medium.

Models developed to predict accumulation and toxicity of cadmium were based on calcium's potential competitive and non-competitive action. Hydrogen was included in modeling of short-term bioaccumulation test data only since it did not improve the fit of the models of long-term data. In total, four modeling scenarios were established for the short-term tests (i.e., competitive and non-competitive action of calcium, with and without hydrogen) and two modeling scenarios were established for the long-term toxicity tests (competitive versus non-competitive action of calcium). Short-term cadmium models indicated that even if steady state conditions could not be confirmed within the test exposure period, the whole-body tissue concentration could still be used in the prediction of toxicity since a relationship could be established with observed toxicity at a given exposure period.

In short-term cadmium exposures the models including calcium as a competitor, rather than an influence on the ligand, predicted accumulation and toxicity best. Hydrogen did not appear to have a significant influence. Although both competitive and non-competitive calcium models were able to predict long-term toxicity within a factor of two of the observed for most tests, the relationship between predicted and observed LC50s was not linear. Instead, for a given calcium concentration, a wide range of LC50s was observed but was associated with a relatively constant predicted LC50 (based on the influence of calcium alone). The predicted LC50s fell roughly in three lateral bands, according to calcium concentrations of approximately 0.3, 1 and 3 mmol/L. The broad range of observed LC50s associated with a relatively constant predicted LC50 reflected variability in organism response in four-week exposures in similar media and/or may have suggested that other factors influencing cadmium toxicity were not accounted for in the models.

Dissolved organic carbon reduced bioaccumulation of total nickel and cadmium in short-term and long-term exposures and reduced toxicity of total cadmium in long-term exposures. The use of the MIINTEQA2 chemical speciation model over-estimated free metal concentrations of both cadmium and nickel in solutions of added DOC, in comparison to accumulation data. Modelling of free cadmium concentrations in various media using MINTEQA2 and Hydroqual's BLM program showed that both models worked

similarly for inorganic media; however binding of cadmium with dissolved organic material was estimated by MINTEQA2 to be much less than indicated by Hydroqual`s BLM Program.

Based on the results of the study, BLM theory may be applied in the prediction of short-term and long-term toxicity of nickel and cadmium to *Hyalella azteca*. Some evidence suggested that calcium changes the biotic ligand and the critical concentration associated with a given effect; however, the overall ratio of the two may remain relatively constant, thereby maintaining the validity of BLM theory (which assumes the two values are constant). Additional work is required to refine the models and to speciate nickel and cadmium in solutions of natural organic material.

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1.0 INTRODUCTION

Luoma (1983) pointed out that "our poor understanding of the processes controlling the biological availability of metals is a major impediment to defining, proving or predicting metals impacts in nature". Since that time, research has significantly increased our understanding of the mechanisms of aquatic metal toxicity. The free metal ion has been widely accepted as the principle form responsible for toxicity in aquatic systems, with the key factors affecting free metal ion toxicity being concentration in solution, competition with other cations and complexation by ligands (Pagenkopf, 1983).

Wood et al. (1997) recommended that as factors affecting metal toxicity are identified, they should be accounted for in the development of water quality criteria, noting that most criteria are based on total, rather than bioavailable, concentrations. Toxicity-modifying factors, including hardness and pH, have been considered in the development of some water quality criteria; however, their inclusion has been based on empirical, rather than mechanistic rationales (Nigoyi and Wood, 2004).

Mechanistic approaches to account for toxicity-modifying factors have more recently been used to predict acute toxicity of metals to aquatic organisms. Such predictive models have a potential for widespread application in risk assessment and in the development of water quality criteria, since they account for the mechanism of toxic action as well as site-specific conditions. A notable example includes the recent application by the United States Environmental Protection Agency (U.S. EPA) of a predictive model, the Biotic Ligand

Model (BLM), in updating its water quality criterion for copper (U.S. EPA, 2007). As noted by Paquin et al. (2002), application of BLM theory is expected to reduce the requirement to apply the precautionary principle while identifying times when insufficient precaution has been applied.

Most BLMs predict acute effects, although chronic effects data are favoured in the assessment of risk and in the development of water quality criteria. Chronic models have been developed for a limited number of metals and aquatic species but have yet to be validated and/or fully implemented as routine tools for evaluating site-specific metal accumulation or toxicity. More work is required to establish predictive tools based on chronic toxicity for a greater number of species and metals. This thesis describes research to evaluate whether the BLM theory may be used to describe long-term metal accumulation and toxicity to the invertebrate *Hyalella azteca* and to develop both short-term and long-term models for nickel and cadmium, two metals of concern in Ontario waters.

2.0 BACKGROUND

2.1 *HYALELLA AZTECA*

Hyalella azteca is an ideal species for the testing of metals and development of a predictive bioaccumulation and toxicity model because it:

- is native to North America, is widely distributed, and represents a major food source for fish in fresh water environments;
- can serve as an indicator of both water and sediment contamination due to its distribution near the sediment-water interface;
- has been found to be one of the most sensitive species to metal toxicity in chronic exposures (Mebane, 2006);
- is reasonably easy to culture in the laboratory; and
- is tolerant of a wide range of environmental conditions (e.g., temperature, hardness, salinity).

2.2 NICKEL IN THE AQUATIC ENVIRONMENT

2.2.1 Occurrence and Speciation

Nickel (Ni) is a common metal in freshwaters. It occurs naturally at concentrations between 1 and 10 µg/L but may reach as high as 1000 µg/L in areas impacted by activities such as mining or smelting (Eisler, 1998). The free nickel ion (Ni^{2+}) represents almost all Ni in

solution at circum-neutral pH, but is replaced by nickel carbonate ($NiCO_3$) as the major form when pH is raised to 9 (Water Environment Research Foundation (WERF), 2002).

2.2.2 Factors Influencing Bioaccumulation and Toxicity

Ni^{2+} has been shown to be predictive of Ni toxicity to *Hyalella azteca*, suggesting that this is the principle form causing toxicity to aquatic organisms (Doig and Liber, 2006). Various studies have shown that water quality characteristics, such as hardness and pH, influence Ni's bioavailability and toxicity. Meyer et al. (1999) found that the acute toxicity of total nickel to fathead minnows decreased 10-fold as calcium increased 10-fold. This finding was supported Hoang et al. (2004), who also found that the acute toxicity of Ni to fathead minnows decreased 10-fold when hardness was increased 7-fold. Hoang et al. (2004) also found that toxicity increased with increasing alkalinity, with a corresponding increase in pH and formation of $NiCO_3$. Keithly et al. (2004) showed that hardness mitigated Ni's effects on survival and reproduction of *Ceriodaphnia dubia* in acute exposures, but that the mitigation was not as significant in chronic exposures. Seven-day exposures of *Hyalella azteca* to Ni also showed a reduction in toxicity when hardness and alkalinity were increased (Borgmann et al., 2005a). Earlier work by Schubauer-Berigan et al. (1993) investigated Ni toxicity to *Ceriodaphnia dubia* and *Hyalella azteca* in acute exposures. They found that Ni toxicity increased as pH increased, with 96-hr LC50s for *Hyalella* of 2,000 and 890 µg/L and 48-hr LC50s for *Ceriodaphnia* of >200 and 13 µg/L at pHs approximating 6.5 and 8.5, respectively.

The addition of dissolved organic material, measured as dissolved organic carbon (DOC), has a variable effect on Ni toxicity and depends on a number of other factors. In studies using fathead minnows, the addition of dissolved organic material to test exposures did not result in a consistent decrease in acute toxicity, and depended on the age of the fish and the ion composition of the water (Hoang et al., 2004). Toxicity was reduced up to 1.8 fold in exposures within the range of 0.5 to 10 mg/L as DOC (Hoang et al., 2004). Doig and Liber (2006) found that concentrations of Ni causing acute lethality to *Hyalella azteca* were higher than those that could be complexed by DOC. However, they proposed that lower Ni concentrations associated with sublethal effects may be influenced by DOC if within its complexing capacity. Mandal et al. (2002) found that in waters of high DOC and high hardness, Ni availability may be increased, due to DOC binding with calcium and magnesium ions, which are in large excess compared to Ni ions. They cautioned that Ni would be released from DOC complexes in waters of high DOC and hardness, resulting in higher concentrations of the toxic form; however, as pointed out by Penttinen et al. (1998), the competitive advantage that large concentrations of hardness ions have over metals for binding sites on dissolved organic material would also likely be the case for binding sites on the organism.

2.2.3 Mode of Action

Pane et al. (2003a) showed that the mode of action for acute Ni toxicity to *Daphnia magna* was from antagonism of magnesium (Mg) uptake, whereas Ni acted as a respiratory toxicant in chronic exposures. Other studies using rainbow trout (*Oncorhynchus mykiss*) showed that Ni is a respiratory toxicant, decreasing the diffusing capacity of the gill and its

ability to function in gas exchange (Pane et al., 2003b). Deleebeeck et al. (2007) noted findings from several studies that Ni and Mg compete for uptake at Mg transport channels, whereas Ca more likely influences membrane permeability. The stabilizing effect on the membrane may protect against Ni-induced Mg loss from the haemolymph.

2.2.4 Water Quality Criteria

Ontario's provincial water quality objective (PWQO) for Ni (for the protection of aquatic life) is 25 µg/L (Ontario Ministry of the Environment (MOE), 1994), which was developed in the 1970s based on chronic effects on *Daphnia magna* (MOE, 1979). PWQOs are intended to protect "all forms of aquatic life and all aspects of the aquatic life cycles during indefinite exposure to the water" (MOE, 1994). They are conservative enough to accommodate different species sensitivities as well as the variation in water characteristics across the province during different seasons. The current Canadian Water Quality Guideline (CWQG), published by the Canadian Council of Ministers of the Environment (CCME) (CCME, 2006), accounts for the hardness of the water body to which it applies. The CWQG for Ni matches the PWQO in waters of hardness below 60 mg/L (as $CaCO_3$) and increases with hardness to a maximum of 150 µg/L at hardness above 180 mg/L as $CaCO_3$ (CCME, 2006).

2.3 CADMIUM IN THE AQUATIC ENVIRONMENT

2.3.1 Occurrence and Speciation

Background concentrations of cadmium (Cd) in North American waters are typically less than 0.1 µg/L, but elevated concentrations may occur due to releases from mining and mineral processing as well as fossil fuel combustion (Mebane, 2006). However, in his review of literature regarding Cd in the environment, Mebane (2006) noted that in North America, concentrations seldom exceed 15 µg/L, even in areas where Cd would be considered "elevated". The free form of cadmium (Cd^{2+}) is considered the dominant species in well-oxygenated freshwater with low DOC (U.S. EPA, 2001).

2.3.2 Factors Influencing Bioaccumulation and Toxicity

When reviewing metal toxicity to fish, Playle (1998) proposed that Cd toxicity was due to the free form (Cd^{2+}) rather than weak complexes such as $CdCl_2$. Mebane (2006) reviewed the toxicity of Cd to aquatic biota for derivation and validation of low-effect criteria values. He noted that both acute and chronic toxicities of Cd were dependent on hardness, especially calcium (Ca), and noted that pH was also important, but less so than Ca in mitigating toxicity. In field studies, Stephenson and Mackie (1988) found that Cd accumulation by *Hyalella azteca* was positively correlated to aqueous Cd concentrations and negatively correlated to the combined effects of hardness and/or alkalinity as well as, but to a lesser degree, DOC. Their subsequent laboratory experiments revealed that the most significant mitigator of toxicity was Ca, with no significant mitigation by alkalinity or its covariant, pH (Stephenson and Mackie, 1989). However, their observations were based

on exposures to Cd below pH 7. In contrast, Schubauer-Berigan et al. (1993) found that acute Cd toxicity to *Hyalella azteca* increased with increasing pH, with 96-hr LC50s of 230 and 5 µg/L at pHs of 6.5 and 8.5, respectively. Tests conducted by Jackson et al. (2000) showed that Mg mitigated Cd acute toxicity to *Hyalella* but to a much lesser extent than Ca. Borgmann et al. (1991) found that hardness had only a minor effect on Cd bioaccumulation and toxicity to *Hyalella azteca* after 4 weeks of exposure. These results were similar to those of Winner and Gauss (1986), who found that bioaccumulation of Cd by *Daphnia magna* in chronic exposures was not reduced in high hardness water exposures, although a reduction was observed in the corresponding toxicity. A study by Penttinen et al. (1998) of the acute toxicity of Cd to *Daphnia magna,* suggested that high concentrations of Ca relative to Cd may effectively outcompete Cd for binding sites on DOC, thereby increasing its bioavailability. However, the Ca may also prevent Cd binding to the organism.

2.3.3 Mode of Action

Cd inhibits Ca uptake across the gills of fish, leading to hypocalcemia, since it is taken up through high-affinity Ca channels (Richards and Playle, 1999). Various studies have explained the protective effect of Ca as either causing a change in gill permeability or competing with Cd for gill binding sites (Hollis et al., 2000).

2.3.4 Water Quality Criteria

The current PWQO for Cd is 0.2 µg/L, developed in the 1970s based on effects on trout and invertebrates (MOE, 1979). However, an interim PWQO has been published, based on more recent information, that accounts for differences in water hardness. Up to a hardness

of 100 mg/L as $CaCO_3$, the interim objective is 0.1 µg/L; above 100 mg/L it is 0.5 µg/L (MOE, 1994). According to MOE guidance, the interim objective is applied when additional protection is required. The CCME interim CWQG is established based on hardness according to the formula: guideline (µg/L) = $10^{\{(0.86*\text{Log}(\text{Hardness (mg/L)}) - 3.2\}}$(CCME, 2006). At a hardness of 100 mg/L, the interim CWQG is 0.03 µg/L.

2.4 MODELS

2.4.1 Free Ion Activity Model

According to Paquin et al. (2002), Morel (1983) was the first to formally conceptualize a model linking the free metal ion with toxicity to aquatic organisms. Morel's Free Ion Activity Model (FIAM) proposed that free metals (aquo-complexes) cause toxicity by exchanging water molecules for organic ligands on a biological membrane in the same manner as ligands in solution. Equilibrium is assumed to be rapidly established between the metal in solution and the metal-biological membrane complex but is qualified as "pseudo-equilibrium" in light of constant uptake of the metal and growth by the organism. The model also assumes that the biological response is proportional to the degree of binding of the metal to the membrane (Morel, 1983). Morel (1983) also proposed that metals do not compete with each other at the biological ligand as long as the sites are in excess and, when aqueous metal is in excess, that binding at the biological ligand does not affect the metal speciation in solution. Although Morel recognized the competitive influence of other cations, he did not include it in the FIAM since much of the early work was done in marine solutions or in defined media, in which concentrations of cations were fairly constant

(Campbell, 1995). The potential toxicity of metal complexes was considered, but accounted for by the hypothesis that the complex would be broken at the plasma membrane where the free metal would favour binding to the biological ligand. This would result in formation of the same metal-ligand complex as if the free metal ion had interacted with the biological ligand directly. Under the FIAM, metals were not believed to cause changes to the biological surface itself, only to its capacity for complexation (Campbell, 1995). Morel (1983) recognized the importance of dissolved organic material in metal binding, thereby mitigating metal effects on aquatic organisms, but noted challenges associated with modelling such reactions due to the heterogeneity of various types of dissolved organic material as well as the potential range of affinities within the same type.

2.4.2 Gill Surface Interaction Model

At the same time that the FIAM was introduced, Pagenkopf (1983) proposed a gill surface interaction model (GSIM) for predicting acute toxicity of metals to fish. As with the FIAM, the GSIM assumed that the biological ligand was defined by a finite number of sites and that reaction of the metal with the biological ligand was rapid. The GSIM differed from the FIAM by specifically addressing fish toxicity, introducing the fish gill as the site of toxic action, and assuming that hardness ions and protons were important competitors with metals at the gill surface. However, the application of the model was limited largely to laboratory toxicity tests since competition by hydrogen (H^+) and complexation by dissolved organic material were intentionally excluded. Pagenkopf expected the amount of gill ligand bound by H^+ or the trace metal of interest to be negligible at pHs above 6 (i.e., negligible in

most laboratory dilution waters) and recognized that laboratory waters used for toxicity tests were low in DOC (Pagenkopf, 1983).

Further concepts regarding the influence of competition on metal toxicity were introduced by Borgmann (1983), who concluded that hardness ions and hydrogen reduced toxicity of the free metal ion through competition for uptake at metal complexing ligands on an animal's surface. He represented the binding of copper and hydrogen to a ligand on an organism through equilibrium partitioning and proposed that toxicity occurs at a constant complexation of copper to the ligand. By combining the equilibrium expressions for copper and hydrogen, the LC50 for free copper could be represented as a linear function of pH, where the free copper LC50 was "y" and pH was "x".

2.4.3 Biotic Ligand Model

Studies investigating metal-ligand interactions, most notably by Playle and colleagues, confirmed the importance of metal complexation and competition to metal accumulation (Paquin et al., 2002). Playle (1998) noted that the interaction of a metal with the fish gill could be described through the use of equilibrium constants in the same way that metal interactions with dissolved ligands were described in readily-available chemical speciation models. Based on the assumption that a given toxic response would occur at a constant tissue concentration of a metal, accumulation and toxicity could be predicted if the concentration of binding sites on the gill and the binding affinities of the gill to various cations in solution were known. By adding chelating agents with known binding affinities to solution, the relative binding affinity of the gill for a metal could be determined based on

the ability of the chelating agent to keep the metal off the gill (Playle, 1998). Similarly, the binding affinity of the gill for competing cations could be established by the concentration of the cations in solution required to keep the metal off the gill. In considering other metal forms associated with toxicity, Playle (1998) noted that any weak metal-ligand complex could be broken if the affinity of the gill for the metal were stronger, and, therefore, some ligands would provide weaker than expected protection from metal toxicity. However, he also acknowledged the existence of some ligands that facilitated uptake at the gill and thereby increased toxicity (e.g., xanthates).

Campbell (1995) noted that the FIAM was insufficient to predict metal toxicity and that competition of the free ion at the binding site with various cations and H^+ also needed to be considered. Meyer et al. (1999) reported on what they believed to be the first published evidence that the metal bound to the biological ligand predicts toxicity rather than the free metal ion. Experiments were conducted using fathead minnows exposed to Ni or copper (Cu). For 96-hr exposures to Ni in waters of increasing hardness, the LA50 (concentration of accumulated metal resulting in mortality to 50% of the test organisms) increased two-fold whereas the LC50 (concentration of metal in solution resulting in mortality to 50% of the test organisms) for the same experiments increased ten-fold. Similarly, experiments with Cu showed up to a four-fold increase in the LC50 with no significant increase in the LA50.

DiToro et al. (2001) brought together the theories of Pagenkopf and Morel and the advances by researchers such as Playle and colleagues to create the Biotic Ligand Model

(BLM) to predict accumulation and toxicity of metals in waters of various physical and chemical characteristics. Existing geo-chemical speciation models were incorporated into the BLM to determine the amount of dissolved metal that would be complexed by inorganic and organic ligands in solution. The remaining free metal ion could then be modeled to predict accumulation and toxicity based on its competition with other cations.

Inputs to the BLM include cations and protons, such as Ca^{2+}, Mg^{2+}, sodium (Na^{+}), potassium (K^{+}) and H^{+}, as well as the major anions, such as sulphate (SO_4^{2-}), chloride (Cl^{-}), and carbonate (CO_3^{2-}). Dissolved organic material is also considered by the BLM, entered as a concentration of DOC in mg/L with a designated percentage as humic acid (Di Toro et al., 2001). As observed by Morel (1983), and more recently by researchers such as Playle et al. (1993), Playle (1998), Richards et al. (2001), Janssen et al. (2003), deSchamphelaere and Janssen (2004) natural organic material is challenging due to its heterogenous nature (many different types and sources) as well as the continuum of binding affinities within one type. Although the model does not consider the various types or binding affinities of DOC, various authors have proposed that the concentration, and not the source, would be more important, especially at concentrations required for acute lethality (Playle, 1998; Doig and Liber, 2006).

The biotic ligand was intended to represent a site of toxic action on any organism; however, the first BLM was developed for acute copper toxicity to fish, using the fish gill as the biotic ligand, since information was already available regarding the binding site density and binding affinities to various cations and metals (DiToro et al., 2001). It was assumed that mortality would occur when binding and resultant accumulation of copper on the gill

exceeded the LA50 (DiToro et al., 2001), in contrast to the LC50, which changed according to the water characteristics. The model also assumed that equilibrium was reached between the metal in solution and the metal bound to the gill. For organisms without a known site of toxic action, the whole-body accumulation could be used as long as accumulation in the whole organism reflected accumulation at the site of toxic action.

Mathematically, the BLM may be described by equilibrium partitioning of the metal in solution to that bound to the biotic ligand in the same way as for metal-ligand binding in solution (equation 1):

$$K_M = \frac{[ML]}{[M][L]} \qquad \text{(equation 1)}$$

where:

K_M = conditional equilibrium constant for metal binding to the biotic ligand (L/μmol)

[M] = concentration of unbound free metal ion in solution (μmol/L)

[ML] = concentration of metal-ligand complex (μmol/g tissue)

[L] = concentration of unbound ligand (μmol/g tissue)

This equation is combined with a mass balance of the total biotic ligand available for binding, represented by the sum of unbound and bound biotic ligand (equation 2):

$$[L_T] = [L] + [ML] \qquad \text{(equation 2)}$$

where:

$[L_T]$ = maximum concentration of binding sites on the ligand available for binding (μmol/g tissue)

The mathematical framework for the BLM, described according to a Langmuir adsorption model (Newman, 1998), thus becomes:

$$[ML] = \frac{[M]*[L_T]*K_M}{1+ K_M*[M]} \qquad \text{(equation 3)}$$

The above equation is a saturation curve, by which the amount of metal adsorbed to the ligand increases with increasing free metal concentration in solution until a maximum amount of binding is reached. Rearrangement of the equation to solve for [M]/[ML] yields a straight line with a slope of $1/L_T$ and an intercept of $1/ (K_M*L_T)$ from which K_M and L_T may be estimated:

$$\frac{[M]}{[ML]} = \frac{1}{[L_T]}*[M] + \frac{1}{K_M*[L_T]} \qquad \text{(equation 4)}$$

Equation 3 describes accumulation in the absence of competing cations. Competing cations, such as Ca^{2+} and H^+, can be accounted for in the model by including the equations:

$$K_{Ca} = \frac{[CaL]}{[Ca][L]}, \; K_H = \frac{[HL]}{[H][L]} \text{ and } [L_T] = [L] + [ML] + [CaL] + [HL]$$

yielding:

$$[ML] = \frac{[M]*[L_T]*K_M}{1+ K_M*[M] + K_{Ca}*[Ca] + K_H*[H]}$$

where:

[CaL] = concentration of calcium-ligand complex (μmol/g)

[Ca] = concentration of unbound calcium ion in solution (μmol/L)

K_{Ca} = equilibrium constant for calcium binding to the biotic ligand (L/μmol)

[HL] = concentration of hydrogen-ligand complex (μmol/g)

[H] = concentration of unbound hydrogen in solution (μmol/L)

K_H = equilibrium constant for hydrogen binding to the biotic ligand (L/μmol)

The BLM as described above assumes one binding site or ligand for a metal. However, Borgmann et al (2005b) found that a two-site model better described short-term toxicity of copper to *Hyalella azteca.* Inclusion of a second ligand requires consideration of competing ions and their competitive influence at both binding sites, increasing the complexity of the model.

De Schamphelaere and Janssen (2002) developed an acute Cu biotic ligand model for *Daphnia magna* based on the one-site model. Due to the small size of the organisms, the whole body concentration of a metal was used as a surrogate for the concentration at the biotic ligand. However, De Schamphelaere and Janssen (2002) proposed an approach for predicting accumulation that would not require determination of the biotic ligand concentration (ML) of a metal. Instead they estimated binding constants for the various competing cations through toxicity data alone.

De Schamphelare and Janssen (2004) used the same approach to develop a model for the prediction of chronic copper toxicity to *Daphnia.* In this study, they noted challenges

associated with modeling accumulation and toxicity, such as differences in mode of action between acute and chronic exposures, accounting for sublethal endpoints, such as reproduction, as well as the apparent toxicity of some Cu-DOC complexes. Similarly, Heijerick et al. (2005) found that Zn toxicity to *Daphnia magna* was not influenced by pH in acute exposures but needed to be considered in chronic exposures. In considering the differences in modes of action between acute and chronic exposures, Borgmann et al. (2005b) noted that in chronic exposures, the estimated K_M would likely reflect not only the external binding of the metal to the organism (e.g., to the gills) but also the transport of the metal through the organism, its binding to internal sites as well as its excretion.

2.4.4 Research Needs

As described above, knowledge regarding the mechanisms of metal complexation and competition has allowed the development of short-term biotic ligand-type models for several metals and species of organism. The early models were developed based on exposures of fish to copper, with prediction of copper toxicity to invertebrates based on an assumed similar mechanism of action. More recent research has provided the mechanistic basis for modeling metal toxicity to invertebrates and has confirmed the suitability of BLM theory for predicting long-term toxicity; however, gaps exist in the metals, exposure duration and/or species selected, which need to be filled before a particular model can be applied with confidence to real-world metal bioavailability and toxicity questions. No model exists to describe long-term toxicity of cadmium or nickel to *Hyalella azteca* and limited data exist regarding the toxicity of nickel to aquatic organisms in general.

3.0 STUDY OVERVIEW

To evaluate the ability of BLM theory to describe uptake and toxicity of Ni and Cd to *Hyalella azteca* and to develop models for the prediction of both short-term and long-term toxicity, the following steps were undertaken. Additional details are provided in Sections 4.0 (Nickel - Methods and Materials) and 7.0 (Cadmium - Methods and Materials).

1) Short-term bioaccumulation tests were completed with adult *Hyalella* to identify which of the common cations present in freshwater compete with Ni or Cd for sites of uptake on the organism and to confirm that steady-state conditions had been achieved within the test exposure period (an assumption of BLM theory).

2) To identify which of the cations investigated in step 1 influence short-term and long-term toxicity of Ni or Cd, seven-day and 28-day toxicity tests were performed using young *Hyalella.* Additional tests were performed with Ni to determine whether pre-acclimation to the exposure conditions (i.e., hardness, alkalinity, pH) would influence the sensitivity of *Hyalella*.

3) The cations shown to influence uptake and toxicity of each metal in both short-term and long-term exposures were included in mathematical expressions to describe bioaccumulation and toxicity. The models were then evaluated by comparison to observed data.

4.0 NICKEL - METHODS AND MATERIALS

4.1 CULTURING

Organisms were originally obtained from Beak International Incorporated (now Ecometrix, Mississauga, ON), and maintained in cultures at the Ontario Ministry of the Environment (MOE) laboratory according to the MOE standard operating procedure HA1: *Hyalella azteca* Culturing (MOE, 2002), based on procedures described by Borgmann et al. (1989) and advice from Dr. Borgmann's laboratory staff. Adjustments to the culturing practice included aging the culture water and cleaning the vessels only periodically to allow growth of organic material and/or micro-organisms, since this was found to support better survival and reproduction than pristine water and culture ware.

Breeding jars were initiated every one to two weeks using mating pairs obtained from mass cultures or "nursery" jars (see below). Approximately ten pairs were placed into 2L plastic containers, containing approximately 1.5L of dechlorinated tap water (characteristics described below) and 2 mg of TetraMin® flakes, ground to a course powder using a mortar and pestle. Cotton gauze (approximately 2 x 4 cm piece, presoaked in culture water) was added to each vessel to provide a substrate for the organisms. The dechlorinated tap water was delivered through the City of Toronto municipal system (from Lake Ontario) and passed through two large carbon filters at the MOE's laboratory, which continuously operated to serve the needs of a rainbow trout holding facility. Before use in the *Hyallela* cultures, the water was first aerated for a minimum of four days in a 200L holding tank.

Cultures were fed two to three times per week and were held at 23 to 25°C under cool white fluorescent lights. Light intensity and photoperiod were approximately 1000 lux and 16 hours light to 8 hours dark, respectively. Culture solutions were replaced weekly when young were counted and removed to nursery containers. Nursery organisms were either used within 10 days in long-term toxicity tests or maintained for several weeks until large enough for use in short-term bioaccumulation tests or for initiating new breeding jars.

Adults and young were separated through gentle pouring of the culture water through two different-sized screens, stacked one on top of the other. Sizes were selected to trap adults on the top screen and young on the bottom screen (approximately 750 µm and 250 µm mesh sizes, respectively). During 2004 and 2005, young were removed from the breeding jars a second time each week to reduce the age range used in long-term tests, although solutions were only renewed weekly. Young born in breeding jars in which there was significant adult mortality (i.e., >20%) in the previous seven days were not transferred to nurseries, but rather were transferred to the mass culture or discarded.

4.2 STEP 1A: SHORT-TERM BIOACCUMULATION TESTS

Seven-day bioaccumulation tests were performed to determine which common freshwater cations influenced the uptake of Ni. The following cations were investigated: Ca^{2+}, Mg^{2+}, Na^{+}, K^{+} and H^{+}. A bioaccumulation test, varying Ni, was also conducted. All subsequent references to cations will be without charge for simplicity.

4.2.1 Media Preparation

In order to observe the effects of a single cation, artifical media were prepared, varying the concentration of one cation at a time. Tests investigating all cations except hydrogen used a mixture of modified standard artificial medium (MSAM), based on Borgmann (1996), dechlorinated tap water and/or deionized water as the test media. MSAM differed from the standard medium by having more sodium bromide (NaBr) present, and was mixed with 20 to 30% dechlorinated tap water. Both the modification of the artificial medium and the addition of dechlorinated tap water were precautions against impairment caused by drastic changes to the ionic composition of the medium, and in response to early problems with organism survival. The addition of dechlorinated tap water to reconstituted water is common practice in some private laboratories in Ontario since it has been shown to enhance survival and reproduction of invertebrates relative to that achieved in reconstituted water alone. Stanley et al. (2005) reported poor survival in hard reconstituted water (hardness of ~160 mg/L), prepared according to the United States Environmental Protection Agency method (EPA /821/R-02/013), when used in long-term tests and found better success using dechlorinated tap water with cotton gauze as a substrate. Borgmann (1996) found that *Hyalella* cannot tolerate high calcium chloride concentrations in the absence of bromide and that 1 to 1000 μM bromide supports similar survival. The bromide concentration selected for the MSAM was within this range at 50 μM but was increased above the standard artificial medium concentration of 10 μM as a preventive measure against potential impairment in exposures of elevated calcium, which were planned for the bioaccumulation and toxicity tests.

Media were prepared in volumes of five to 20 litres, depending on the requirement for the test(s), using acid-washed glass or plastic (polypropylene) beakers or plastic (high density polyethylene) carboys as containers. To prepare 20 litres of MSAM, 14 litres of deionized water were filled into a carboy. Then, five salts were added according to Table 4.1. If the influence of one or more cation was under investigation, the amount of corresponding salt was either increased or reduced accordingly. Salts were first dissolved in an aliquot of the deioinized water before being added to the carboy. Once the salts were added, enough dechlorinated tap water (DC) was added to make up approximately 30% of the final volume. The DC used in media preparation was the same as that used in culturing (aerated for a minimum of four days before use). Low hardness medium (LHM) was prepared in the same manner as MSAM, except that no salts were added to the solution.

Table 4.1: Addition of Salts to Prepare MSAM

Salt	mmol/L
$CaCl_2.2H_2O$	1
$NaHCO_3$	1
NaBr	0.05
$MgSO_4.7H_2O$	0.25
KCl	0.05

Tests investigating the effects of H^+ were performed in 100% DC since early trials with the MSAM and LHM showed poor pH stability. Media were adjusted to the desired pH by addition of nitric acid (HNO_3) or sodium hydroxide (NaOH). Once pH-adjusted, DC was covered and left for at least six hours or overnight before use in tests.

Media were also prepared using salts other than those listed in Table 4.1. In one test, Ca was added as calcium sulphate ($CaSO_4$), rather than calcium chloride ($CaCl_2$) and in another, Na was varied using sodium nitrate ($NaNO_3$) rather than NaOH to isolate effects of Na from alkalinity. All media were aerated at least 24 hours before use in tests. However, pH-adjusted DC was not aerated after the addition of acid or base. Table 4.2 provides a list of the tests performed and the characteristics of the test media.

4.2.2 Test Set-up and Maintenance

For the Ni, Ca, Mg, Na and K tests, the cation of interest was omitted from the preparation of the test medium. Then, a subsample of the medium was collected, to which enough cation was added to make up the highest test concentration. Using a graduated cylinder, the high-cation subsample was serially diluted with the low cation medium to make up a concentration range of the cation of interest. All test solutions were then spiked with the same concentration of Ni (except in the test varying Ni). Subsamples of the solutions containing the highest and lowest cation concentration, without Ni, served as controls.

To investigate the effects of H^+ on the uptake of Ni, a graduated pH test was performed using dechlorinated tap water, spiked with HNO_3 or NaOH to lower or raise the pH, respectively. Solutions were spiked, then covered and left at least six hours or over night. Just before testing, the solutions were readjusted to the desired pH, spiked with a single concentration of Ni, and then divided into replicate containers. A small headspace was left at the top of all vessels, which were covered with plastic film.

Table 4.2: List of Short-term Bioaccumulation Tests and Composition of Test Media

Test	Base		Medium Characteristics (mg/L)									
#	Medium	Adjustment	pH*	Cl	Ca	Mg	Na	K	SO_4	ALK	DOC	NO_3
1	MSAM	Vary Ni	7.9	62	40	7.3	22	1.9	27	66	0.4	
2	LHM	Vary Ni	7.9	5.6	14	2.1	2.8	0.4	7.5	38	0.2	
3	LHM	high Ca, vary Ni	7.9	230	140	3.1	5.6	0.6	11	36	0.2	
4	MSAM	vary Ca	7.6	16	v	8.1	25	2.3	v	75	0.4	
5	DC	vary H	v	33	35	9.0	17	1.8	35	84	0.2	
6	MSAM	vary Ca	7.9	v	v	7.2	23	2.0	26	73	0.2	
7	MSAM	vary K	7.5	v	42	7.7	23	v	31	68	0.2	
8	MSAM	vary Mg	7.9	63	45	v	24	2.0	v	83	0.2	
9	MSAM	low Ca, vary Mg	7.7	14	17	v	24	2.0	v	76	0.2	
10	MSAM	vary Mg	7.7	63	43	v	24	2.0	v	76	0.2	
11	LHM	vary Mg	7.6	7.9	11	v	3.9	0.5	v	25	0.2	
12	MSAM	vary Mg	7.8	62	39	v	22	2.0	v	63	0.2	
13	LHM	vary Mg	7.7	8.4	10	v	4.1	0.5	v	25	0.2	
14	MSAM	vary Mg	7.9	63	34	v	21	2.0	v	61	0.2	
15	MSAM	vary Na	7.2	63	41	7.4	v	2.0	28	66	0.2	v
16	MSAM	vary Na	7.5	59	39	7.3	v	2.0	29	27	0.2	v
17	LHM	vary Ni	7.7	11	17	3.1	6.1	0.7	11	41	0.2	

v= varied concentrations; ALK = alkalinity; *pH represents average exposure conditions during testing
MSAM = modified standard artificial medium; LHM = low hardness medium; DC = dechlorinated tap water

The above seven-day tests were conducted using two to four replicates, with five to ten organisms per replicate, depending on organism availability. Organisms were either transferred directly to randomly selected test vessels or were first transferred to small plastic cups containing test medium and then transferred at random to test vessels. Tests vessels were either 400 mL or 500 mL glass containers, containing 300 mL or 400 mL solution, respectively. To each vessel, a small piece of gauze (1 x 2 cm^2) was added along with 2 mg of ground up TetraMin® flakes. Tests were performed in a water bath maintained at 23 to 25°C by an immersion heater/circulator. Test temperature was monitored daily and organisms were fed again on day 4 of testing. For the H^+ tests only, pH was monitored daily and adjusted to within 0.2 from the desired pH using dilute acid (nitric acid) or base (sodium hydroxide). Table 4.3 provides a summary of the bioaccumulation test design.

4.2.3 Test Termination and Organism Collection

On the seventh day of testing, temperature, pH and mortality were recorded. Surviving organisms from each replicate were removed from the test vessels and placed in a shallow dish containing deionized water charged (saturated) with carbon dioxide (CO_2). Organisms were removed from this dish and rinsed in an identical container of CO_2 –charged water before being transferred to labeled weighing paper (one paper per replicate). No depuration period was included in the test procedure since minimal gut uptake of Ni was anticipated in water-only exposures.

Test organisms and weighing paper were then placed in a 60°C drying oven and left for at least six hours before being removed to a dessicator. Once cooled, each replicate was weighed in grams to four or five decimal places using a Mettler balance.

4.2.4 Acid Digestion and Measurement of Ni Bioaccumulation

After weighing, test organisms were transferred to 2-mL acid-washed polypropylene cryovials for acid digestion. The digestion method was based on that described by Borgmann et al. (1991) as adapted from Stephenson and Mackie (1988). HNO_3 (100 µL of 70% solution for trace metal analysis) was added to each vial. The vials were then capped and left for a minimum of seven days, at which time reagent-grade hydrogen peroxide (H_2O_2) (100 µL of 30% solution) was added. The vials were recapped and left overnight before a final addition of deionized water (1800 µL). A blank, containing HNO_3, H_2O_2 and deionized water, was included with each batch.

Table 4.3: Short-term Bioaccumulation Test Design and Conditions

Short-term (seven-day) *Hyalella azteca* Bioaccumulation Test	
Medium:	Varied (see Table 4.2)
Temperature:	23-25°C
Organism Age:	6 – 12 weeks
Organisms/vessel:	5 to 10
Test vessels:	400 mL or 500 mL glass jars
Test solution:	300 mL or 400 mL
Replicates:	2 to 4
Feeding:	2 mg TetraMin® flakes on Day 0 and 4
Aeration:	None
Gauze:	Approximately 1 cm x 2 cm
Lighting:	16 hours light / 8 hours dark
Lighting Intensity:	~1000 lux
Monitoring:	pH Day 0 and 7, temperature daily, mortality Day 7
Endpoint:	Survival and accumulation of Ni

Analysis of Ni in the digested tissue solutions was carried out at the National Water Research Institute (NWRI) in Burlington, Ontario using a Zeeman® Graphite Furnace Atomic Absorption (GFAA) Spectrophotometer. A blank solution (acidified deionized water) and standard solutions of Ni were first prepared to develop a standard curve for each run of samples. Every ten samples, the blank and a single standard were remeasured. Additionally, a method blank, containing the same amount of acid, hydrogen peroxide and deionized water as the tissue samples was prepared along with each set of tissue samples to account for any Ni added from the experimental method. Samples were diluted with deionized water to bring the Ni concentration in the range of the program calibration.

4.2.5 Chemical Analysis and Chemical Modelling

Subsamples of the highest test concentrations were collected on the day of preparation to confirm nominal additions. Additionally, a few samples were collected on the last day of testing to confirm the recovery of Ni from the exposure vessels. All samples were submitted to the MOE's laboratory for analysis of major cations, anions and Ni. Analytical methods were as follows:

- Ni: inductively coupled plasma-atomic emission spectrophotometry using ultra-sonic nebulization;
- major cations: atomic absorption spectrophotometry;
- chloride and nitrate: colourimetry;
- sulphate: automated ion chromatography;
- alkalinity: potentiometry;
- TOC: combustion and colourimetry; and
- DOC: precipitation and colourimetry.

From the physical and chemical characteristics of the test media, the speciation of Ni could be estimated using the geochemical speciation model, MINTEQA2 (Hydrogeologic Inc. and Allison Geoscience Consultants Inc., 1998). Inputs to the model, in addition to Ni, were as follows: 1) cations: hydrogen (H^+), sodium (Na^+), calcium (Ca^{2+}), magnesium (Mg^+), potassium (K^+); and 2) anions: hydroxide (OH^-), chloride (Cl^-), sulphate (SO_4^{2-}), and carbonate (CO_3^{2-}), entered as total alkalinity in mg/L as $CaCO_3$). Additionally, nitrate (NO_3^-) was added as a component when required to balance the solution charge from $NaNO_3$ additions. MINTEQA2 estimated the percentage of Ni and the major cations in

solution that were present in the free ion form and these percentages were converted to concentrations as free ion for subsequent analyses. The model corrects for ionic strength through the use of the Davies equation.

Nominal concentrations of Ni (as long as measured concentrations were within 25% of the nominal values) and measured concentrations of major cations were used in MINTEQA2 modelling. Nominal concentrations of Ni were favoured over measured concentrations since measured concentrations were not available for every concentration tested.

4.2.6 Data Analysis

The whole-body concentration of Ni (µg/ L) measured by the GFAA was divided by the dry weight of the sample (g) to obtain the concentration of Ni (µg/g) in *Hyalella*. Then, curves were fit to relationship of Ni in tissue (y, as µmol/g) to the cation or Ni in the test medium (x, as µmol/L) through non-linear regression in SigmaPlot 4.00 (SPSS, 1997) using the equation $y = a*x /(b+ x)$ to determine whether increases in the cation or Ni concentration influenced Ni uptake.

4.3 STEP 1B: TIME-SERIES TESTS

Short-term bioaccumulation tests were also carried out to confirm that Ni uptake by *Hyalella* reached steady state within seven days, since steady state is an assumption of the BLM. Procedures followed those described in Section 4.2, with the following exceptions:

- Each test included identical test solutions, prepared as subsamples of a single large volume of solution spiked with Ni;
- Organisms were collected from replicate vessels throughout the exposure period to determine Ni uptake over time; and
- Test duration was 48 hours.

In total, three tests were conducted: two at ~40 µmol Ni/L in either LH or DC medium and one at ~5 µmol Ni/L in DC. Since all the exposure solutions were identical, and since the purpose of the tests was to confirm only that steady state had been reached, no chemical analyses to confirm nominal concentrations were carried out. Organisms from two replicate vessels were collected every few hours (i.e., 0, 4, 8, 18, 26, 34 and 48 hours) then dried, weighed and digested as described above. After analysis by GFAA, whole-body concentrations of Ni were calculated and plotted against time using Sigma Plot 4.0 (SPSS, 1997).

4.4 STEP 2A: SHORT-TERM TOXICITY TESTS

Short-term toxicity tests were performed in different media to determine whether different cations influenced the toxicity of Ni to young organisms. Tests were performed with MSAM medium, in which the concentration of the cation of interest was adjusted. For each test, MSAM was prepared, excluding the cation of interest. Then the medium was split into two subsamples. The cation of interest was added to one subsample at approximately four times the amount normally added to prepare MSAM and served as the "high concentration"

solution. The other subsample was not amended (i.e., without any addition of the cation of interest) to serve as the "low concentration" solution. Table 4.4 lists the short-term toxicity tests performed and the characteristics of the media.

For toxicity testing, subsamples of the test medium, prepared at least one day before test initiation, were spiked with Ni to create the highest test concentration and this solution was serially diluted with the test medium to create a concentration series. A solution, containing medium without added Ni, served as a control. TetraMin® and gauze were added to all solutions as described above, which were left at test temperature for at least four hours before use. The tests were initiated when ten organisms (6-12 weeks) were transferred to each test vessel. Solutions were monitored for pH on day 0 and again on day 7, when the test was terminated. Mortality data from each test were used to calculate LC50s for Ni. Table 4.5 describes the toxicity test design.

4.4.1 Chemical Analysis

Subsamples of selected test solutions were collected and submitted to the MOE's laboratory to confirm the nominal concentrations of Ni as well as the cation of interest. Measured concentrations for the major cations and anions were used except for the concentration of NO_3 in the high Na exposure, with a measured concentration of 59 mg/L, which was below that possible with the addition of Na (confirmed by chemical analysis). Nominal Ni concentrations were used in all calculations as long as they were within 25% of the measured concentrations.

Table 4.4: List of Short-term Toxicity Tests and Composition of Test Media

Medium		Medium Characteristics (mg/L)									
Base	Adjustment (compared to MSAM)	pH*	Cl	Ca	Mg	Na	K	SO_4	ALK	DOC	NO_3
MSAM	-	7.8	60	40	7	21	2	30	70	0.4	0.2
MSAM	Low K (no addition)	7.8	60	40	7.1	21	0.5	27	68	0.4	0.16
MSAM	High K (4x)	7.8	66	40	7.2	21	8.6	28	68	0.4	0.17
MSAM	Low Na (no addition)	7.6	60	41	7.2	4.6	1.8	27	33	0.4	0.16
MSAM	High Na (4x)	7.3	61	40	7.1	100	1.9	28	33	0.4	260**
MSAM	Low Mg (no addition)	7.6	78	45	2.7	21	1.9	10	59	0.4	0.19
MSAM	High Mg (4x)	7.6	79	47	29	21	1.9	110	58	0.4	0.21
MSAM	Low Ca (no addition)	7.7	11	10	9.4	21	1.9	37	58	0.4	0.18
MSAM	High Ca (4x)	7.7	298	170	9.4	21	1.9	38	59	0.4	0.20

*based on test exposure; ** concentration calculated from Na addition

Table 4.5: Short-term Toxicity Test Design and Conditions

Short-term (seven-day) *Hyalella azteca* Toxicity Tests	
Medium:	Varied
Temperature:	23-25°C
Organism Age:	2 –10 days
Organisms/vessel:	10
Test vessels:	400 mL or 500 mL glass jars
Test solution:	300 mL or 400 mL
Replication:	None
Feeding:	2 mg TetraMin® flakes on Day 0 and 4
Gauze:	Approximately 1 cm x 2 cm Added on day 0 and replaced as needed (i.e., if deteriorated)
Aeration:	None
Lighting:	16 hours light / 8 hours dark
Lighting Intensity:	~1000 lux
Monitoring:	pH - minimum Day 0 and 7; Temperature daily, mortality Day 7
Endpoint:	Survival

4.4.2 LC50 Determination

The MOE's TOXSTATS (MOE, 1995) program was used to calculate LC50s. Selection of a method to calculate the LC50 depended on the nature of the dose response and followed the guidance of the U.S. EPA (2002). For example, if the dose response was monotonic and included at least two partial mortalities, the Probit method was always selected. If more than one partial mortality was observed but the dose response was not monotonic, the Moving Average method was selected and, when the dose response included only one partial mortality bracketed by complete and no mortality, the Spearman-Karber method was selected (0% trim). A manual calculation by the Binomial method was carried out for all other tests (i.e., those with two concentrations bracketing a response of 50% mortality). LC50s from each paired set of tests were compared according to the Litchfield-Wilcoxon method, as described in Environment Canada's guidance document for statistical analysis of ecotixicity data (Environment Canada, 2005). Environment Canada (2005) notes that the method is analogous to to the procedure for obtaining a single pooled estimate of variance from the variances of two distributions and that it is likely valid for comparing pairs of tests with similar distributions of data.

4.5 STEP 2B: ACCLIMATION TESTS

Since so many different test media were used during the study, several short-term bioaccumulation and toxicity tests were carried out to determine whether Ni uptake and toxicity would be influenced by the pre-acclimation of *Hyalella azteca* to the different test media. In the environment, aquatic organisms are already acclimated to the conditions of

the water they inhabit when responding to contaminants; however, invertebrate test organisms are not commonly pre-acclimated in the laboratory if water other than the standard laboratory water is used for dilution.

A minimum period of 48 hours was selected for pre-acclimation based on the assumption that steady state with major cations could be achieved during that period (see Section 5.4 below). For these tests, breeding jars were maintained in dechlorinated tap water and young were removed each week to media of different hardness, pH and/or alkalinity. After 48 to 72 hours acclimation, toxicity tests were initiated using the same medium spiked with Ni. For bioaccumulation tests, adults were also transferred from the main culture to solutions of different hardness, pH and/or alkalinity and used in tests after 48 to 72 hours pre-acclimation. Both bioaccumulation and toxicity tests were carried out as concurrent exposures of pre-acclimated and non-acclimated organisms (i.e., those reared in the dechlorinated tap water).

4.5.1 Acclimation Toxicity Tests

Table 4.6 describes the design of the short-term Ni toxicity tests. The hardness or alkalinity of MSAM was adjusted by increasing or decreasing the addition of calcium chloride and magnesium sulphate or sodium bicarbonate, respectively. The pH of the DC solutions was adjusted by the addition of nitric acid. The characteristics of the test media are provided in Table 4.7. All solutions were aerated at room temperature until use except for the pH-adjusted DC, which was adjusted, covered and left for several hours before readjustment and solution preparation. No precipitate was observed in any of the media used for testing.

On the first day of testing, a subsample of the medium was spiked with Ni to create the highest test concentration and this solution was serially diluted with the test medium to create a concentration series. A solution, containing medium without added Ni, served as a control. Two solutions were prepared of each concentration for the pre-acclimated and non-acclimated exposures. TetraMin® and gauze were added to all solutions, which were left at test temperature for at least four hours before use.

The MOE's TOXSTATS program was used to calculate LC50s as described for the short-term toxicity tests above. LC50s for the pre-acclimated and non-acclimated tests were compared by their 95% confidence limits. An overlap of the confidence limits was accepted as similarity in response and, conversely, no overlap was considered potential dissimilarity in response.

Table 4.6: Acclimation Toxicity Test Design and Conditions

Short-term (seven-day) *Hyalella azteca* Toxicity Tests	
Media:	DC – adjusted to pH 7 using nitric acid MSAM – modified to create low/high alkalinity by decreasing or increasing sodium bicarbonate addition; MSAM – modified to create low/high hardness by decreasing or increasing calcium chloride and magnesium sulphate additions
Temperature:	23-25°C
Organism Age:	2 –10 days
Organisms/vessel:	10 – 20
Acclimated Organisms:	Young 0-7 days old transferred to test medium (without Ni) 48 to 72 hours prior to testing
Non-acclimated Organisms:	Young 0-7 days old maintained in dechlorinated tap water 48 to 72 hours prior to testing
Test vessels:	400 mL or 500 mL glass jars
Test solution:	300 mL or 400 mL
Replication:	None
Feeding:	2 mg TetraMin® flakes on Day 0 and 4 (300 mL solutions)
Gauze:	Approximately 1 cm x 2 cm Added on day 0 and replaced as needed
Aeration:	None
Lighting:	16 hours light / 8 hours dark
Lighting Intensity:	~1000 lux
Monitoring:	pH - minimum Day 0 and 7 Temperature daily, mortality Day 7
Endpoint:	Survival

Table 4.7: Characteristics of Media used for Culturing and Testing

Media	**Hardness mg/L $CaCO_3$**	**Initial Alkalinity mg/L $CaCO_3$**	**pH**
PH7	137	90	7.0
MSAM - low hardness	47	67	8.1
MSAM - high hardness	930	67	8.1
MSAM - low alkalinity	130	33	8.1
MSAM - high alkalinity	130	440	8.3

4.5.2 Acclimation Bioaccumulation Tests

For the bioaccumulation tests, organisms were exposed to a nominal total Ni concentration of 2500 µg/L in LHM for a period of 48 hours. Half the organisms exposed in the LHM were pre-acclimated to those conditions 48 hours before addition of Ni. Tests procedures are summarized in Table 4.8.

Table 4.8: Acclimation Bioaccumulation Test Design and Conditions

48-hr *Hyalella azteca* Acclimation Bioaccumulation Test	
Medium:	Low Hardness Medium (LHM)
Temperature:	23-25°C
Organism Age:	6 – 8 weeks
Organisms/vessel:	6
Acclimated Organisms:	Adults transferred to LHM (without Ni) 48 hours prior to testing
Non-acclimated: Organisms:	Adults maintained in dechlorinated tap water prior to testing
Test vessels:	400 mL glass jars
Test solution:	250 mL
Replicates:	2
Feeding:	2 mg TetraMin® flakes on Day 0
Aeration:	None
Gauze:	Approximately 1 cm x 2 cm
Lighting:	16 hours light / 8 hours dark
Lighting Intensity:	~1000 lux
Monitoring:	pH day 0, temperature daily
Endpoint:	Accumulation of Ni

4.6 STEP 2C: LONG-TERM TOXICITY TESTS

4.6.1 Media Preparation

Different media were prepared to test the influence of various water characteristics on the long-term toxicity of Ni. MSAM and LHM were used for tests with varied additions of calcium, magnesium, sodium, potassium, and sodium bicarbonate. Dechlorinated tap water was used to test differences in pH. All media were aerated prior to use in toxicity tests.

Several tests were also carried out with added natural organic material, measured as DOC, from the Suwannee River (SR) (obtained from the International Humic Acid Society as a freeze-dried powder) and from the Luther Marsh (LM) (obtained from Wilfried Laurier University as a liquid concentrate). Both samples of organic material were extracted from their source waters through reverse osmosis.

Media with added DOC were aerated for several days before test solution preparation to allow full equilibrium of solutions and were not filtered before use. The characteristics of the test media for the long-term toxicity tests performed are provided in Table 4.9.

Table 4.9: List of Long-term Toxicity Tests and Composition of Test Media

Test	Base Medium	Adjustment	Medium Characteristics (mg/L) pH	Cl	Ca	Mg	Na	K	SO_4	ALK	DOC
18	DC	low pH	6.4	33	35	9.0	17	1.8	35	84	0.2
19	DC	med pH	7.4	33	35	9.0	17	1.8	35	84	0.2
20	DC	high pH	8.7	33	35	9.0	17	1.8	35	84	0.2
21	MSAM	low Ca	8.1	10	10	6.9	24	1.9	28	68	0.2
22	DC		8	33	35	9.0	17	1.8	35	84	0.2
23	MSAM	High Ca	7.8	230	130	7.0	24	1.8	29	58	0.4
24	DC	low pH	6.4	33	35	9.0	17	1.8	35	84	0.2
25	DC	med pH	7.3	33	35	9.0	17	1.8	35	84	0.2
26	DC	high pH	8.4	33	35	9.0	17	1.8	35	84	0.2
27	DC	high pH	8.4	33	35	9.0	17	1.8	35	84	0.2
28	MSAM	low Ca	8.1	10	10	6.9	24	1.9	28	68	0.2
29	DC		8.2	33	35	9.0	17	1.8	35	84	0.2
30	MSAM	high Ca	8	230	130	7.0	24	1.8	29	58	0.4
31	DC	low pH	6.7	33	35	9.0	17	1.8	35	84	0.2
32	MSAM	low alk/low Ca	7.9	10	10	7.1	21	1.8	27	26	0.2
33	MSAM	low alk	7.8	63	40	7.0	21	1.8	27	25	0.2
34	MSAM	high alk	8.5	60	30	7.0	72	1.8	27	145	0.2
35	MSAM	high Ca	8.1	280	157	10	32	2.8	38	48	0.6
36	DC	high pH	8.9	40	35	10	29	1.9	38	100	0.7
37	MSAM	high Hard	8	260	160	26	30	2.2	98	64	0.5
38	MSAM	high Mg	8.2	64	42	22	24	1.8	81	70	0.3
39	MSAM	all high	8.2	398	190	36	133	10	130	212	0.4
40	LHM		7.7	8	14	2.7	4	0.5	16	30	0.5
41	MSAM	high Hard	7.9	550	300	47	22	1.8	185	66	0.6
42	MSAM	high Mg	8.1	61	37	47	22	1.9	185	63	0.5
43	MSAM	high alk*	8.8	64	43	7.0	220	2.0	29	600	0.4
44	MSAM		7.9	59	34	6.9	21	1.9	28	60	0.4

Table 4.9 cont'd: List of Long-term Toxicity Tests and Composition of Test Media

	Medium		Medium Characteristics (mg/L)								
Test	Base	Adjustment	pH	Cl	Ca	Mg	Na	K	SO_4	ALK	DOC
45	LHM	DOC SR5	7.7	8.0	11	2.5	3.9	0.5	9.1	26	4.8
46	MSAM	DOC SR5	8.2	65	40	7.1	22	2.1	28	63	4.7
47	LHM	DOC SR10	7.7	8.5	9.5	2.4	4.0	0.5	9.8	23	8.5
48	LHM	DOC LM10	8	10	14	3.3	4.0	0.5	10	27	10
49	LHM	DOC SR20	7.4	10	11	2.7	4.3	0.5	10	19	16
50	LHM		7.7	8.5	10	2.6	3.9	0.5	9.4	25	0.4
51	MSAM		8.1	63	38	7.2	21	2.1	28	61	0.5
52	LHM	high Ca	7.7	271	157	2.8	5.5	0.6	10	30	0.4

MSAM = modified standard artificial medium; LHM = low hardness medium; DC = dechlorinated tap water
SR = Suwannee River organic material; SR5, SR10, SR20 = 5, 10, 20 mg/L as DOC, respectively
LM10 = Luther Marsh organic material with 10 mg/L as DOC
*nominal, rather than measured Ca and ALK are provided in the table due to problems with chemical measurements

4.6.2 Test Set-up and Maintenance

The long-term toxicity tests were carried out in different media to estimate LC50s for one, two, three and four weeks of exposure. Subsamples of the test medium, prepared at least one day before test initiation, were spiked with Ni to create the highest test concentration and this solution was serially diluted with the test medium to create a concentration series. A solution, containing medium without added Ni, served as a control. TetraMin® and gauze were added to all vessels, which were left at test temperature for at least four hours after Ni addition before organisms were introduced.

Organisms within an age range of 2 to 10 days were used in four-week tests. In 2004, this range was further restricted by using organisms born within four days of each other. Organisms were either directly transferred to randomly-selected test vessels or were first transferred to small cups containing a small volume of test medium and then transferred to

randomly selected test vessels. Depending on organism availablility, 15 to 20 organisms were used per concentration.

Solutions were monitored for pH on Day 0, periodically throughout the exposure period and again on Day 7, 14, 21 and 28. Every seven days, fresh solutions were prepared, as described above. Mortality and pH were monitored and surviving organisms were transferred to the fresh solutions. Table 4.10 describes the toxicity test design. Of note is that smaller plastic (polystyrene) test vessels replaced glass beakers when tests using added DOC were carried out, due to limited sample volumes available.

4.6.3 Test Termination, Acid Digestion and Measurement of Ni Bioaccumulation

On the last day of testing, mortality, temperature and pH were recorded and test solutions were discarded. All LC50 tests conducted in 2004 (tests 35 to 51) included measurement of Ni accumulation by the test organisms. Organisms were collected, weighed, acid digested and analysed according to the procedures described for the bioaccumulation tests (Section 4.2).

4.6.4 Chemical Analysis

Subsamples of highest test concentrations were collected and submitted to the MOE's laboratory to confirm the nominal concentrations of major cations and Ni. Additionally, samples were also collected from tests 36, 41, 42, 43, and 44 at solution exchange in order to confirm that Ni remained in solution throughout the exposure period. If the measured concentrations of Ni were within 25% of the nominal, the nominal was used in all

calculations, rather than averaging the four different measurements taken over the course of the test. Major cations and anions were measured only once in the test medium and measured values were used for all analyses.

Table 4.10: Long-term Toxicity Test Design and Conditions

Four-week *Hyalella azteca* Toxicity Tests	
Media:	Varied
Temperature:	23-25°C
Organism Age:	2 –10 days
Organisms/vessel:	15 to 20
Test vessels:	400 mL or 500 mL glass jars (2003 to 2004)
	290 mL plastic (polystyrene) cups (fall 2004)
Test solution:	300 mL or 400 mL (2003 t0 2004)
	200 mL (fall 2004)
Replication:	None
Feeding:	2 mg TetraMin® flakes on Day 0 and 4 (300 mL solutions)
	1 mg TetraMin® flakes on Day 0 and 4 (200 mL solutions)
Gauze:	Approximately 1 cm x 2 cm
	Added on day 0 and replaced as needed
Aeration:	None
Lighting:	16 hours light / 8 hours dark
Lighting Intensity:	~1000 lux
Monitoring:	pH - minimum Day 0 and 7
	Temperature daily, mortality Day 7, 14, 21, 28
Solution Exchange:	Once per week
Endpoint:	Survival and accumulation of Ni

For most tests conducted in media without added organic material, only total concentrations were measured and were assumed to represent dissolved concentrations. However, both total and dissolved concentrations were measured in all tests performed with added organic material. For these tests, subsamples were collected from the highest test concentration when solutions were prepared each week and periodically from the test solutions before solution exchange (i.e., day 7, 14, 21 or 28).

4.6.5 Chemical Speciation Modelling

Chemistry data characterizing the test media were used to speciate Ni in each solution using MINTEQA2 as described above for the short-term bioaccumulation tests. For solutions with added dissolved organic material, DOC was entered as a component. In MINTEQA2, there are a total of six components serving as DOC, each with identical binding affinities and site densities (unless changed by the user in a separate database file). DOC is treated as a continuous Gaussian distribution, in which the "concentrations of individual ligands of the complex dissolved organic material mixture are normally distributed with respect to their log K values" (Hydrogeologic Inc. and Allison Geoscience Consultants Inc., 1998). Allison and Perdue (1994) point out that other models may be used and that it is unlikely that any substance conforms completely to the model selected for MINTEQA2; however, it is expected that the model can "reasonably represent the complexation behaviour of protons and metals" to dissolved organic material. Each component of dissolved organic material represents a potential acidic functional group, such as carboxyl, phenols, and alcohols, to which metals or other cations may bind in competition with protons (Perdue, 1985, as cited in Hydrogeologic Inc. and Allison Geoscience Consultants Inc., 1998). Assumptions of the model include a 1:1 stoichemistry between the complexing cation and the ligand and a constant ratio between the equilibrium constants for the metal and protons binding to the ligand (i.e., constant K_{ML} / K_{HL}) (Allison and Perdue, 1994). The mean log K value for the Ni-DOC complex is 3.3 (Allison and Perdue, 1994).

For the current study, a two-site type (bimodal) model was selected for modelling the solutions with added organic material. This decision was based on studies by Tipping

(1994) and Serkiz (1991) who used or postulated two site types for Suwannee River organic material, respectively (as cited by Hydrogeologic Inc. and Allison Geoscience Consultants Inc., 1998). If more than one site type is used in modelling, the percentage of metal that binds to each is presented separately as two unimodal distributions.

4.6.6 Data Analysis

LC50s were calculated as described above for the short-term toxicity tests and were converted to a concentration of Ni as Ni^{2+} using MINTEQA2. The influence of a cation on the LC50 over time was assessed by a comparison of the slopes of the regression lines of LC50s vs cations for days 7, 14, 21 and 28 (Zar, 1999). Additionally, LA50s (accumulated concentration of Ni associated with 50% mortality) were calculated for those tests with tissue Ni data for exposures bracketing 50% mortality.

4.7 STEP 3: MODEL DEVELOPMENT

Models for the uptake of Ni were developed based on the cations that influenced Ni uptake and toxicity.

Plots of bioaccumulation tests, in which the concentration of Ni was varied, were used to determine preliminary estimates of L_T and K_{Ni}. Specifically, from linear plots of Ni/NiL (y) versus the concentration of free Ni in solution (x), L_T was calculated from 1/slope and K_{Ni} was calculated from the intercept ($1/K_{Ni}*L_T$). These estimates were valid for the individual exposures for which they were calculated but did not include consideration of the binding

affinities of competitor cations. Competitor cations were incorporated by modeling with Systat®11(SYSTAT Software Inc., 2004) using multiple regression. Each competitor was added sequentially to the mathematical expression and the model(s) that best fit the available data (least squares analysis) from the short-term bioaccumulation tests and long-term toxicity tests were selected for prediction of accumulation and toxicity.

For example, the equation including H, Ca and Mg as competitors was analysed by least squares as :

$$[NiT] = \frac{[Ni]*[L_T]*K_{Ni}}{1+ K_{Ni}*[Ni] + K_{Ca}*[Ca] + K_H*[H] + K_{Mg}*[Mg]}$$

but was simplified to:

$$[NiT] = [Ni] / a + IL_T*[Ni] + b*[Ca] + c*[H] + d*[Mg]$$

where:

[NiT] = whole-body Ni concentration

[Ni] = concentration of Ni^{2+} in solution

$a = 1/K_{Ni}*L_T$

$b = K_{Ca} / K_{Ni}*L_T$

$c = K_H / K_{Ni}*L_T$

$d = K_{Mg} / K_{Ni}*L_T$

$ILT = 1/L_T$

If accumulation (NiT) does not saturate within the concentration series tested, then L_T is infinite and cannot be computed. However, $1/L_T$ approximates zero in such cases, which can be determined.

From this first step, constants from the model(s) of the best fit were used to predict LA50s (predicted NiL) at observed LC50s ([Ni]). By rearranging the equation and solving for [Ni], the LC50 could be predicted at a given LA50. To illustrate,

$$[NiT] = \frac{[Ni]*[L_T]*K_{Ni}}{1+ K_{Ni}*[Ni]}$$ is re-arranged in the following steps:

$$[NiT]*(1+ K_{Ni}*[Ni]) = [Ni]*[L_T]*K_{Ni}$$

$$[NiT] + [NiT]*K_{Ni}*[Ni]) = [Ni]*[L_T]*K_{Ni}$$

$$[NiT] = [Ni]*[L_T]*K_{Ni} - ([NiT]*K_{Ni}*[Ni])$$

$$[NiT] = [Ni]*K_{Ni} ([L_T]-[NiT])$$

to: $$[Ni] = \frac{[NiT]}{([L_T]-[NiT])*K_{Ni}}$$

In addition to the above competitive model, a non-competitive mechanism for inhibition of accumulation and toxicity of both Ni and cadmium was investigated based on the idea that the amount of ligand available for binding may be reduced if an enzyme required for ligand synthesis is rendered inactive when bound to a cation. In such a case L_T, is replaced by L_{T0}, representing the rate of synthesis and degradation of L_T multiplied by the total concentration of the enzyme that makes L_T. Further details on the development and evaluation of non-competitive models are provided in Sections 6.0 and 9.0.

5.0 NICKEL - RESULTS AND DISCUSSION

5.1 STEP 1A: SHORT-TERM BIOACCUMULATION TESTS

Appendix A provides data regarding the chemistry, speciation and accumulation data for each of the bioaccumulation tests.

5.1.1 Chemistry

Chemical analysis confirmed the nominal additions of Ni to solutions in all tests except for test 5, for which no data are available (Appendix A). The average measured nickel concentration on the day of solution preparation was 94% of the nominal addition, with a range of 83 – 109%. Analysis of solutions collected from the exposure vessels on day 7 of testing averaged 91% of the nominal concentration, with a range of 78-102%. However, when compared to the corresponding concentration measured when solutions were prepared, the recovery ranged from 94 – 107%, supporting the assumption that Ni concentrations were maintained in solution throughout the test exposure. Modelling with MINTEQA2 indicated that the majority of Ni was present in the free (Ni^{2+}) form but was replaced by other forms ($NiCO_3$) at pH above 8.

5.1.2 Bioaccumulation

Visual inspection of the data indicated that Ni uptake by *Hyalella* increased with increasing Ni (as Ni^{2+}) concentration in water until a maximum uptake was reached. Non-linear regression of Ni in tissue (y) versus Ni^{2+} in solution (x) according to the model $y= a*x/b+x$ accounted for between 56 and 78 percent of the variation observed (based on adjusted r^2

values) Figure 5.1). In this model, *a* represented L_T and *b* represented $1/K_{Ni}$. Using estimates for *a* and *b* estimated from the uptake models, the accumulation of Ni in tissue was compared among exposures at a Ni^{2+} concentration of 30 µmol/L. Accumulation was similar in LHM and MSAM exposures, as shown by modeled tissue concentrations of 2.78 and 2.35 µmol/g dw in LHM (tests 2 and 17, respectively) versus 2.39 µmol/g dw in MSAM (test1). Increasing the Ca concentration to 3.6 mmol/L (test 3) reduced the accumulation of Ni in LHM by 46 to 55% (compared to tests 2 and 17, respectively).

When Ni concentration was held constant and Ca was varied, Ni uptake (*y*) was reduced with increasing calcium concentration (*x*) (Tests 4 and 6, Figure 5.2). The reduction in uptake was modeled using the equation $y = a*c/c + x$, with the *c* term incorporating [Ni] as a constant and Ca replacing Ni as the *x* value. Ni accumulation was estimated at a Ca concentration of 3.6 mmol/L and compared to that estimated in test 3 at the same Ni concentration (~4 µmol/L). The estimated accumulation in tests 4 and 6 was 0.30 and 0.45 µmol/g dw, respectively, compared to 0.53 µmol/g dw in test 3. The models reflecting the decreased accumulation with increasing Ca accounted for 66% and 49% of the observed variation in accumulation.

Increasing H^+ concentrations (decreasing pH) while holding the nominal Ni concentration constant reduced Ni uptake (Figure 5.3), with 85% of the observed variation accounted for by non-linear fitting of Ni uptake (*y*) to H^+ concentration (*x*). In contrast, no significant influence on uptake was observed in exposures of increasing K or Na (Figures 5.4 and 5.5). The influence of Mg was more difficult to determine as showed by tests in MSAM and low-

Ca MSAM (MSAM without added Ca) (Figure 5.6) and in LHM media (Figure 5.7). Accumulation in MSAM, with and without the addition of Ca, showed no effect of magnesium (Figure 5.6). However, exposures in LHM did indicate that Mg had an influence (Figure 5.7), with reductions in accumulation between 32 and 46%, for tests 13 and 11, respectively.

Figure 5.1: Ni in Tissue (NiT; µmol/g dw) versus Ni^{2+} (µmol/L) in Different Media: A: Test 1 (MSAM: r^2=0.78; p=<0.0001); B: Test 2 (LHM: r^2=0.66; p=0.0002); C: Test 3: LHM-High Ca: r^2=0.56; p=<0.0001); and D: Test 17 (LHM: r^2=0.67; p=<0.0001)

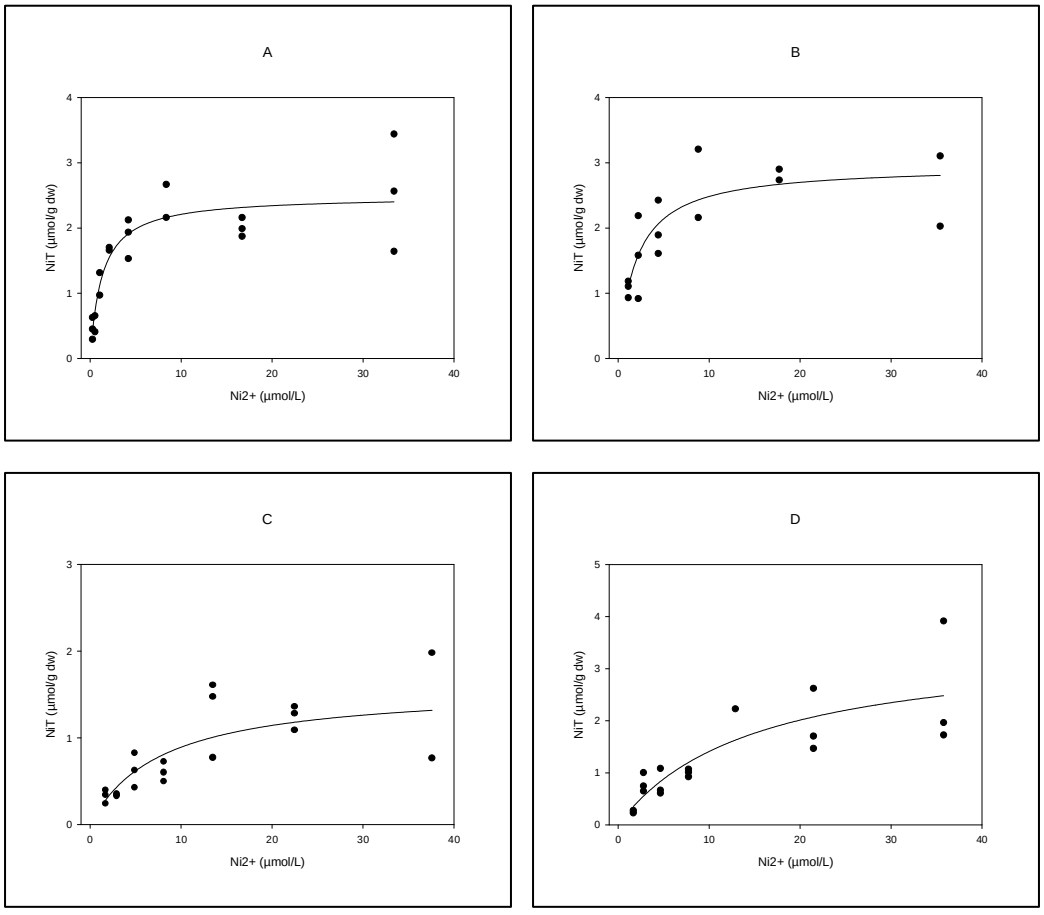

Figure 5.2: Ni in Tissue (NiT; µmol/g) versus Ca (µmol/L) in MSAM:
A: Test 4 (r^2=0.66, p=<0.0001); B: Test 6 (r^2=0.49; p=0.0007)

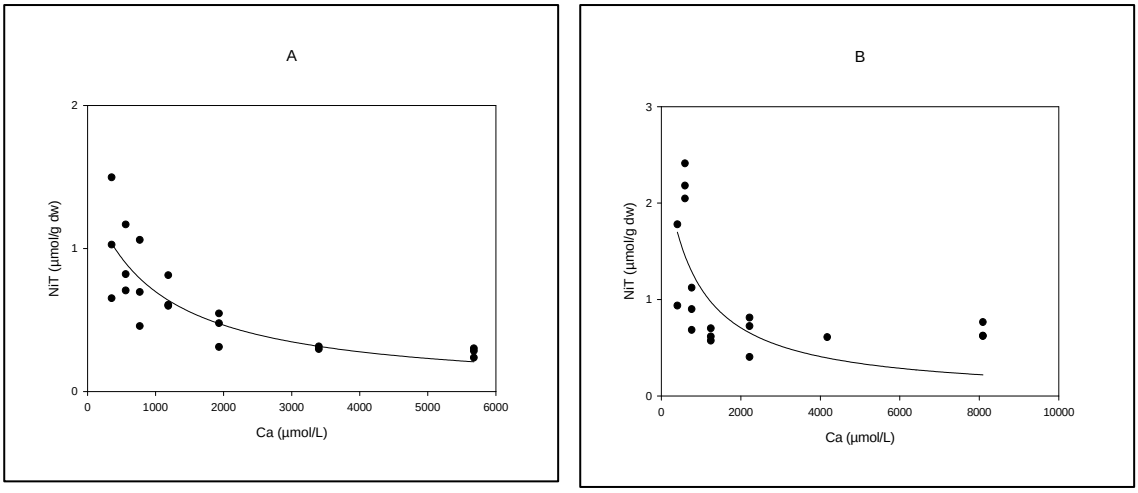

Figure 5.3: Ni in Tissue (NiT; µmol/g) versus H^+ (µmol/L) in DC:
Test 5 (r^2=0.85; p=<0.0001).

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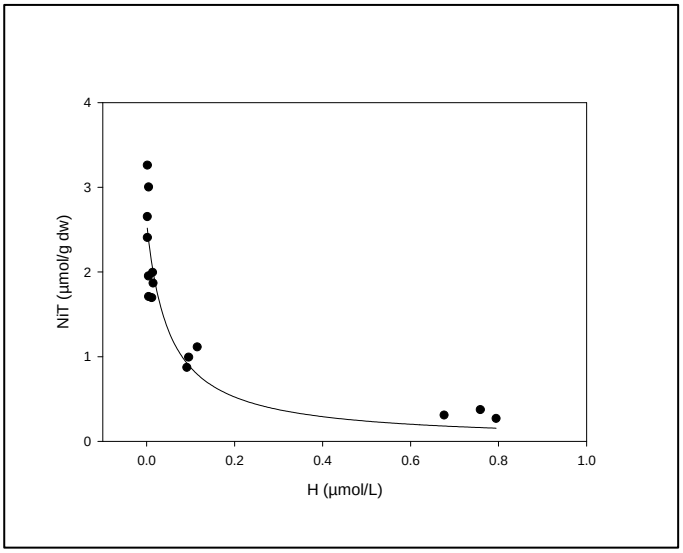


Figure 5.4: Ni in Tissue (NiT; µmol/g) versus K (µmol/L) in MSAM: Test 7 (r^2=0.02; p=0.272).

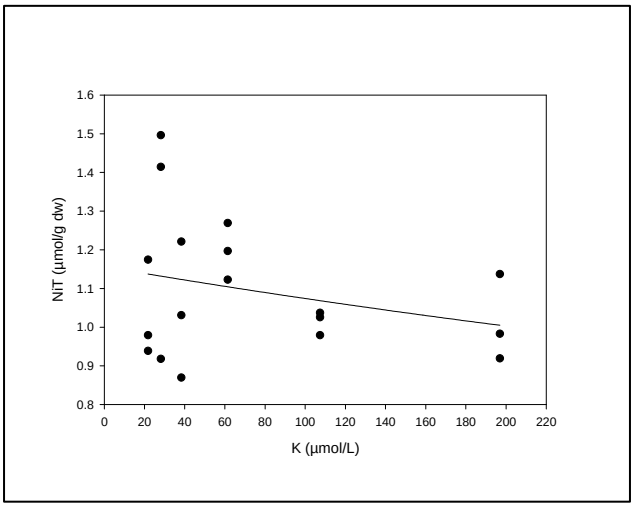


Figure 5.5: Ni in Tissue (NiT; µmol/g) versus Na (µmol/L) in MSAM: A: Test 15 (r^2~0; p=0.530) and B: Test 16 (r^2~0; p=0.751).

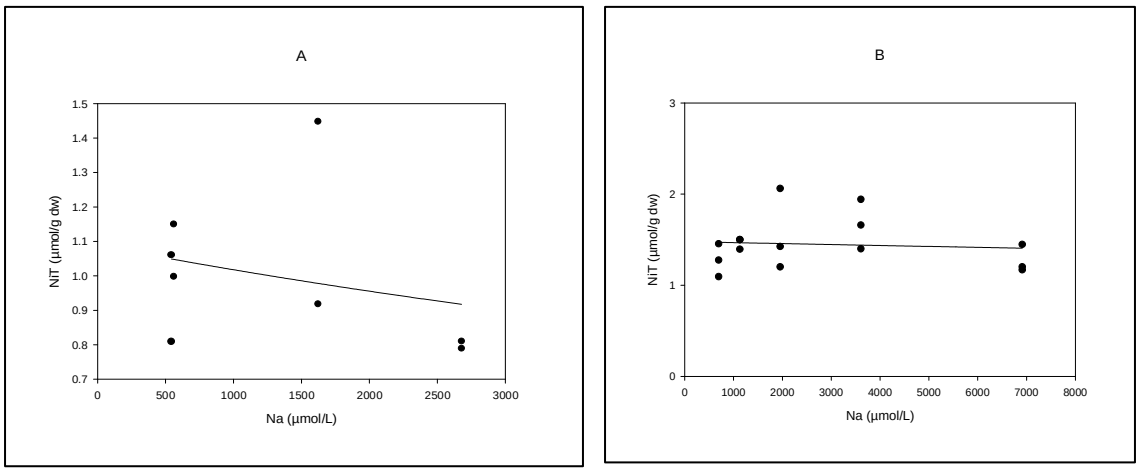


Figure 5.6: Ni in Tissue (NiT; µmol/g) versus Mg (µmol/L) in MSAM: A: Test 8 (r^2=0.04; p=0.210); B: Test 10 (r^2~0; p=0.740); C: Test 12 (r^2=0.016; p=0.275); D: Test 14 (r^2=0.099; p=0.180); and in Low Ca MSAM: (E: Test 9 (r^2~0; p=0.999))

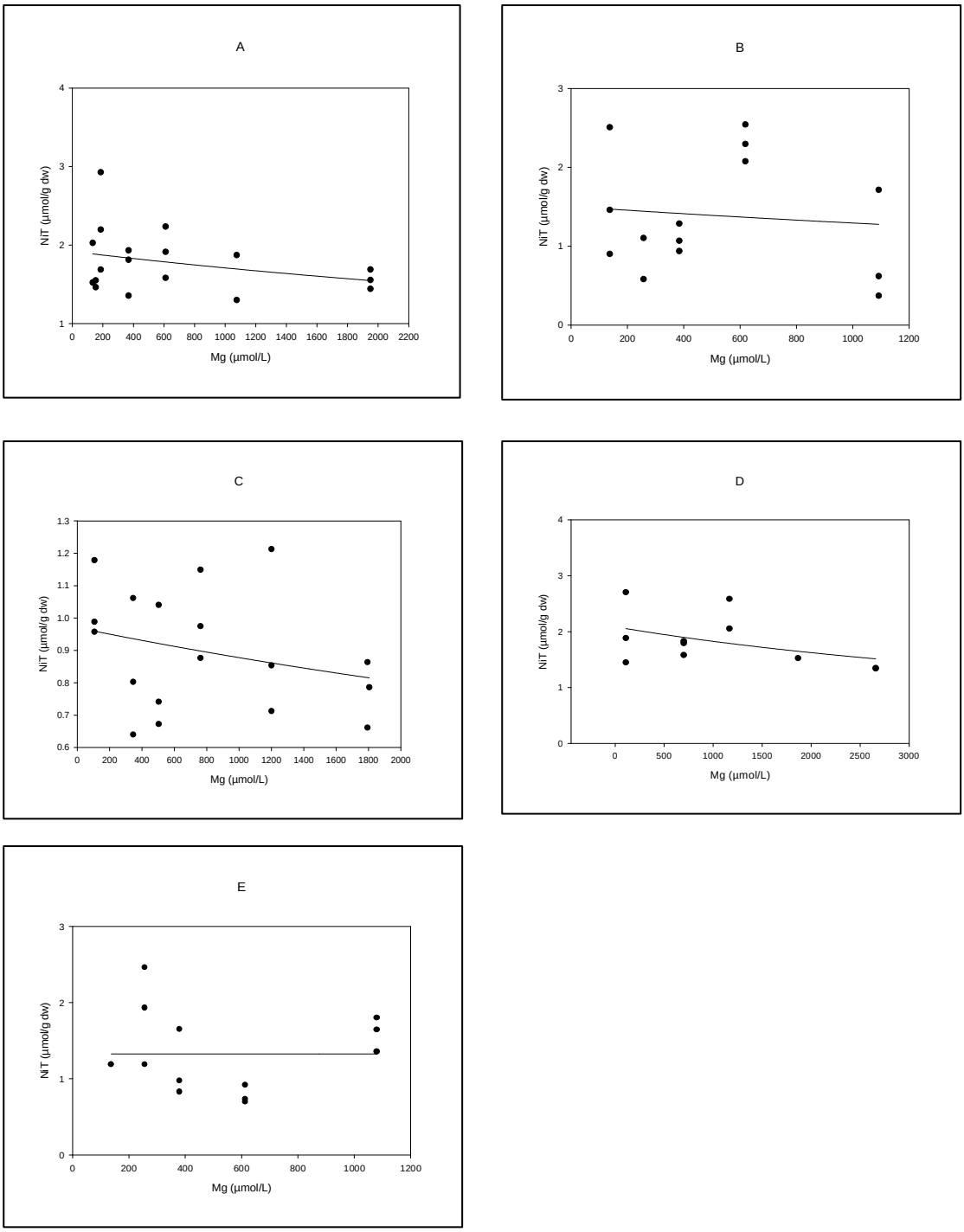
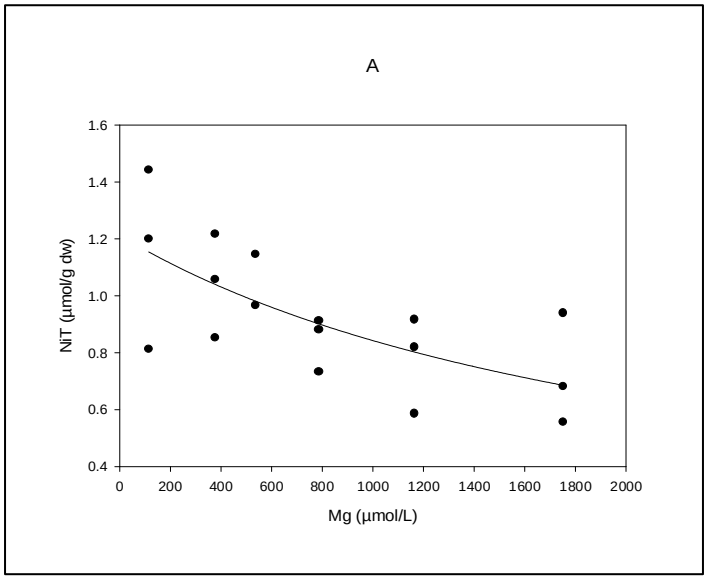
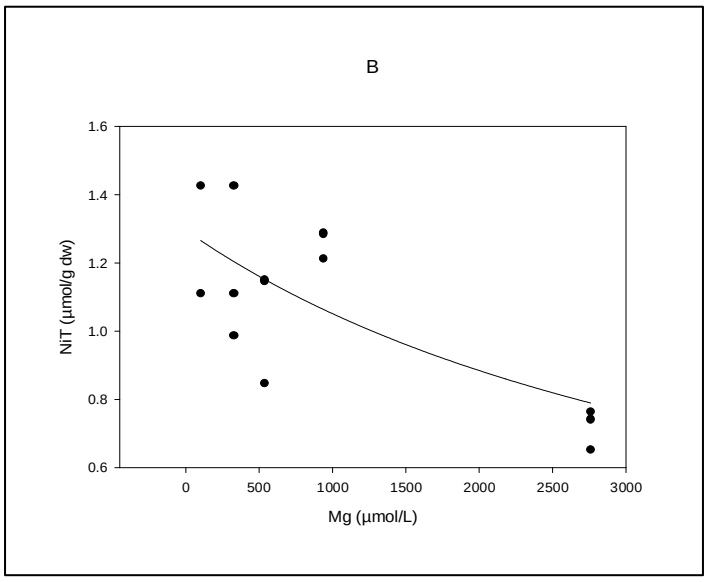


Figure 5.7: Ni in Tissue (NiT; µmol/g) versus Mg (µmol/L) in LHM: A: Test 11 (r^2=0.44; p=0.0022) and B: Test 13 (r^2=0.45; p=0.005).





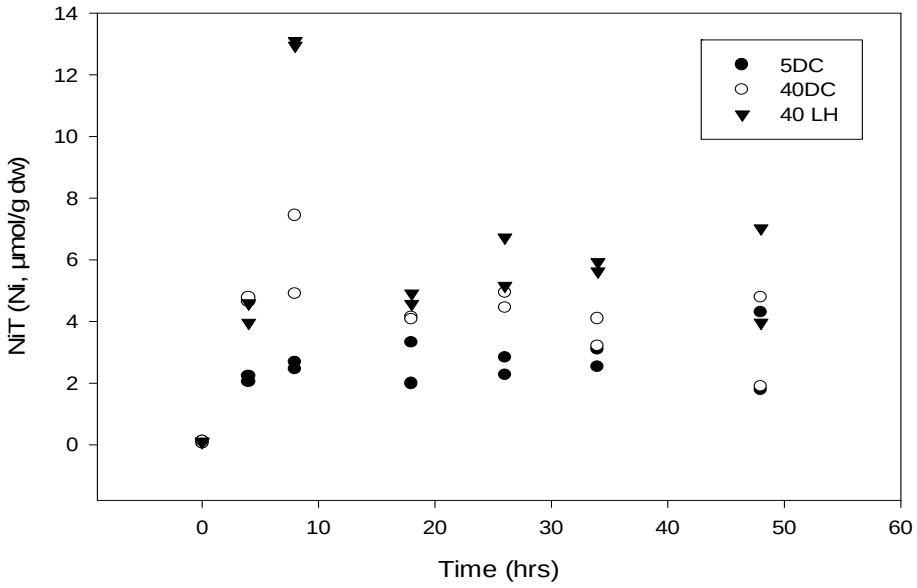
5.1.3 Toxicity

Since the primary purpose of the seven-day bioaccumulation tests was to assess uptake in different media, Ni was added at concentrations below those expected to cause lethality. The only exceptions were the tests in which Ni was varied (tests 1, 2, and 3), which had sufficient mortality to calculate LC50s; however, mortality did not exceed 56% in test 1 or 3, reducing the certainty of the LC50 estimates. LC50s calculated from tests 1, 2 and 3 were 31, 24 and 32 μmol (Ni^{2+})/L, respectively. Of note is that all LC50s had overlapping confidence limits. Similarly, LA50s could not be calculated with confidence from the limited mortality and tissue data available; however, LA50s were estimated as approximately 2.6, 2.5 and 1.2 μmol/g dw in tests 1, 2 and 3, respectively.

5.2 STEP 1B: TIME-SERIES TESTS

The results of the time-series tests are presented in Figure 5.8. All tests confirmed that uptake of Ni by *Hyalella* reached steady state within 48 hours. These tests supported the BLM assumption of steady state in the seven day bioaccumulation tests described in Section 5.1. Of note is that after approximately eight hours of exposure, Ni in tissue spiked in exposures to total Ni concentrations of 40 μmol/L (in both LHM and DC) before stabilizing at lower concentrations. Therefore, prediction of toxicity would likely be best achieved by using body concentrations measured after at least 24 hours in order to increase the likelihood that body concentrations have stabilised.

Figure 5.8: Accumulation of Ni by Adult *Hyalella* over 48 Hours in DC (5 µmol/L and 40 µmol/L (5DC and 40DC)) and LHM (40 µmol/L (40LH)) Exposures



5.3 STEP 2A: SHORT-TERM TOXICITY TESTS

For all the paired toxicity tests in exposures of high and low concentrations of selected cations, the LC50 for the test with the high cation concentration was always higher than that of the low cation concentration; however, based on comparison of the paired tests using the Litchfield-Wilcoxon method, none of the higher LC50s was significantly different from its corresponding lower LC50 except for the test with higher Ca (Table 5.1). Deleebeeck et al. (2007) also found that acute Ni toxicity was not influenced by Na or K, based on acute lethality tests conducted with *Daphnia magna.*

Table 5.1: Seven-day LC50s (Ni^{2+}, µmol/L) in Different Media and Comparison of LC50s according to the Litchfield-Wilcoxon Method (as described by Environment Canada, 2005).

Exposure	pH	LC50 (Ni^{2+} µmol/L)	95% Confidence Limits		Ratio of LC50s*	$f_{1,2}$**
			LCL	UCL		
Low K	7.8	2.8	2.4	2.8		
High K	7.8	3.6	2.5	3.6	1.3	1.4
Low Na	7.6	4.6	3.4	4.6		
High Na	7.2	6.0	4.5	6.0	1.3	1.5
Low Mg	7.6	5.3	3.8	5.3		
High Mg	7.5	5.6	3.8	5.6	1.1	1.7
Low Ca	7.7	5.0	3.7	5.0		
High Ca	7.7	8.0	5.8	8.0	1.6	1.5

ucl = 95% upper confidence limit; lcl = lower 95% confidence limit
*Ratio of LC50s = higher LC50/lower LC50
**$f_{1,2}$ = antilog $\sqrt{((\log f_1)^2 + (\log f_2)^2)}$ where f_1 (lower LC50) = ((UCL/LC50 + LC50/LCL)/2) and f_2 (higher LC50) = ((UCL/LC50 + LC50/LCL)/2). No significant difference if ratio of LC50s < $f_{1,2}$

5.4 STEP 2B: ACCLIMATION TESTS

5.4.1 Acclimation Toxicity Tests

Results of the toxicity tests using acclimated and non-acclimated organisms are presented in Table 5.2. *Hyalella* response in the toxicity tests was consistent with the findings of the bioaccumulation tests in Section 5.1 and the toxicity tests in Section 5.3 in that toxicity decreased at higher hardness and increased with higher pH (high alkalinity exposure). Preacclimation to the test medium before exposure to Ni did not influence the sensitivity of *Hyalella* to Ni in seven-day exposures. These data indicated that *Hyalella* may be cultured in moderately hard laboratory water and transferred to various test media without a pre-acclimation step, at least for tests with exposure of at least seven days duration. Deleebeeck et al. (2007) observed similar results in their tests, exposing several cladoceran species to Ni over a period of 16 to 21 days. Organisms that were collected from and

reared in soft water were no more or less sensitive to Ni in hard water exposures than organisms collected and reared from hard water exposures. In their study, hardness ranged from 5 to 53 mg/L as $CaCO_3$.

Table 5.2: Seven-day LC50s (Total Ni, µmol/L) from Tests Using Organisms Pre-acclimated or Not Acclimated to the Exposure Medium.

Test	Seven-day LC50 (Total Ni µmol/L) and 95% Confidence Limits					
	Pre-acclimated			Non-acclimated		
	LC50	LCL	UCL	LC50	LCL	UCL
MSAM – Low Hardness	3.7	2.4	5.5	5.0	3.6	7.0
MSAM – High Hardness	36	27	52	37	30	45
	26*	18	42			
MSAM –Low Alkalinity	5.9	5.0	7.0	6.7	4.8	9.0
MSAM -High Alkalinity / High pH	2.2	1.4	3.0	2.5	1.9	3.3
DC pH 7	8.9	5.9	14	7.1	4.7	11
	7.7*	5.0	11			

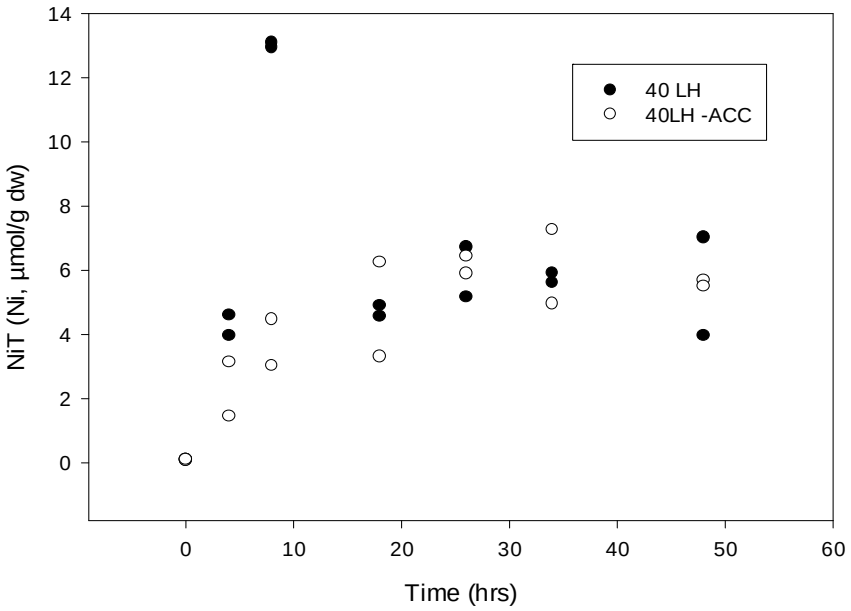
*replicate; lcl = lower 95% confidence limit, ucl = upper 95% confidence limit

5.4.2 Acclimation Bioaccumulation Tests

Results of the bioaccumulation tests supported the acclimation-toxicity results, demonstrating similar uptake with and without pre-acclimation to the exposure medium. The uptake of Ni by acclimated organisms did not appear to spike after eight hours of exposure, as did the uptake in the non-acclimated organisms; however, no difference was apparent by 18 hours of exposure (Figure 5.9). In acute and chronic exposures, Deleebeeck et al. (2007) found that cladocerans reared in soft water were generally no more sensitive to Ni that those reared in hard water, when tested in the same medium. This further supports

the conclusion that organisms need not be pre-acclimated before short-term toxicity tests; however, further evaluation would be warranted for exposures less than 12 hours.

Figure 5.9: Accumulation of Ni in Tissue in LH Medium With and Without Preacclimation to LH Medium



5.5 STEP 2C: LONG-TERM TOXICITY TESTS WITHOUT DOC

Appendix B provides data regarding the chemistry, speciation and accumulation data for each of the 28-day toxicity tests.

5.5.1 Chemistry

Chemical analysis of selected test solutions confirmed nominal additions except in pH-adjusted tests, for which data are unavailable (Appendix B). Measured Ni concentrations were on average 92% of nominal concentrations for samples collected on the day of solution preparation, confirming the accuracy of Ni additions. Additionally, Ni

concentrations measured in test vessels after seven days of exposure were on average 88% of the nominal, confirming that Ni stayed in solution during the seven days between solution exchange. Ni, measured in a few filtered samples, was within 10% of concentrations measured in corresponding unfiltered samples, indicating that essentially all Ni was dissolved in test solutions. Of note is that recovery of calcium was low in the test 43 sample, less than half the concentration expected from the known addition of DC to the medium and below that indicated by the corresponding concentration of chloride (from calcium chloride addition). Measured alkalinity was also below nominal in spite of concentrations of sodium that were consistent with nominal additions of sodium bicarbonate. Nominal concentrations of calcium and alkalinity were used for this test but the data were used with caution. As shown for the bioaccumulation tests, modeling with MINTEQA2 indicated that the majority of Ni was in the free form in most solutions (above 75%) but was reduced significantly when pH rose above 8.5 or when salts were elevated (Appendix B).

5.5.2 Bioaccumulation

The tissue data from the 28-day toxicity tests were limited but could be fitted to the model ($y = x*a/(b + x)$) using non-linear regression. Estimates for parameters a (L_T) and b ($1/K_{Ni}$) (with adjusted r^2values >0.7) were used to estimate accumulation at a single exposure concentration. As shown in Table 5.3, at a Ni^{2+} exposure concentration of 0.5 µmol/L, accumulation of Ni was generally lower in solutions with higher Ca and H^+.

Table 5.3: Modelled Ni in Tissue (NiT) (µmol/g dw) at 0.5 µmol/L Ni^{2+} in Long-term Toxicity Tests (NiT = a*Ni^{2+}/(b+Ni^{2+}))

	Medium	Coefficients			Ca	Mg	NiT
Test	Base	a	b	pH	mg/L	mg/L	µmol/g dw
35	MSAM	381069	315719	8.1	160	10	0.6
36	DC	6.1118	0.7216	8.9	35	10	2.5
37	MSAM	1.0941	0.7210	8.1	160	26	0.45
39	MSAM	0.9005	0.2961	8.2	190	26	0.57
40	LHM	94712	65242	7.7	14	3	0.73
41	MSAM	70588	107172	7.9	300	47	0.33
42	MSAM	132584	52062	8.1	37	47	1.3
43	MSAM	3.0153	0.1891	8.8	38	7	2.2
44	MSAM	0.8041	0.3655	7.9	34	7	0.46
51	MSAM	8.4333	3.6930	8.1	38	7	1.0

$a = L_T$; $b = 1/K_{Ni}$

Keithly et al. (2004) measured wet-weight accumulation in 14-day exposures of *Hyalella* to Ni in solutions of low Ca and Mg. Using a dry/wet-weight conversion factor of 0.19 (Borgmann, 2002), the observed wet-weight accumulation of Ni in the Keithly et al. (2004) study may be estimated as 0.52 and 0.89 µmol/g dry weight at dissolved Ni concentrations of approximately 0.5 and 1.0 µmol/L, respectively. These values are consistent with the observed 28-day body concentrations in LH medium from this study of 0.55 and 1.42 µmol/g dw in Ni^{2+} concentrations of approximately 0.5 and 1.0, respectively. Although the exposure duration is different, the body concentration of Ni would be expected to be similar since steady state is reached within 48 hours.

5.5.3 Toxicity

Control survival in most 28-day toxicity tests exceeded 80%. However, some adjustments to the media caused impairment to the control organisms, forcing the discontinuation of testing or the omission of test data from later model development. Problematic media

included the following: dechlorinated tap water adjusted to pHs below 7; solutions of MSAM without added Ca; solutions of LH medium with Ca above 3 mmol/L; and some tests of LH medium.

Results of the 28-day toxicity tests for all media are presented in Tables 5.4 (total Ni) and 5.5 (Ni^{2+}). Generally, the observed toxicity was consistent with the bioaccumulation data in that higher LC50s were observed with lower pH and higher Ca concentrations and were also consistent with trends published by others, such as Schubauer-Berigan et al. (1993), although differences were noted in the LC50s themselves. For example, Borgmann et al. (2005) reported seven-day LC50s for Ni of 1.3 and 2.5 µmol/L in media similar to LH and MSAM/DC, respectively, which are lower than corresponding LC50s from this study (5.2 and 7.8 µmol/L, respectively). However, one LH LC50 from this study, using younger organisms than other tests, was 1.8 µmol/L. Differences in toxicity within the same exposures of this study and between different studies may be linked to sensitivity difference among age ranges tested.

The tests conducted in DC showed a clear linear relationship between the LC50 for Ni^{2+} and pH over the seven-day to 28-day exposure periods (Figure 5.10) suggesting that hydrogen competition may be important in the mitigation of Ni toxicity although, as indicated by tests initiated below pH 7, hydrogen itself likely exerts a toxic effect under acidic conditions. A comparison of the slopes of the regression lines showed no decrease in slope between days 7 and 14 but a decrease by 21 days of exposure (Appendix B),

suggesting that in longer-term exposures the influence of pH is reduced. However, this decrease was not reflected by a decrease in the ratio of maximum to minimum LC50s. A linear relationship was also observed between the seven-day LC50 for Ni^{2+} and Ca in MSAM or DC tests of pH 7.9 ± 0.2 (Figure 5.11). All correlation coefficients (r^2) were below 0.6, however, which may reflect the influence of other factors, such as pH, on toxicity and/or increased sensitivity to Ni due to ionic changes of the medium. A decrease in slope of the regression line was observed after 7 days of exposure, suggesting that Ca's influence may be more important in short-term exposures. (Appendix B). This corresponded with a decrease in the ratio of maximum to minimum LC50s over the 28 days and may reflect a change in mode of action as noted by Pane et al. (2003a) for Ni toxicity to *Daphnia magna*.

Sufficient data were available to regress LC50s against Mg in exposures of Ca ~1mmol/L and pH of 8.0 to 8.2. As shown in Figure 5.12, a linear relationship was observed in the seven-day exposures, based on a result from one high LC50 for Ni in MSAM. However, the concentration of Mg tested (1600 µmol/L) was far above those expected in waters of southern Ontario and, as observed for Ca, the influence of Mg on toxicity decreased with the duration of the exposure as shown by a decrease in slope after 7 days of exposure and a corresponding decrease in the ratio of maximum to minimum LC50 (Appendix B). Deleebeeck et al. (2007) noted several studies that suggested Mg^{2+} and Ni^{2+} competed for uptake at Mg transport channels but these studies investigated fish rather than invertebrates. Nigoyi and Wood (2004) proposed that Mg would likely only be important in the mitigation of toxicity to invertebrates rather than fish, based on the findings of Pane et al. (2003 a,b)

that in short-term exposures Ni inhibited Mg uptake in daphnids but was a respiratory toxicant to fish.

DeSchamphelaere and Janssen (2004) and Pane et al. (2003a) found that the influence of various cations on Ni toxicity to *Daphnia magna* decreased with exposure duration. To explain this, Nigoyi and Wood (2003) proposed that high affinity – low capacity sites, which are affected by Ca, are filled first in acute exposures. Low affinity – high capacity sites, which are less affected by Ca, are filled later (chronic exposures). In contrast, Deleebeeck et al. (2007) proposed that the protective effects of hardness ions are more important in chronic than in acute exposures. They noted that since there is a large acute to chronic ratio for Ni toxicity to *Daphnia magna*, the concentrations of Ni associated with chronic toxicity may low enough to bind to high affinity –low capacity sites.
However, the decreased influence of Ca on Ni toxicity to *Hyalella* observed in this study support the Nigoyi and Wood (2003) theory.

Table 5.4: 28-day LC50s (Total Ni, µmol/L) excluding DOC Tests

Test	Medium				7-d LC50s (µmol/L)			14-d LC50s (µmol/L)			21-d LC50s (µmol/L)			28-d LC50s (µmol/L)		
#	Base	pH	Ca	Mg	LC50	lcl	ucl	LC50	lcl	ucl	LC50	lcl	ucl	LC50	lcl	ucl
18	DC	6.4	35	9.0	10	7.2	17									
19	DC	7.4	35	9.0	14	10	29									
20	DC	8.7	35	9.0	4.1	3.0	5.7	1.1	0.6	1.6						
21	MSAM	8.1	9.9	6.9	1.8	1.0	2.7									
22	DC	8	35	9.0	6.2	4.9	8.0	2.1	1.7	2.6						
23	MSAM	7.8	130	7.0	13	10	23	2.2	1.4	3.2	1.6	1.3	1.8			
24	DC	6.4	35	9.0	13	11	18	6.9	5.5	8.5						
25	DC	7.3	35	9.0	10	7.1	15	5.2	4.2	6.4	2.4	2.0	2.7	2.0	1.7	2.5
27	DC	8.4	35	9.0	3.1	2.4	3.9	0.7	0.5	0.8	0.5	0.4	0.6	0.5	0.4	0.6
28	MSAM	8.1	9.9	6.9	3.1	2.3	4.1	1.0	0.8	1.4	0.9	0.7	1.1			
29	DC	8.2	35	9.0	5.5	3.8	10	1.6	1.2	2.1	0.9	0.7	1.2	0.7	0.6	0.9
30	MSAM	8.0	130	7.0	7.3	5.6	9.6	2.7	2.1	3.5	1.9	1.6	2.4			
31	DC	6.7	35	9.0	11	9.8	13	5.9	4.9	7.1	3.3	2.6	4.1			
32	MSAM	7.9	12	7.2	7.8	6.2	12	2.6	2.1	3.1	1.5	1.2	1.8			
33	MSAM	7.8	40	7.2	10	8.1	15	3.8	3.2	4.6	2.1	1.7	2.5			
34	MSAM	8.5	30	7.2	8.8	6.3	21	1.8	1.6	2.0	1.0	0.8	1.3			
35	MSAM	8.1	160	10	16	13	22	1.5	1.0	2.1	0.8	0.5	1.2			
36	DC	8.9	35	9.6	3.8	2.8	7.7	0.9	0.7	1.1	0.6	0.5	0.8	0.5	0.4	0.6
37	MSAM	8	160	26	6.4	4.5	9.4	1.7	1.4	2.0	1.3	1.1	1.6	1.3	1.1	1.6
38	MSAM	8.2	42	22	13	9.7	17	2.7	1.2	7.4	1.6	0.8	3.3	1.1	0.9	1.5
39	MSAM	8.2	190	36	11	8.8	15	3.1	2.4	4.1	1.0	0.7	1.3	0.7	0.5	0.9
40	LH	7.7	14	2.7	5.2	3.8	8.3	1.6	1.3	2.0	1.2	1.0	1.5	1.1	0.9	1.3
41	MSAM	7.9	300	47	32	22	53	6.0	4.6	8.1	3.0	2.3	3.9	1.7	0.3	8.3
42	MSAM	8.1	37	47	24	18	31	3.8	2.9	5.2	1.5	1.1	2.0	1.2	0.9	1.5
43	MSAM	8.8	42	6.5	5.8	0.4	66	1.1	0.8	1.5	0.5	0.4	0.7	0.4	0.3	0.5
44	MSAM	7.9	34	6.9	7.8	6.1	10	2.3	1.8	2.9	1.2	0.9	1.5	0.9	0.7	1.1
50	LH	7.7	10	2.6	1.8	1.6	2.0	0.6	0.5	0.8	0.5	0.4	0.7	0.3	0.3	0.4
51	MSAM	8.1	38	7.2	13	9.7	17	2.7	1.2	7.4	1.6	0.8	3.3	1.1	0.9	1.5

Table 5.5: 28-day LC50s (Ni^{2+}, µmol/L) excluding DOC Tests

Test	Medium				7-d LC50s (µmol/L)			14-d LC50s (µmol/L)			21-d LC50s (µmol/L)			28-d LC50s (µmol/L)		
#	Base	pH	Ca	Mg	LC50	lcl	ucl	LC50	lcl	ucl	LC50	lcl	ucl	LC50	lcl	ucl
18	DC	6.4	35	9.0	8.4	6.1	14									
19	DC	7.4	35	9.0	12	8.3	23									
20	DC	8.7	35	9.0	1.9	1.4	2.7	0.5	0.3	0.7						
21	MSAM	8.1	9.9	6.9	1.3	0.7	2.0									
22	DC	8	35	9.0	4.5	3.5	5.8	1.5	1.2	1.9						
23	MSAM	7.8	130	7.0	11	8.5	20	1.8	1.2	2.7						
24	DC	6.4	35	9.0	11	8.9	15	5.8	4.6	7.1						
25	DC	7.3	35	9.0	7.8	5.8	12	4.2	3.4	5.2	1.9	1.7	2.2	1.7	1.4	2.0
27	DC	8.4	35	9.0	1.8	1.4	2.3	0.4	0.3	0.5	0.3	0.2	0.4	0.3	0.2	0.4
28	MSAM	8.1	9.9	6.9	2.2	1.7	2.9	0.8	0.6	1.0	0.6	0.5	0.8			
29	DC	8.2	35	9.0	3.6	2.5	6.6	1.1	0.8	1.4	0.6	0.5	0.8	0.5	0.4	0.6
30	MSAM	8	130	7.0	6.1	4.7	8.1	2.3	1.8	3.0	1.6	1.3	2.0			
31	DC	6.7	35	9.0	9.5	8.1	11	4.9	4.1	5.9	2.7	2.2	3.4			
32	MSAM	7.9	12	7.2	6.8	5.4	10	2.3	1.9	2.7	1.3	1.1	1.6			
33	MSAM	7.8	40	7.2	8.7	7.2	14	3.4	2.8	4.1	1.8	1.5	2.2			
34	MSAM	8.5	30	7.2	3.9	2.8	9.2	0.8	0.7	0.9	0.5	0.4	0.6			
35	MSAM	8.1	160	10	13	11	18	1.2	0.8	1.7	0.7	0.4	1.0			
36	DC	8.9	35	9.6	1.3	1.0	2.7	0.3	0.2	0.4	0.2	0.2	0.3	0.2	0.1	0.2
37	MSAM	8	160	26	5.1	3.6	7.4	1.4	1.1	1.6	1.1	0.9	1.3	1.1	0.9	1.3
38	MSAM	8.2	42	22	8.8	6.8	12	1.9	0.9	5.2	1.1	0.5	2.3	0.8	0.6	1.0
39	MSAM	8.2	190	36	5.9	4.8	8.1	1.7	1.3	2.2	0.5	0.4	0.7	0.4	0.3	0.5
40	LH	7.7	14	2.7	4.6	3.4	7.4	1.5	1.2	1.8	1.1	0.9	1.3	1.0	0.8	1.2
41	MSAM	7.9	300	47	26	18	43	4.8	3.6	6.5	2.4	1.9	3.1	1.4	0.2	6.6
42	MSAM	8.1	37	47	17	13	22	2.7	2.0	3.7	1.1	0.8	1.4	0.8	0.6	1.1
43	MSAM	8.8	42	6.5	0.7	0.1	8.1	0.1	0.1	0.2	0.1	0.1	0.1	0.0	0.0	0.1
44	MSAM	7.9	34	6.9	6.1	4.7	8.1	1.8	1.4	2.3	0.9	0.7	1.2	0.7	0.5	0.9
50	LH	7.7	10	2.6	1.6	1.4	1.8	0.6	0.4	0.7	0.5	0.4	0.6	0.3	0.2	0.4
51	MSAM	8.1	38	7.2	9.5	7.3	13	2.0	0.9	5.6	1.2	0.6	2.4	0.8	0.7	1.1

Figure 5.10: LC50s (Ni $^{2+}$, µmol/L) versus pH in DC (7d r^2=0.76, p = 0.0006; 14d r^2=0.95 p = 0.00002; 21d r^2 = 0.95 p = 0.003; 28d r^2 = 0.88 p=0.04)

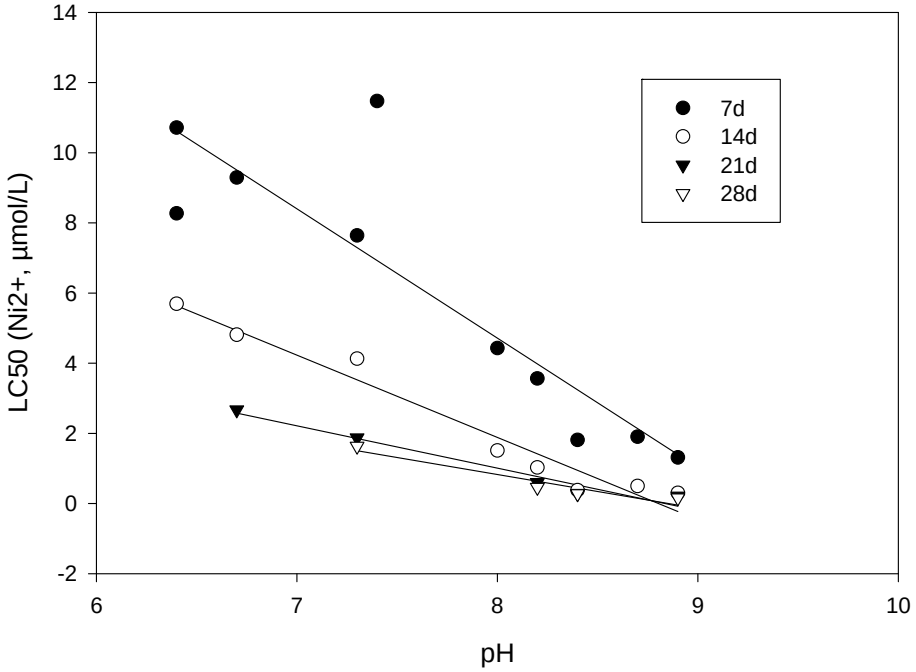


Figure 5.11: LC50s (Ni $^{2+}$, µmol/L) versus Ca (µmol/L) in pH of 7.9 ± 0.2 (7d r^2 = 0.51, p = 0.002; 14d r^2 = 0.24, p = 0.04; 21d r^2= 0.34, p = 0.05; 28d r^2 = 0.55, p = 0.03)

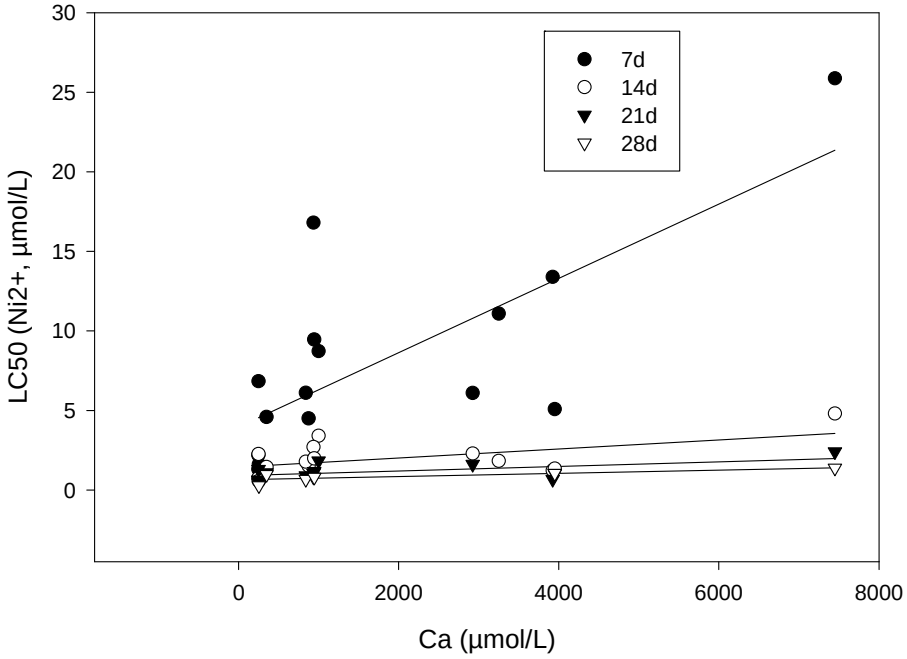
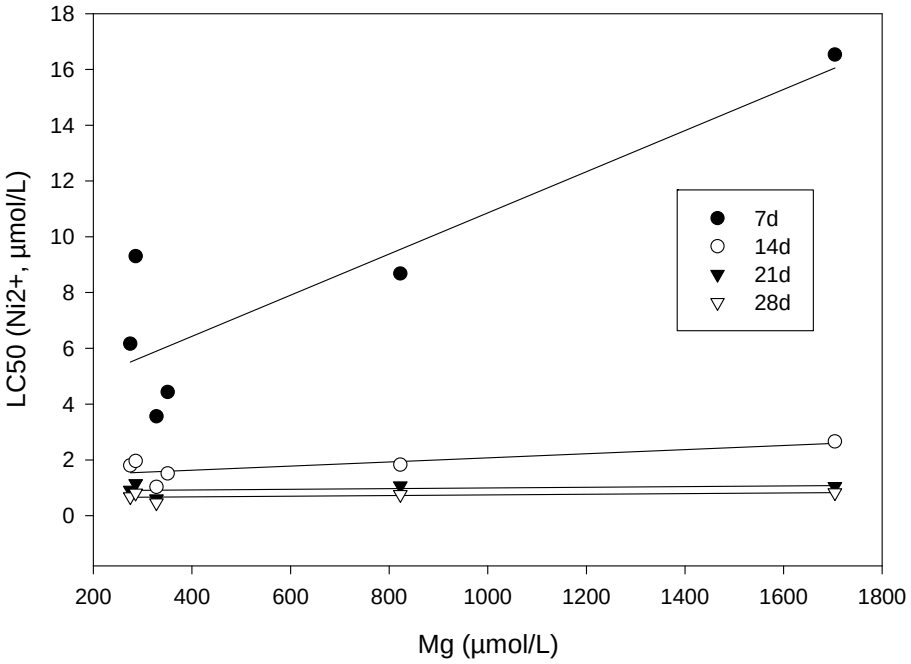


Figure 5.12: LC50s (Ni^{2+}, µmol/L) versus Mg (µmol/L) in exposures of pH 8.0-8.2, Ca ~ 1 mmol/L (7d r^2=0.74, p = 0.02; 14d r^2=0.52 p = 0.07; 21d r^2~0 p = 0.59; 28d r^2~0 p = 0.40).



Meyer (1999) proposed that the slope of ln LC50 to ln hardness of transition metals would be approximately "1" within a hardness range of 20 to 200 mg/L as $CaCO_3$, based on acute lethality tests with fathead minnows. Slopes estimated from linear plots of the seven-day LC50s (as total Ni) to hardness within this range were 0.4 and 0.02 for seven and 28 days, respectively, but increased to 1 and 0.5 when the pH range was restricted from 7.8 to 8.2 (Figures 5.13 and 5.14). The relationship between Ln LC50s and Ln hardness was only reflected in the short- term exposures within a narrow pH range (Figure 5.14). Based on plots of LC50s vs Ca and LC50s vs Mg, it is likely the majority of the influence on toxicity expressed by hardness is due to Ca.

Figure 5.13: LC50s (Total Ni, µg/L) versus Hardness in the Range of 20-200 mg/L as $CaCO_3$ – all pHs (7d r^2=0.069, p = 0.1; 28d r^2~0 p = 0.9)

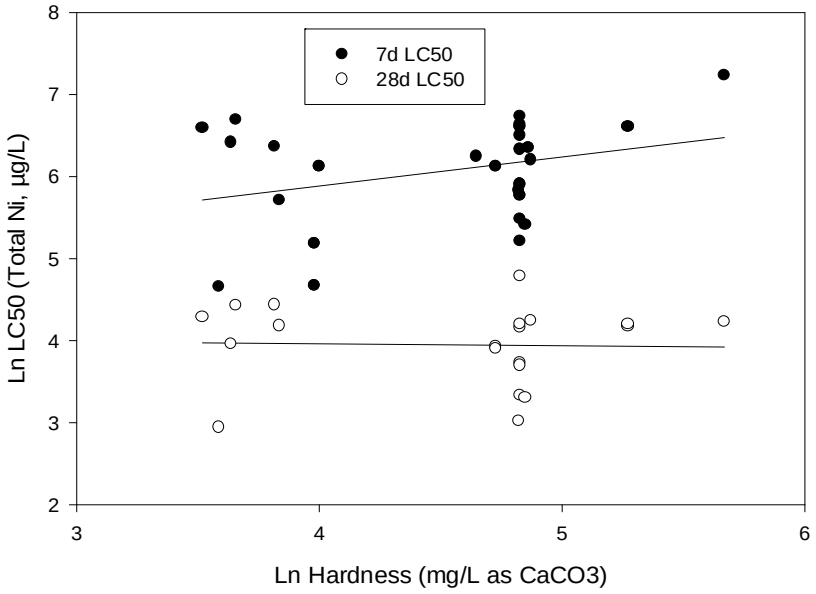


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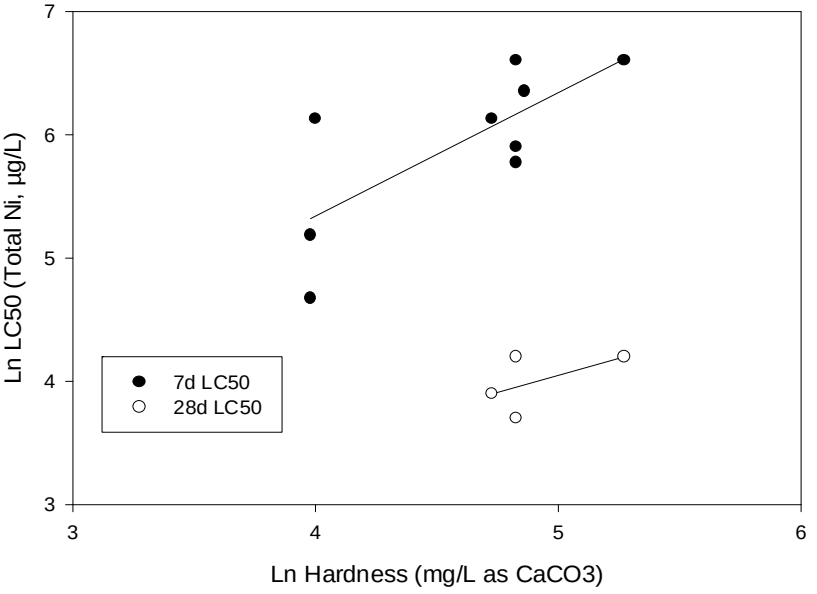
Figure 5.14: LC50s (Total Ni, µg/L) versus Hardness in the Range of 20-200 mg/L as $CaCO_3$ – pHs 7.8 – 8.2 (7d r^2 = 0.48, p = 0.02 slope =1; 28d r^2~0, p = 0.46 slope = 0.5)



5.6 STEP 2C: LONG-TERM TOXICITY TESTS WITH ADDED DOC

5.6.1 Chemistry

The average recovery of nickel in samples collected after solution preparation and after 7 days of exposure was 1.0 and 0.99, respectively, confirming nominal additions and that Ni remained in solution between solution transfers (Appendix B). However, an analysis of filtered test solutions, sub-sampled during transfers on day 7, 14, 21, or 28, indicated that Ni concentrations were reduced relative to total Ni. In the LHM-DOC exposures, dissolved Ni, as a fraction of total Ni, was 0.78 (test 45), 0.56 (test 47), 0.62 (test 48) and 0.44 (test 49) for nominal concentrations of DOC equal to 5, 10, 10 and 20 mg/L, respectively. The dissolved Ni in the MSAM test solution, with a nominal concentration of 5 mg/L DOC (test 46), was 0.85 of the total measured concentration.

Decreases in dissolved Ni concentrations with DOC may reflect adsorption of the metal to fine particulates in the organic material. However, if Ni had adsorbed to the organic material, total Ni concentrations would have been expected to decline after standing for 7 days due to precipitation in the test vessels. Notably, solutions filtered immediately after preparation had lower concentrations of Ni (<50%). However, these analyses were not considered reflective of the exposure conditions since the solutions were left for at least 24 hours (most often 48 to 72 hours) after the addition of Ni before use in testing.

The addition of DOC to LHM and MSAM did not significantly reduce the proportion of Ni^{2+} in solution. The MINTEQA2 model estimated that in LHM solutions, between 86 and

91% of the dissolved Ni concentration was in the free form for the range in DOC tested (tests 45, 47, 48 and 49). The estimated free concentration in the MSAM solutions (nominal DOC of 5 mg/L, test 46) was 73% of the dissolved concentration, compared to 78% without added DOC.

5.6.2 Bioaccumulation

Figures 5.15 and 5.16 show the accumulation of Ni in tissue versus total Ni in solution for the limited tissue data available from the DOC test exposures. Based on estimates from the non-linear regression of $y = a*x/ b+x$, Ni accumulation was decreased by approximately 40 to 50% in LH exposures of 5 to 10 mg/L added DOC (tests 45, 47 and 48), using a total Ni concentration of 1.0 µmol/L (the approximate 28-day LC50) (Figure 5.15). Based on a single data point from the LH exposure with 20 mg/L added DOC (test 49), accumulation appeared to be reduced by 86 %. In the MSAM test, no reduction was apparent by the addition of 5 mg/L DOC and in fact accumulation appeared to be enhanced (Figure 5.16).

Bioaccumulation based on the free ion concentrations was similar to that based on total concentrations, since MINTEQA2 predicted that the vast majority of Ni in the LH medium would be present in the free form, regardless of added DOC. However, in theory, bioaccumulation at the same concentration of Ni^{2+} should be the same regardless of the DOC concentration and, therefore, tissue concentrations should be the same in all solutions of the same Ni^{2+} concentration. If the measured dissolved concentrations are taken into account, the resulting accumulation of free Ni (based on the measured dissolved

concentrations) is more consistent but does not completely resolve the accumulation differences (Figures 5.17 and 5.18).

Figure 5.15: Ni in Tissue (NiT) versus Total Ni in LHM with Added DOC (test 40 (LH): r^2 = 0.97, p=0.001; test 45 (LH-SR5): r^2 = 0.87, p = 0.04; test 47 (LH-SR10): r^2 = 0.79, p=0.028; tests 48 (LH-LM10): r2 = 0.97, p = 0.001; and 49 (LH-SR20) – insufficient data for regression).

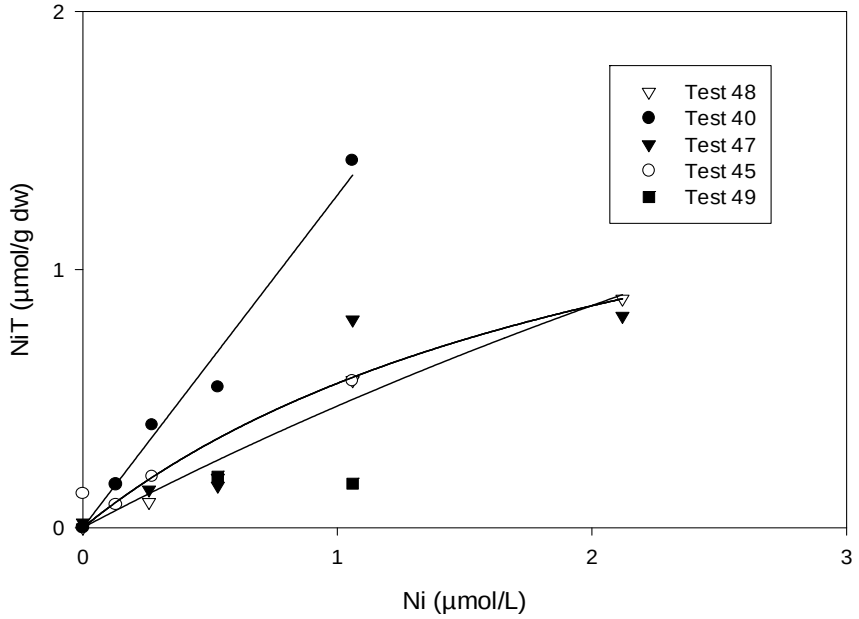


Figure 5.16: Ni in Tissue (NiT) versus Total Ni in MSAM with Added DOC (test 44 (MSAM): r^2 = 0.84, p=0.02; test 46 (MSAM-SR5): r^2 = 0.99, p=0.0002)

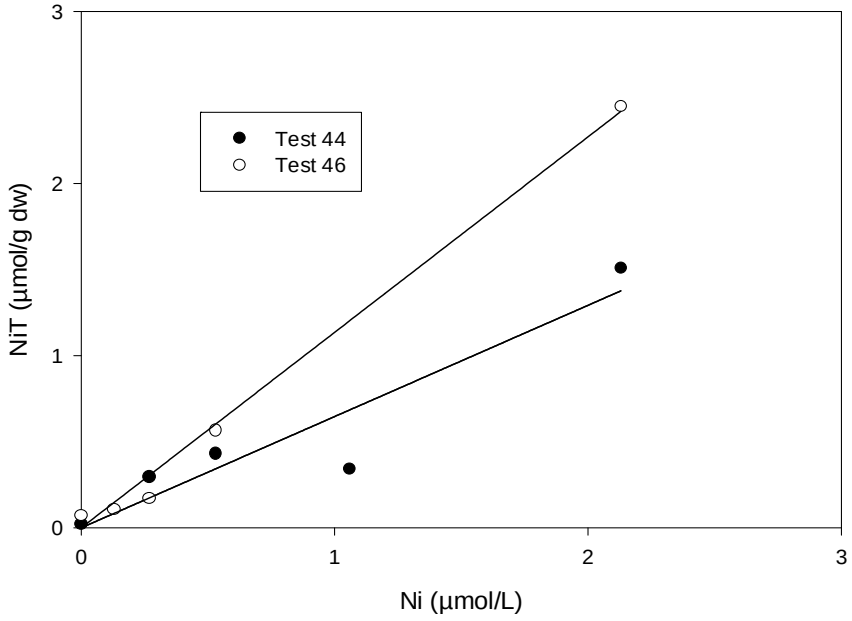


Figure 5.17: Ni in Tissue (NiT) versus Ni^{2+} in LHM with Added DOC (test 40 (LH): r^2 = 0.97, p=0.001; test 45 (LH-SR5): r^2 = 0.87, p = 0.04; test 47 (LH-SR10): r^2 = 0.79, p=0.03; tests 48 (LH-LM10): r^2 = 0.81, p=0.02; and test 49 (LH-SR20) – insufficient data for regression)

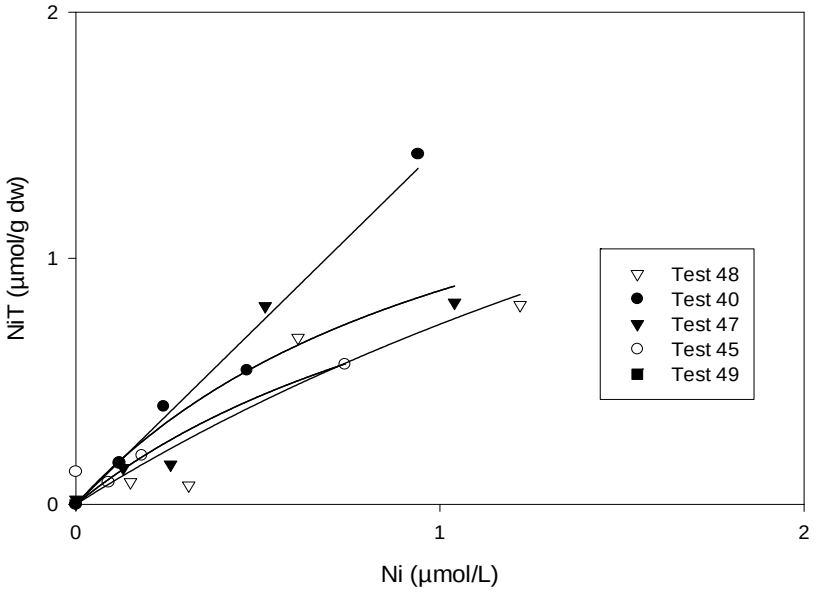
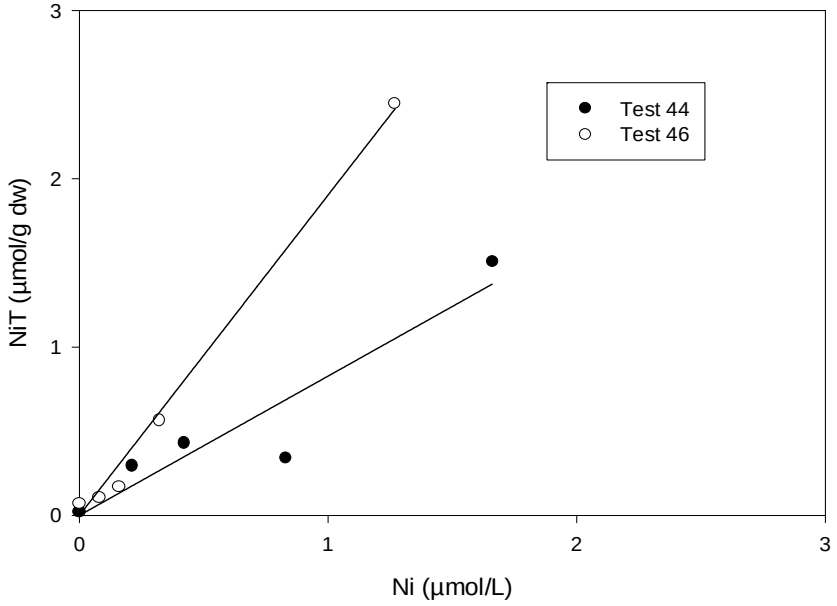


Figure 5.18: Ni in Tissue (NiT) versus Ni^{2+} in MSAM with Added DOC (test 44 (MSAM): r^2 = 0.84, p=0.04; test 46 (MSAM-SR5): r^2 = 0.99, p=0.0003)



Doig and Liber (2007) investigated the speciation of Ni when present with DOC in various ratios. They added increasing amounts of Ni to solutions (similar in hardness, alkalinity and pH to MSAM) containing a constant concentration of DOC (10 mg/L), and measured the concentration of free Ni using a miniature ion exchange technique. The range of Ni concentrations covered the observed seven-day and 28-day LC50s from this study (roughly 1 mM Ni/g DOC and 0.1 mmM Ni/g DOC, respectively). At concentrations above 1 mM Ni/g DOC, neither Suwannee River fulvic acid nor humic acid significantly influenced Ni speciation. However, humic acid was found to reduce the free Ni concentration by roughly 50% at concentrations below 1 mM/g DOC. The Windermere Humic Acid Model (WHAM, Tipping (1998) as cited in Doig and Liber, 2007), used to predict the speciation of Ni, overestimated the free Ni concentration of the humic acid samples at concentrations below those associated with acute toxicity. Doig and Liber (2007) found that the Ni:DOC ratio influences the Ni:DOC stability constant as well and noted findings of other studies that additional factors, such as pH, salinity and competing ion concentrations also influenced the stability constant. They also noted that different sources of fulvic and humic acids are not as important as their concentrations in predicting Ni speciation. However, differences in fulvic and humic acid content within various sources of organic material would hamper comparison and predictability using speciation models.

5.6.3 Toxicity

As shown by LC50s based on total Ni, the addition of DOC to LHM reduced toxicity by between 2 and 2.6 times after seven days of exposure (Table 5.6). This influence was reduced with increasing exposure duration, however, and by 28 days of exposure, no

significant difference was discernable among the LC50s even though accumulation after 28 days was clearly reduced (Fig 5.15). The accumulation of Ni observed after 28 days of exposure was, therefore, consistent with the observed seven-day toxicity but not the 28-day toxicity.

No difference in Ni toxicity was observed between the Suwannee River (SR) and Luther Marsh (LM) DOC, tested with nominal concentrations of 10 mg/L as DOC (Table 5.6). This is consistent with Doig and Liber (2006) finding that at concentrations approximating those of North American surface waters, the ratio of Ni to DOC was important in determining bioavailability and toxicity rather than the absolute concentration or type. The one concentration of DOC tested in MSAM (5 mg/L as DOC) showed no mitigation of Ni toxicity compared with a MSAM test without added DOC. This was consistent with the observed accumulation (Table 5.6).

Since dissolved organic material complexes rather than competes with Ni, LC50s based on free Ni concentrations are expected to be the same in the same media. However, the relative difference among LC50s of the same exposure duration (based on the ratio of maximum to minimum LC50) was decreased in LHM only in the seven-day exposures and in MSAM only in the 21- and 28-day exposures (Tables 5.6 and 5.7).

5.7 LA50s

For the 28-day toxicity tests in which Ni accumulation was measured, nine had sufficient Ni data for calculation of 28-day LA50s (Table 5.8). The mean LA50 was calculated as 0.91 μmol/g dw, with a ratio of maximum to minimum of 4. DOC test data were included with the other tests since the LA50 is derived from tissue concentrations and does not consider speciation in solution. The corresponding range in LC50s was 35. If DOC tests are omitted, the mean LA50 is reduced to 0.81, and the ratio of maximum to minimum is reduced to 2. The corresponding ratio of maximum to minimum LC50s remains the same. These findings support those of Meyer et al. (1999) who compared accumulation of Ni in fathead minnow gills after 24-hours exposure to 96-hr toxicity tests. They found that the 96-hr Ni^{2+} LC50 increased 7-fold as the concentration of Ca increased whereas the LA50 increased only 2-fold and concluded that accumulated Ni was a reliable predictor of acute toxicity in media of increasing Ca.

Table 5.6: 28-day LC50s (Total Ni, µmol/L) from Tests with Added DOC

			LC50s - Total Ni (µmol/L)											
Test	**DOC (mg/L)**	**pH**	**7-day**	**lcl**	**ucl**	**14-day**	**lcl**	**ucl**	**21-day**	**lcl**	**ucl**	**28-day**	**lcl**	**ucl**
40	0 (LH)	7.7	5.2	3.8	8.3	1.6	1.3	2.0	1.2	1.0	1.5	1.1	0.9	1.3
45	5 (LH-SR)	7.7	10	4.9	33	2.7	2.2	3.5	1.2	1.0	1.6	0.9	0.7	1.2
47	10 (LH-SR)	7.7	12	9.4	17	2.4	2.0	2.9	1.4	1.2	1.7	1.2	1.0	1.5
48	10 (LH-LM)	8	9.9	6.9	15	1.9	1.5	2.4	1.6	1.3	2.0	1.4	1.2	1.8
49	20 (LH-SR)	7.4	14	10	20	2.9	2.3	3.8	1.7	1.3	2.3	1.4	1.1	1.9
44	0 (MSAM)	7.9	7.8	6.1	10	2.3	1.8	2.9	1.2	0.9	1.5	0.9	0.7	1.1
46	5 (MSAM-SR)	8.1	8.4	6.3	12	2.1	1.7	2.7	1.7	1.3	2.1	1.2	1.0	1.5

LH = LH medium; SR = Suwannee River; LM = Luther Marsh; MSAM = modified standard artificial medium; lcl/ucl = lower/upper 95% confidence limits

Table 5.7: 28-day LC50s (Ni^{2+}, µmol/L) from Tests with Added DOC (based on measured dissolved concentrations)

			LC50s –Ni^{2+} (µmol/L)											
Test	**DOC**	**pH**	**7-day**	**lcl**	**ucl**	**14-day**	**lcl**	**ucl**	**21-day**	**lcl**	**ucl**	**28-day**	**lcl**	**ucl**
40	0 (LH)	7.7	4.6	3.4	7.4	1.5	1.2	1.8	1.1	0.9	1.3	1.0	0.8	1.2
45	5 (LH-SR)	7.7	7.3	3.4	23	1.9	1.6	2.4	0.9	0.7	1.2	0.6	0.5	0.8
47	10 (LH-SR)	7.7	6.0	4.6	8.3	1.2	1.0	1.4	0.7	0.6	0.8	0.6	0.5	0.7
48	10 (LH-LM)	8	5.7	4.0	8.6	1.1	0.9	1.4	0.9	0.8	1.2	0.8	0.7	1.0
49	20 (LH-SR)	7.4	5.5	4.1	8.0	1.2	0.9	1.5	0.7	0.5	0.9	0.6	0.4	0.8
44	0 (MSAM)	7.9	6.1	4.7	8.1	1.8	1.4	2.3	0.9	0.7	1.2	0.7	0.5	0.9
46	5 (MSAM-SR)	8.2	5.2	3.9	7.4	1.3	1.0	1.7	1.0	0.8	1.3	0.7	0.6	0.9

LH = LH medium; SR = Suwannee River; LM = Luther Marsh; MSAM = modified standard artificial medium; lcl/ucl = lower/upper 95% confidence limits

Table 5.8: Observed 28-day LA50s (µmol/g dw) and LC50s (Ni^{2+}, µmol/L)

	Observed LA50s			Observed LC50s		
Test	**LA50**	**lcl**	**ucl**	**LC50**	**lcl**	**ucl**
39	0.7	0.39	1.4	0.36	0.25	0.49
41	1.1	0.65	1.6	1.4	0.24	6.6
43	0.51	0.41	0.68	0.04	0.03	0.06
44	0.72	0.53	0.94	0.68	0.52	0.88
46 (SR)	1.3	1	1.8	0.74	0.59	0.91
47 (SR)	0.61	0.5	0.74	0.6	0.5	0.73
48 (LM)	0.8	0.44	2.8	0.83	0.66	1.0
49 (SR)	1.9	0.74	18	0.58	0.44	0.76
51	1.2	0.96	1.6	0.82	0.66	1.1
Mean including DOC	0.91			-		
Max/min including DOC	4			35		
Mean excluding DOC	0.81					
max/min excluding DOC	2			35		

Highlighted text indicates Tests with Added DOC; SR = Suwanee River, LM = Luther Marsh; ucl/lcl = upper/lower 95% confidence limits

5.8 SUMMARY OF KEY FINDINGS

- *Short-term Tests:*
    - Ni accumulation (in adults) and toxicity (to young) varied with increasing Ni and was mitigated by Ca and H^+. It is possible that Mg also mitigates Ni accumulation but only at very high concentrations and in low concentrations of Ca. Mg did not reduce toxicity in seven-day tests in which Ca was ~1mmol/L.
    - Steady-state whole-body concentrations of Ni were achieved within 48 hours of exposure.

- Acclimation to the hardness, alkalinity and/or pH conditions of the exposure medium before testing did not influence the short-term bioaccumulation or toxicity of Ni, suggesting that organisms need not be acclimated to different exposure media before seven-day tests.

- *Long-term Tests*:
  - Long-term tests supported the results of short-term tests, in that toxicity and bioaccumulation were influenced by Ca and H^+. However, based on comparisons of slopes, Ca's influence appeared to decrease after seven days of exposure and the influence of pH appeared to decrease after 14 days of exposure although not to the same degree as the decrease in Ca's influence.
  - The addition of DOC to test media reduced toxicity after 7 days and bioaccumulation after 28 days. However, toxicity was not reduced after 28 days exposure, based on overlapping confidence limits of the total Ni LC50s.
  - Bioaccumulation based on Ni^{2+} in solution was similar to that based on total concentrations since MINTEQA2 predicted that the vast majority of Ni would be present in the free form, regardless of added DOC. However, in theory, bioaccumulation at the same concentration of Ni^{2+} should be the same regardless of the DOC concentration and, therefore, tissue concentrations should be the same in all solutions of the same Ni^{2+} concentration (assuming all other solution characteristics are the same).
  - Mean 28-day LA50s were 0.91 and 0.81 μmol/g dw for tests including and excluding DOC, respectively. The range in LA50s (4 times and 2 times,

respectively) was an order of magnitude lower than that of the LC50s (35 times) calculated as either total or free Ni.

6.0 NICKEL – STEP 3: MODEL DEVELOPMENT

6.1 SHORT-TERM BIOACCUMULATION TESTS

6.1.1 Preliminary Estimates of L_T

Preliminary estimates for L_T were calculated from linear transformations of the uptake model as 2.6, 3.1, 2.4 and 1.2 for the LH (tests 2 and 17), MSAM (test 1) and LH-High Ca (test 3) exposures, respectively (Figures 6.1 to 6.4). The similarity in L_T estimates (based on comparison slopes of the regressions of Ni/NiL vs Ni) in the DC and LH exposures supported the competitive model. However, the reduced apparent L_T (or elevated slope) in the high Ca exposure (test 3) does not support the competitive model and may suggest a different mechanism depending on the concentration of Ca (Appendix B). Of note is that the estimated L_Ts were similar to the LA50s approximated from the limited tissue data in tests 1 and 2 but the LA50s in those tests were estimated from concentrations at which accumulation had reached a plateau and therefore, their LA50s may be over-estimated. From the intercepts, preliminary estimates for log K_{Ni} (conditional values) were also calculated as 5.9, 4.9, 5.9 and 5.3 for LH (test 2 and 17), MSAM (test 1) and LH-high Ca (test 3) exposures, respectively.

Figure 6.1: Test 1 (MSAM), Ratio of Ni in Water / Ni in Tissue (Ni/NiT, g tissue dw/L) versus Ni^{2+} in Water (µmol/L) (r^2 = 0.87, p=<0.00001). Regression line: y = 0.42x + 0.56

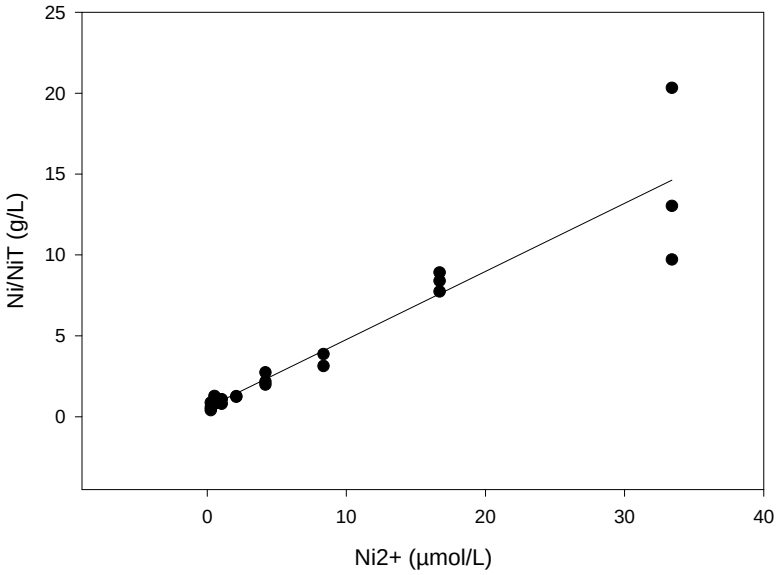


Figure 6.2: Test 2 (LHM), Ratio of Ni in Water / Ni in Tissue (Ni/NiT, g tissue dw/L) versus Ni^{2+} (µmol/L) (r^2 = 0.92, p=<0.00001). Regression line: y = 0.38x + 0.48.

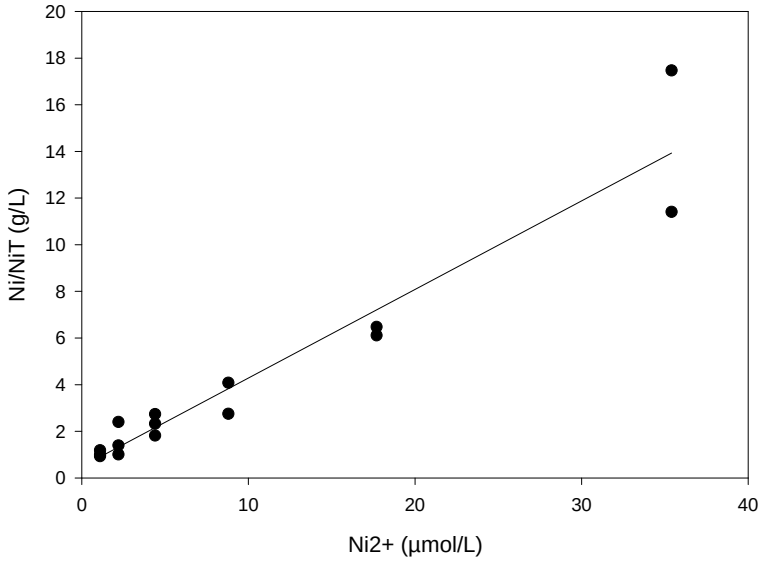


Figure 6.3: Test 17 (LHM), Ratio of Ni in Water / Ni in Tissue (Ni/NiT, g tissue dw/L) versus Ni^{2+}(µmol/L) (r^2 = 0.66, p=<0.00001). Regression Line: y = 0.32x + 4.4.

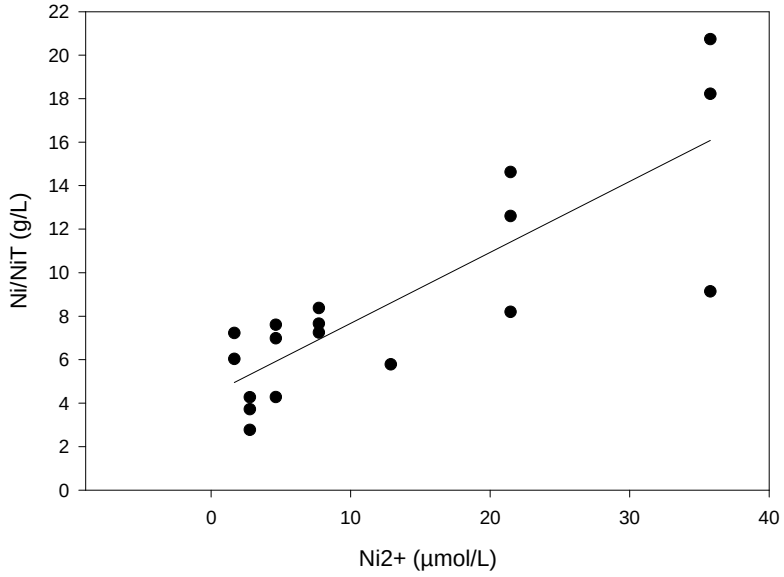
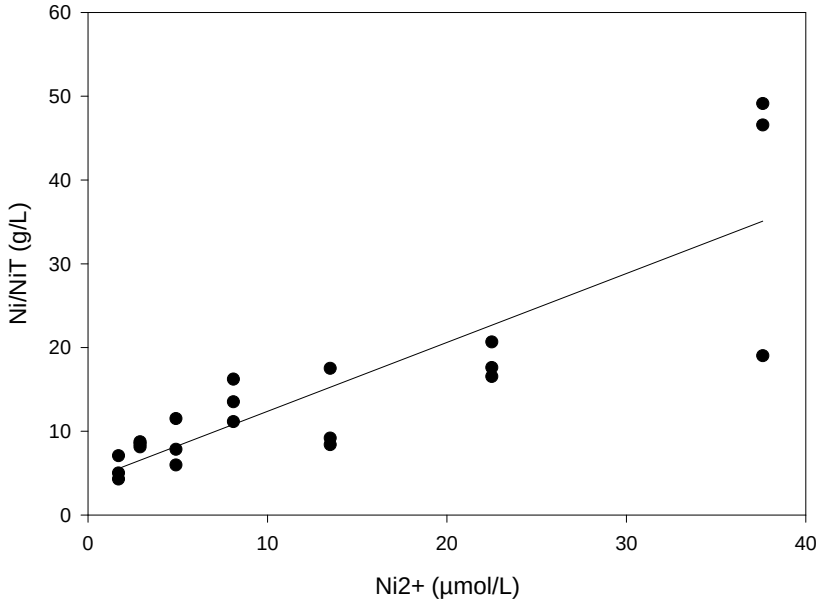


Figure 6.4: Test 3: (LHM-HCa), Ratio of Ni in Water / Ni in Tissue (Ni/NiT, g tissue dw/L) versus Ni^{2+} (µmol/L) (r^2 = 0.70, p=<0.00001). Regression line: y = 0.82x + 4.4.



6.1.2 Defining Models

Based on the accumulation observed in the seven-day bioaccumulation tests, a competitive model for the uptake of Ni was developed, including Ni^{2+}, Ca^{2+} and H^{+}:

$$NiT = \frac{K_{Ni}*Ni*L_T}{1+ K_{Ni}*Ni + K_{Ca}*Ca + K_H*H}$$

This model assumed a constant L_T in spite of the indications that L_T was influenced by Ca. L_T may change and thereby influence the prediction of LA50s; however, if the proportion of LA50/L_T is constant, the predicted LC50 should not be affected.

The model was log transformed to equalize the variance and fitted to the bioaccumulation data using Systat®11 as the following expression (as described in Section 4.7):

$$\text{Log (NiT)} = \text{Log [Ni / } (a + b*\text{Ca} + c*\text{H} + IL_T*\text{Ni})]$$

where:

NiT = whole body concentration of Ni (µmol/g dw)

$a = 1/(K_{Ni}*L_T)$

$b = K_{Ca}/(K_{Ni}*L_T)$

$c = K_H/(K_{Ni}*L_T)$

$IL_T = 1/ L_T$

Multiple regressions were run with sequential addition of the other cations. As described by Borgmann et al. (2004), the log values and their 95% confidence limits were obtained

using the "funpar" (function of the parameter) command, providing log-based confidence limits consistent with log transformation of the bioaccumulation data.

When data from all tests were included, no model fitted the data well (all r^2 values ≤ 0.4). Therefore, those tests varying cations without significant effect on Ni accumulation (determined by non-linear regression) were omitted to assess whether Ni, Ca, and H^+ would be sufficient to develop a predictive model. Mg was also omitted since its inclusion reduced the fit of the model and since the weight of toxicity and bioaccumulation data indicated that this cation did not influence bioaccumulation or toxicity, with the potential exception at concentrations above 1000 μmol/L and in low concentrations of other cations.

Significantly better fits were obtained using only those tests in which Ni^{2+} was varied (tests 1, 2, 3, 5 and 17). Although a single concentration of total Ni was used in test 5 (varying pH), predicted free concentrations at the pHs tested produced a range of free Ni concentrations. Estimates of *b*, *c* and ILT were determined from Systat®11 for the various iterations. However, no positive estimate for *a* could be obtained for any model that included the competitive effect of Ca. The three best fitting models are described below and summarized in Table 6.1.

Model A: In model A, *a* was set equal to zero. If *a* is zero, then it may be assumed that virtually all the ligand is bound up by one or more of the competing cations. That is,

$L_T = L + NiL + CaL + HL$ becomes $L_T \sim NiL + CaL + HL$, and

$L_T \sim K_{Ni} * Ni * L + K_{Ca} * Ca * L + K_H * H * L$

$$\sim L(K_{Ni}*Ni + K_{Ca}*Ca + K_H*H)$$

The resulting model is:

$$Log\ (NiT) \sim Log\ [Ni / (b*Ca + c*H + IL_T*Ni)] \qquad \text{(model A)}$$

Model B: To account for the potential influence of Ca on L_T in addition to competition with Ni for binding, the above BLM-style expression was modified to:

$$Log\ (NiT) \sim Log\ [Ni / ((b'*Ca + c'*H + IL_{T0}*Ni)(1 + b2*Ca))] \qquad \text{(model B)}$$

Here Ca is included as both a competitor and as either a non-competitive modulator or an inhibitor of ligand synthesis and b2 represents the binding strength of Ca to a hypothetical enzyme involved in ligand synthesis. If Ca binds to the enzyme, it is no longer available for ligand synthesis. Appendix C provides further details regarding the derivation of b2. Model B provided a better fit of the data than model A (r^2 of 0.59 versus 0.52). Consequently, the constants *b* and *c* are now replaced by *b'* and *c'* where *b'* = $K_{Ca}/(K_{Ni}*L_{T0})$ and *c'* = $K_H/(K_{Ni}*L_{T0})$.

The reduction in estimated L_T values from the preliminary plots of Ni/NiT to Ni as well as findings of several authors (below) support a physiological effect of Ca on the ligand (model B). Paquin et al. (2002) described a hypothesis for Ca mitigation of silver toxicity to fish, not only by competition for uptake at the gill but also by "tightening" the gill epithelium, thereby reducing loss of Na and Cl via paracellualer pathways. Nigoyi and Wood (2003) proposed that the branchial transport of Ca is up-regulated in Ca poor waters, resulting in an increase in binding sites over that observed in waters of higher Ca

concentration. Additionally, in their 2004 paper, Nigoyi and Wood summarized findings from different studies that Ca and Mg decreased ionic losses at the gill by stabilizing the paracellular junctions in the gill epithelium (Nigoyi and Wood, 2004). This is supported by several additional studies cited by Deleebeeck et al. (2007), which suggested that the protective effect of Ca was due to it stabilizing effect on membrane permeability.

Model C: By excluding the competitive effect of Ca and including its effect on the total ligand, a positive estimate for *a'* (*a'* = $1/(K_{Ni}*L_{T0})$. The fit of this non-competitive model was not as good as when both competitive and non-competitive action were included (0.56 for Model C vs 0.59 for Model B).

$$\text{Log (NiT)} = \text{Log [Ni / ((}a\text{'} + c\text{'}*\text{H} + IL_{T0}*\text{Ni})(1 + b2*\text{Ca }))] \qquad \text{(model C)}$$

Table 6.1: Short-term Bioaccumulation Model Outputs and 95% Confidence Limits

Constant	Model A ($r^2 = 0.52$)	Model B ($r^2 = 0.59$)	Model C ($r^2 = 0.56$)
a'	-	-	0.313392 (0.070396, 0.556387)
b	0.000824 (0.000490, 0.001158)	-	-
b'	-	0.000367 (0.000069, 0.000666)	-
c	18.16 (6.848, 29.47)	-	-
c'	-	20.62 (10.39, 30.85)	16.69 (6.701, 26.68)
IL_T	0.501056 (0.407947, 0.594164)	-	-
IL_{T0}	-	0.416730 (0.328384, 0.505075)	0.356026 (0.000221, 0.000789)
b2	-	0.000258 (0.000043, 0.000473)	0.000505 (0.000221, 0.000789)

Note: Ni, H and Ca = µmol/L; L_T = µmol/g dw

L_T (total ligand) and L_{T0} (total ligand as influenced by Ca, equal to L_T when Ca = 0)) were calculated from Table 6.1 as 2.0, 2.4 and 2.8 for models A, B, and C, respectively, and were consistent with those estimated from linear regressions of the MSAM and LHM experiments (Section 6.1).

Binding constants could also be estimated from the model constants. From model C, log K_{Ni} and log K_H were calculated as 6.1 and 7.7, respectively, from *a'*, *c'* and IL_{T0}. These constants should be valid, if the model is correct, but they might differ somewhat from what would be calculated if it were assumed that L_T is constant. Compared to estimates reported by Nigoyi and Wood (2004) for the fish gill, the log K_H is similar (7.7 versus 7.5) but the log K_{Ni} estimate is 100 times higher (6.1 versus 4.0). The log K_H values from this study are higher than those estimated by De Schamphelaere and Janssen (2004) from chronic *Daphnia magna* exposures to copper (6.1 to 6.7) in exposures of pH 6.8. The lower K_H may reflect binding of H^+ to both low affinity and high affinity sites, as noted by Playle (1993) for exposures to high concentrations of metals.

Because no other positive estimate for "a" was obtained in any of the other models, binding constants could only be estimated as ratios (i.e., 1: K_{Ca}/K_{Ni}, 2: K_H/K_{Ni} and 3: K_{Ca}/K_H). From model A, the ratios 1, 2 and 3 were estimated as 1.64×10^{-3}, 36.2, and 4.54×10^{-5}, respectively. Similarly, ratios 1, 2 and 3 were estimated from model B as 8.81×10^{-4}, 49.5 and 1.78×10^{-5}. From model C, ratio 2 was calculated as 46.88. The ratios of K_H to K_{Ni} are significantly smaller than the corresponding ratio that can be calculated from Nigoyi and Wood (2004) estimates of K_H and K_{Ni} (~3200). Deleebeeck et al. (2007) reported estimated log values for K_{Mg} and K_{Ca} from chronic exposures of cladocerans to Ni. Estimates for both

Ca and Mg were identical and ranged from 3.5 to 4.7, depending on the model used. If the log K_{Ni} and log K_H from model C are used as starting points, the log K_{Ca} can be estimated as 3.7 and 3.0, for models A and B, respectively.

6.2 LONG-TERM TOXICITY TESTS

Estimates for a, b, c, ILT and b2 from the short-term bioaccumulation tests were used as starting points in modelling the LC50 test data in the same manner as described for the short-term bioaccumulation tests. Data from DOC tests were not included in this step of the model development since Ni speciation in the presence of the added organic material was not resolved by the MINTEQA2 model. Also, the data from test 43 (high alkalinity) were also omitted due to questionable test solution chemistry.

Non-blank corrected tissue data were used since some correction for blanks resulted in negative tissue concentrations. Additionally, the data were screened to remove outliers prior to modelling in Systat. Outliers were visually identified from plots of the Ni in tissue against Ni in water, and indicated contamination in the analysis vials.

As was found for the bioaccumulation tests, the best fit of the data was in Models A, B and C. However, different estimates for the constants were obtained as shown in Table 6.2. Models derived from the long-term toxicity tests using young were identified as A(Y), B(Y) and C(Y) to distinguish them from the models developed from the adult data.

From the IL_T values in Table 6.2, L_T and L_{T0} were calculated as 3.5, 4.0 and 7.2 µmol/g dw for models A(Y), B(Y) and C(Y), respectively, higher than those obtained from tests with adults (Table 6.3). These estimates and the estimates derived from the adult tests were used in subsequent steps of model development to determine which estimates and models predicted tissue uptake and toxicity.

Table 6.2: Model Outputs and 95% Confidence Limits for the Long-term Toxicity Tests, excluding DOC

Constant	Model A(Y) (r^2 = 0.69)	Model B(Y) (r^2 = 0.70)	Model C(Y) (r^2 = 0.71)
a'	-	-	0.047364 (-0.021838, 0.116567)
b	0.000117 (0.000048, 0.000186)	-	-
b'	-	0.000058 (-0.000036, 0.000151)	-
c	38.68 (19.75, 57.61)	-	-
c'	-	40.82 (23.19, 58.46)	39.12 (21.51, 56.74)
IL_T	0.288931 (-0.038003, 0.615865)	-	-
IL_{T0}	-	0.250627 (-0.022922, 0.524177)	0.139683 (-0.069268, 0.348633)
b2	-	0.000092 (-0.000092, 0.000275)	0.000289 (0.000049, 0.000530)

Note: Ni, H and Ca = µmol/L; L_T = µmol/g dw

Table 6.3: Comparison of L_Ts and L_{T0}s (µmol/g dw) Estimated by Adult and Young Models

seven-day Adult Data	Model A	Model B	Model C
L_T or L_{T0}	2.0	2.4	2.8
28-day Young Data	**Model A(Y)**	**Model B(Y)**	**Model C(Y)**
L_T or L_{T0}	3.5	4.0	7.2

Estimated Log K values for Ni and hydrogen from model C(Y) were higher than those estimated from model C (6.5 versus 6.1 and 8.9 versus 7.7, respectively). From model A(Y), the ratios of K values for 1: Ca to Ni, 2: H^+ to Ni and 3: Ca to H^+ were estimated as $4.05x10^{-4}$, 134 and $3.02x10^{-6}$, respectively, and ratios 1, 2 and 3 were estimated from model B(Y) as $2.31x10^{-4}$, 163 and $1.42x10^{-6}$, respectively. From model C(Y), ratio 2 was calculated as 280. Although larger than the ratios calculated from the adult data, the ratios of K_H to K_{Ni} from the young models were still significantly smaller than corresponding ratio calculated from Nigoyi and Wood (2004) estimates of K_H and K_{Ni} (~3200).

6.2.1 Prediction of LA50s

Estimates from exposures with pH less than 7 consistently gave very low estimates of the LA50 compared to the other tests. Therefore, those predicted LA50s were excluded from the mean predicted LA50. It is likely that below pH 7, H^+ exerts a toxic effect on *Hyalella*, confounding the inclusion of this proton as a protective competitor in the models. Another possibility is that increased CO_2 at lower pH may have made the organisms more sensitive to Ni.

28-Day Data

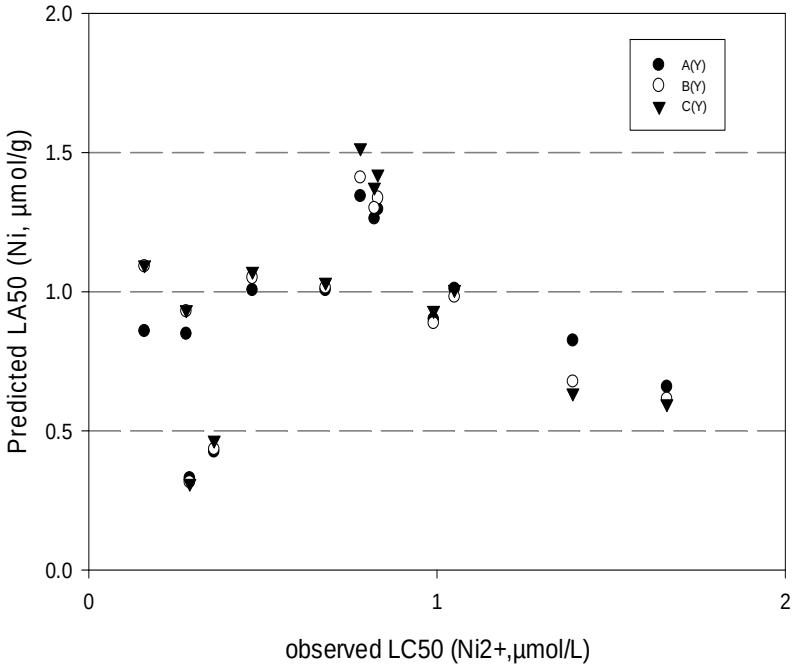
The mean predicted LA50s from all models were within 10% of the observed mean LA50 of 0.91 for all tests, and within 7% of the observed mean LA50 of 0.81 for those tests excluding added DOC. As shown in Table 6.4 and Figure 6.5, the mean LA50s were less variable than the corresponding LC50s.

Table 6.4: Predicted Mean 28-day LA50s (µmol/g dw) from Models A(Y), B(Y) and C(Y) using Toxicity Data from Tests with Young

	LA50s Model A(Y)	LA50s Model B(Y)	LA50s Model C(Y)	LA50s Observed	LC50s Observed
Include DOC					
mean	0.82	0.83	0.85	0.91	na
max/min	4.1	4.5	4.9	4	42
Exclude DOC					
mean	0.84	0.85	0.87	0.81	na
max/min	4.1	4.5	4.9	2	42

na = not applicable

Figure 6.5: Model-Predicted 28-day LA50s (µmol/g dw) from 28-day LC50 Data (µmol/L), from Tests Without Added DOC



Of the nine 28-day toxicity tests with a calculated (observed) LA50, the models predicted LA50s within a factor of two of the all tests except one, test 49 (20 mg/L DOC added to LH) (Table 6.5).

Table 6.5: Predicted vs Observed LA50s (Ni, µmol/g dw) from Long-term Toxicity Tests

	Observed LA50s			predicted LA50s		
Test	**LA50**	**lcl**	**ucl**	**A(Y)**	**B(Y)**	**C(Y)**
39	0.70	0.39	1.4	0.42	0.43	0.47
41	1.1	0.65	1.6	0.82	0.68	0.64
43	0.51	0.41	0.68	0.26	0.33	0.29
44	0.72	0.53	0.94	1.0	1.0	1.0
46 (SR)	1.3	1.0	1.8	1.3	1.4	1.5
47 (SR)	0.61	0.5	0.74	0.62	0.60	0.62
48 (LM)	0.80	0.44	2.8	1.2	1.3	1.4
49 (SR)	1.9	0.74	18	0.40	0.38	0.39
51	1.2	0.96	1.6	1.26	1.3	1.4

Shading represents tests with added DOC; lcl = lower 95% confidence limit; ucl = upper 95% confidence limit
SR = Suwanee River; LM = Luther Marsh

6.2.2 Prediction of LC50s

By rearranging the uptake models to solve for Ni at the NiT at which 50% mortality occurs, LC50s could be predicted:

$$[Ni^{2+}] = ([NiT]*(b*[Ca] + c*[H]) / (1-([NiT]*[IL_T]) \quad A(Y)$$

$$[Ni^{2+}] = \frac{([NiT]*(b'*[Ca] + c'*[H] + b2*[Ca]*b'*[Ca] + b2*[Ca]*c'*[H])}{(1- ([NiT]*(IL_{T0} + b2*[Ca]* [IL_{T0}]))} \quad B(Y)$$

$$[Ni^{2+}] = \frac{([NiT]*(a' + c'*[H] + b2*[Ca]*a' + b2*[Ca]*c'*[H])}{(1- ([NiT]*([IL_{T0}] + b2*[Ca]* [IL_{T0}])))} \quad C(Y)$$

where:

[NiT] = Observed mean LA50 (μmol/g)

$[Ni^{2+}]$ = predicted LC50 (μmol/L)

The observed mean LA50, including DOC data, was used to predict LC50s by substituting the LA50 for NiT in models A(Y), B(Y) and C(Y). All LC50s except three (39, 49 and 50) were predicted within a factor of two of the observed (Table 6.6). The lower observed versus predicted LC50 in test 39 (all MSAM salts elevated by ~5 times) suggested that the elevated salts in the test medium contributed to toxicity. Since the model only accounts for toxicity due to Ni, the test conditions of test 39 are likely outside the range of the model. The poor predictability of Test 49, with 20 mg/L added DOC, reflects problems with MINTEQA2, rather than the toxicity model. Finally, test 50 was conducted in identical conditions as test 40, for which toxicity was well predicted. Therefore, the poor predictability of test 50 could also reflect a problem with the test rather than the model.

Figures 6.6 and 6.7 present plots of the predicted versus observed LC50s, with and without tests with added DOC. The three parallel lines in the figure represent the line of perfect

agreement (slope of 1 through the origin), bounded by the upper and lower limits of 2x and ½ of the line of perfect agreement. All three models produced similar LC50 estimates, preventing differentiation between competitive and non-competitive action of Ca in long-term exposures.

Table 6.6: Predicted versus Observed 28-day LC50s (Ni^{2+}, µmol/L)

				Observed	Predicted LC50		
Test	**pH**	**Ca**	**Mg**	**LC50**	**A(Y)**	**B(Y)**	**C(Y)**
25	7.3	830	350	1.7	2.5	2.7	2.7
27	8.4	820	350	0.28	0.31	0.27	0.27
29	8.2	750	330	0.47	0.41	0.39	0.39
36	8.9	780	360	0.16	0.17	0.13	0.13
37	8.1	3700	1000	1.1	0.91	0.94	0.91
38	8.2	950	820	0.78	0.44	0.41	0.41
39	8.2	4300	1400	0.36	0.92	0.94	0.84
40	7.7	340	100	0.99	1.0	1.0	0.96
41	7.9	6800	1800	1.4	1.6	2.2	2.4
42	8.1	800	1700	0.83	0.49	0.48	0.48
44	8	800	280	0.68	0.59	0.59	0.58
45	7.7	270	100	0.63	0.99	1.0	0.94
46	8.2	960	280	0.74	0.44	0.41	0.41
47	7.7	230	97	0.6	0.99	1.0	0.93
48	8	340	100	0.83	0.53	0.52	0.51
49	7.5	260	110	0.57	1.6	1.6	1.5
50	7.7	250	110	0.29	0.99	1.0	0.93
51	8.1	900	290	0.82	0.51	0.49	0.49

Shading represents tests with added DOC

Figure 6.6: Predicted versus Observed 28-day LC50s (Ni^{2+}, µmol/L), including DOC Tests

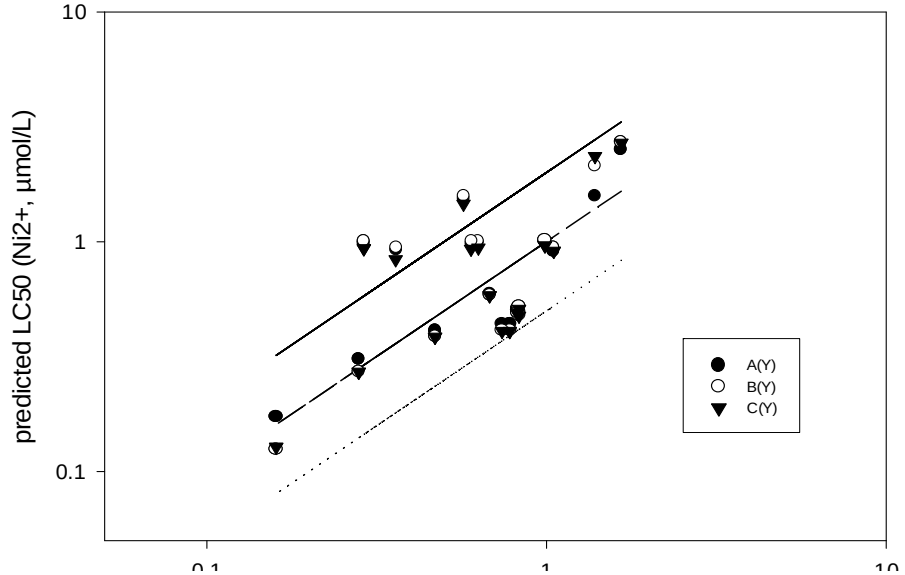
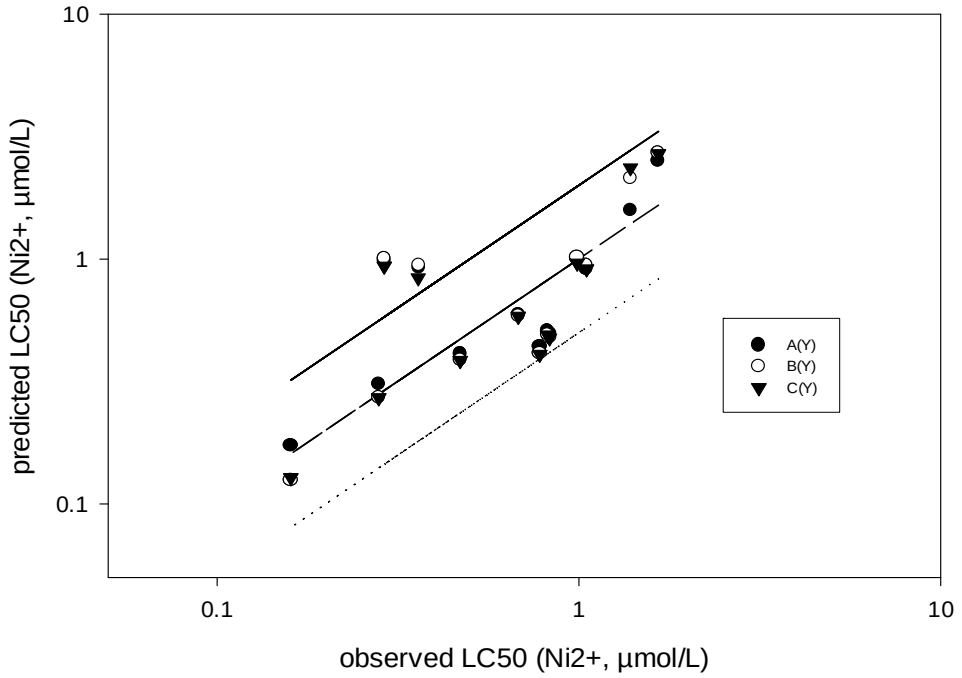


Figure 6.7: Predicted versus Observed 28-day LC50s (Ni^{2+}, µmol/L), excluding DOC Tests



The reduction in L_T observed with increasing Ca may not be sufficient to impair the function of the competitive model and/or the influence of the Ca on the ligand may be subtle and difficult to observe in the present data set. However, as L_T changes the corresponding LA50 should change as well, maintaining the constant proportion of sites that are associated with a given effect, and thereby maintaining the predictive ability of the competitive model.

Because H^+ influences Ni speciation and competes for sites on the biotic ligand, pH control of the test solutions would likely have reduced the variability observed in the data set. Some of the variability may also be related to changes in the test media, which in turn could have enhanced the sensitivity of the test organisms to Ni. Some authors have suggested that $NiCO_3$ may also be bioavailable (e.g., Hoang et al., 2004). If so, then this form of Ni will need to be included in the model for exposures above pH 8. With the exception of test 43, this study does not indicate any reduced predictability at higher pH, suggesting reduced competition with H^+, rather than the formation of toxic $NiCO_3$, as the most likely explanation for increased toxicity at higher pH.

As noted by De Schamphelaere and Janssen (2004), development of a mechanistic toxicity model for a metal requires that accurate speciation data are obtained from the physiochemical analyses of solutions. This posed a challenge for all of the test solutions with added DOC as well as some of the more extreme changes to the test media, which hampered chemical analysis and subsequent modeling using MINTEQA2.

6.3 PREDICTING 28-DAY TOXICITY FROM SEVEN-DAY TEST DATA

Models based on the seven-day adult bioaccumulation data were evaluated for their ability to predict toxicity in the 28-day toxicity tests with young. Estimates from Models A, B and C were used to predict LA50s and LC50s in the long-term toxicity tests. In addition to the tests excluded from the modeling using Models A(Y), B(Y) and C(Y), test 39 was excluded from this exercise since it was identified as a problem above and its inclusion increased the range in predicted 28-day LA50s by approximately 41% to 124%.

6.3.1 Predicted LA50s

LA50s were predicted from each model using the toxicity data from the various LC50 tests and are presented in Table 6.7. For the seven-day data, the range in predicted values was lowest in model A and highest in model C. In contrast, model C had the lowest range in predicted 28-day LA50s. The ratio of maximum to minimum values was approximately 2 to 3.5 for the seven-day data and approximately 3 to 6 for the 28-day data (compared to a range of 4 to 10 for the 28-day data if test 39 was included).

Figures 6.8 and 6.9 present the predicted LA50s versus LC50s for the seven-day and 28-day data, respectively. As shown in Figure 6.9, a linear relationship between the 28-day LA50s and LC50s is apparent for all tests except three (25, 37 and 41), suggesting a change in L_T with medium. This relationship was not apparent in the young models.

Table 6.7: Predicted Mean LA50s (µmol/g dw) for Long-term Tests (with Young) using Models A, B and C (derived from seven-day tests with Adults)

	Model A	Model B	Model C
Seven-day Data			
mean LA50	1.4	1.4	1.3
max/min	2.0	2.9	3.5
28-day Data			
mean LA50	0.45	0.53	0.54
max/min	4.1	6.4	2.9

Figure 6.8: Predicted Seven-day LA50s (µmol/g dw) from Adult models versus Seven-day LC50s (Ni^{2+}, µmol/L) from Tests with Young

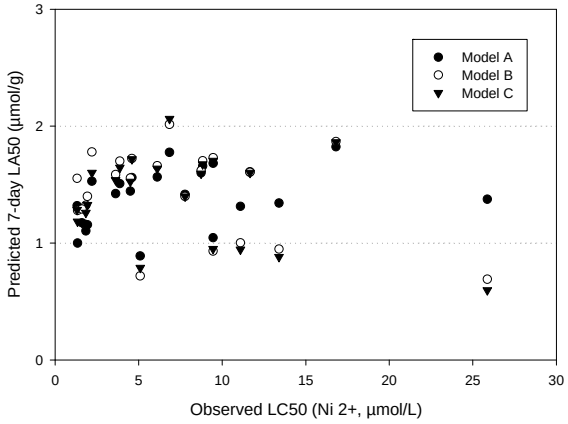


Figure 6.9: Predicted 28-day LA50s (µmol/g dw) from Adult Models versus 28-day LC50s (Ni^{2+}, µmol/L) from Tests with Young

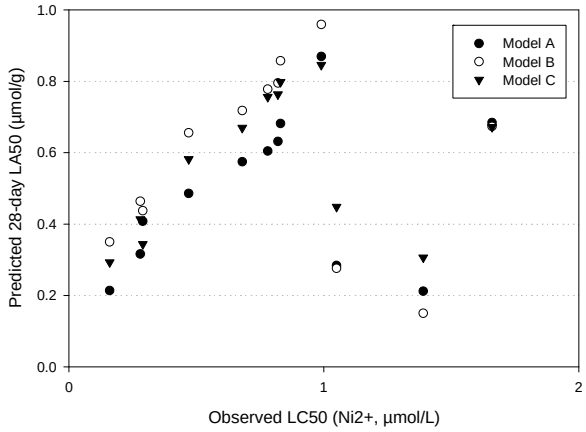


Table 6.8 compares 28-day LA50s predicted from the adult models (A, B and C) and young models (models A(Y), B(Y) and C(Y)) as proportions of L_T. Although the LA50s predicted by the adult models are lower than those of both the observed and predicted mean LA50s from the young models, the LA50s as a proportion of the L_T are similar.

Table 6.8: Comparison of Predicted 28-day LA50s (µmol/g dw) from the Adult and Young Models

	Model A	**Model B**	**Model C**
mean LA50 (µmol/g)	0.45	0.53	0.54
L_T (µmol/g)	2.0	2.4	2.8
Ratio LA50/L_T	0.23	0.22	0.19
	Model A(Y)	**Model B(Y)**	**Model C(Y)**
mean LA50 (µmol/g)	0.84	0.85	0.87
L_T (µmol/g)	3.5	4.0	7.2
Ratio LA50/L_T	0.24	0.21	0.12

6.3.2 Predicted LC50s

The above mean predicted LA50s were used to predict seven-day and 28-day LC50s for young. For the seven-day data, Model A predicted LC50s within a factor of two of the observed values in 19 out of 24 cases (79%) and was clearly a better fit than Models B and C, which predicted negative LC50s in five of the 24 LC50s and predicted LC50s within a factor of two of the observed LC50 in less than 50% of the 24 cases (Table 6.9). Of note is that negative LC50s were predicted by Models B and C for all tests with Ca concentrations

above 2 mmol/L. Figure 6.10 shows a plot of the seven-day LC50s as predicted by Model A with wide scatter in the data points.

Table 6.9: Comparison of Predicted Seven-day LC50s (Ni^{2+}, µmol/L) from Models A, B and C to Observed LC50s from Tests with Young.

				Observed	Predicted LC50s		
test	**pH**	**Ca**	**Mg**	**LC50**	**Model A**	**Model B**	**Model C**
19	7.4	830	350	12	6.3	7.5	5.6
20	8.7	810	350	1.9	3.1	2.2	2.0
21	8.1	230	270	1.3	1.5	0.97	1.4
22	8	820	350	4.5	3.8	3.4	2.8
23	7.8	3200	280	11	13	-15	-8.8
25	7.3	830	350	7.8	7.1	9.0	6.6
27	8.4	820	350	1.8	3.3	2.5	2.2
28	8.1	230	270	2.2	1.5	0.97	1.4
29	8.2	750	330	3.6	3.3	2.5	2.2
30	8	2800	280	6.1	11	-16	-10
31	6.7	830	350	9.4	19	30	21
32	7.9	240	280	6.8	1.9	1.46	2.0
33	7.8	960	280	8.7	4.8	5.2	4.0
34	8.5	690	270	3.9	2.8	1.8	1.8
35	8.1	3800	400	13	15	-11	-4.6
36	8.9	780	360	1.3	3.0	2.0	1.8
37	8.1	3700	1000	5.1	14	-11	-4.8
38	8.2	950	820	8.8	4.0	3.6	2.8
40	7.7	340	110	4.6	2.9	2.3	2.3
41	7.9	6800	1800	26	26	-9.7	-2.8
42	8.1	800	1700	17	3.6	2.9	2.5
44	8	800	280	6.1	3.8	3.2	2.7
50	7.7	250	110	1.6	2.5	2.0	2.1
51	8.1	900	290	9.5	4.0	3.5	2.8

Figure 6.10: Model A Predicted vs Observed Seven-day LC50s (Ni^{2+}, µmol/L)

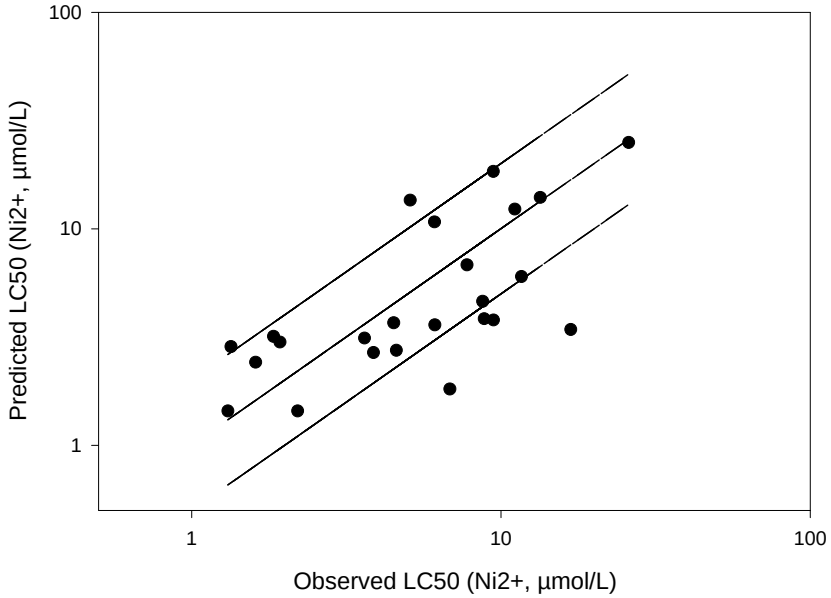


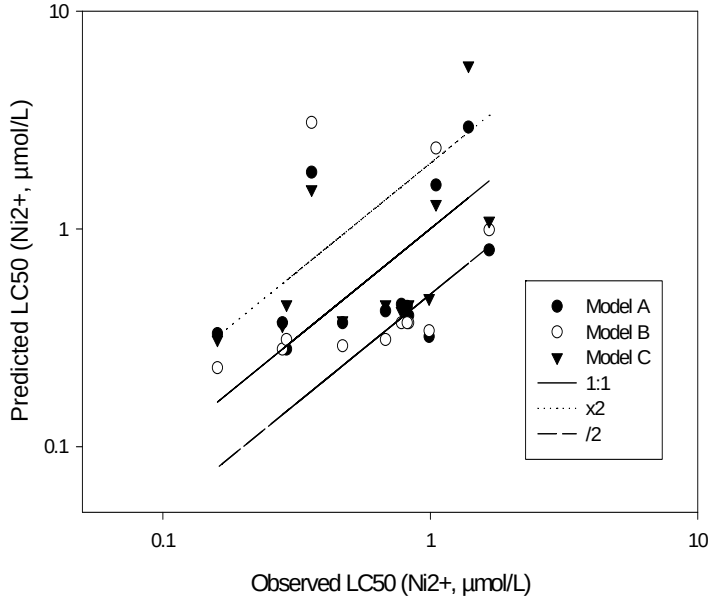
Table 6.10 lists the 28-day LC50s predicted using the adult models (A, B and C) compared to those predicted by the young models (A(Y), B(Y) and C(Y)). The adult models did not appear to reflect all factors mitigating Ni's effects, with the result that many predicted LC50s were similar along a wide range of observed LC50s. This is shown in Figure 6.11 by the horizontal band of predicted LC50s of approximately 0.4 µmol/L. Of the three adult models, model C predicted toxicity best, with the most predicted LC50s within a factor of two of the observed. The poor agreement between predicted and observed toxicity using the short-term model reflects the reduced influence of Ca in long-term compared to short-term exposures. Alternatively, the data may suggest a different mode of action between short-term and long-term exposures and/or that the data used for the development of the adult models were insufficient to develop a long-term model. Additional work would be required in order to refine the adult model as a predictive tool for young tests. As noted by

Meador (2006), the mechanism and mode of action may change over time for a given toxicant and may be reflected by changes in fits of the various models.

Table 6.10: Comparison of 28-day LC50s (Ni^{2+}, µmol/L) Predicted by the Adult Models (A, B, and C) and Young Models (A(Y), B(Y) and C(Y))

				Observed	Predicted LC50s					
Test	**pH**	**Ca**	**Mg**	**LC50**	**A(Y)**	**A**	**B(Y)**	**B**	**C(Y)**	**C**
25	7.3	830	350	1.7	2.5	0.93	2.7	1.2	2.7	1.2
27	8.4	820	350	0.28	0.31	0.43	0.27	0.34	0.27	0.4
29	8.2	750	330	0.47	0.41	0.43	0.39	0.35	0.39	0.42
36	8.9	780	360	0.16	0.17	0.39	0.13	0.27	0.13	0.34
37	8.1	3700	1000	1.1	0.91	1.8	0.94	3.1	0.91	1.5
38	8.2	950	820	0.78	0.44	0.52	0.41	0.44	0.41	0.47
39	8.2	4300	1400	0.36	0.92	2.1	0.94	4.2	0.84	1.8
40	7.7	340	110	0.99	1.0	0.37	1.0	0.41	0.96	0.53
41	7.9	6800	1800	1.4	1.6	3.4	2.2	17	2.4	8.8
42	8.1	800	1700	0.83	0.49	0.47	0.48	0.4	0.48	0.46
44	8	800	280	0.68	0.59	0.49	0.59	0.44	0.58	0.50
50	7.7	250	110	0.29	0.99	0.33	1.0	0.37	0.93	0.50
51	8.1	900	290	0.82	0.51	0.52	0.49	0.45	0.49	0.49

Figure 6.11: Predicted versus Observed 28-day LC50s (Ni^{2+}, µmol/L) from Models A, B and C.



6.4 SUMMARY OF KEY FINDINGS

Short-term Tests:

- Linear regression of Ni^{2+}/NiT vs Ni^{2+} produced preliminary estimates of L_T of 1.22 to 3.12 µmol/g dw and conditional logK_{Ni} of 4.9 to 5.9. The estimated L_T was lowest in high Ca solutions, suggesting that Ca decreased L_T, consistent with non-competitive models.
- Based on least squares non-linear regression, three models were identified as best fitting the accumulation data:
  - Model A: assumed absence of free L and that Ni accumulation was influenced by competition with Ca and H^+.
  - Model B: assumed absence of free L and that Ni accumulation was influenced by competition with Ca and H^+ as well as by non-competitive action of Ca on the ligand.
  - Model C: assumed some free L and that Ni accumulation was influenced by competition with H^+ as well as by non-competitive action of Ca on the ligand.
- Model C allowed calculation of the log K values for Ni and H^+ whereas model A and B allowed only calculation of ratios of the K values due to the absence of free ligand. Table 6.11 table below summarizes the output from the models:

Table 6.11: Summary of Output from Adult Models A, B and C

Parameter	Model A	Model B	Model C
L_T (µmol/g dw)	2.0	2.4	2.8
Log K_{Ni}			6.1
Log K_H			7.7
Log K_{Ca}*	3.7	3.0	
K_{Ca}/K_{Ni}	1.64×10^{-3}	8.81×10^{-4}	
K_H/K_{Ni}	36.2	49.5	46.88
K_{Ca}/K_H	4.54×10^{-5}	1.78×10^{-5}	

*using Model C Log K_{Ni} and Log K_H

Long-term Tests:

- As observed with the short-term test data, models A, B and C also fit the young data best. The output from the models (designated with (Y) to distinguish them from the adult models) is summarized in Table 6.12:

Table 6.12: Summary of Output from Adult Models A(Y), B(Y) and C(Y)

Parameter	**Model A(Y)**	**Model B(Y)**	**Model C(Y)**
L_T (µmol/g dw)	3.5	4.0	7.2
Log K_{Ni}			6.5
Log K_H			8.9
Log K_{Ca}*	2.03	2.86	
K_{Ca}/K_{Ni}	4.04×10^{-4}	2.31×10^{-4}	
K_H/K_{Ni}	134	163	280
K_{Ca}/K_H	3.02×10^{-6}	1.42×10^{-6}	

- The models predicted similar LA50s between 0.82 and 0.87 µmol/g dw, whether DOC data were included or not. The range in LA50s was between 4.0 and 4.5, compared to the range in corresponding LC50s of 42.
- The predicted LA50s were within a factor of two of the observed values for eight of the nine tests for which data were available.
- All three models predicted similar LC50s, supporting both competitive and non-competitive action of Ca.
- Models developed from seven-day adult bioaccumulation tests did not reliably predict long-term toxicity to younger *Hyalella*. However, ratios of predicted LA50/L_T were similar between adult and young models. Model A worked best for prediction of seven-day LC50s but all three models performed similarly in predicting 28-day LC50s. Poor fit was observed for exposures with Ca above 2 mmol/L.

7.0 CADMIUM – METHODS AND MATERIALS

7.1 CULTURING

Culturing of organisms used in experiments with Cd was carried out as described in Section 4.1.

7.2 STEP 1A: SHORT-TERM BIOACCUMULATION TESTS

Seven-day bioaccumulation tests were performed to determine which common cations of freshwater influenced the uptake of Cd. In contrast to the experiments with Ni, in which a single cation was varied while the concentration was held constant, Cd tests involved varying the concentration of Cd in different media, while all other cations were held constant. This approach was taken in order to estimate L_T from each of the exposures. The following cations were investigated: Ca^{2+}, Mg^{2+},Na^{+}, K^{+}, and H^{+}.

7.2.1 Media Preparation

As carried out for the Ni experiments, MSAM, DC and/or DI were used as the test media and were prepared in five to 20 litre volumes, using beakers or plastic carboys as containers. Salts were first dissolved in an aliquot of the deioinized water before being added to the carboy, to which DC was added to make up 30% of the final volume. LHM was prepared in the same manner as MSAM, except that no salts were added to the solution. In contrast to the Ni tests, all salts were added to each medium at the same time,

since Cd, rather than the cation of interest, was varied in each experiment. Table 7.1 lists the tests performed and the characteristics of the test media.

7.2.2 Test Set-up and Maintenance

A 100 mg/L Cd stock was used to prepare all of the solutions in the experiments listed above. The stock was prepared in an acid-washed 100 ml volumetric flask, with 10 ml of an acidified 1000 mg/L Cd standard (VWR, BDH Inc.) diluted to 100 ml with deionized water. Test solutions were prepared by spiking a subsample of the medium with a sufficient volume of the Cd stock to prepare the highest test concentration. This solution was then serially diluted with medium using a graduated cylinder to prepare the other test solutions. All other aspects of the test set up and maintenance were the same as described in Section 4.2.

7.2.3 Test Termination and Organism Collection

All Cd bioaccumulation tests were terminated as described in Section 4.2.3.

7.2.4 Acid Digestion and Measurement of Cd Bioaccumulation

After weighing, test organisms were transferred to either 2-ml or 4-ml cryovials for acid digestion, as was performed using the Ni samples. When 4-ml vials were selected, the added volumes of HNO_3, H_2O_2 and DI were doubled. A blank, containing HNO_3 and DI, was included with each batch.

Analysis of Cd in the digested tissue solutions was carried out at NWRI in Burlington, Ontario using a GFAA. Ammonium phosphate is required as a modifier for the analysis of Cd and was included in each GFAA run. Solutions were diluted with deionized water to bring the Cd concentration within the range of the program (0-20 µg/L).

Table 7.1: List of Experiments and Media Characteristics for Seven-day Bioaccumulation Tests using Cd

Medium			Medium Characteristics (mg/L)								
Test	**Base**	**Adjustment**	**pH**	**Cl**	**Ca**	**Mg**	**Na**	**K**	**SO4**	**ALK**	**DOC**
1	DC		8.3	34	32	8.3	28	1.9	36	95	1.0
2	LH		7.8	8.6	14	2.9	4.4	0.5	11	34	0.5
5	LH	High Ca	7.5	270	150	2.7	4	0.5	11	27	0.5
6	LH	High Mg	7.6	7.9	11	25	4	0.5	100	29	0.5
7	LH	High K	7.5	15	11	2.6	4.8	7.4	10	26	0.5
8	LH	High Na*	7.5	9	11	2.6	87	0.5	10	26	0.5
10	LH		7.0	8.1	11	2.6	4	0.5	9.5	26	0.5
11	DC		7.0	34	32	8.3	28	1.9	36	95	1.0
12	LH	High Ca	7.4	160	97	2.9	4.5	0.5	10	28	0.5
13	LH	High Ca	7.6	85	56	2.9	5	0.5	10	39	0.5
14	LH		7.6	9.6	13	2.7	4.9	0.5	10	35	0.5
41	DC		7	34	32	8.3	28	1.9	36	95	1.0
42	DC		9.1	34	32	8.3	28	1.9	36	95	1.0
46	SAM	High Mg	8.1	62	37	25	22	2.0	100	61	0.5
49	SAM		7.8	63	38	7.2	21	2.1	28	61	0.5
50	LH	High Ca	7.3	260	150	3	4.9	0.6	11	28	0.4

*added as $NaNO_3$

7.2.5 Chemical Analysis and Chemical Modelling

Subsamples of selected test solutions were collected and submitted to the MOE's laboratory to confirm the nominal additions of Cd, cations and anions to the test media. As carried out

in the Ni experiments, nominal concentrations of Cd were used for calculations as long as they were within 25% of the measured concentrations but measured concentrations of major cations and anions were used to describe each medium. Total and dissolved concentrations of metals were measured in solutions containing added organic material but total measured concentrations were used to represent dissolved concentrations for all solutions without added organic material. Mebane (2006), based on studies by others reporting differences of less than 5% between filtered and unfiltered water samples, suggested that the distinction between total and dissolved Cd is not of practical importance since Cd is highly soluble.

Chemistry data characterizing the test media were used to speciate Cd in solution using MINTEQA2. Inputs to the model, in addition to Cd, were as follows: 1) cations: hydrogen (H^+), sodium (Na^+), calcium (Ca^{2+}), magnesium (Mg^+), potassium (K^+); and 2) anions: hydroxide (OH^-), chloride (Cl^-), sulphate (SO_4^{2-}), and carbonate (CO_3^{2-}), entered as total alkalinity in mg/L as $CaCO_3$). Additionally, nitrate (NO_3^-) was added as a component when required to balance the solution charge from $NaNO_3$ additions.

7.2.6 Data Analysis

The whole-body concentration of Cd per litre measured by the GFAA was divided by the dry weight of the sample to obtain the concentration of Cd per gram in *Hyalella*. Then, this tissue concentration was plotted against the water concentration of Cd to determine the influence of the cation concentration in the medium on Cd accumulation.

7.3 STEP 1B: TIME-SERIES TESTS

A total of seven bioaccumulation experiments, lasting eight to 21 days, were carried out to investigate the time required to reach maximum Cd accumulation (steady state). Test design, set up and maintenance followed those described in Section 4.3 for Ni, with Cd spiked into a single solution of medium divided into replicated containers. Table 7.2 summarizes the time-series experiments that were performed. In total, four sets of experiments were initiated in LH medium. Three of the four sets included parallel exposures to Cd in another medium (MSAM or DC). Five to ten organisms were randomly assigned to each replicate vessel and were harvested periodically during the exposure period for analysis of tissue Cd. One to four replicate containers were sampled at each time period. Solutions were exchanged after seven days of exposure in the last two runs of the time-series experiments (tests of 14 and 21 days in duration) but not in the first (tests of eight and 14 days in duration). Nominal additions of Cd were not confirmed by chemical analysis since all solutions were identical and since the absolute concentration of Cd was not critical to the assessment of time to steady state.

Once the organisms were sampled from the test vessels, they were dried, weighed and analysed for whole-body Cd content as described in Section 4.2.4. The whole-body dry weight of Cd was then plotted against time to determine the time to steady state, and the bioaccumulation data were modeled in order to estimate the uptake rate constant and predicted steady-state concentration.

Table 7.2: List of Time-Series Tests Performed with Cd

Test #	Medium	Duration (days)	pH*	nominal addition total Cd (µg/L)	Solution Exchange
9	LHM	8	7.8	5	No
26	DC	14	8	2	No
27	LHM	14	7.8	2	No
34	LHM	14	7.6	0.8	yes, D7
35	MSAM	14	8	0.8	yes, D7
39	LHM	21	7.6	2	yes, D7,14
40	DC	21	7.7	2	yes, D7,14

*average pH measured in exposure vessels

7.4 STEP 2: LONG-TERM TOXICITY TESTS

Most of the long-term toxicity tests with Cd were conducted in parallel with long-term toxicity tests with Ni using the same media and batch of organisms and media preparation, test set-up and termination were as described in Section 4.6. As carried out for Ni, different media were prepared to test the influence of various water characteristics on the long-term toxicity of Cd. MSAM and LH were used for tests with varied additions of Ca, Mg, Na, K, and $NaHCO_3$. DC was used to test differences in pH. All media were aerated before use in toxicity tests.

Several experiments were also carried out with natural organic matter from the Suwannee River and from Luther Marsh. All aspects of solution preparation and storage are as

described for Ni, and the characteristics of the test media for the long-term toxicity tests performed with Cd are listed in Table 7.3.

7.4.1 Chemical Analysis

Subsamples of selected test solutions were collected and submitted to the MOE's laboratory to confirm the nominal concentrations of major cations and Cd. If the measured concentrations of Cd were within 25% of the nominal, the nominal was used in all calculations, rather than averaging several measurements taken over the course of the test. Major cations and anions were measured only once in the test medium, and measured values were used for all analyses.

For most experiments conducted in media without added organic matter, only total concentrations were measured and were assumed to represent dissolved concentrations. However, both total and dissolved concentrations were measured in all tests performed with added organic material. For these tests, subsamples were collected from the highest test concentration when solutions were prepared each week and periodically from the test solutions before solution exchange (i.e., day 7, 14, 21 or 28). On the last day of testing, mortality, temperature and pH were monitored, and test solutions were discarded.

7.4.2 Chemical Speciation Modelling

Chemistry data characterizing the test media were used to speciate Cd in each solution using MINTEQA2 in the same way as described for the tests with Ni.

Table 7.3: Characteristics of Media used in Long-Term Cd Toxicity Tests

Test	Medium			mg/L								
	Base	Adjustment	pH	Cl	Ca	Mg	Na	K	SO_4	HARD	ALK	DOC
3	DC		8	34	32	8.3	28	1.9	36	120	95	1
4	LH		7.7	8.1	11	2.6	4	0.5	9.5	38	26	0.5
15	LH		7.8	11	12	2.4	5.9	0.5	9.1	41	33	0.5
16	LH		7.8	210	120	2.8	6.2	0.5	11	310	45	0.5
18	MSAM	high Ca	7.9	280	160	10	32	2.8	38	430	48	0.6
19	DC	high pH	8.9	34	31	9.6	31	1.9	38	117	94	0.7
21	MSAM	High Mg	8.2	64	42	22	24	1.8	81	190	70	0..3
22	MSAM	all high	8.2	400	190	36	130	10	130	620	212	0.4
23	LH		7.7	7.7	14	2.7	4	0.5	16	46	30	0.5
28	MSAM	high alk	8.8	64	43	6.5	220	2	29	37	600	0.4
29	MSAM		7.9	59	34	6.9	21	1.9	28	110	60	0.4
30	LH	DOC - SR5	7.7	8	11	2.5	3.9	0.5	9.1	38	26	4.8
31	MSAM	DOC - SR5	8.1	65	40	7.1	22	2.1	28	130	63	4.7
32	LH	DOC – SR10	7.7	8.5	9.5	2.4	4.0	0.5	9.8	33	23	8.5
33	LH	DOC - LM10	7.9	8	9.4	2.5	4	0.5	10	40	25	10
36	MSAM	high Mg	7.9	61	38	47	22	2.1	180	290	61	0.7
37	LH			8.1	11	2.6	4	0.5	9.5	38	26	0.5
38	LH	DOC - SR20	7.4	10	11	2.7	4.3	0.5	10	38	19	16

DOC- SR5 = Suwannee River, 5 mg/L nominal DOC, DOC-SR10 = Suwannee River, 10 mg/L nominal DOC
DOC- SR20 = Suwannee River, 20 mg/L nominal DOC, DOC-LM10, Luther Marsh, 10 mg/L nominal DOC

7.4.3 Data Analysis

LC50s were calculated as described above for Ni and were converted to a concentration of Cd as Cd^{2+} using MINTEQA2. Additionally, LA50s (accumulated concentration of Cd associated with 50% mortality) were calculated for those tests with tissue Cd data for exposures bracketing 50% mortality.

7.5 STEP 3: MODEL DEVELOPMENT

Models for Cd were developed in a similar way to the Ni models, based on the cations that influenced uptake and toxicity. Linear plots of the ratio of Cd in solution to Cd in tissue (as *y*) versus the concentration of Cd^{2+} (as *x*) in solution were used to calculate initial estimates of L_T and K_{Cd}, and least squares regression of various cations with Cd accumulation was carried out in in Systat 11 to determine the model that best fit the observed accumulation. Further details on model development are provided in Section 9.0.

8.0 CADMIUM – RESULTS AND DISCUSSION

8.1 STEP 1A: SHORT-TERM BIOACCUMULATION TESTS

Appendix D provides details regarding the seven-day bioaccumulation tests performed with Cd.

8.1.1 Chemistry

Chemical analysis confirmed the nominal additions of Cd to solutions in all experiments except two (experiments 7 and 12), for which no data were available (Appendix D). Therefore, nominal concentrations were used in all subsequent analyses of the bioaccumulation test data. Although the nominal addition was confirmed in the pH9 DC test (test 42) on day 0, an analysis of the highest test concentration after seven days of exposure suggested that the aqueous concentration of Cd had been reduced to 23% of the nominal addition. This finding was similar to that found in two studies by Borgmann et al. (1991, 2005), who tested Cd in low hardness water and tap water at circum-neutral pH. In the 1991 study, the mean recovery reported for Cd in dechlorinated tap water after 7 days was 31% and in the 2005 study, recovery was reported between 26% and 36% of nominal additions in low hardness and dechlorinated tap water exposures, respectively. Based on the findings of Borgmann et al. (1991, 2005), it is likely that the losses observed in the pH9 exposures of this study are reflective of similar losses in the other bioaccumulation tests.

Of note is that the concentration of Cd^{2+} was reduced in solutions of elevated Ca chloride from an estimated 83% at approximately 1 mmol/L as Ca to 66% in the 4 mmol/L solution in contrast to additions of the other salts, which did not influence Cd speciation, according to the MINTEQA2 model.

8.1.2 Bioaccumulation

Figures 8.1 to 8.16 show Cd accumulation in adult *Hyalella* in the different test media after seven days of exposure. Visual inspection of the data indicated that Cd uptake by *Hyalella* increased with increasing Cd^{2+} concentration in water but did not appear to reach a well defined plateau (steady state). Lines fitted to the Cd in tissue (y) vs Cd in solution (x) by non-linear regression (according to the model $y = (a*x)/(b+x)$) accounted for between 79 and 98 percent of the variation observed.

Using the coefficients estimated from the uptake models, the accumulation of Cd was compared among exposures at a Cd^{2+} concentration of 0.04 µmol/L (Table 8.1). Generally, accumulation was highest in the LHM exposures at 0.734 to 0.941 µmol/g dw (tests 14 and 2, respectively); however, the addition of Mg, Na or K to LHM increased uptake of Cd, as shown by a range of accumulation from 1.2 to 1.4 µmol/g (Tests 6, 7 and 8). The two experiments conducted using MSAM (MSAM (test 49) and MSAM with Mg added (test 46)) showed similar accumulation to that observed in the LHM tests in that the addition of Mg increased rather than decreased Cd accumulation (0.429 and 0.608 µmol/g dw, respectively).

When pH of LHM was reduced to 7, accumulation was decreased to 0.497 µmol/g dw (Test 10). Accumulation in the DC medium was similar to that in the LHM at pH 7, with accumulation of 0.440 µmol/g dw at 0.04 µmol/L (Test 11). Accumulation in DC increased to 0.644 µmol/g dw when pH was raised to 8.3 (Test 1) but decreased at pH 9 (Test 42) to 0.263 µmol/g dw. The reduced accumulation in LHM and DC at pH 7 suggested that H^+ competed with Cd^{2+} for uptake and is consistent with results of Schubauer-Berigan et al. (1993) and Craig et al. (1999), who considered that competition with hydrogen decreases uptake of Cd. A possible explanation for the apparent reduced uptake in DC at pH 9 is that the concentration of Cd^{2+} was overestimated. Insoluble Cd precipitates are thought to form between pH 9 and 11 (Van Sprang and Janssen, 2001) removing Cd from solution, thereby explaining the reduced Cd concentration measured in the pH 9 test solution at test termination. Although the average pH of the test solutions was 9.1 over the 7 days exposure, the initial pH of the solutions was closer to 9.4, which may have influenced speciation beyond that indicated by the average pH of 9.1.

Generally, the lowest Cd accumulation was observed in the exposures with elevated Ca. In tests 5 and 50 (4 mmol/L Ca), Cd accumulation was 0.293 and 0.182 µmol/g dw, respectively, and 0.366 to 0.387 µmol/g dw in tests 12 and 13, with Ca concentrations of 1.4 and 2.5 mmol/L, respectively. These results are consistent with those published by Craig et al (1999), who concluded that Cd was accumulated in *Chironomus staegeri* through Ca channels and by Stephenson and Mackie (1988) who showed that *Hyalella azteca* accumulated less Cd in lakes with higher Ca. Taylor (1986) (as cited by Craig, 1999) proposed that Ca-Cd interactions may be more important in crustaceans than in

aquatic insects due to the Ca demands of this class of organism. In chronic experiments conducted with *Daphnia magna*, Winner and Gauss (1986) did not see a significant difference in the bioaccumulation of Cd (as measured by whole body concentrations) between exposures to two different hardnesses (115 mg/L versus 230 mg/L as $CaCO_3$) although a decrease in toxicity at the higher hardness was observed.

8.1.3 Toxicity

Of the 16 bioaccumulation tests performed, five had sufficiently high mortality to calculate LC50s and had sufficient tissue data to calculate LA50s. As shown in Table 8.2, toxicity based on water concentrations of Cd was consistent with bioaccumulation, in that higher LC50s were associated with higher Ca concentrations (experiments 13, 46 and 50). The five LC50s ranged from 0.032 to 0.193 µmol/L as Cd^{2+}, a six-fold difference. The range in LA50s calculated from the whole body concentrations was less than two-fold, from 0.66 to 1.17 µmol/g dw (mean 0.86 µmol/g dw), supporting the BLM assumption of a single whole-body concentration associated with a given effect level. The LA50s corresponded well to the those observed by Borgmann et al. (1991) in 28-day sediment tests with Ni of 0.77 and 0.87 µmol/g dw.

Figure 8.1: Test 1- DC: Accumulation of Cd (CdT µmol/g dw) versus Cd^{2+} in solution (µmol/L); $r^2 = 0.83$, p = <0.0001.

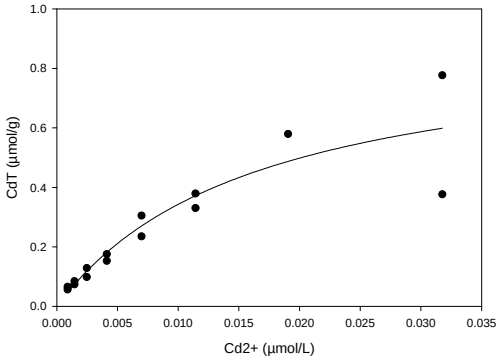


Figure 8.2: Test 2- LHM: Accumulation of Cd (CdT µmol/g dw) versus Cd^{2+} in solution (µmol/L); $r^2 = 0.79$, p = <0.0001.

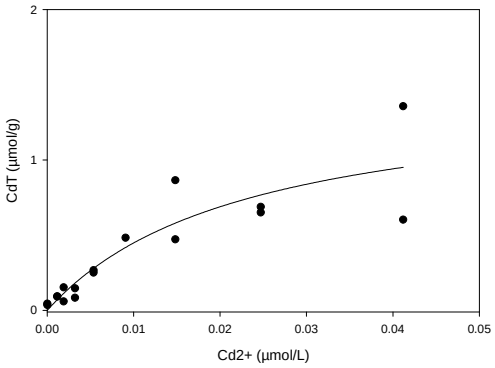


Figure 8.3: Test 5 - LH-HCa (4 mmol/L): Accumulation of Cd (CdT µmol/g dw) versus Cd^{2+} in solution (µmol/L); $r^2 = 0.98$, p = <0.0001.

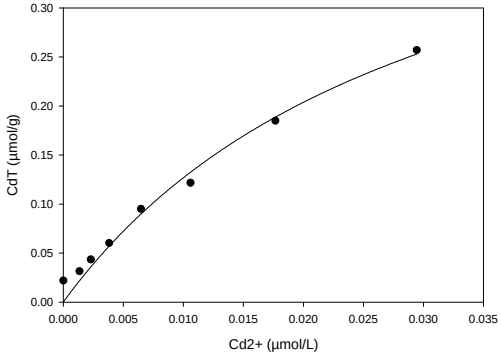


Figure 8.4: Test 6 - LH-HMg (1 mmol/L): Accumulation of Cd (CdT µmol/g dw) versus Cd^{2+} in solution (µmol/L); $r^2 = 0.86$, p = 0.0002.

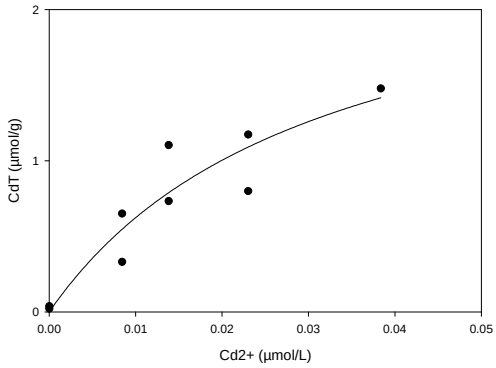


Figure 8.5: Test 7 – LH-HK (0.2 mmol/L): Accumulation of Cd (CdT µmol/g dw) versus Cd^{2+} in solution (µmol/L); $r^2 = 0.93$, $p = 0.0012$.

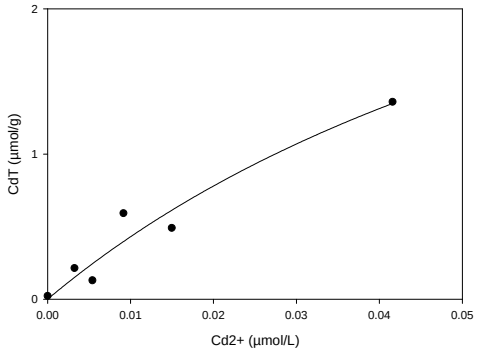


Figure 8.6: Test 8 – LH-HHNa (4 mmol/L): Accumulation of Cd (CdT µmol/g dw) versus Cd^{2+} in solution (µmol/L); $r^2 = 0.93$, $p = 0.0012$.

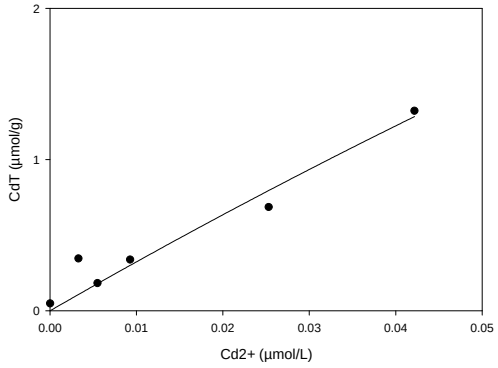


Figure 8.7: Test 10 – LH-pH7: Accumulation of Cd (CdT µmol/g dw) versus Cd^{2+} in solution (µmol/L); $r^2 = 0.93$, $p = <0.0001$.

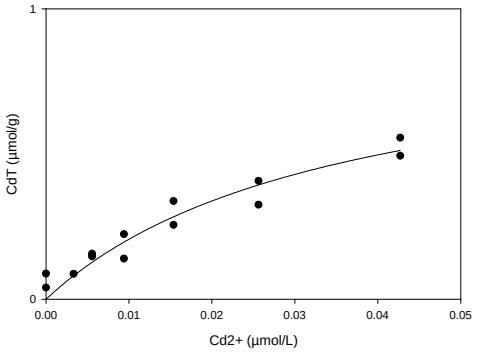


Figure 8.8: Test 11 – DC-pH7: Accumulation of Cd (CdT µmol/g dw) versus Cd^{2+} in solution (µmol/L); $r^2 = 0.96$, $p = <0.0001$.

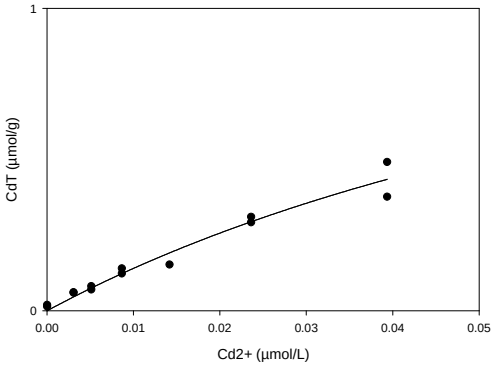


Figure 8.9: Test 12 – LH-HCa (2.5 mmol/L): Accumulation of Cd (CdT µmol/g dw) versus Cd^{2+} in solution (µmol/L); $r^2 = 0.92$, p = <0.0001.

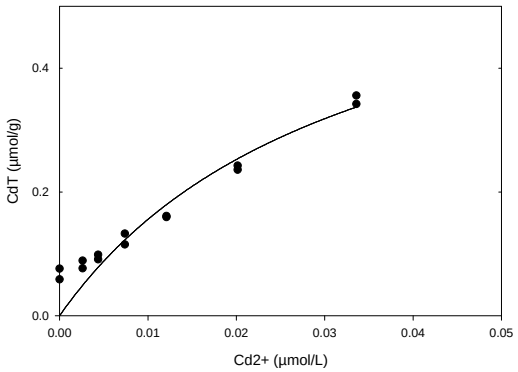


Figure 8.10: Test 13 – LH-HCa (1.4 mmol/L): Accumulation of Cd (CdT µmol/g dw) versus Cd^{2+} in solution (µmol/L); $r^2 = 0.97$, p = <0.0001.

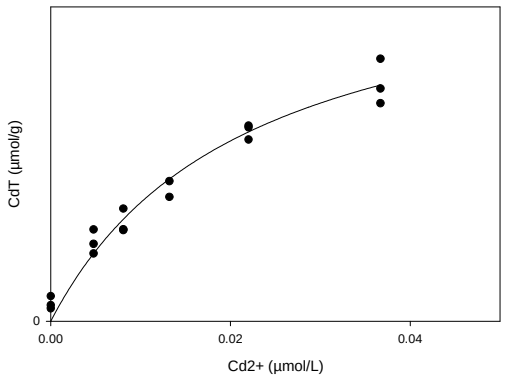


Figure 8.11: Test 14 – LHM: Accumulation of Cd (CdT µmol/g dw) versus Cd^{2+} in solution (µmol/L); $r^2 = 0.93$, p = <0.0001.

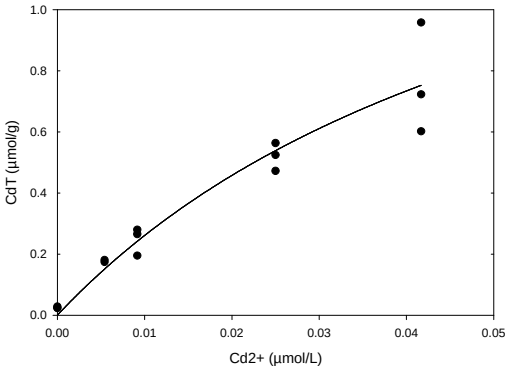


Figure 8.12: Test 41 – DC-pH 7: Accumulation of Cd (CdT µmol/g dw) versus Cd^{2+} in solution (µmol/L); $r^2 = 0.94$, p = <0.0001.

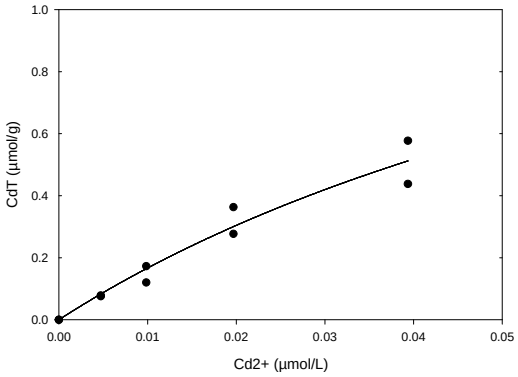


Figure 8.13: Test 42 – DC-PH9: Accumulation of Cd (CdT µmol/g dw) versus Cd^{2+} in solution (µmol/L); r^2 = 0.90, p = <0.0001.

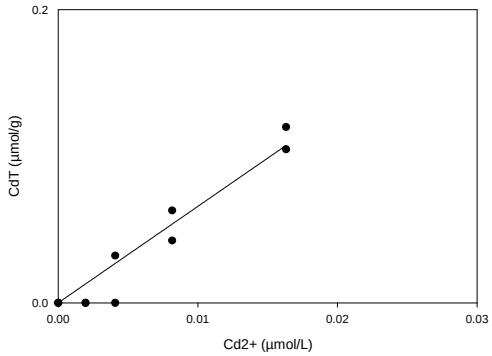


Figure 8.14: Test 46 – MSAM-HMg (4 mmol/L): Accumulation of Cd (CdT µmol/g dw) versus Cd^{2+} in solution (µmol/L); r^2 = 0.97, p = <0.0001.

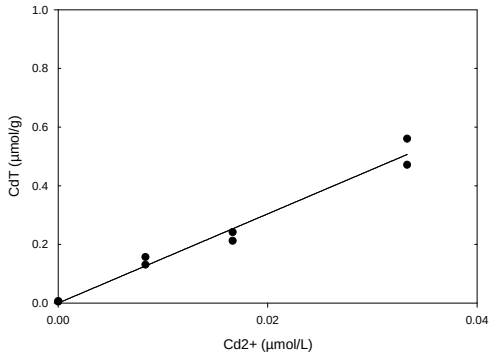


Figure 8.15: Test 49 – MSAM: Accumulation of Cd (CdT µmol/g dw) versus Cd^{2+} in solution (µmol/L); r^2 = 0.95, p = <0.0001.

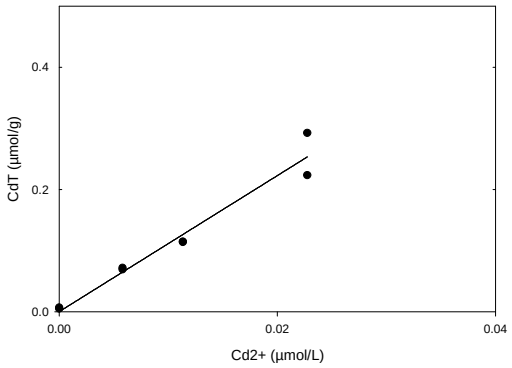


Figure 8.16: Test 50 – LH-HCa (4 mmol/L): Accumulation of Cd (CdT µmol/g dw) versus Cd^{2+} in solution (µmol/L); r^2 = 0.93, p = <0.0001.

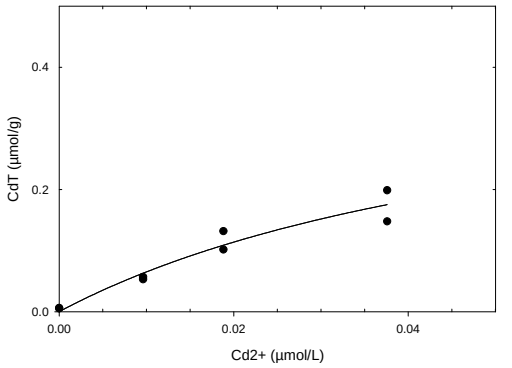


Table 8.1: Estimated Accumulation of Cd (µmol/g dw) by *Hyalella azteca* in Seven-day Bioaccumulation Tests at Exposure Concentration of 0.04 µmol/L Cd^{2+} using the uptake model $CdT = (a*Cd^{2+})/(b + Cd^{2+})$ ($a = L_T$ and $b = 1/(K_{Cd}*L_T)$).

Test	Medium	pH	Ca mg/L	Mg mg/L	Coefficients *a*	Coefficients *b*	Predicted CdT @ 0.04 Cd^{2+}
1	DC	8.3	32	8.3	0.9116	0.017	0.64
2	LH	7.8	14	2.9	1.4842	0.023	0.94
5	LH-HCa	7.5	150	2.7	0.5179	0.031	0.29
6	LH-HMg	7.6	11	25	2.5677	0.031	1.4
7	LH-HK	7.5	11	2.6	4.1546	0.087	1.3
8	LH-HNa	7.5	11	2.6	16.475	0.499	1.2
10	LH	7	11	2.6	0.9365	0.035	0.50
11	DC	7	32	8.3	1.5265	0.099	0.44
12	LH-HCa	7.4	97	2.9	0.6671	0.033	0.37
13	LH-HCa	7.6	56	2.9	0.5975	0.022	0.39
14	LH	7.6	13	2.7	1.8555	0.061	0.73
41	DC	7	32	8.3	1.7381	0.094	0.52
42	DC	9.1	32	8.3	2184.2	332	0.26
46	MSAM-HMg	8.1	37	25	5163.8	0.195	0.61
49	MSAM	7.8	38	7.2	7.2279	4535	0.43
50	LH-HCa	7.3	150	3	0.4526	0.309	0.18

Table 8.2: LC50s (Cd^{2+}, µmol/L) and LA50s (Cd, µmol/g dw) Calculated from Seven-day Bioaccumulation Tests

Test	**Base**	**Add**	**LC50 (µmol/L)**			**LA50 (µmol/g dw)**		
			LC50	**LCL**	**UCL**	**LA50**	**LCL**	**UCL**
7	LHM	K (0.2mmol/L)	0.037	0.032	0.044	NA		
8	LHM	Na (3.5 mmol/L)	0.032	0.022	0.04	0.93	0.62	2.2
13	LHM	Ca (1.4 mmol/L)	0.092	0.074	0.128	1.17	0.9	1.8
14	LHM		0.033	0.029	0.038	0.71	0.57	1.1
46	MSAM	Mg (1 mmol/L)	0.074	0.059	0.093	0.92	0.78	1.1
50	LHM	Ca (4mmol/L)	0.193	0.133	0.361	0.66	0.51	1.1

lcl = lower 95% confidence limit, ucl = upper confidence limit, NC – not calculable

8.2 STEP 1B: TIME-SERIES TESTS

The results of the time-series experiments are presented in Figures 8.17 to 8.23, which show uptake in µmol/g dw (CdT) versus time of exposure in hours (hr). None of the tests confirmed that uptake of Cd by *Hyalella* reached steady state within the exposure time period, although some tests appeared close to steady state (tests 26 and 35). This may reflect a longer time to reach steady state required by Cd or sequestration (incorporation into the carapace). For example, Wright (1980) found that after rinsing *Gammarus* in deionized water, Cd on the carapace still accounted for approximately 24% of the total body concentration and noted findings from several studies demonstrating the ability of crustaceans to sequester Cd in tissues adjacent to the alimentary canal. Additionally, Winner and Gauss (1986) reported that whole body concentrations of Cd in *Daphnia magna* continued to increase over a 28-day test period. Similarly, Heugens et al. (2003) found that steady state was not reached after 25 hours of exposure of *Daphnia magna* to Cd. Of note is that test organisms in these experiments were rinsed in deionized water, as were organisms in this study. In contrast, Munger et al. (1999) rinsed *Ceriodaphnia dubia* in EDTA rather than deionized water after exposure to Cd, and did not detect a significant difference in body concentrations between organisms exposed for one day and those exposed for 60 days.

In his review of the tissue residue approach, Meador (2006) noted that although metals bioaccumulate, many factors may obscure a whole-body dose-response relationship. These include: induction of metallothionein, formation of detoxified granules, specific tissue

affinity and homeostatic regulation. Meador (2006) also cites work by Rainbow and Dallinger (1993) who proposed some invertebrates may regulate metals at the tissue level reducing the validity of using a whole-body tissue concentration as a surrogate for tissue accumulation at the site of toxic action. Cd is not an essential metal for *Hyalella* but could be regulated to some degree by induction of metallothionein (Ball et al., 2005; Newman, 1998).

If the whole-body tissue concentration does not represent steady state conditions, it may still be used in the prediction of toxicity if a relationship can be established between the tissue concentration of a given exposure period and toxicity observed at a different time period. Nigoyi and Wood (2004) noted findings from various researchers measuring uptake at the gills in short-term exposures that equilibrium conditions did not truly exist, in that prolonged exposure would have resulted in greater uptake of metal. However, the assumption was that reactions at the gill occurred much faster than the corresponding pathological response, thereby accommodating the use of equilibrium modelling.

The uptake in the various time series exposures at time "t" was compared using the integration of the rate of accumulation, as described by Borgmann et al. (2008):

Rate of accumulation (uptake over time): $d[CdT]/dt = k_a*[CdL] - k_e*CdB$

Integration (uptake at any one time): $[CdT] = k_a/k_e*[CdL]*(1- (exp^{-ke*t}))$

At infinite time $[CdT] = [Cdss]$ and $[Cdss] = K_a/K_e*[CdL]$

therefore:

$[CdT] = [Cd_{ss}]*(1- (exp^{-ke*t}))$

Where:

CdT = concentration of Cd in tissue (B) (µmol/g dw)

d = change in …

t = time (hrs)

k_a = rate of transfer of Cd to tissue (B)

k_e = rate of Cd elimination from tissue

CdL = concentration of Cd adsorbed onto the ligand (µmol/g dw)

Cdss = steady state concentration absorbed to the organism

Using SigmaPlot to model CdT versus time (t), Cd_{ss} and k_e were estimated and used to: 1) compare CdT in the times series tests at seven days of exposure to the CdT in the seven-day bioaccumulation tests; and 2) estimate the relative proportion of Cd_{ss} achieved after seven and 28 days exposure in the time series tests. Accumulation of Cd after 7 days (168 hours) in 0.04 µmol/L Cd^{2+} (test 9) was estimated as 1.07 µmol/g dw (Table 8.3). Reduction of the exposure Cd concentration by approximately 60% resulted in a reduction in accumulation by 40% (test 27) over the same exposure period. Accumulation in the time-series tests after 7 days generally matched that observed in exposures of the same pH and medium in the seven-day bioaccumulation tests. Accumulation was highest in the LHM exposures and was consistently lower in both the time-series and seven-day bioaccumulation tests at lower pH exposures (Table 8.3).

Figure 8.17: Test 9 (Cd^{2+} = 0.04 µmol/L in LHM), Cd Accumulation (CdT, µmol/g dw) versus Time (hrs); r^2 = 0.95, p = < 0.0001.

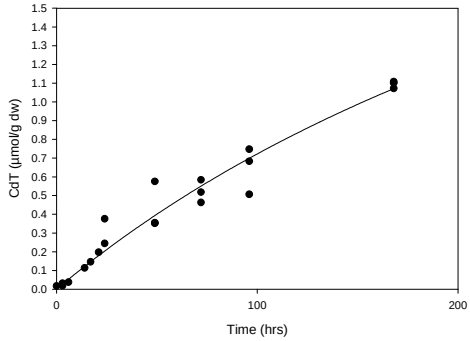


Figure 8.18: Test 26 (Cd^{2+} = 0.014 µmol/L in DC), Cd Accumulation (CdT, µmol/g dw) versus Time (hrs); r^2 = 0.92, p = < 0.0001.

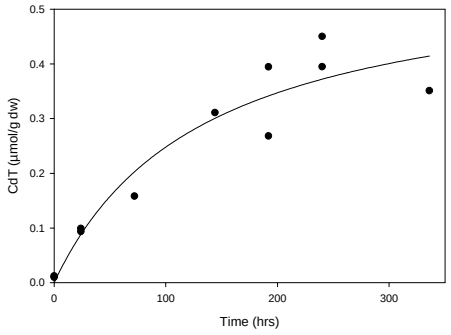


Figure 8.19: Test 27 (Cd^{2+} = 0.016 µmol/L in LHM), Cd Accumulation (CdT, µmol/g dw) versus Time (hrs), r^2 = 0.93, p = < 0.0001.

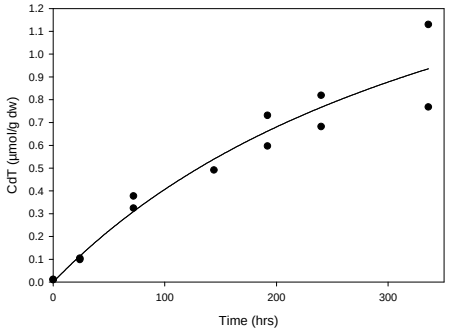


Figure 8.20: Test 34 (Cd^{2+} = 0.007 µmol/L in LHM), Cd Accumulation (CdT, µmol/g dw) Versus Time (hrs), r^2 = 0.82, p = < 0.0001.

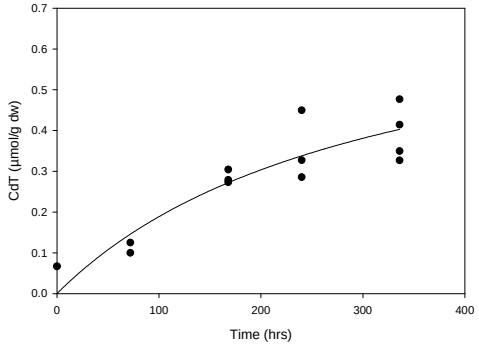


Figure 8.21: Test 35 (Cd^{2+} = 0.006 µmol/L in MSAM), Cd Accumulation (CdT, µmol/g dw) versus Time (hrs), $r^2 = 0.72$, p = 0.0002**.**

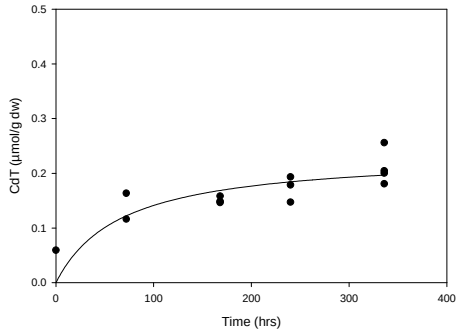


Figure 8.22: Test 39 (Cd^{2+}=0.016 µmol/L in LHM), Cd Accumulation (CdT, µmol/g dw) versus Time (hrs), $r^2 = 0.96$, p = < 0.0001.

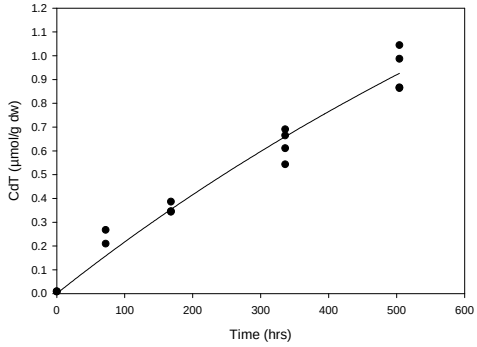


Figure 8.23: Test 40 (Cd^{2+} = 0.015 µmol/L in DC), Cd Accumulation (CdT, µmol/g dw) versus Time (hrs), $r^2 = 0.95$, p = < 0.0001.

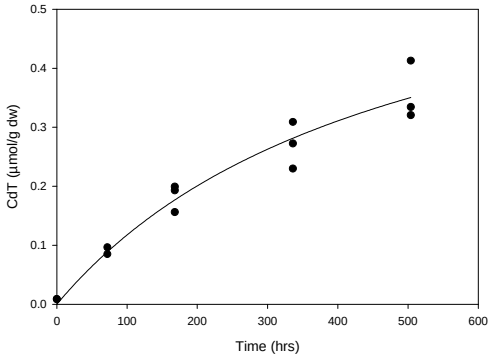


Table 8.3: Comparison of Estimated Accumulation between Time-series (TS) and Seven-day Bioaccumulation Tests (BIO) in Various Media using Models CdT = Cd_{ss}*(1- exp(-k_e*time) and CdT = *a**Cd/(*b*+Cd), respectively**.**

Test #	Test Type	Medium	pH	Cd2+ (µmol/L)	Estimated CdT (µmol/g dw)
9	TS	LH	7.8	0.04	1.1
2	BIO	LH	7.8	0.04	0.94
14	BIO	LH	7.6	0.04	0.73
27	TS	LH	7.8	0.016	0.61
39	TS	LH	7.6	0.016	0.35
2	BIO	LH	7.8	0.016	0.61
14	BIO	LH	7.6	0.016	0.38
34	TS	LH	7.6	0.007	0.28
2	BIO	LH	7.8	0.007	0.34
14	BIO	LH	7.6	0.007	0.19
35	TS	MSAM	8	0.006	0.17
49	BIO	MSAM	7.8	0.006	0.07
40	TS	DC	7.7	0.015	0.18
1	BIO	DC	8.3	0.015	0.43
26	TS	DC	8	0.014	0.33
1	BIO	DC	8.3	0.014	0.42

LH = low hardness medium; DC = dechlorinated tap water; MSAM = modified standard artificial medium
TS = time-series experiment; BIO = seven-day bioaccumulation tests

Table 8.4 summarizes the estimated CdT concentration as a percentage of Cdss for each test after seven days and 28 days of exposure. The two tests closest to steady state were tests 26 (DC) and 35 (MSAM), with estimated tissue Cd concentrations of 77% and 86% relative to steady state. After 28 days of exposure, all time-series test data, except for test 39 (LHM), indicated that steady state would essentially be met by 28 days of exposure.

Table 8.4: Estimated CdT (µmol/g dw) as a Percentage of Cd_{ss} (µmol/g dw) After Seven days and 28 days of Exposure.

Test	Cd_{ss}	ke	CdT at 7 days	% of Cd_{ss}	CdT at 28 days	% of Cd_{ss}
9	2.1919	0.004	1.1	49	2.0	93
26	0.4255	0.0088	0.33	77	0.42	100
27	1.3344	0.0036	0.61	45	1.22	91
34	0.5054	0.0047	0.28	55	0.48	96
35	0.1981	0.0119	0.17	86	0.20	100
39	2.7654	0.0008	0.35	13	1.2	42
40	0.4555	0.0029	0.18	39	0.39	86

8.3 STEP 2: LONG-TERM TOXICITY TESTS WITHOUT DOC

Detailed chemical speciation and bioaccumulation information for the long-term tests is provided in Appendix E.

8.3.1 Chemistry

Chemical analyses confirmed the measured concentrations of Cd spiked into test solutions matched nominal additions. No samples were collected during the preparation of pH 8.9 solutions (test 19). However, analyses of total Cd in solutions just before exchange on days seven, 14 and 21 indicated that an average of 50% was lost, possibly due to precipitation or adsorption (as was discussed for the seven-day bioaccumulation tests). Data from solutions collected just before solution exchange during experiment 28 (high alkalinity, pH 8.8) also showed loss of Cd, even though total concentrations were confirmed during solution preparation. Of note is that the measured concentration of alkalinity as well as the recovery of Ca was low in the experiment 28 sample, less than half the concentration expected from

the known addition of DC to the medium and below that indicated by the corresponding measured concentration of chloride (added as Ca chloride). This was also observed in the nickel test 43, which was performed using the same medium and suggests a problem with the chemical analysis. All data from this test were used with caution. As noted above for the bioaccumulation tests, it is possible that the poor recovery observed in the high pH tests reflected the loss of Cd that occurred in all test vessels as the Cd adhered to the gauze and/or test vessel walls during the exposures.

Only three samples were collected from non-DOC test solutions for comparison of dissolved and total Cd. In experiment 36 (MSAM with high Mg), the highest concentration was analysed for total and dissolved Cd immediately after preparation on Day 0. The Cd concentration in the dissolved (filtered) sample was only 48 % of the nominal concentration, whereas the total concentration was 90% of the nominal. However, as discussed for the nickel tests with added DOC, the filtering of samples immediately after preparation may not be reflective of the equilibrium/exposure conditions since all solutions were left for several hours before test initiation, and more of the metal would likely have been solubilized in solution (as indicated by the dissolved concentrations measured from the exposure vessels). Therefore, no correction for the low dissolved concentration was applied. Test exposure solutions sampled just before solution exchange in experiments 28 and 29 also indicated loss of Cd with measured dissolved Cd concentrations of 30 and 39%, respectively, compared to nominals. As noted above, the total concentration measured in the corresponding test 28 sample was also low (53%) whereas the total concentration in test 29 was 72% of nominal (Appendix E).

8.3.2 Bioaccumulation

Detailed bioaccumulation data are provided in Appendix E. As observed in the seven-day bioaccumulation tests, the highest overall accumulation of Cd after 4-weeks of exposure was observed in LHM. The average accumulation in experiments 4 and 15 (LH medium without additions) was 1.1 and approximately 0.9 µmol/g dw, respectively, in approximately 0.01 µmol/L as Cd^{2+}. Exposures to 0.01 µmol/L Cd^{2+} in LHM spiked with 3 mmol/L Ca reduced accumulation of Cd by *Hyalella*, as shown by a whole-body tissue concentration of 0.55 µmol/g dw (test 16).

In DC, Cd accumulation observed in the pH 8.0 (test 3) and pH 8.9 (test 19) exposures to 0.01 µmol/L Cd^{2+} was 0.55 and 0.97µmol/g dw. The increased uptake at pH 8.9 was in contrast to the seven-day bioaccumulation test, which showed lower uptake. However, the pH in the bioaccumulation test was slightly higher, and modelling in MINTEQA2 indicated changes to Cd speciation may be significant in this pH range. For example, between pHs of 8.8 and 9.2, the estimated fraction of total Cd as Cd^{2+} drops by approximately 40%, with a corresponding increase in the fractions present as $CdCO_3$ and $Cd(CO_3)_2^{2-}$.

The limited tissue data from the 28-day toxicity tests were fitted to the Langmuir uptake model ($y = a*x/(b+x)$) using non-linear regression and estimates for a and b were used to calculate the expected uptake at an exposure Cd^{2+} concentration of 0.01 µmol/L (Table 8.5). As observed in the short-term bioaccumulation tests, the highest accumulation was observed in the LH exposures, with estimated tissue concentrations at 0.01 µmol/L Cd^{2+}

exposures of 1.40, 1.39 and 0.87 µmol/g for tests 23, 4 and 15, respectively. Accumulation was similar in DC and MSAM with pHs of 8.9 and 8.8 at 0.93 and 1.29 (tests 19 and 28, respectively). The lowest accumulation was observed in MSAM with elevated Ca, with similar tissue concentrations estimated whether or not other cations were elevated (tests 18 and 22).

Table 8.5: Modelled Cd in Tissue (CdT, µmol/g dw) at 0.01 µmol/L Cd^{2+} in Long term Toxicity Tests (based on $CdT = a*Cd^{2+}/(b+Cd^{2+})$)

Test	Medium	pH	Ca	Mg	Estimated CdT
			mg/L	mg/L	µmol/g dw
3	DC	8	32	8.3	0.44
4	LH	7.7	11	2.6	1.4
15	LH	7.8	12	2.4	0.87
16	LH	7.8	120	2.8	0.45
18	MSAM	7.9	160	10	0.31
19	DC	8.9	31	9.6	0.93
22	MSAM	8.2	190	36	0.29
23	LH	7.7	14	2.7	1.4
28	MSAM	8.8	43	6.5	1.3
29	MSAM	7.9	34	6.9	0.75

8.3.3 Toxicity

LC50s calculated from the long-term toxicity test data, for all media except the DOC experiments, are presented as total Cd (Table 8.6) and as Cd^{2+} modeled using MINTEQA2 (Table 8.7).

Most of the variation in LC50s (based on total or free Cd) could be explained by Ca alone, with a clear linear relationship evident between LC50 and Ca concentrations in both the

short-term and long-term exposures, regardless of pH (Figure 8.24). The influence of Ca on Cd toxicity decreased after 14 days, based on a comparison of slopes even though the ratio of maximum to minimum LC50 did not decrease (Appendix E). The decreased influence against Cd toxicity did not appear to be as significant as its decreased influence in Ni exposures, which may reflect a more consistent mode of action of Cd from short-term to long-term exposures. No relationship was evident between LC50 and H^+ or Mg (Figures 8.25 and 8.26). These results are consistent with those of Jackson et al. (2000) who exposed *Hyalella* to Cd for 96 hours. They noted a clear influence of Ca on Cd toxicity and, although they also noted an increase in total Cd LC50s with Mg concentration, this influence was not discernable when Cd LC50s were based on free ion activity. Of note is that the range of Mg tested in the Jackson et al. (2000) tests was wider than used in this study (~1 to 83 mg/L versus ~3 to 23 mg/L).

The competitive action of other cations, such as Ca, is supported by findings and theories of Barata et al. (1998) who studied Cd toxicity to *Daphnia magna.* They noted that between pH 7 and 8, the vast majority of Cd would be present in the free form and that if the FIAM were correct, toxicity would not vary if the free ion concentration did not change significantly. However, because toxicity varied widely in exposures of the same free ion concentration, they proposed that toxicity was mitigated by competitive action of other cations present and/or through physiological responses by *Daphnia* to increasing water hardness.

Plots of long-term LC50s versus Ca and Mg, expressed as hardness, showed a clear linear relationship, which was most likely due to the Ca present (Figures 8.27 and 8.28). Jackson et al. (2000) cited a study by Davis et al. (1993) that found little mitigation of Cd toxicity to rainbow trout when hardness was increased by magnesium sulphate additions.

Table 8.6: 28-day LC50s (Total Cd, µmol/L), excluding DOC Tests

Test	Medium				day 7			day 14			day 21			day 28		
#	Base	Adjustment	pH	Ca (mg/L)	LC50	lcl	ucl	LC50	lcl	ucl	LC50	lcl	ucl	LC50	lcl	ucl
44	MSAM	HCA7	7.9	260	0.35	0.29	0.44									
47	DC	pH7	7.1	32	0.084	0.058	0.11									
3	DC		8	32	>0.044			0.036	0.025	0.072	0.019	0.015	0.023	0.015	0.011	0.021
4	LH		7.7	11	>0.044			0.025	0.019	0.037	0.011	0.009	0.014	0.008	0.007	0.01
15	LH		7.8	12	0.031	0.026	0.035	0.028	0.024	0.032	0.023	0.024	0.027	0.022	0.018	0.026
16	LH	Ca3	7.8	120	0.18	0.15	0.35	0.13	0.11	0.16	0.12	0.097	0.15	0.09	0.068	0.13
17	LH	HH	7.8	160	0.31	0.27	0.36									
18	MSAM	Ca4	7.9	160	0.16	0.11	0.18	0.11	0.097	0.13	0.1	0.092	0.12	0.095	0.082	0.11
19	DC	pH9	8.9	31	0.12	0.1	0.13	0.093	0.079	0.11	0.084	0.071	0.1	0.079	0.065	0.096
21	MSAM	Mg1	8.2	42	0.1	0.09	0.11	0.084	0.073	0.1	0.079	0.067	0.093	0.066	0.055	0.079
22	MSAM	MSAM4	8.2	190	>0.89			0.42	0.36	0.48	0.27	0.235	0.32	0.24	0.21	0.28
23	LH		7.7	14	0.026	0.022	0.031	0.014	0.012	0.017	0.008	0.006	0.009	0.006	0.005	0.007
24	MSAM	HH	7.9	300	0.67	0.59	0.77	0.41	0.35	0.49						
28	MSAM	Alk5	8.8	36	0.085	0.071	0.1	0.065	0.061	0.07	0.046	0.04	0.054	0.04	0.032	0.048
29	MSAM		7.9	34	0.086	0.07	0.11	0.053	0.045	0.063	0.042	0.034	0.052	0.041	0.032	0.051
36	MSAM	Mg1	7.9	38	0.064	0.051	0.077	0.027	0.022	0.033	0.023	0.019	0.027	0.02	0.017	0.023
37	LH		7.5	11	0.033	0.027	0.039	0.017	0.013	0.023						

lcl/ucl = lower/upper 95% confidence limits

Table 8.7: 28-Day LC50s (Cd^{2+}, µmol/L), excluding DOC Tests

Test	Medium				day 7			day 14			day 21			day 28		
#	Base	Adjustment	pH	Ca (mg/L)	LC50	lcl	ucl	LC50	lcl	ucl	LC50	lcl	ucl	LC50	lcl	ucl
44	MSAM	HCA7	7.9	260	0.2	0.16	0.24									
47	DC	pH7	7.1	32	0.074	0.051	0.094									
3	DC		8	32		0	0	0.028	0.02	0.057	0.015	0.012	0.019	0.012	0.009	0.017
4	LH		7.7	11		0	0	0.023	0.018	0.035	0.011	0.008	0.013	0.008	0.006	0.01
15	LH		7.8	12	0.028	0.024	0.033	0.026	0.022	0.03	0.021	0.022	0.025	0.02	0.016	0.024
16	LH	Ca3	7.8	120	0.12	0.102	0.25	0.087	0.073	0.11	0.081	0.067	0.101	0.062	0.047	0.093
17	LH	HH	7.8	160	0.19	0.169	0.22									
18	MSAM	Ca4	7.9	160	0.11	0.069	0.12	0.071	0.063	0.081	0.067	0.059	0.075	0.061	0.053	0.071
19	DC	pH9	8.9	31	0.054	0.048	0.062	0.042	0.036	0.05	0.039	0.033	0.046	0.036	0.03	0.044
21	MSAM	Mg1	8.2	42	0.074	0.066	0.083	0.062	0.053	0.073	0.057	0.049	0.068	0.048	0.04	0.058
22	MSAM	MSAM4	8.2	190		0	0	0.21	0.18	0.24	0.13	0.12	0.16	0.12	0.11	0.14
23	LH		7.7	14	0.025	0.021	0.029	0.013	0.011	0.016	0.007	0.006	0.009	0.005	0.004	0.007
24	MSAM	HH	7.9	300	0.34	0.3	0.39	0.21	0.18	0.25						
28	MSAM	Alk5	8.8	36	0.022	0.019	0.027	0.017	0.016	0.018	0.012	0.01	0.014	0.01	0.008	0.013
29	MSAM		7.9	34	0.07	0.057	0.085	0.043	0.036	0.051	0.034	0.027	0.042	0.033	0.026	0.042
36	MSAM	Mg1	7.9	38	0.047	0.038	0.058	0.02	0.017	0.024	0.017	0.014	0.02	0.015	0.013	0.017
37	LH		7.5	11	0.031	0.026	0.037	0.016	0.013	0.021						

lcl/ucl = lower/upper 95% confidence limits

Figure 8.24: LC50 (Cd^{2+}, µmol/L) Versus Ca (µmol/L) in Various Media: (7d r^2 = 0.88, p = <0.0001; 14d r^2 = 0.85, p = <0.0001; 21d r^2 = 0.80, p = <0.0001; 28d r^2 = 0.80, p = <0.0001)

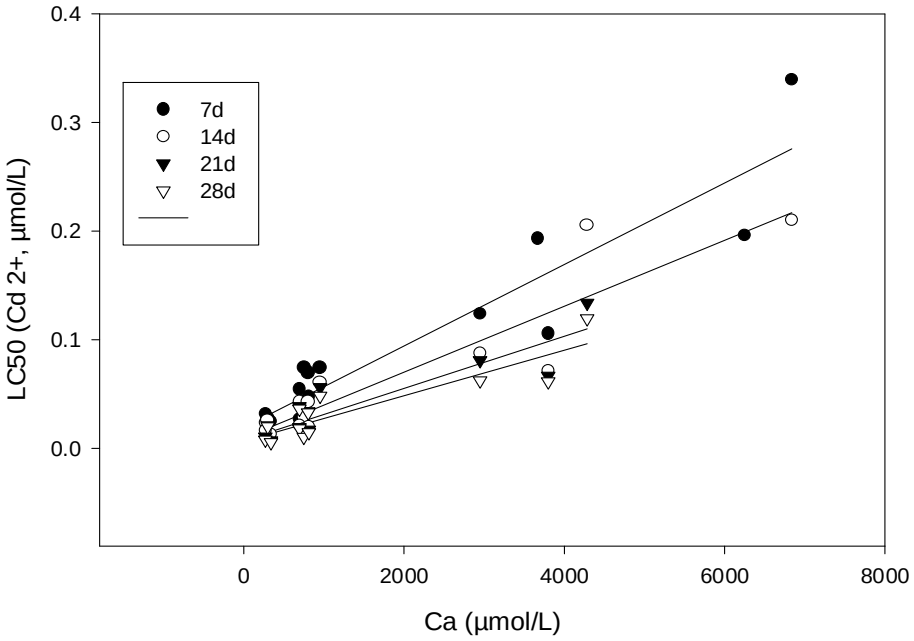


Figure 8.25: LC50 (Cd^{2+}, µmol/L) Versus pH in Various Media: (7d r^2 = -0.07, p = 0.68; 14d r^2 = -0.07, p = 0.66; 21d r^2 = -0.09, p = 0.82; 28d r^2 = -0.09, p = 0.77)

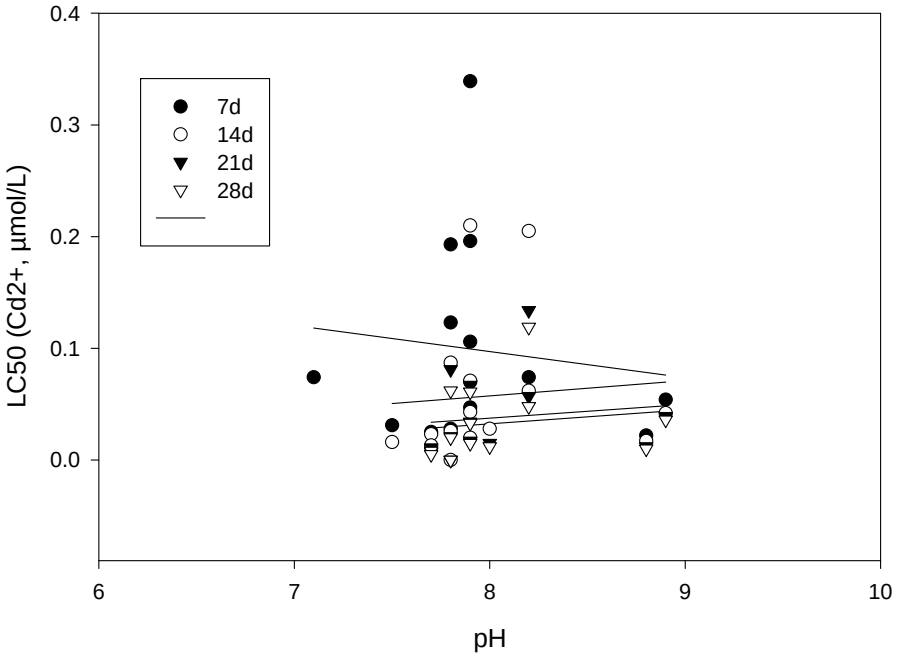
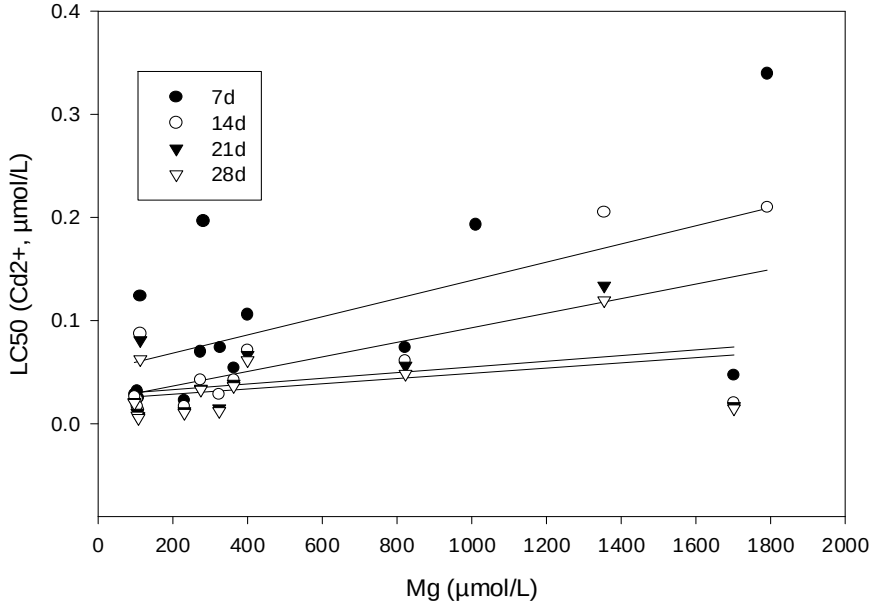


Figure 8.26: LC50 (Cd^{2+}, µmol/L) Versus Mg (µmol/L) in Various Media: (7d $r^2 = 0.26$, $p = 0.04$; 14d $r^2 = 0.39$, $p = 0.01$; 21d $r^2 = 0.06$, $p = 0.21$; 28d $r^2 = 0.06$, $p = 0.19$)



Meyer (1999) proposed that the slope of ln LC50 to ln hardness of transition metals would be approximately 1 within the hardness range of 20 to 200 mg/L as $CaCO_3$. A review by Mebane (2006) showed a slope of 0.65 to 0.68 for ln LC50 versus ln hardness from pooled exposures of *Hyalella azteca* of between 14 and 42 days. Additionally, Mebane (2006) found that hardness accounted for more than 90% of the variability in chronic test results from five different species.

When the LC50s (as total Cd) from this study were (natural) log transformed and plotted against ln hardness, the slopes were 0.94 (R^2 of 0.87) for the seven-day data (from the 28-day LC50 tests) (Figure 8.27) and 1.2 (R^2 of 0.18) for the

28-day data (Figure 8.28), excluding DOC tests and hardnesses outside the range of 20 - 200 mg/L.

Figure 8.27: Seven-day Ln LC50 (Total Cd, µg/L) Versus Ln Hardness in the range of 20 – 200 mg/L as $CaCO_3$ ($r^2 = 0.87$, $p = 0.0004$)

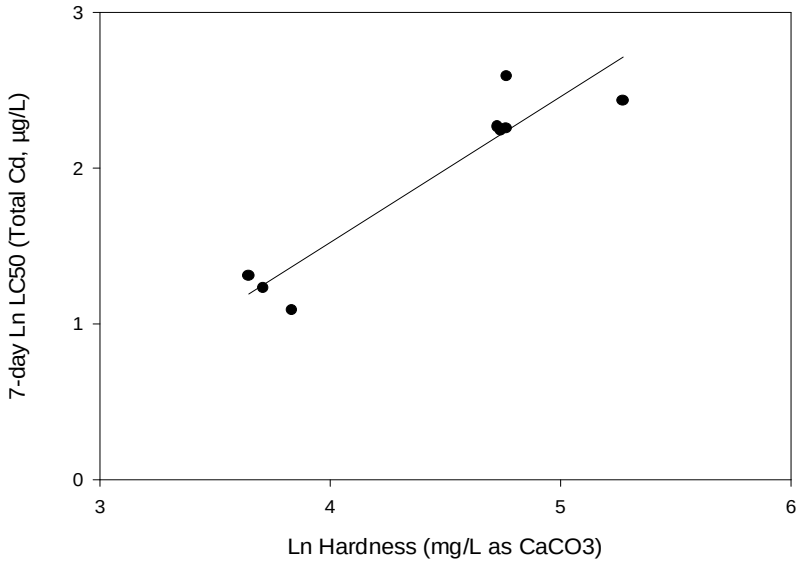
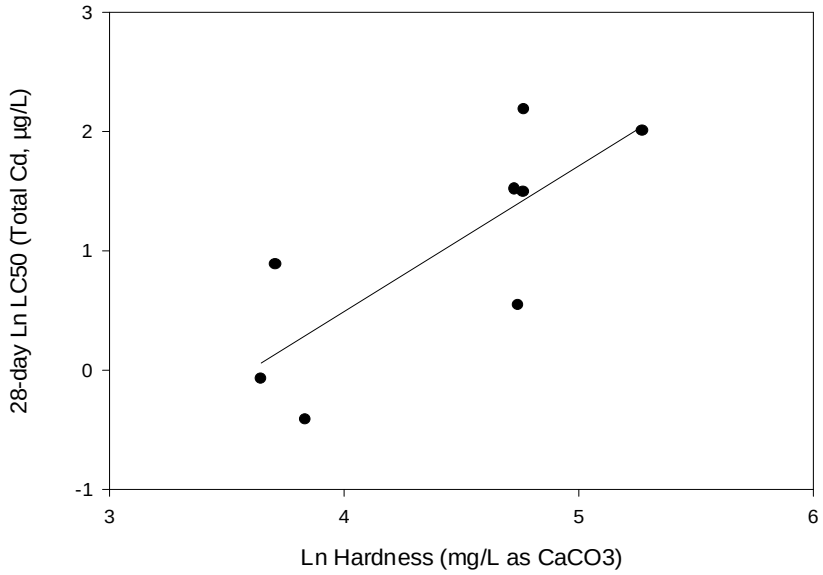


Figure 8.28: 28-day Ln LC50 (Total Cd, µg/L) Versus Ln Hardness in the range of 20 – 200 mg/L as $CaCO_3$ ($r^2 = 0.18$, $p = 0.22$).



Based on total Cd, the lowest LC50s were observed in tests using LHM and highest in exposures with elevated Ca. Although accumulation after 28 days in the pH 9 and high alkalinity (pH 8.8) exposures was elevated relative to the same/similar media at ~pH 8, toxicity based on total Cd was either reduced (pH 8.9) or similar (pH 8.8) to that of the MSAM (pH7.9) exposure and reduced relative to the DC (pH 8) exposure. However, if LC50s based on modeled free Cd^{2+} are compared, the high alkalinity exposure is more toxic than the MSAM exposure (test 29) and the pH 8.9 (test 19) exposure. Comparison of seven-day data shows that the toxicity (as Cd^{2+}) in DC at pH 7 was similar to that of MSAM at pH 7.9 (LC50s of 0.074 versus 0.069, respectively) and was significantly lower than observed in the pH 9 exposure (LC50 of 0.027 μmol/L).

Erten-Unal et al. (1998) noted that fathead minnows were more sensitive than *Daphnia magna* in exposures to cadmium carbonate ($CdCO_3$) and proposed that it was due to the more intimate contact of the fish to particulate material at the bottom of the test vessel. It is possible that *Hyalella azteca*, like the fathead minnows, could have ingested particulate Cd carbonate, formed at the bottom of the test vessel at high pH, adding a second exposure route of Cd in the long-term test. However, Ball et al. (2005) found that Cd toxicity did not vary with accumulation when the route of exposure was through food. Similarly, Golding et. al. (2006) observed no relationship between Cd-contaminated food and observed toxicity in water-only exposures of *Hyalella*. Barata et al. (2002)

studied the relative importance of food and water as sources of Cd toxicity to *Daphnia magna* and found that more Cd was taken up from water than from food and that Cd was more toxic in water-only exposures. However, their comparisons of Cd toxicity to three different daphnid clones suggested that linking laboratory tests with field results may be problematic due to the fact that more tolerant species accumulate more Cd.

If the increased toxicity at higher pH was due to less competition by hydrogen, the accumulation of Cd at higher pH would be expected to be elevated relative to the lower pH exposures. Similarly, the seven-day LC50 at pH 7 (test 47) would have been higher than that of similar exposures of pH 8 (test 29). Van Sprang and Janssen (2001) proposed that significant precipitation of cadmium sulphates and carbonates occurs at pH 9 but in the pH range of 6.5 to 8.5, the speciation of Cd does not change significantly. They noted an increase in acute Cd toxicity to *Daphnia magna* of 33% when pH was raised from 7.5 to 8.5 and a reduction of 76% when pH was reduced to 6.5, suggesting that the decreased toxicity at the lower pH was due to competition from increased H^+ concentration. No tests were performed with Cd at pH below 7 in this study and no difference in toxicity was observed in the range of pH 7 to 8.

8.4 STEP 2: LONG-TERM TOXICITY TESTS WITH DOC

8.4.1 Chemistry

Measured concentrations of total Cd and DOC added to the test solutions matched nominal concentrations in samples collected both at preparation and at solution exchange, unlike the analyses of solutions without added DOC. However, loss of available Cd was indicated by a reduction in dissolved Cd (Appendix E). No correction was made for the Cd concentration here since the adsorption or complexation of Cd in these exposures would have a similar impact on Cd availability as the adsorption of Cd to gauze or vessels as observed in the non-DOC exposures.

No chemical data regarding major cations and anions in the Luther Marsh medium were available. Therefore, medium characteristics of the Suwannee River dissolved organic material were used, since they showed that the DOC did not alter the major cations and anions concentrations of the LHM. Data from Glover et al. (2005) supported this assumption, indicating that no Ca, Mg or Na was contributed from the Luther Marsh dissolved organic material source. The concentration of chloride in the Luther Marsh dissolved organic material reported by Glover et al. (2005) was 29 μmol/g carbon or 10 mg/L (in a solution with nominal DOC concentration of 10 mg/L), and was consistent with the chloride value of 8.5 mg/L used to characterize the Luther Marsh solutions.

MINTEQA2 Modelling

According to MINTEQA2, increasing concentrations of DOC in the test media had a very minor effect on the speciation of Cd in solution. The Cd^{2+} in solution was 87 to 92 percent in the LHM, compared to 93 percent when no DOC was added. In MSAM, Cd^{2+} was reduced to 76 percent compared to 81 per cent with no added DOC.

Hydroqual Cd BLM

The speciation mode of Hydroqual's BLM program (Hydroqual, 2006) for prediction of acute Cd toxicity was also used to estimate free Cd in solution. According to the BLM program, the percentage of Cd present in the free form was significantly lower than that predicted by MINTEQA2. The free Cd in solution was 39 to 17 percent in the LHM with added DOC of 5 to 20 mg/L, compared to 84 to 82 percent when no DOC was added. In the MSAM, free Cd was reduced to 41 percent compared to 80 per cent with no added DOC.

8.4.2 Bioaccumulation

Figures 8.29 and 8.30 show Cd accumulation in LHM and MSAM, respectively. In LHM, the addition of 5 mg/L DOC did not significantly affect the accumulation of Cd whereas additions of 10 mg/L (Luther Marsh) and 20 mg/L (Suwannee R.) DOC decreased accumulation by approximately 40% and 30%, respectively, at an exposure concentration of 0.01 μmol/L as Cd^{2+}. Unfortunately, no comparison could be made between accumulation in the

Luther Marsh and Suwannee River exposures at 10 mg/L DOC since poor survival and high accumulation in the Suwannee River test suggested contamination of the lowest exposure concentrations.

In contrast to the LH exposure, accumulation in MSAM was reduced by approximately 45% when 5 mg/L DOC was added. Bioaccumulation was also evaluated based on the free ion concentration, as estimated by MINTEQA2 and the BLM program (Figures 8.31 to 8.34). In theory, bioaccumulation at the same concentration of Cd^{2+} should be the same regardless of the DOC concentration (assuming all other water characteristics are the same) and, therefore, tissue concentrations should be the same in all solutions of the same Cd^{2+} concentration. Accumulation of Cd, based on MINTEQA2 solution concentrations of Cd^{2+}, was approximately the same to that based on total concentrations, since the majority of Cd was predicted to be in the free form (Figures 8.31 and 8.32). In contrast, the BLM program predicted accumulation of free Cd in DOC solutions above that of the LHM without added DOC (Figures 8.33) but predicted approximately the same accumulation in the MSAM with and without DOC (Figure 8.34). In summary, it appeared that MINTEQA2 underestimated the amount of Cd bound to DOC and that the BLM overestimated the amount of Cd bound to DOC. However, the BLM program was able to resolve the bioaccumulation difference in the MSAM with and without DOC.

Figure 8.29: Accumulation of Cd in Tissue versus Total Cd in LHM with Added DOC. LH (4, 5, 23): $r^2 = 0.83$, $p = <0.0001$; LH-SR5 (30) $r^2 = 0.82$, $p = 0.063$; LH-LM10(33): $r^2 = 0.98$, $p = <0.0001$. LH-SR20 (38) insufficient data for regression.

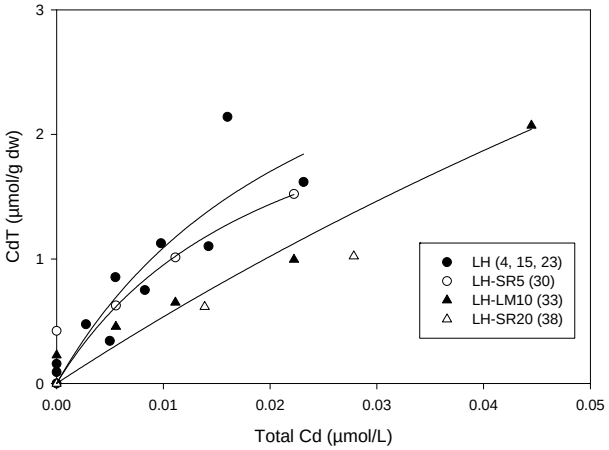


Figure 8.30: Accumulation of Cd in Tissue versus Total Cd in MSAM with DOC. MSAM (29): $r^2 = 0.97$, $p = 0.0002$; MSAM -SR5 (31) $r^2 = 0.99$, $p = <0.0001$.

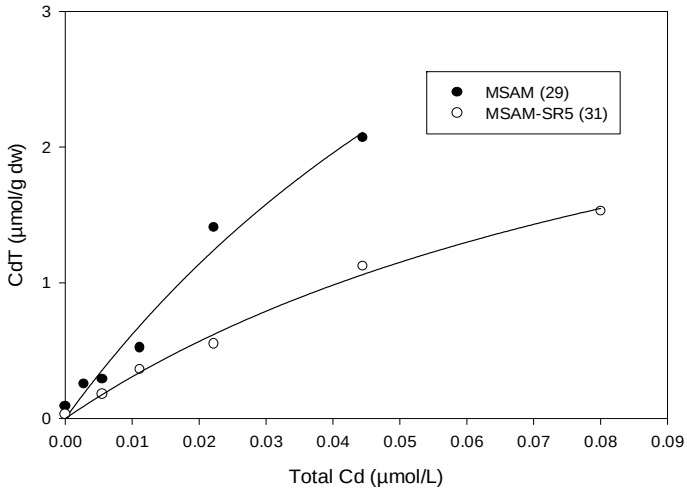


Figure 8.31: Accumulation of Cd in Tissue versus Cd^{2+} (MINTEQA2 speciation) in LHM with Added DOC. LH (4, 5, 23): $r^2 = 0.84$, $p = <0.0001$; LH-SR5 (30) $r^2 = 0.82$, $p = 0.063$; LH-LM10(33): $r^2 = 0.95$, $p = 0.0029$. LH-SR20 (38) insufficient data for regression.

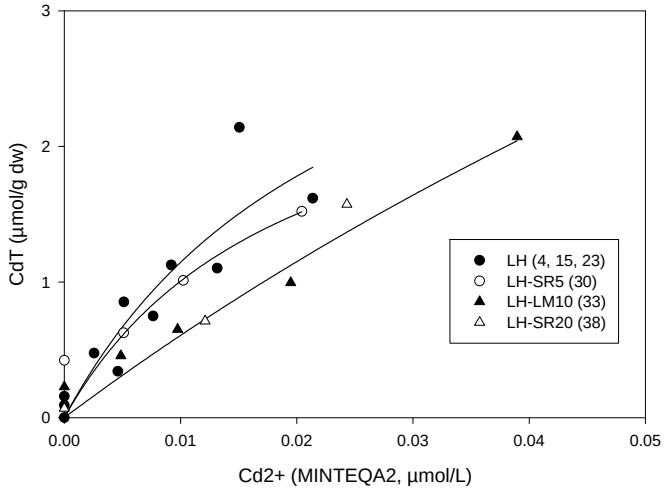


Figure 8.32 Accumulation of Cd in Tissue versus Cd^{2+} (MINTEQA2 speciation) in MSAM with Added DOC. MSAM (29): $r^2 = 0.97$, $p = 0.0002$; MSAM -SR5 (31) $r^2 = 0.99$, $p = <0.0001$.

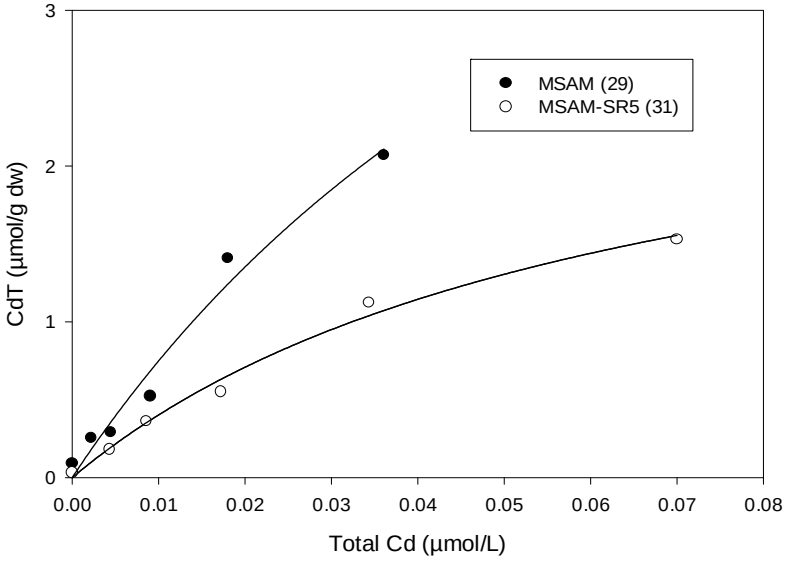


Figure 8.33: Accumulation of Cd in Tissue versus Cd^{2+} (BLM speciation) in LHM with Added DOC. LH (4, 5, 23): $r^2 = 0.84$, $p = <0.0001$; LH-SR5 (30) $r^2 = 0.82$, $p = 0.063$; LH-LM10(33): $r^2 = 0.99$, $p = <0.0001$. LH-SR20 (38) insufficient data for regression.

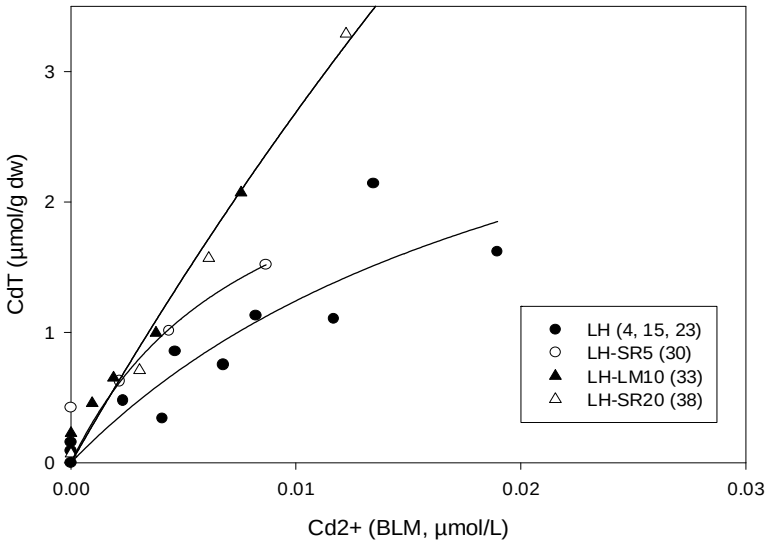
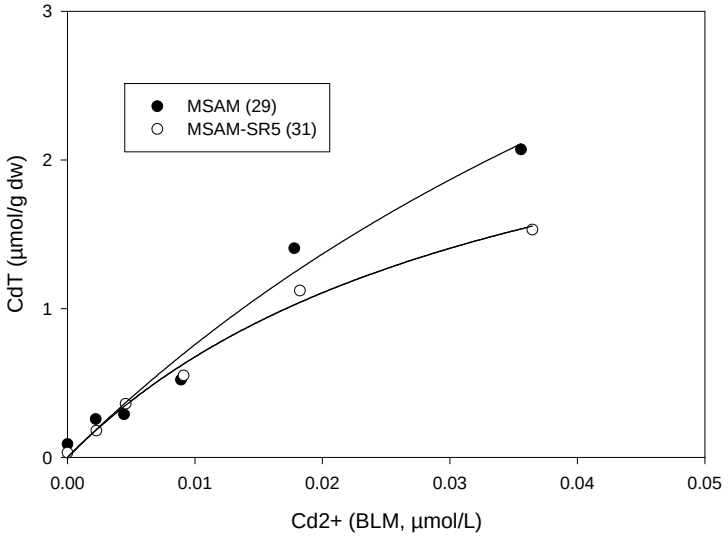


Figure 8.34: Accumulation of Cd in Tissue versus Cd^{2+} (BLM speciation) in MSAM with Added DOC. MSAM (29): $r^2 = 0.97$, $p = 0.0002$; MSAM -SR5 (31) $r^2 = 0.99$, $p = <0.0001$.



8.4.3 Toxicity

Because several tests were carried out using LHM, the range in response of all LH tests (test 4, 15, 23 and 37) was used as a baseline of comparison to those tests in LHM with added DOC. The seven-day LC50s were consistent among the LH tests at approximately 0.03 μmol/L total Cd but became more variable as exposure duration increased, as illustrated by 28-day LC50s ranging from 0.006 to 0.022 μmol/L total Cd (Table 8.7).

Some of the tests conducted with Cd in LHM with added DOC showed non-dose related toxicity. Specifically, control survival was only 60% in the Luther Marsh test (10 mg/L DOC), although survival exceeded 90% in the lowest two concentrations. Also, high mortality was observed in the lowest two concentrations of the 10 mg/L Suwannee River test although control survival was above 90%. Since the observed mortality in the control and lower concentrations was not related to Cd concentration, these exposures were omitted from the calculation of the LC50s for these tests.

Results of the long-term toxicity tests with DOC are presented as total Cd (Table 8.8) and Cd^{2+}, modelled using both MINTEQA2 (Table 8.8) and the BLM program (Table 8.10).

As shown in Table 8.8, the addition of 5 mg/L DOC did not result in a significant reduction in toxicity in either the LH or the MSAM medium, in spite

of the reduced bioaccumulation in MSAM. However, the addition of DOC concentrations of 10 and 20 mg/L reduced toxicity by approximately 1.5 to 2 times. Although the highest LC50 was observed in the Luther Marsh (10 mg/L DOC) exposure, it was not significantly different from the Suwannee River LC50s (10 and 20 mg/L DOC), based on overlapping confidence limits.

LC50s estimated from solutions based on the Cd^{2+} concentration, as modeled using MINTEQA2 (Table 8.9), were similar to those based on total Cd since MINTEQA2 did not indicate a strong influence of DOC on Cd binding. Additionally, the use of MINTEQA2's Cd^{2+} concentrations did not reduce the difference in LC50s among tests with and without added DOC, suggesting that MINTEQA2 may underestimate the binding of Cd to DOC. However, the modeling results supported the toxicity and accumulation observed in the LH tests with 5 mg/L added DOC, since neither bioaccumulation nor toxicity were reduced with the addition of 5 mg/L DOC from Suwannee River DOC.
When the BLM program was used for predicting Cd^{2+} concentrations, the LC50s for tests with added DOC were more consistent among themselves but decreased below those of the tests without added DOC (Table 8.10), suggesting that the BLM program may overestimate the binding of Cd to DOC.

Table 8.8: 28-Day LC50s (Total Cd, µmol/L) from Tests with Added DOC

Test	Medium				day 7			day 14			day 21			day 28		
#	Base	Add	pH	Ca (mg/L)	LC50	lcl	ucl	LC50	lcl	ucl	LC50	lcl	ucl	LC50	lcl	ucl
4	LH		7.7	11				0.025	0.019	0.037	0.011	0.009	0.014	0.008	0.007	0.01
15	LH		7.8	12	0.031	0.026	0.036	0.028	0.024	0.032	0.023	0.024	0.027	0.022	0.018	0.026
23	LH		7.7	14	0.026	0.022	0.031	0.014	0.012	0.017	0.008	0.006	0.009	0.006	0.005	0.007
37	LH		7.5	11	0.033	0.027	0.039	0.017	0.013	0.023						
30	LH	SR5	7.7	11	0.033	0.028	0.038	0.024	0.02	0.03	0.02	0.016	0.025	0.02	0.016	0.025
32	LH	SR10	7.7	9.5	0.051	0.041	0.063	0.048	0.038	0.059	0.037	0.032	0.042	0.034	0.029	0.038
33	LH	LM10	7.9	9.4	0.069	0.057	0.084	0.054	0.045	0.067	0.049	0.04	0.061	0.039	0.031	0.049
38	LH	SR20	7.4	11	0.052	0.044	0.061	0.044	0.039	0.046	0.043	0.036	0.049	0.039	0.034	0.046
29	SAM		7.9	34	0.086	0.07	0.105	0.053	0.045	0.063	0.042	0.034	0.052	0.041	0.032	0.051
31	SAM	SR5	8.1	40	0.098	0.081	0.12	0.061	0.051	0.073	0.059	0.049	0.072	0.057	0.047	0.069

lcl/ucl = lower/upper 95% confidence limits

Table 8.9: 28-Day LC50s (Cd^{2+}, µmol/L, MINTEQA2) from Tests with Added DOC

Test	Medium				day 7			day 14			day 21			day 28		
#	Base	Add	pH	Ca (mg/L)	LC50	lcl	ucl	LC50	lcl	ucl	LC50	lcl	ucl	LC50	lcl	ucl
4	LH		7.7	11				0.023	0.018	0.035	0.011	0.008	0.014	0.008	0.006	0.01
15	LH		7.8	12	0.028	0.024	0.033	0.026	0.022	0.03	0.021	0.022	0.025	0.02	0.016	0.024
23	LH		7.7	14	0.025	0.021	0.029	0.013	0.011	0.016	0.007	0.006	0.009	0.006	0.004	0.007
37	LH		7.5	11	0.031	0.026	0.037	0.017	0.013	0.021						
30	LH	SR5	7.7	11	0.03	0.026	0.035	0.022	0.018	0.027	0.018	0.014	0.023	0.018	0.014	0.023
32	LH	SR10	7.7	9.5	0.046	0.037	0.056	0.043	0.034	0.053	0.033	0.029	0.038	0.03	0.026	0.034
33	LH	LM10	7.9	9.4	0.061	0.05	0.074	0.048	0.039	0.058	0.043	0.035	0.053	0.034	0.027	0.043
38	LH	SR20	7.4	11	0.045	0.039	0.053	0.039	0.034	0.04	0.037	0.032	0.043	0.034	0.029	0.04
29	MSAM		7.9	34	0.07	0.057	0.085	0.043	0.036	0.051	0.034	0.027	0.042	0.033	0.026	0.042
31	MSAM	SR5	8.1	40	0.076	0.062	0.093	0.047	0.039	0.057	0.046	0.038	0.055	0.044	0.036	0.054

lcl/ucl = lower/upper 95% confidence limits

Table 8.10: LC50s (Cd2+, µmol/L, BLM) from Tests with Added DOC

Test	Medium				day 7			day 14			day 21			day 28		
#	Base	Add	pH	Ca (mg/L)	LC50	lcl	ucl	LC50	lcl	ucl	LC50	lcl	ucl	LC50	lcl	ucl
4	LH		7.7	11				0.021	0.016	0.031	0.01	0.007	0.012	0.007	0.006	0.009
15	LH		7.8	12	0.025	0.022	0.029	0.023	0.02	0.026	0.019	0.02	0.022	0.018	0.015	0.021
23	LH		7.7	14	0.022	0.019	0.026	0.012	0.01	0.014	0.006	0.005	0.008	0.005	0.004	0.006
37	LH		7.5	11	0.029	0.024	0.034	0.015	0.012	0.02						
30	LH	SR5	7.7	11	0.013	0.011	0.015	0.009	0.008	0.012	0.008	0.006	0.01	0.008	0.006	0.01
32	LH	SR10	7.7	9.5	0.013	0.01	0.016	0.012	0.01	0.015	0.009	0.008	0.011	0.008	0.007	0.01
33	LH	LM10	7.9	9.4	0.012	0.01	0.014	0.009	0.008	0.011	0.008	0.007	0.01	0.007	0.005	0.008
38	LH	SR20	7.4	11	0.011	0.01	0.013	0.01	0.009	0.01	0.009	0.008	0.011	0.009	0.007	0.01
29	MSAM		7.9	34	0.069	0.056	0.084	0.042	0.036	0.05	0.033	0.027	0.042	0.033	0.026	0.041
31	MSAM	SR5	8.1	40	0.04	0.033	0.049	0.025	0.021	0.03	0.024	0.02	0.029	0.023	0.019	0.029

lcl/ucl = lower/upper 95% confidence limits

8.5 LA50s

Sufficient tissue data were available to calculate LA50s for nine of the long-term toxicity tests performed. The mean LA50 for the nine tests was 1.4 µmol/g dw, with a three fold range between highest and lowest. LC50s calculated from the same group of tests ranged approximately 22-fold, based on Cd^{2+} (modeled using MINTEQA2) (Table 8.11). If tests with added DOC are excluded, the average LA50 is 1.23 µmol/g dw but the range in LA50s and LC50s does not change.

Table 8.11: 28-day LA50s (µmol/g dw) Compared to 28-day LC50s (Cd^{2+}, µmol/L, MINTEQA2)

Test	LC50	lcl	ucl	LA50	lcl	ucl
3	0.012	0.009	0.017	0.70	0.55	0.97
16	0.062	0.047	0.093	1.5	1.0	4.0
18	0.061	0.053	0.071	1.1	0.97	1.1
19	0.036	0.030	0.044	2.3	2.0	3.1
22	0.12	0.11	0.14	1.8	1.5	2.6
23	0.005	0.004	0.007	0.70	0.48	0.85
31	0.044	0.036	0.054	1.2	1.0	1.4
33	0.034	0.027	0.044	1.8	1.4	2.3
38	0.034	0.029	0.040	2.3	1.9	2.7
Mean including DOC	24			1.4		
max/min including DOC				3.4		
Mean excluding DOC	24			1.2		
max/min excluding DOC				3.4		

lcl = lower 95% confidence limit; ucl = upper 95% confidence limit; shaded rows indicate tests with added DOC

8.6 SUMMARY OF KEY FINDINGS

- *Short-term Tests:*
    - Chemical analysis of test solutions after seven-days of exposure suggested that most of the Cd would be lost from solution within this time frame.
    - Cd accumulation in tests with adults varied with increasing Cd and was mitigated by Ca and H^+. Toxicity data for a limited number of tests supported the bioaccumulation data, in that most toxicity could be explained by changes in Ca.
    - The mean seven-day LA50 was calculated as 0.86 µmol/g dw, with a less than two-fold difference between minimum and maximum values, compared to a six-fold difference between minimum and maximum LC50s for the same tests.
- *Time-series Tests:*
    - Time-series tests conducted for up to 21 days did not confirm steady state in any exposure. However, accumulation of Cd was estimated to be at 80% of steady state in MSAM and DC exposures after 7 days and essentially reached in all exposures after 28 days of exposure.

- *Long-term tests:*
    - Chemical analysis of the test solutions immediately before solution exchange every 7 days supported the findings in the short-term tests that most of the Cd was lost from solution in that time frame.
    - Long-term tests supported the results of short-term tests, in that bioaccumulation was influenced by Ca and H^+. However, only Ca showed a clear linear relationship with toxicity over the 28-days exposure period.
    - Based on total Cd, the addition of at least 10 mg/L DOC to LHM decreased bioaccumulation and toxicity. This influence on toxicity was maintained throughout the exposure period. No significant impact on toxicity was observed from the addition of 5 mg/L DOC in either LHM or MSAM. However, bioaccumulation in MSAM was reduced.
    - Comparison of estimated Cd^{2+} in the various DOC tests suggested that MINTEQA2 overestimated the proportion of Cd present in the free form and that the Hydroqual BLM program underestimated the proportion as free Cd.
    - The mean observed 28-day LA50 was calculated as either 1.4 or 1.2 µmol/g dw, depending on whether DOC tests were included or excluded, respectively. The range in LA50s was 3.4 times compared to the corresponding range in LC50s of 24 times.

9.0 CADMIUM - MODEL DEVELOPMENT

9.1 SHORT-TERM BIOACCUMULATION TESTS

9.1.1 Preliminary Estimates of L_T

Preliminary estimates for L_T were calculated for each short-term bioaccumulation test from linear transformations of the uptake model (Cd/CdT vs Cd). Estimated L_Ts for tests with correlation coefficients above 0.5 (all except 7, 8, 41, 42, 46, 49) ranged from 0.20 to 1.5 µmol/g dw, but generally the tests did not fit the linear model as well as the nickel data(Appendix F), since steady state was not apparent after 7 days of exposure. Estimated L_Ts decreased with increasing calcium concentrations (Figure 9.1) but were not influenced by pH (Figure 9.2).

The estimated L_Ts for Cd were within the range of observed LA50s from the seven-day tests (range of 0.66 to 1.2 µmol/g, mean of 0.86 µmol/g dw). However, the lack of saturation and apparent influence of Ca on the L_T value may have hampered the establishment of a reliable L_T value from the linear transformation of the accumulation model. From the intercepts of the tests with correlation coefficients above 0.5, a preliminary mean estimate for the conditional log K_{Cd} was calculated as 7.9 (range of 7.6 to 8.1).

Figure 9.1: Estimated L_T (from Plots of Cd/CdT vs Cd) versus Ca for Seven-day Bioaccumulation Data ($r^2 = 0.48$, $p = 0.015$)

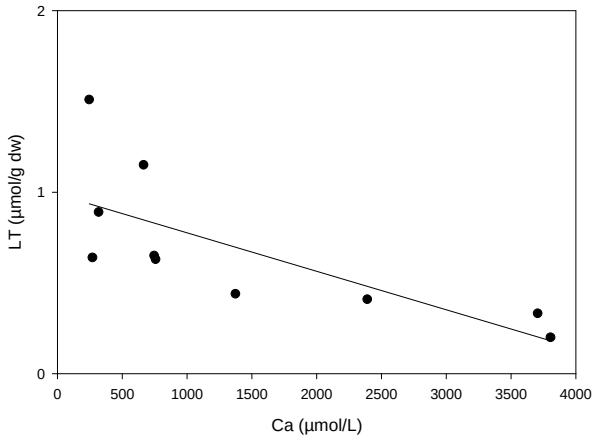
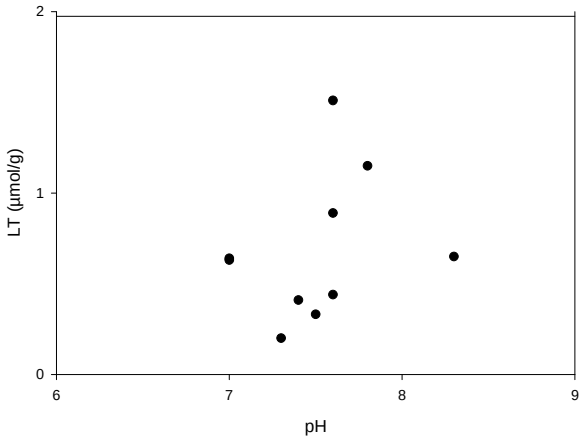


Figure 9.2: Estimated L_T (from Plots of Cd/CdT vs Cd) versus pH for seven-day Bioaccumulation Data.



9.1.2 Defining a Model

Based on the experimental results, a preliminary model was developed for the uptake of Cd, based only on competition with calcium:

$$[CdT] = \frac{[Cd]}{a + b*[Ca] + ILT*[Cd]} \qquad \text{(Model A)}$$

where:

[CdT] = whole body concentration of Cd (μmol/g dw)

$a = 1/(K_{Cd}*L_T)$

$b = K_{Ca}/(K_{Cd}*L_T)$

$c = K_H/(K_{Cd}*L_T)$

$IL_T = 1/L_T$

As was the case for Ni, this model assumed a constant L_T in spite of the indications that L_T was influenced by Ca. L_T may change and thereby influence the prediction of LA50s; however, if the proportion of $LA50/L_T$ is constant, the predicted LC50 should not be affected.

Jackson et al. (2000) concluded that competition with Ca ions was the reason that LC50s for Cd, based on the free ion, increased with increasing Ca. This theory is consistent with model A; however, an increase in LC50s would also have been observed if calcium exerted a physiological, rather than competitive influence, consistent with the change in L_T noted above. Therefore, the model was modified to a non-competitive scenario with the inclusion of the hypothetical constant b2 (model B) as was investigated for nickel:

$$CdT = \frac{[Cd]}{(a' + IL_{T0}*[Cd])} * \frac{1}{(1 + b2*Ca)} \quad \text{(Model B)}$$

where $a' = 1/(K_{Cd}*L_{T0})$

As described for the nickel models, *b2* represents the binding strength of Ca to a hypothetical enzyme involved in ligand synthesis. The enzyme is rendered inactive when bound to Ca, thereby reducing the amount of ligand available for binding to any of the competitor cations. Here L_T is replaced by L_{T0}, which includes the synthesis and degration rates for L_T multiplied by the total concentration of the enzyme that makes L_T (Appendix C). Alternatively, *b2* could also represent simple non-competitive binding of Ca to the Cd uptake site.

Multiple regressions of the above models were log transformed to equalize the variance and run in least-square regressions, with the sequential addition of other cations, to determine if any other cations influenced uptake.

Both Models A and B fitted the data equally well, with correlation coefficients of 0.77. The inclusion of hydrogen as a competitor into Models A and B improved the fit of both models and, therefore, two additional models (Models C and D) were included in subsequent steps of the analysis (Table 9.1):

$$[CdT] = ([Cd] / (a + b*[Ca] + c*[H] + IL_T*[Cd])) \quad \text{(model C)}$$

$$[CdT] = ([Cd] / (a' + c'*[H] + IL_{T0}*[Cd])/(1 + b2*[Ca])) \quad \text{(model D)}$$

where $c = K_H/(K_{Cd}*L_T)$ and $c' = K_H/(K_{Cd}*L_{T0})$.

Table 9.1: Model Outputs and 95% Confidence Limits for the Short-term Toxicity Tests

Constant	**Model A** (R^2 of 0.77)	**Model C** (R^2 of 0.83)
a	0.014277	0.004345
	(0.009620, 0.018934)	(-0.000053, 0.008743)
b	0.000022	0.000023
	(0.000017, 0.000028)	(0.000018, 0.000028)
c	-	0.293694
		(0.198590, 0.388797)
IL_T	0.862813	0.799415
	(0.648197, 1.077428)	(0.620730, 0.978101)
Constant	**Model B** (R^2 of 0.77)	**Model D** (R^2 of 0.82)
a'	0.020284	0.013461
	(0.016726, 0.023841)	(0.010567, 0.016355)
c'	-	0.197970
		(0.129806, 0.266134)
IL_{T0}	0.487908	0.460458
	(0.335600, 0.640216)	(0.333453, 0.587463)
b2	0.000755	0.000773
	(0.000476, 0.001035)	(0.000524, 0.001022)

Note: Cd, H, and Ca = µmol/L and L_T = µmol/g dw

From the IL_T and IL_{T0} values in Table 9.1, L_T and L_{T0} were calculated for models A, B, C and D as 1.2, 2.1, 1.2 and 2.2 µmol/g dw. Log K_{Cd} was estimated as 7.8, 7.4, 8.3 and 7.5 for models A to D, respectively. Models A and C allowed estimation of Log K_{Ca} as 3.2 and 3.7, respectively and models C and D allowed estimation of Log K_H as 7.8 and 7.2, respectively. Estimated log K values for Cd and Ca are lower than those developed by Playle et al. (1993) using fathead minnows exposed in low-hardness water to concentrations of Cd in the same range as those of the seven-day bioaccumulation tests of this study. Their estimated log values for K_{Cd}, K_H and K_{Ca} were 8.6, 6.7 and 5.0, respectively. Influences on the estimation of the binding affinity may be related

to species-specific factors or to the duration of the exposure or the exposure concentration. For example, in exposures to higher metal concentrations, binding occurs with both low-affinity as well as high-affinity sites on the ligand, whereas only high-affinity sites bind at low metal concentrations (Playle et al., 1993).

9.1.3 Prediction of LA50s

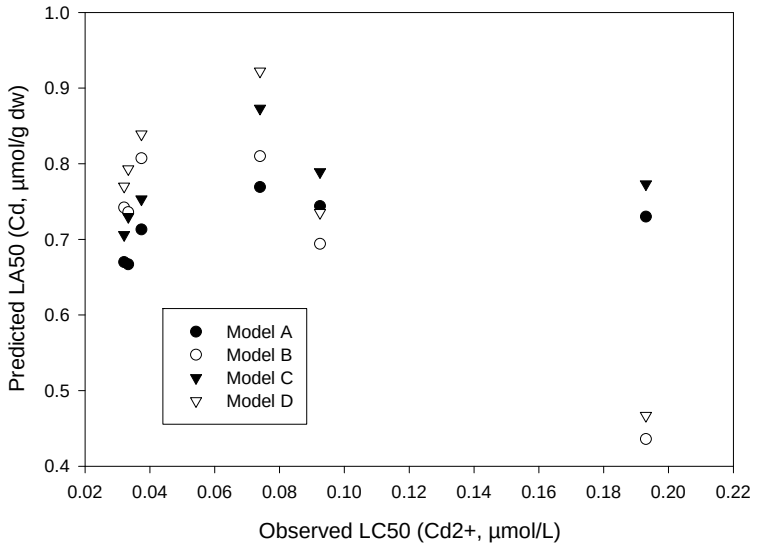
All four models were used to predict LA50s at the LC50, for all tests for which a reliable LC50 had been calculated. Mean LA50s of all tests for which an LC50 could be calculated were estimated from models A, B, C and D as 0.71, 0.69, 0.77 and 0.74 µmol/g dw, respectively, which were all within 20% of the observed mean LA50 of 0.86 µmol/g dw. For each test, the four models predicted similar LA50s except for test 50 (Ca=4 mmol/L). In that case, the competitive models (A and C) predicted LA50s closer to the observed value of 0.66 µmol/g dw than did non-competitive models (B and D) (Table 9.2).

Table 9.2: Comparison of Calculated LA50s (µmol/g dw) to Predicted LA50s (µmol/g dw) from Seven-day Bioaccumulation Tests

			Observed	Predicted LA50			
Test	**Base**	**Add**	**LA50**	**Model A**	**Model B**	**Model C**	**Model D**
8	LH	Na3.5	0.93	0.67	0.74	0.71	0.77
13	LH	Ca1.4	1.2	0.74	0.69	0.79	0.74
14	LH		0.71	0.67	0.74	0.73	0.79
46	SAM	Mg1	0.92	0.77	0.81	0.87	0.92
50	LH	Ca4	0.66	0.73	0.44	0.77	0.47

Based on the limited dataset, LA50s estimated from the four models demonstrated greater consistency (ranging from 1.2 to 2 times) than observed LC50s (range of 6 times) (Figure 9.3). The lower LA50 predicted at higher LC50 may be reflective of the potential influence of calcium on the ligand, reducing the total amount of ligand available for binding.

Figure 9.3: Predicted Seven-day LA50s (Cd, µmol/g dw) for Models A, B, C, and D versus Observed Seven-day LC50s (Cd^{2+}, µmol/L).



9.1.4 Prediction of LC50s

Mean LA50s from the four models were combined with the estimates for the model constants in the model expressions, which were reorganized to solve for Cd at the LA50 (CdL). The mean observed LA50 of 0.86 µmol/g dw was also used to predict LC50s. Predicted LC50s derived from the four models using the mean observed LA50s and mean predicted LA50s are presented in Tables 9.3 and 9.4, respectively. As shown in Figures 9.4 and 9.5, the use of the mean

observed LA50 predicted LC50s less accurately than the use of the mean predicted LA50.

Table 9.3: Predicted vs Observed LC50s (Cd^{2+}, µmol/L) using Mean Observed LA50s

Test	Observed	Predicted LC50s			
	LC50	Model A	Model B	Model C	Model D
7	0.037	0.067	0.042	0.055	0.039
8	0.032	0.067	0.042	0.054	0.039
13	0.092	0.15	0.24	0.12	0.18
14	0.033	0.071	0.045	0.052	0.039
46	0.074	0.11	0.088	0.070	0.060
50	0.19	0.33	-0.11	0.26	-0.096

Table 9.4: Predicted vs Observed LC50s (Cd^{2+}, µmol/L) using Mean Predicted LA50s

Test	Observed	Predicted LC50s			
	LC50	Model A	Model B	Model C	Model D
7	0.037	0.037	0.028	0.040	0.030
8	0.032	0.037	0.028	0.040	0.030
13	0.092	0.082	0.091	0.087	0.094
14	0.033	0.039	0.030	0.038	0.029
46	0.074	0.059	0.050	0.051	0.041
50	0.19	0.18	-0.18	0.19	-0.14

Excluding test 50, all models (using both observed and predicted mean LA50s) estimated LC50s within a factor of two of the observed. Using the predicted mean LA50, the mean ratio of predicted to observed LC50s was 0.98 and 0.99 for models A and C, and 0.83 and 0.82 for models B and D, respectively. As shown in Figures 9.4 and 9.5, the inclusion of H^+ did not improve the prediction of LC50s, since estimates from model A versus C and model B versus D were almost identical.

Figure 9.4: Predicted versus Observed Seven-day LC50s (Cd^{2+}, µmol/L) for Models A, B, C, and D Based on the Mean Observed LA50 (µmol/g dw).

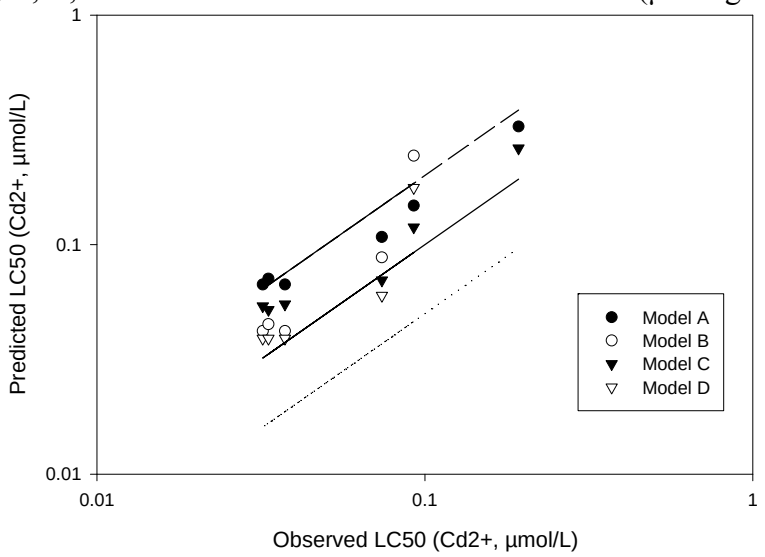
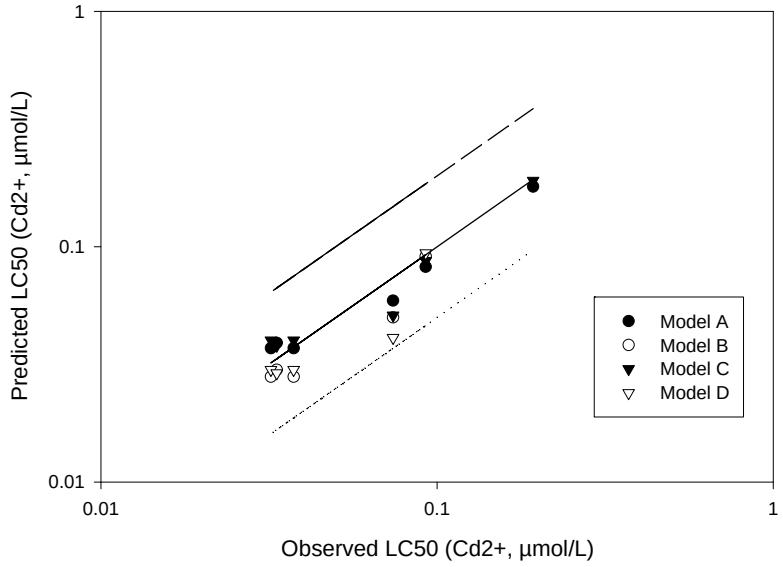


Figure 9.5: Predicted versus Observed seven-day LC50s (Cd^{2+}, µmol/L) for Models A, B, C, and D Based on Mean Predicted LA50s (µmol/g dw).



9.2 LONG-TERM TOXICITY TESTS

Long-term test data were modeled in the same manner as the short-term tests. That is, the candidate models were log transformed to equalize the variance, and then multiple regressions were run with the sequential addition of other cations to determine if any other cations influenced uptake. Modelling was carried out excluding data from the DOC tests and test 28, which had problematic chemistry.

The models that fit the long-term test data best were Models A and B. In contrast to the seven-day bioaccumulation data, models including H^+ as a competitor (models C and D) did not fit the data well and, therefore, were not considered further. Estimates of each constant from the models A and B are summarized in Table 9.5.

From the values in Table 9.5, L_T and L_{T0} were calculated for Model A(Y) and B(Y) as 3.80 and 9.27 μmol/g dw, respectively. These estimates were over three times higher than those estimated from Models A and B (1.2 and 2.1 μmol/g dw, respectively). Log K_{Cd} estimates were consistent with those of the adult models at 7.6 (versus 7. 8) for model A(Y) and 7.1 (versus 7.4) for model B(Y). Similarly, the log K_{Ca} estimated from model A(Y) of 3.0 was consistent with the 3.2 estimated from model A.

Table 9.5: Model Outputs and 95% Confidence Limits for the Long-term Toxicity Tests, excluding DOC

Constant	Estimates from Model A(Y) (R^2 of 0.88)	Estimates from Model B(Y) (R^2 of 0.89)
a	0.005892 (0.003894, 0.007891)	-
a'	-	0.007745 (0.006116, 0.009374)
b	0.000006 (0.000004, 0.000008)	-
$\mathbf{IL_T}$	0.262938 (0.144228, 0.381648)	
$\mathbf{IL_{T0}}$		0.107839 (0.041119, 0.174560)
b2		0.000604 (0.000314, 0.000893)

Note: Cd, H, and Ca = µmol/L and L_T = µmol/g dw

9.2.1 Prediction of LA50s

As shown in Table 9.6 the mean predicted LA50s from Models A(Y) and B(Y) were within 10% of the mean observed LA50s for datasets including and excluding DOC tests. Most predicted LA50s were in agreement with observed values, as reflected by an average ratio of observed to predicted LA50s of 0.93 and 0.98 for Model A(Y) and 0.88 and 0.99 for Model B(Y), including and excluding DOC tests, respectively (Table 9.7). Figure 9.6 presents predicted LA50s from Models A(Y) and B(Y) versus observed LC50s for the data set excluding DOC tests.

Table 9.6: Predicted Mean 28-day LA50s (µmol/g dw) from Models A(Y) and B(Y) using Toxicity Data from Tests with Young

	Model A(Y)	Model B(Y)	Observed LA50	Observed LC50
Include DOC tests				
mean	1.4	1.5	1.4	na
max/min	3.6	4.8	3.4	24
Exclude DOC tests				
mean	1.2	1.3	1.2	na
max/min	3.4	4.4	3.4	24

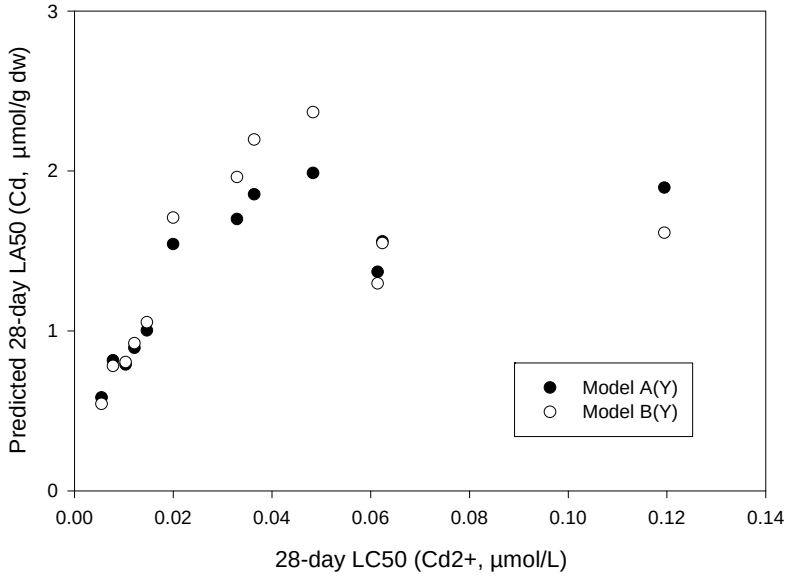
na = not applicable

Table 9.7: Comparison of Observed 28-day LA50s (µmol/g dw) to Those Predicted by Models A(Y) and B(Y)

Test	Observed LA50	lcl	ucl	Model A(Y)	Model B(Y)
3	0.7	0.55	0.97	0.89	0.92
16	1.5	1.0	4.0	1.6	1.6
18	1.1	0.97	1.1	1.4	1.3
19	2.4	2.0	3. 1	1.8	2.2
22	1.8	1.5	2.6	1.9	1.6
23	0.7	0.48	0.85	0.58	0.54
31	1.2	1.1	1.4	1.9	2.2
33	1.8	1.4	2.3	2.1	2.6
38	2.3	1.9	2.7	2.1	2.6

lcl: 95% lower confidence limits; ucl: 95% upper confidence limits; Shading indicates tests with DOC

Figure 9.6: Predicted 28-day LA50s (µmol/g dw) from 28-day LC50 Data, excluding DOC tests



9.2.2 Prediction of LC50s

Models A(Y) and B(Y) were used to predict 28-day LC50s using the mean observed LA50 by rearranging the model formulae to solve for Cd. LC50s predicted from models A(Y) and B(Y) were similar and are presented in Table 9.8 and Figure 9.7. Of the 12 tests without added DOC, Model A(Y) predicted LC50s within a factor-of-two range for all but tests 23 and 28, and Model B(Y) predicted LC50s within a factor-of-two range for all except tests 21 and 28. Test 21 was conducted in MSAM with added magnesium and had lower observed toxicity than predicted by the model, suggesting that the elevated magnesium may have exerted toxicity, which is not accounted for by the model. Test 23 was an LH exposure and was lower than another LC50 conducted in this medium; therefore the lack of correspondence between predicted and observed toxicity may be due to a problem with the test rather than the model. This is

likely also the case with Test 28, which was previously identified as having questionable chemistry data.

Of particular note is that instead of a clear 1:1 linear relationship between predicted and observed values, the predicted LC50s fell roughly in three lateral bands, according to calcium concentrations of approximately 0.3, 1 and 3 mmol/L (Figure 9.8). The broad range of observed LC50s associated with a single predicted value reflected variability in organism response in four-week exposures to similar media and/or may have suggested that other factors influencing Cd toxicity were not accounted for in the models.

Table 9.8: Predicted versus Observed 28-day LC50s (Cd^{2+}, µmol/L) Based on Mean Observed LA50 (µmol/g dw) for Models A(Y) and B(Y)

Test	Observed LC50	Model A(Y)	Model B(Y)
3	0.012	0.022	0.020
4	0.008	0.016	0.015
15	0.020	0.016	0.015
16	0.062	0.051	0.050
18	0.061	0.061	0.068
19	0.036	0.022	0.019
21	0.048	0.025	0.022
22	0.12	0.068	0.081
23	0.005	0.017	0.016
28	0.010	0.022	0.020
29	0.033	0.023	0.020
30	0.018	0.016	0.015
31	0.044	0.025	0.022
32	0.030	0.016	0.015
33	0.034	0.016	0.015
36	0.015	0.023	0.020
38	0.034	0.016	0.015

Shaded rows represent tests with added DOC

Figure 9.7: Predicted vs Observed 28-day LC50s (Cd^{2+}, µmol/L) from Models A(Y) and B(Y), excluding DOC tests

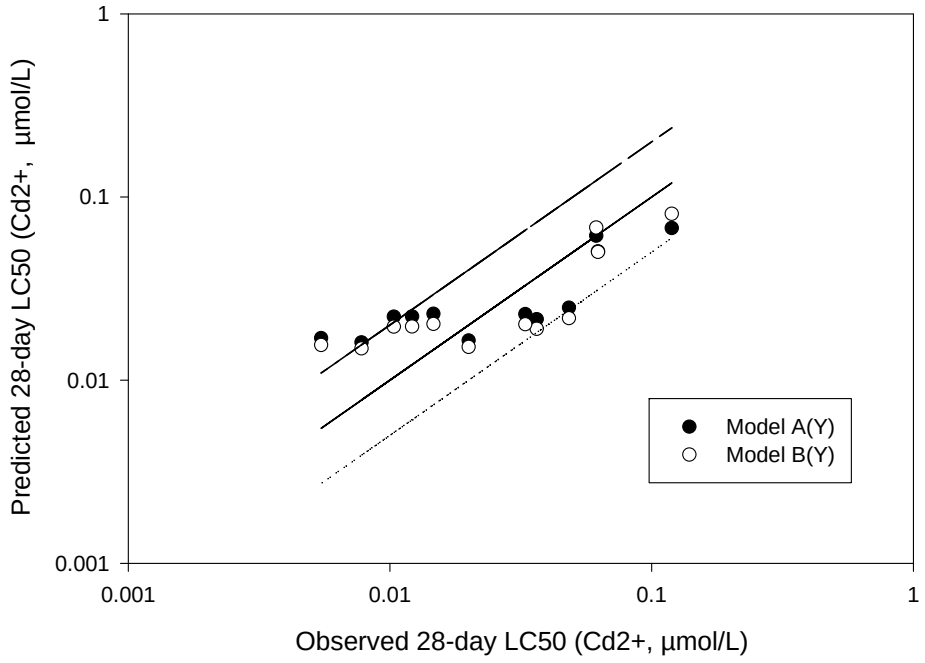
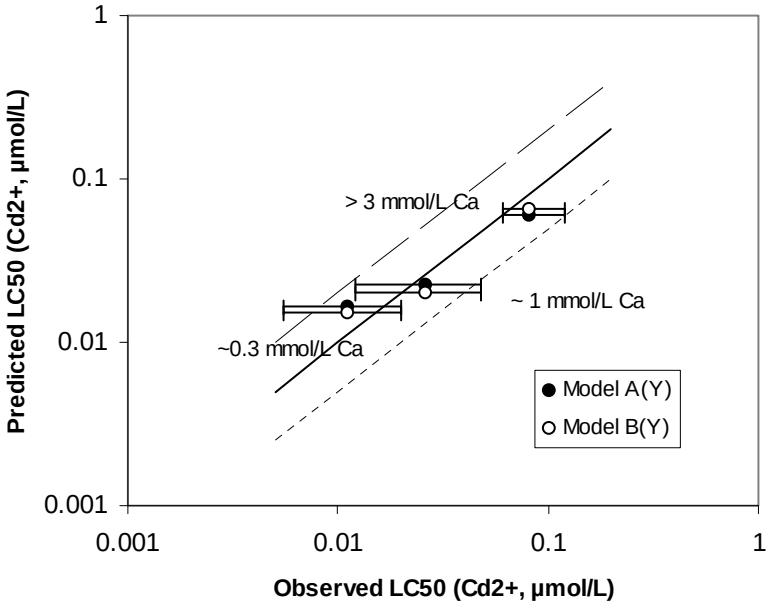


Figure 9.8: Range of Mean Observed 28-day LC50s (Cd^{2+}, µmol/L) at Three Different Calcium Concentrations Compared to Predicted 28-day LC50s (Cd^{2+}, µmol/L) from Models A(Y) and B(Y), (excluding DOC tests)



As proposed for Ni, organism response to Cd may have been influenced by the media in which they were exposed. That is, even if the organisms were not visibly impaired by the medium itself, it is possible that their sensitivity was enhanced when exposed to Cd. This is not accounted for in the model. However, media in which organisms were clearly stressed (based on poor control survival), were not used in model development.

The challenges associated with Ni speciation modeling were also experienced with Cd. MINTEQA2 did not predict the speciation of Cd in solutions of added DOC with consistent accuracy, hampering the use of the DOC data in model development. More research is required on metal interactions with dissolved organic material in order to develop models that can consistently predict metal speciation in solutions of various types of dissolved organic material and concentrations.

In contrast to the Ni data, H^+ did not influence the toxicity of Cd in Long-term exposures; therefore, pH control is not as important and likely did not influence the modeling. This was shown clearly by the significant relationship between calcium and Cd toxicity and the lack of a visible relationship for Ni except at the same exposure pH.

Unlike Ni, steady state of Cd accumulation was not apparent for any test exposure period but was predicted by kinetic modeling to be achieved within 28

days. This may be due to slow kinetics or sequestering of Cd within tissues of the organism where it does not exert a toxic effect. The increasing concentrations could also reflect adsorption to the carapace, since some researchers, who rinsed test organisms in EDTA before digestion, observed steady state.

9.3 PREDICTING 28-DAY TOXICITY FROM SEVEN-DAY DATA

Models based on the seven-day adult bioaccumulation data were evaluated for their ability to predict toxicity in the 28-day toxicity tests with young.

9.3.1 Prediction of LA50s

Mean LA50s predicted from each of the four adult models using the toxicity data from the various LC50 tests with young ranged from 0.55 to 0.72 µmol/g dw for the seven-day data and 0.37 to 0.46 µmol/g dw for the 28-day data (Table 9.9). The mean predicted 28-day LA50s were roughly one third of the observed mean 28-day LA50 and the 28-day LA50s predicted by the young models. However, when compared as proportions of their respective L_T estimates, the mean LA50s were consistent (Table 9.10).

Robinson et al. (2003) compared Cd accumulation in *Ceriodaphnia dubia* and *Daphnia magna* neonates in 10-minute exposures and found that adsorption was related to surface area. The larger *Daphnia* neonates adsorbed approximately five times more Cd than the smaller *Ceriodaphnia dubia*; however, after

adjustment for surface area of the carapace, the accumulation was similar. The authors noted that although larger individuals tend to accumulate more metal on a per organism basis, they tend to have lower whole-body concentrations based on weight due to their lower surface to volume ratios than smaller organisms. This is consistent with the young *Hyalella* models, which had higher predicted LA50s (on a per gram basis) than the adult models.

For the seven-day exposures, LA50s predicted by the non-competitive models (B and D) varied inversely with LC50 (Figure 9.9) but this trend was not apparent in models of the 28-day data (Figure 9.10), although more variation was evident in all models in the longer exposure. As shown in Figure 9.10, a linear relationship between the 28-day LA50s and LC50s is apparent for all tests except three (test 16, 18 and 22), suggesting a change in L_T with medium. This relationship was also observed in the nickel data (also with three outlier tests but different exposures) but was not apparent in the young models for either metal.

Table 9.9: Predicted Mean LA50s (µmol/g dw) for Long-term Tests (with Young) using Models A, B, C and D (derived from seven-day tests with adults)

	Model A	**Model B**	**Model C**	**Model D**
seven-day Data				
mean LA50	0.65	0.55	0.72	0.61
max/min	1.8	2.8	1.5	2.9
28-day Data				
mean LA50	0.40	0.37	0.46	0.44
max/min	3.0	3.4	3.0	3.6

Table 9.10: Comparison of Predicted 28-day LA50s (µmol/g dw) from the Adult and Young Models

	Model A	**Model B**
mean LA50 (µmol/g)	0.40	0.37
L_T (µmol/g)	1.2	2.1
Ratio LA50/L_T	0.25	0.18
	Model A(Y)	**Model B(Y)**
mean LA50 (µmol/g)	1.2	1.3
L_T (µmol/g)	3.8	9.3
Ratio LA50/L_T	0.32	0.14

Figure 9.9: Predicted seven-day LA50s (µmol/g dw) (from Adult Models) versus Seven-day LC50s (Cd^{2+}, µmol/L) from Tests with Young

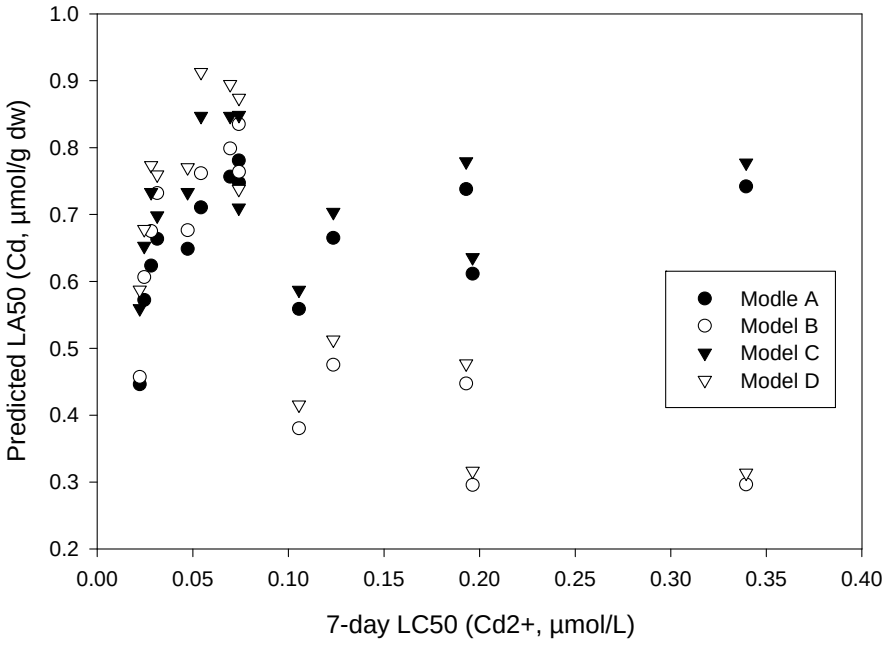
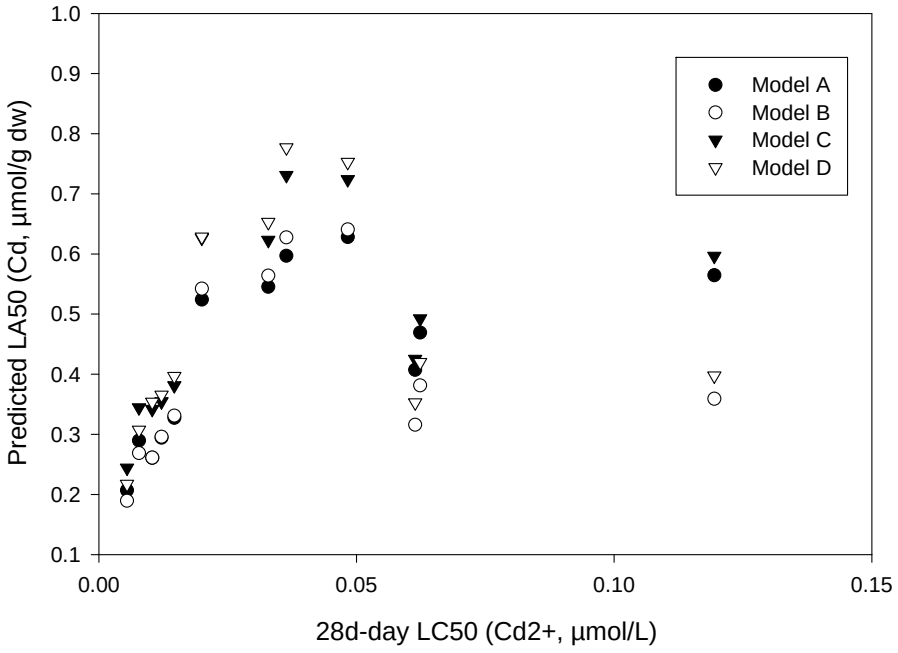


Figure 9.10: Predicted 28-day LA50s (µmol/g dw) from Adult Models versus Seven-day LC50s (Cd^{2+}, µmol/L) from Tests with Young



9.3.2 Prediction of LC50s

Using the predicted LA50s from each model, seven-day and 28-day LC50s were predicted. Models B and D produced several negative estimates for seven-day LC50s (Table 9.11, Figure 9.11), but worked equally well to the competitive models in the 28-day exposures (Table 9.12, Figure 9.12). These results are consistent those of the young models. Also as observed in the young models, the predicted 28-day toxicity from the adult models fell across three lateral bands consistent with three different concentrations of calcium (Figure 9.12).

Table 9.11: Comparison of Predicted Seven-day LC50s (Cd^{2+}, µmol/L) from Models A, B, C and D to Observed Seven-day LC50s (Cd^{2+}, µmol/L) from Tests with Young.

	Observed	Predicted LC50s			
Test	**LC50**	**Model A**	**Model B**	**Model C**	**Model D**
44	0.20	0.23	-0.12	0.26	-0.089
47	0.074	0.046	0.03	0.076	0.051
15	0.028	0.031	0.02	0.027	0.019
16	0.12	0.12	0.27	0.13	0.42
17	0.19	0.14	-3.5	0.16	-0.50
18	0.11	0.15	-1.1	0.16	-0.36
19	0.054	0.044	0.029	0.035	0.023
21	0.074	0.052	0.036	0.048	0.030
23	0.025	0.032	0.021	0.031	0.021
24	0.34	0.24	-0.11	0.28	-0.080
28	0.022	0.045	0.03	0.037	0.024
29	0.07	0.047	0.031	0.045	0.029
30	0.03	0.03	0.02	0.028	0.019
31	0.076	0.052	0.036	0.049	0.031
32	0.046	0.029	0.019	0.026	0.019
33	0.061	0.029	0.019	0.023	0.017
36	0.047	0.048	0.032	0.045	0.029
37	0.031	0.030	0.020	0.034	0.022
38	0.045	0.030	0.020	0.037	0.024

shaded rows represent tests with added DOC

Figure 9.11: Predicted vs Observed Seven-day LC50s (Cd^{2+}, µmol/L) for Young from Adult Models A and C.

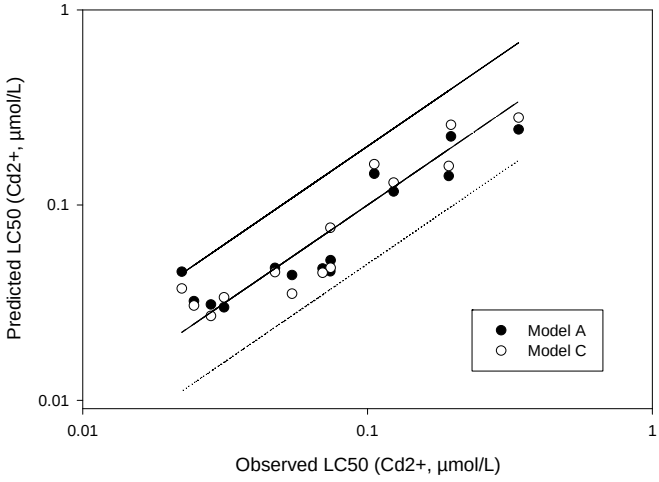
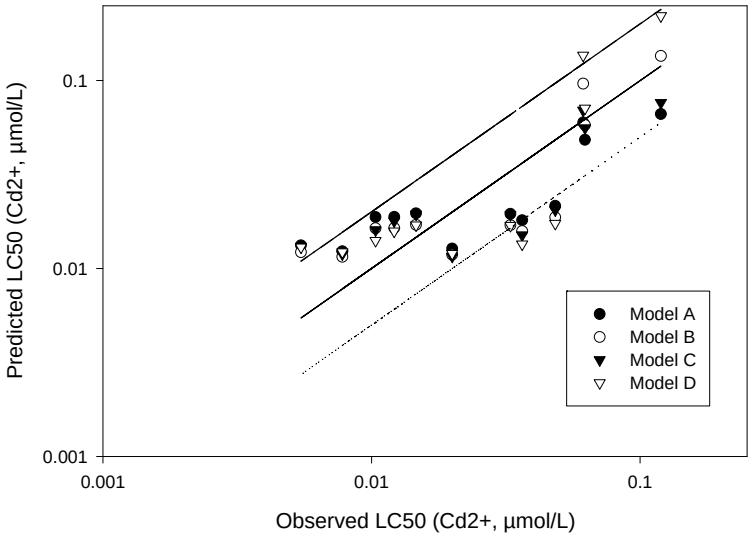


Table 9.12: Comparison of Predicted 28-day LC50s (Cd^{2+}, µmol/L) from Models A, B, C and D to Observed 28-day LC50s (Cd^{2+}, µmol/L) from Tests with Young.

	Observed	Predicted LC50s			
Test	**LC50**	**Model A**	**Model B**	**Model C**	**Model D**
3	0.012	0.019	0.016	0.018	0.016
4	0.008	0.012	0.012	0.012	0.012
15	0.020	0.013	0.012	0.012	0.012
16	0.062	0.048	0.058	0.056	0.071
18	0.061	0.060	0.096	0.069	0.14
19	0.036	0.018	0.016	0.015	0.014
21	0.048	0.022	0.019	0.021	0.017
22	0.12	0.066	0.14	0.076	0.22
23	0.006	0.013	0.012	0.013	0.013
28	0.010	0.019	0.016	0.016	0.014
29	0.033	0.020	0.017	0.019	0.017
30	0.018	0.012	0.012	0.012	0.012
31	0.044	0.022	0.019	0.021	0.018
32	0.030	0.012	0.011	0.011	0.012
33	0.034	0.012	0.011	0.010	0.011
36	0.015	0.020	0.017	0.019	0.017
38	0.034	0.012	0.012	0.016	0.015

shaded rows represent tests with added DOC

Figure 9.12: Predicted vs Observed 28-day LC50s (Cd^{2+}, µmol/L) for Young from Adult Models, (excluding tests with added DOC)



9.4 SUMMARY OF KEY FINDINGS

Short-term Tests:

- For data fitting a linear regression of Cd^{2+}/CdT vs Cd^{2+}, preliminary estimates were calculated for L_T (0.66 to 1.2 µmol/g dw) and conditional $logK_{Cd}$ (7.6 to 8.1). The estimated L_T was lowest in high Ca solutions, suggesting that increasing Ca decreased L_T, consistent with non-competitive models.
- Based on least-squares non-linear regression, four models were identified as best fitting the accumulation data:
  - Model A: assumed some free L and that Cd accumulation was influenced by competition with Ca.
  - Model B: assumed some free L and that Cd accumulation was influenced by non-competitive action of Ca on the ligand.
  - Model C: assumed some free L and that Cd accumulation was influenced by competition with Ca as well as H^+.
  - Model D: assumed some free L and that Cd accumulation was influenced by competition with H^+ as well as non-competitive action of Ca on the ligand.
- Model outputs are summarized in Table 9.13:

Table 9.13: Summary of Output from Adult Models A, B, C and D

Parameter	Model A	Model B	Model C	Model D
L_T (μmol/g dw)	1.2	2.1	1.2	2.2
Log K_{Cd}	7.8	7.4	8.3	7.5
Log K_H			7.8	7.2
Log K_{Ca}	3.2		3.7	

- The mean predicted LA50s from the four models were similar, ranging from 0.69 to 0.77 μmol/g dw. Toxicity was best predicted by the competitive models; however, the inclusion of H^+ did not improve the performance of the either model A or B.

Long-term Tests:

- In contrast to the seven-day bioaccumulation data, models including H^+ as a competitor (models C and D) did not fit the data well and were dropped in the analysis of the long-term test data.
- Model outputs are summarized in Table 9.14:

Table 9.14: Summary of Output from Adult Models A(Y) and B(Y)

Parameter	Model A(Y)	Model B (Y)
L_T (μmol/g dw)	3.8	9.3
Log K_{Cd}	7.6	7.1
Log K_{Ca}	3.0	

- The mean predicted LA50s from Models A(Y) and B(Y) were 1.24 and 1.28 μmol/g dw, and were within 10% of the mean observed LA50s for datasets including and excluding DOC tests.
- The two models predicted LC50s within a factor-of-two range for 10 of the 12 tests. Instead of a clear 1:1 linear relationship between predicted

and observed values, the predicted LC50s fell roughly in three lateral bands, according to calcium concentrations of approximately 0.3, 1 and 3 mmol/L (Figures 9.7 and 9.8). The broad range of observed LC50s associated with a single predicted value reflected variability in organism response in four-week exposures to similar media and/or may have suggested that other factors influencing Cd toxicity were not accounted for in the models.

- Prediction of toxicity in young tests using the adult models was consistent to that predicted by the young models. Model A fit the seven-day data best but all models were able to predict 28-day toxicity similarly. As observed in the young models, the predicted toxicity from the adult models fell across three lateral bands consistent with three different concentrations of Ca.

10.0 CONCLUSIONS

1. BLM theory assumes that response is a function of accumulation. This is supported by this study, which showed that increasing toxicity corresponded with whole-body concentrations of both Ni and Cd and with increasing concentrations of the individual metals in solution. In Ni exposures, steady state was apparent within 48 hours, consistent with an assumed thermodynamic equilibrium at the biotic ligand within the test exposure period. This was not the case with Cd; however, accumulation and toxicity could still be predicted, consistent with a condition of steady state between the metal in solution and the metal bound to the biotic ligand (which is assumed to be reached much faster than between the metal at the ligand and the metal within the organism).
2. The BLM predicts accumulation and toxicity based on concentration, complexation and competition of a metal with other ions in solution. In this study, Ca mitigated both short-term and long-term toxicity and bioaccumulation of Ni and Cd. H^+ mitigated short-term and long-term toxicity of Ni but did not mitigate the toxicity of Cd other than by influencing its speciation. There was limited evidence to suggest that Mg also mitigated the short-term bioaccumulation and toxicity of Ni but only at high concentrations and in exposures of low Ca concentrations.
3. Despite the apparent physiological effect of Ca on the ligand, short-term Ni toxicity was best predicted by a competitive model including Ca and H^+

(classic BLM theory). This suggests that the physiological effect of Ca is not as great as its competitive effect in short-term exposures. Another explanation is that maintenance of an approximately constant LA50/L_T ratio maintained the validity of the competitive model in spite of individual changes in LA50 or L_T.

4. In long-term (28-day) exposures, Ni toxicity was explained equally well by both competitive and non-competitive models that included Ca and H^+. However, the wide variation in the data set may have hampered detection of a subtle but important distinction in the models. Because H^+ is an important competitor with Ni at the biotic ligand, tighter pH control during testing would be warranted.
5. Short-term Cd toxicity was also best predicted by a competitive model including Ca. The non-competitive model including Ca worked best for prediction of long-term Cd toxicity; however, predicted LC50s did not demonstrate a clear linear relationship with observed LC50s, but rather tended to aggregate in lateral bands according to Ca concentration. This model should be refined or adjusted with additional test data for establishment of clear linear relationship between predicted and observed toxicity.
6. Based on comparison of slopes of LC50s against Ca for each exposure period, the influence of Ca against Ni and Cd toxicity appears to decrease over time. However, this influence appears to decrease sooner and to a greater extent in Ni exposures than in Cd exposures. This finding may

explain the poor performance of Ni models developed from short-term tests with adults in predicting toxicit y to young in long-term Ni exposures. In contrast, short-term Cd models developed from adult tests did predict both short-term and long-term toxicity of Cd to young equally well to the models developed from the long-term data. It is possible that the mode of action for Ni toxicity to *Hyalella* may change between short-term and long-term exposures, as noted by Pane et al. (2003a) for Ni toxicity to *Daphnia magna*.

7. The influence of dissolved organic material as a complexing ligand appears to be more limited with Ni than with Cd. Within a DOC range of 0-20 mg/L, bioaccumulation of total Ni and Cd was reduced in short-term and long-term exposures and Cd toxicity, but not Ni toxicity, was reduced in long-term exposures (LC50s of 0.01 to 0.06 µmol/L total Cd and LC50s ~ 1 µmol/L total Ni).
8. Evaluation of dissolved organic material in mitigating Ni and Cd toxicity was hampered by limitations in geochemical speciation models and in the chemistry data available. For inorganic solutions, MINTEQA2 worked similarly to Hydroqual's BLM program. However, the BLM appeared to over-predict the binding of Cd to dissolved organic material whereas the MINTEQA2 program appeared to underestimate it. Reliable binding constants for a number of types of dissolved organic material should be established and validated for application in geochemical models.
9. The low variability associated with observed LA50s from this study, in comparison with LC50s, provides some support to the BLM assumption of a

single body concentration for a given effect. However, this study indicated that the LA50 as a proportion of L_T, rather than the LA50 itself, remains constant, since modeling of both Ni and Cd indicated that Ca might have a physiological effect on L_T, an effect that is not considered by BLM theory.

11.0 RESEARCH APPLICATION

"All models are wrong, some of them are useful" (Box, 1979).

The current study illustrates some of the challenges in modeling toxicity as well as potential for application of toxicity models.

The short-term and long-term models for Ni and Cd developed in this study were able to predict toxicity within a factor of two in most cases; however, high variability in observed LC50s under similar exposure conditions sometimes obscured a relationship between predicted and observed toxicity, as illustrated in long-term exposures to Cd. Another challenge was a potential change in mode of action as the exposure period increases. This was not as much of an issue for Cd toxicity, but appeared to influence the ability of short-term Ni models to predict long-term toxicity. Additionally, the poor predictability of metal speciation in the presence of dissolved organic material limits the applicability of the Cd and Ni models developed in this study.

Niyogi and Wood (2004) noted that the BLM improves our ability to generate site-specific water quality criteria for metals beyond the currently applied adjustment for hardness. The BLM approach "explicitly and quantitatively" accounts for water quality parameters that influence metal bioavailability and is more cost effective than other non-mechanistic methods (U.S. EPA, 2007). Once a model is validated for a range of water quality conditions, it may be used

to adjust a generic water quality criterion, by accounting for site specific influences on competition with, and complexation of, a metal. Water quality criteria are sometimes criticized for being too conservative, especially if they incorporate a large safety factor because of limited toxicity data. Conversely, they may not be protective if they do not account for factors related to increased vulnerability of a specific receiving environment or a resident species. Interestingly, the data from this study suggest that some water bodies (especially low hardness, low DOC) would benefit by a lower water quality objective for Ni than the current 25 µg/L given a range in 28-day LC50s for *Hyalella* of 18 to 120 µg/L total Ni.

A long-term BLM-type model could be used to develop a site-specific water quality objective that would consider the characteristics and vulnerability of a specific area. For example, if detailed information from a water body were available, seasonal variation in water quality could be modeled and the maximum estimated availability of a specific metal could be used to set a site-specific criterion (Paul Welsh, MOE, Toronto, personal communication December 13, 2007). DiToro et al. (2001) described the potential for using the BLM to estimate the frequency of exceedance of a water quality criterion as well as a method for developing the probability distribution of a metal and other chemistry variables for a specific water body using Monte Carlo simulations.

Another application could be in making adjustments to a criterion developed through a species sensitivity distribution. If characteristics of a specific site were adequately described, and models were available for several species of interest, a species sensitivity curve could be "site-adjusted" in the same manner as distributions are currently sometimes hardness-adjusted (Tim Fletcher, MOE, Toronto, personal communication December 13, 2007). Such adjustments could be applied not only to relax overly conservative objectives but also to provide an additional level of protection where characteristics of the water body and/or sensitivities of the resident species may not be reflected by a generic criterion.

Models that can reliably predict toxicity represent an important advance in aquatic toxicology and, as noted above, have a number of potential applications. However, caution must be applied in the application of models in assessment and management of the aquatic environment since no model can completely reflect the range or variation in environmental conditions. Further, the response of laboratory organisms may not reflect the increased vulnerability or resistance of the same species of organism in the field.

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APPENDIX A

SEVEN-DAY NICKEL BIOACCUMULATION TEST DATA

Test		Medium			% Free (MINTEQA2)			MINTEQA2 µmol/L			survival (%)	blank corr	
#	date	Base	Add	pH	Ni	Ca	Mg	Ni	Ca	Mg	7-d	NiT (µg/g)	weight/bug (mg)
1.0	30-Mar-01	MSAM		7.9	78.5	95.8	98	0.000	958	298	86	.	0.2600
1.0	30-Mar-01	MSAM		7.9	78.5	95.8	98	0.000	958	298	86	0.1029	0.4375
1.0	30-Mar-01	MSAM		7.9	78.5	95.8	98	0.000	958	298	86	0.0919	0.1860
1.0	30-Mar-01	MSAM		7.9	78.5	95.8	98	0.254	958	298	86	0.4511	0.2633
1.0	30-Mar-01	MSAM		7.9	78.5	95.8	98	0.254	958	298	86	0.6285	0.2775
1.0	30-Mar-01	MSAM		7.9	78.5	95.8	98	0.254	958	298	86	0.2929	0.2660
1.0	30-Mar-01	MSAM		7.9	78.5	95.8	98	0.522	958	298	86	0.6558	0.3060
1.0	30-Mar-01	MSAM		7.9	78.5	95.8	98	0.522	958	298	71	0.4088	0.4525
1.0	30-Mar-01	MSAM		7.9	78.5	95.8	98	0.522	958	298	71	.	0.2780
1.0	30-Mar-01	MSAM		7.9	78.5	95.8	98	1.043	958	298	86	.	0.2920
1.0	30-Mar-01	MSAM		7.9	78.5	95.8	98	1.043	958	298	86	0.9707	0.2583
1.0	30-Mar-01	MSAM		7.9	78.5	95.8	98	1.043	958	298	71	1.3144	0.2380
1.0	30-Mar-01	MSAM		7.9	78.5	95.8	98	2.09	958	298	71	1.6575	0.2500
1.0	30-Mar-01	MSAM		7.9	78.5	95.8	98	2.09	958	298	86	1.7017	0.2133
1.0	30-Mar-01	MSAM		7.9	78.5	95.8	98	2.09	958	298	86	.	
1.0	30-Mar-01	MSAM		7.9	78.5	95.8	98	4.19	958	298	86	2.1230	
1.0	30-Mar-01	MSAM		7.9	78.5	95.8	98	4.19	958	298	86	1.9357	
1.0	30-Mar-01	MSAM		7.9	78.5	95.8	98	4.19	958	298	86	1.5311	
1.0	30-Mar-01	MSAM		7.9	78.5	95.8	98	8.36	958	298	86	.	
1.0	30-Mar-01	MSAM		7.9	78.5	95.8	98	8.36	958	298	86	2.6685	0.2275
1.0	30-Mar-01	MSAM		7.9	78.5	95.8	98	8.36	958	298	86	2.1609	0.2650
1.0	30-Mar-01	MSAM		7.9	78.5	95.8	98	16.7	958	298	57	2.1595	0.1825
1.0	30-Mar-01	MSAM		7.9	78.5	95.8	98	16.7	958	298	86	1.8727	0.2567
1.0	30-Mar-01	MSAM		7.9	78.5	95.8	98	16.7	958	298	86	1.9894	0.2360
1.0	30-Mar-01	MSAM		7.9	78.5	95.8	98	33.4	958	298	29	2.5644	0.1800
1.0	30-Mar-01	MSAM		7.9	78.5	95.8	98	33.4	958	298	29	1.6431	0.3250
1.0	30-Mar-01	MSAM		7.9	78.5	95.8	98	33.4	958	298	57	3.4395	0.2567
2.0	15-Jan-02	LH		7.9	83	97.9	98.2	0.0	348	84	100	0.1533	0.2840
2.0	15-Jan-02	LH		7.9	83	97.9	98.2	0.0	348	84	83	0.1241	0.3420
2.0	15-Jan-02	LH		7.9	83	97.9	98.2	0.0	348	84	67	.	0.0000
2.0	15-Jan-02	LH		7.9	83	97.9	98.2	1.1	348	84	83	0.9308	0.3575
2.0	15-Jan-02	LH		7.9	83	97.9	98.2	1.1	348	84	100	1.1834	0.3160
2.0	15-Jan-02	LH		7.9	83	97.9	98.2	1.1	348	84	100	1.1061	0.3260
2.0	15-Jan-02	LH		7.9	83	97.9	98.2	2.2	348	84	100	2.1884	0.2400
2.0	15-Jan-02	LH		7.9	83	97.9	98.2	2.2	348	84	100	1.5813	0.3683
2.0	15-Jan-02	LH		7.9	83	97.9	98.2	2.2	348	84	83	0.9172	0.2420
2.0	15-Jan-02	LH		7.9	83	97.9	98.2	4.4	348	84	100	1.6082	0.3767
2.0	15-Jan-02	LH		7.9	83	97.9	98.2	4.4	348	84	100	2.4254	0.2580
2.0	15-Jan-02	LH		7.9	83	97.9	98.2	4.4	348	84	100	1.8925	0.2640
2.0	15-Jan-02	LH		7.9	83	97.9	98.2	8.8	348	84	67	2.1589	0.2667
2.0	15-Jan-02	LH		7.9	83	97.9	98.2	8.8	348	84	83	3.2072	0.3140
2.0	15-Jan-02	LH		7.9	83	97.9	98.2	8.8	348	84	100	.	0.3167
2.0	15-Jan-02	LH		7.9	83	97.9	98.2	17.7	348	84	83	2.8996	0.2580
2.0	15-Jan-02	LH		7.9	83	97.9	98.2	17.7	348	84	50	1.4485	0.2300
2.0	15-Jan-02	LH		7.9	83	97.9	98.2	17.7	348	84	100	2.7342	0.2917
2.0	15-Jan-02	LH		7.9	83	97.9	98.2	35.4	348	84	50	3.1049	0.3900
2.0	15-Jan-02	LH		7.9	83	97.9	98.2	35.4	348	84	17	.	0.3100
2.0	15-Jan-02	LH		7.9	83	97.9	98.2	35.4	348	84	33	2.0269	0.2700
2.0	15-Jan-02	LH		7.9	83	97.9	98.2	70.7	348	84	0	.	
2.0	15-Jan-02	LH		7.9	83	97.9	98.2	70.7	348	84	0	.	
2.0	15-Jan-02	LH		7.9	83	97.9	98.2	70.7	348	84	0	.	
2.0	15-Jan-02	LH		7.9	83	97.9	98.2	141.4	348	84	0	.	

	Test	Medium			% Free (MINTEQA2)			MINTEQA2 µmol/L			survival (%)	blank corr	
#	date	Base	Add	pH	Ni	Ca	Mg	Ni	Ca	Mg	7-d	NiT (µg/g)	weight/bug (mg)
2.0	15-Jan-02	LH		7.9	83	97.9	98.2	141.4	348	84	0	.	
2.0	15-Jan-02	LH		7.9	83	97.9	98.2	141.4	348	84	0	.	
3.0	15-Mar-04	LH	Ca	7.9	88.2	98.5	98.7	0.0	3571	128	114	0.1293	0.9029
3.0	15-Mar-04	LH	Ca	7.9	88.2	98.5	98.7	0.0	3571	128	86	.	0.8267
3.0	15-Mar-04	LH	Ca	7.9	88.2	98.5	98.7	0.0	3571	128	86	0.0277	0.7883
3.0	15-Mar-04	LH	Ca	7.9	88.2	98.5	98.7	1.7	3571	128	71	0.3390	0.7029
3.0	15-Mar-04	LH	Ca	7.9	88.2	98.5	98.7	1.7	3571	128	86	0.2384	0.8740
3.0	15-Mar-04	LH	Ca	7.9	88.2	98.5	98.7	1.7	3571	128	86	0.3957	0.6657
3.0	15-Mar-04	LH	Ca	7.9	88.2	98.5	98.7	2.9	3571	128	71	0.3407	0.8233
3.0	15-Mar-04	LH	Ca	7.9	88.2	98.5	98.7	2.9	3571	128	86	0.3298	1.0200
3.0	15-Mar-04	LH	Ca	7.9	88.2	98.5	98.7	2.9	3571	128	100	0.3556	0.7529
3.0	15-Mar-04	LH	Ca	7.9	88.2	98.5	98.7	4.9	3571	128	57	0.4245	0.9625
3.0	15-Mar-04	LH	Ca	7.9	88.2	98.5	98.7	4.9	3571	128	100	0.8217	0.6914
3.0	15-Mar-04	LH	Ca	7.9	88.2	98.5	98.7	4.9	3571	128	86	0.6242	0.7517
3.0	15-Mar-04	LH	Ca	7.9	88.2	98.5	98.7	8.1	3571	128	71	0.5980	0.7020
3.0	15-Mar-04	LH	Ca	7.9	88.2	98.5	98.7	8.1	3571	128	86	0.7264	0.7517
3.0	15-Mar-04	LH	Ca	7.9	88.2	98.5	98.7	8.1	3571	128	57	0.4976	0.8550
3.0	15-Mar-04	LH	Ca	7.9	88.2	98.5	98.7	13.5	3571	128	71	1.6048	0.6575
3.0	15-Mar-04	LH	Ca	7.9	88.2	98.5	98.7	13.5	3571	128	57	1.4691	0.5575
3.0	15-Mar-04	LH	Ca	7.9	88.2	98.5	98.7	13.5	3571	128	71	0.7695	0.6800
3.0	15-Mar-04	LH	Ca	7.9	88.2	98.5	98.7	22.5	3571	128	29	1.3563	0.6800
3.0	15-Mar-04	LH	Ca	7.9	88.2	98.5	98.7	22.5	3571	128	71	1.2760	0.5880
3.0	15-Mar-04	LH	Ca	7.9	88.2	98.5	98.7	22.5	3571	128	71	1.0871	0.6700
3.0	15-Mar-04	LH	Ca	7.9	88.2	98.5	98.7	37.6	3571	128	29	1.9733	0.8150
3.0	15-Mar-04	LH	Ca	7.9	88.2	98.5	98.7	37.6	3571	128	43	0.7602	0.7350
3.0	15-Mar-04	LH	Ca	7.9	88.2	98.5	98.7	37.6	3571	128	71	0.8007	1.0700
4.0	11-Jun-02	MSAM	vCa	7.6	80.3	96	96.5	4.3	355	326	60	1.0264	0.1367
4.0	11-Jun-02	MSAM	vCa	7.6	80.3	96	96.5	4.3	355	326	40	1.4974	0.1800
4.0	11-Jun-02	MSAM	vCa	7.6	80.3	96	96.5	4.3	355	326	40	0.6518	0.1550
4.0	11-Jun-02	MSAM	vCa	7.6	79.2	93.6	94.5	4.2	562	320	60	0.8201	0.1967
4.0	11-Jun-02	MSAM	vCa	7.6	79.2	93.6	94.5	4.2	562	320	60	0.7059	0.2000
4.0	11-Jun-02	MSAM	vCa	7.6	79.2	93.6	94.5	4.2	562	320	60	1.1676	0.2500
4.0	11-Jun-02	MSAM	vCa	7.6	78.4	91.7	93	4.2	768	315	80	0.4576	0.1225
4.0	11-Jun-02	MSAM	vCa	7.6	78.4	91.7	93	4.2	768	315	80	0.6960	0.1875
4.0	11-Jun-02	MSAM	vCa	7.6	78.4	91.7	93	4.2	768	315	100	1.0595	0.1740
4.0	11-Jun-02	MSAM	vCa	7.6	76.8	88.5	90.3	4.1	1186	306	100	0.5984	0.2000
4.0	11-Jun-02	MSAM	vCa	7.6	76.8	88.5	90.3	4.1	1186	306	100	0.6053	0.1900
4.0	11-Jun-02	MSAM	vCa	7.6	76.8	88.5	90.3	4.1	1186	306	100	0.8127	0.2120
4.0	11-Jun-02	MSAM	vCa	7.6	74.4	83.7	86.4	4.0	1933	292	80	0.3112	0.2050
4.0	11-Jun-02	MSAM	vCa	7.6	74.4	83.7	86.4	4.0	1933	292	80	0.4777	0.1350
4.0	11-Jun-02	MSAM	vCa	7.6	74.4	83.7	86.4	4.0	1933	292	100	0.5452	0.1840
4.0	11-Jun-02	MSAM	vCa	7.6	71.1	77.7	81.2	3.8	3399	275	100	0.2962	0.2450
4.0	11-Jun-02	MSAM	vCa	7.6	71.1	77.7	81.2	3.8	3399	275	100	.	0.1150
4.0	11-Jun-02	MSAM	vCa	7.6	71.1	77.7	81.2	3.8	3399	275	80	0.3145	0.1425
4.0	11-Jun-02	MSAM	vCa	7.6	66.5	70.3	74.7	3.5	5677	253	100	0.2361	0.1940
4.0	11-Jun-02	MSAM	vCa	7.6	66.5	70.3	74.7	3.5	5677	253	100	0.2833	0.2100
4.0	11-Jun-02	MSAM	vCa	7.6	66.5	70.3	74.7	3.5	5677	252	100	0.3006	0.2260
5.0	14-Aug-01	DC		6.12	84	95	96	4.5	831	359	80	0.3738	0.3833
5.0	14-Aug-01	DC		6.17	84	95	96	4.5	831	359	100	0.3091	0.2620
5.0	14-Aug-01	DC		6.10	84	95	96	4.5	831	359	60	0.2703	0.0000
5.0	14-Aug-01	DC		7.02	82	95	96	4.4	831	359	100	0.9938	0.3060
5.0	14-Aug-01	DC		6.94	83	95	96	4.5	831	359	100	1.1145	0.2400
5.0	14-Aug-01	DC		7.04	82	95	96	4.5	831	359	100	0.8729	0.3850
5.0	14-Aug-01	DC		7.84	75	95	95	4.1	831	355	100	1.8684	0.3240
5.0	14-Aug-01	DC		7.87	74	95	95	4.1	831	355	100	1.9949	0.4120
5.0	14-Aug-01	DC		7.94	74	95	95	4.1	831	355	80	1.6964	0.2600
5.0	14-Aug-01	DC		8.35	58.5	94	94	3.2	823	352	100	3.0033	0.2280
5.0	14-Aug-01	DC		8.38	58.5	94	94	3.2	823	352	100	1.9524	0.5767

	Test	Medium			% Free (MINTEQA2)			MINTEQA2 µmol/L			survival (%)	blank corr	
#	date	Base	Add	pH	Ni	Ca	Mg	Ni	Ca	Mg	7-d	NiT (µg/g)	weight/bug (mg)
5.0	14-Aug-01	DC		8.35	58.5	94	94	3.2	823	352	100	1.7087	0.2740
5.0	14-Aug-01	DC		8.79	41	91	93	2.3	796	348	100	2.4058	0.3950
5.0	14-Aug-01	DC		8.81	41.4	91	93	2.3	796	348	80	3.2607	0.2320
5.0	14-Aug-01	DC		8.89	37	91	93	2.1	796	348	100	2.6532	0.3320
6.0	30-Mar-01	MSAM	vCa	7.9	75.9	95.1	95.8	4.05	404	287	83	0.5734	0.2700
6.0	30-Mar-01	MSAM	vCa	7.9	75.9	95.1	95.8	4.05	404	287	100	.	0.2267
6.0	30-Mar-01	MSAM	vCa	7.9	75.9	95.1	95.8	4.05	404	287	100	0.9201	0.2167
6.0	30-Mar-01	MSAM	vCa	7.9	76.3	95.3	95.3	4.07	596	286	100	1.1062	0.1250
6.0	30-Mar-01	MSAM	vCa	7.9	76.3	95.3	95.3	4.07	596	286	100	0.9908	0.1317
6.0	30-Mar-01	MSAM	vCa	7.9	76.3	95.3	95.3	4.07	596	286	100	1.0315	0.1750
6.0	30-Mar-01	MSAM	vCa	7.9	76.7	95.5	95.5	4.09	764	287	100	0.3710	0.1850
6.0	30-Mar-01	MSAM	vCa	7.9	76.7	95.5	95.5	4.09	764	287	100	0.4557	0.2633
6.0	30-Mar-01	MSAM	vCa	7.9	76.7	95.5	95.5	4.09	764	287	100	0.2581	0.2083
6.0	30-Mar-01	MSAM	vCa	7.9	77.6	95.9	95.9	4.14	1247	288	83	0.2597	0.2517
6.0	30-Mar-01	MSAM	vCa	7.9	77.6	95.9	95.9	4.14	1247	288	100	0.2234	0.3067
6.0	30-Mar-01	MSAM	vCa	7.9	77.6	95.9	95.9	4.14	1247	288	100	0.1998	0.1717
6.0	30-Mar-01	MSAM	vCa	7.9	78.9	96.5	96.5	4.21	2220	290	100	0.2388	0.2817
6.0	30-Mar-01	MSAM	vCa	7.9	78.9	96.5	96.5	4.21	2220	290	100	0.1309	0.2817
6.0	30-Mar-01	MSAM	vCa	7.9	78.9	96.5	96.5	4.21	2220	290	83	0.2519	0.1500
6.0	30-Mar-01	MSAM	vCa	7.9	80.7	97.1	97	4.3	4175	291	100	.	0.1233
6.0	30-Mar-01	MSAM	vCa	7.9	80.7	97.1	97	4.3	4175	291	100	0.1750	0.2833
6.0	30-Mar-01	MSAM	vCa	7.9	80.7	97.1	97	4.3	4175	291	100	.	0.1917
6.0	30-Mar-01	MSAM	vCa	7.9	82.7	97.8	97.8	4.41	8093	293	100	0.1640	0.1667
6.0	30-Mar-01	MSAM	vCa	7.9	82.7	97.8	97.8	4.41	8093	293	83	0.1880	0.1517
6.0	30-Mar-01	MSAM	vCa	7.9	82.7	97.8	97.8	4.41	8093	293	100	0.1456	0.2467
7.0	3-Jul-02	MSAM	vK	7.5	83.9	97.6	97.8	4.47	1025	314	100	0.9383	0.3880
7.0	3-Jul-02	MSAM	vK	7.5	83.9	97.6	97.8	4.47	1025	314	100	1.1743	0.2680
7.0	3-Jul-02	MSAM	vK	7.5	83.9	97.6	97.8	4.47	1025	314	100	0.9794	0.3600
7.0	3-Jul-02	MSAM	vK	7.5	83.9	97.6	97.8	4.47	1025	314	100	1.4144	0.3400
7.0	3-Jul-02	MSAM	vK	7.5	83.9	97.6	97.8	4.47	1025	314	100	0.9179	0.3440
7.0	3-Jul-02	MSAM	vK	7.5	83.9	97.6	97.8	4.47	1025	314	100	1.4963	0.3420
7.0	3-Jul-02	MSAM	vK	7.5	83.9	97.6	97.8	4.47	1025	314	100	1.0309	0.2950
7.0	3-Jul-02	MSAM	vK	7.5	83.9	97.6	97.8	4.47	1025	314	100	1.2211	0.2560
7.0	3-Jul-02	MSAM	vK	7.5	83.9	97.6	97.8	4.47	1025	314	100	0.8693	0.3280
7.0	3-Jul-02	MSAM	vK	7.5	83.9	97.6	97.8	4.47	1025	314	100	1.2692	0.2967
7.0	3-Jul-02	MSAM	vK	7.5	83.9	97.6	97.8	4.47	1025	314	100	1.1226	0.3700
7.0	3-Jul-02	MSAM	vK	7.5	83.9	97.6	97.8	4.47	1025	314	100	1.1967	0.2660
7.0	3-Jul-02	MSAM	vK	7.5	83.9	97.6	97.8	4.47	1025	314	100	0.9794	0.4050
7.0	3-Jul-02	MSAM	vK	7.5	83.9	97.6	97.8	4.47	1025	314	60	1.0372	0.3200
7.0	3-Jul-02	MSAM	vK	7.5	83.9	97.6	97.8	4.47	1025	314	100	1.0254	0.3000
7.0	3-Jul-02	MSAM	vK	7.5	83.9	97.6	97.8	4.47	1025	314	100	0.9191	0.2900
7.0	3-Jul-02	MSAM	vK	7.5	83.9	97.6	97.8	4.47	1025	314	100	0.9828	0.2200
7.0	3-Jul-02	MSAM	vK	7.5	83.9	97.6	97.8	4.47	1025	314	60	1.1372	0.3400
8.0	13-Apr-01	MSAM	vMg	7.9	75.5	96.8	97.2	4.02	1089	134	83	2.0277	0.4150
8.0	13-Apr-01	MSAM	vMg	7.9	75.5	96.8	97.2	4.02	1089	134	100	1.5235	0.3150
8.0	13-Apr-01	MSAM	vMg	7.9	75.5	96.8	97.2	4.02	1089	134	83	.	0.3300
8.0	13-Apr-01	MSAM	vMg	7.9	75.5	96.8	97.2	4.02	1089	154	100	1.4637	0.3417
8.0	13-Apr-01	MSAM	vMg	7.9	75.5	96.8	97.2	4.02	1089	154	67	1.5506	0.2633
8.0	13-Apr-01	MSAM	vMg	7.9	75.5	96.8	97.2	4.02	1089	154	100	.	0.3567
8.0	13-Apr-01	MSAM	vMg	7.9	75.3	96.2	96.7	4.01	1082	187	100	2.9271	0.2240
8.0	13-Apr-01	MSAM	vMg	7.9	75.3	96.2	96.7	4.01	1082	187	83	1.6879	0.2800
8.0	13-Apr-01	MSAM	vMg	7.9	75.3	96.2	96.7	4.01	1082	187	83	2.1971	0.2975
8.0	13-Apr-01	MSAM	vMg	7.9	74.8	94.7	95.5	3.99	1065	368	83	1.3549	0.2850
8.0	13-Apr-01	MSAM	vMg	7.9	74.8	94.7	95.5	3.99	1065	368	100	1.8123	0.2683
8.0	13-Apr-01	MSAM	vMg	7.9	74.8	94.7	95.5	3.99	1065	368	83	1.9322	0.3375
8.0	13-Apr-01	MSAM	vMg	7.9	74.1	92.8	93.9	3.95	1044	610	67	1.9134	0.2400
8.0	13-Apr-01	MSAM	vMg	7.9	74.1	92.8	93.9	3.95	1044	610	100	1.5812	0.3100
8.0	13-Apr-01	MSAM	vMg	7.9	74.1	92.8	93.9	3.95	1044	610	83	2.2353	0.2940

Test		Medium			% Free (MINTEQA2)			MINTEQA2 µmol/L			survival (%)	blank corr	
#	date	Base	Add	pH	Ni	Ca	Mg	Ni	Ca	Mg	7-d	NiT (µg/g)	weight/bug (mg)
8.0	13-Apr-01	MSAM	vMg	7.9	72.9	89.5	91.2	3.89	1007	1075	83	1.8718	0.2900
8.0	13-Apr-01	MSAM	vMg	7.9	72.9	89.5	91.2	3.89	1007	1075	83	1.3002	0.4000
8.0	13-Apr-01	MSAM	vMg	7.9	72.9	89.5	91.2	3.89	1007	1075	67	.	0.2225
8.0	13-Apr-01	MSAM	vMg	7.9	71.1	84.8	87.3	3.79	954	1950	67	1.4420	0.3375
8.0	13-Apr-01	MSAM	vMg	7.9	71.1	84.8	87.3	3.79	954	1950	83	1.5553	0.3200
8.0	13-Apr-01	MSAM	vMg	7.9	71.1	84.8	87.3	3.79	954	1950	67	1.6886	0.3775
9.0	23-Apr-02	MSAM	LCvMg	7.7	79	97	97	4.21	412	137	100	1.1873	0.2660
9.0	23-Apr-02	MSAM	LCvMg	7.7	79	97	97	4.21	412	137	100	1.1907	0.2625
9.0	23-Apr-02	MSAM	LCvMg	7.7	79	97	97	4.21	412	137	100	.	0.3475
9.0	23-Apr-02	MSAM	LCvMg	7.7	78	95	96	4.16	404	256	100	1.9332	0.1940
9.0	23-Apr-02	MSAM	LCvMg	7.7	78	95	96	4.16	404	256	100	1.1870	0.2420
9.0	23-Apr-02	MSAM	LCvMg	7.7	78	95	96	4.16	404	256	100	2.4626	0.1950
9.0	23-Apr-02	MSAM	LCvMg	7.7	78	94	95	4.16	400	380	100	1.6484	0.2933
9.0	23-Apr-02	MSAM	LCvMg	7.7	78	94	95	4.16	400	380	100	0.8285	0.2100
9.0	23-Apr-02	MSAM	LCvMg	7.7	78	94	95	4.16	400	380	100	0.9765	0.3725
9.0	23-Apr-02	MSAM	LCvMg	7.7	77	92	93	4.1	391	612	100	0.7351	0.2575
9.0	23-Apr-02	MSAM	LCvMg	7.7	77	92	93	4.1	391	612	100	0.9181	0.1720
9.0	23-Apr-02	MSAM	LCvMg	7.7	77	92	93	4.1	391	612	100	0.6985	0.2150
9.0	23-Apr-02	MSAM	LCvMg	7.7	76	88	90	4.05	374	1080	100	1.6423	0.2175
9.0	23-Apr-02	MSAM	LCvMg	7.7	76	88	90	4.05	374	1080	100	1.3553	0.3000
9.0	23-Apr-02	MSAM	LCvMg	7.7	76	88	90	4.05	374	1080	100	1.8001	0.2760
10.0	23-Apr-02	MSAM	vMg	7.7	80	97	97	4.26	1043	137	100	1.4608	0.3050
10.0	23-Apr-02	MSAM	vMg	7.7	80	97	97	4.26	1043	137	100	0.9034	0.1433
10.0	23-Apr-02	MSAM	vMg	7.7	80	97	97	4.26	1043	137	60	2.5085	0.1950
10.0	23-Apr-02	MSAM	vMg	7.7	80	96	96.5	4.26	1032	257	180	1.1047	0.2214
10.0	23-Apr-02	MSAM	vMg	7.7	80	96	96.5	4.26	1032	257	160	0.5833	0.2350
10.0	23-Apr-02	MSAM	vMg	7.7	80	96	96.5	4.26	1032	257	80	.	0.1200
10.0	23-Apr-02	MSAM	vMg	7.7	79	95	96	4.21	1021	384	100	1.0695	0.2900
10.0	23-Apr-02	MSAM	vMg	7.7	79	95	96	4.21	1021	384	100	0.9393	0.2400
10.0	23-Apr-02	MSAM	vMg	7.7	79	95	96	4.21	1021	384	80	1.2868	0.3050
10.0	23-Apr-02	MSAM	vMg	7.7	78	93	94	4.16	1000	619	100	2.2957	0.1780
10.0	23-Apr-02	MSAM	vMg	7.7	78	93	94	4.16	1000	619	100	2.0768	0.3350
10.0	23-Apr-02	MSAM	vMg	7.7	78	93	94	4.16	1000	619	100	2.5427	0.3475
10.0	23-Apr-02	MSAM	vMg	7.7	77	90	91	4.1	968	1092	100	1.7151	0.1980
10.0	23-Apr-02	MSAM	vMg	7.7	77	90	91	4.1	968	1092	60	0.3726	0.2033
10.0	23-Apr-02	MSAM	vMg	7.7	77	90	91	4.1	968	1092	100	0.6210	0.3125
11.0	26-Jul-04	LH	vMg	7.6	90.9	97.7	98.1	4.84	269	115	67	0.8126	0.7150
11.0	26-Jul-04	LH	vMg	7.6	90.9	97.7	98.1	4.84	269	114	67	1.4432	0.4250
11.0	26-Jul-04	LH	vMg	7.6	90.9	97.7	98.1	4.84	269	114	50	1.1995	0.5000
11.0	26-Jul-04	LH	vMg	7.6	88.7	94.4	95.4	4.73	260	378	100	0.8528	0.4575
11.0	26-Jul-04	LH	vMg	7.6	88.7	94.4	95.4	4.73	260	378	117	1.0571	0.4329
11.0	26-Jul-04	LH	vMg	7.6	88.7	94.4	95.4	4.73	260	378	83	1.2165	0.4280
11.0	26-Jul-04	LH	vMg	7.6	87.5	92.7	94	4.66	255	537	100	0.9667	0.3783
11.0	26-Jul-04	LH	vMg	7.6	87.5	92.7	94	4.66	255	537	100	1.1459	0.4133

Test		Medium			% Free (MINTEQA2)			MINTEQA2 µmol/L			survival (%)	blank corr	
#	date	Base	Add	pH	Ni	Ca	Mg	Ni	Ca	Mg	7-d	NiT (µg/g)	weight/bug (mg)
11.0	26-Jul-04	LH	vMg	7.6	87.5	92.7	94	4.66	255	537	100	.	0.5225
11.0	26-Jul-04	LH	vMg	7.6	86.0	90.4	92.2	4.58	249	788	67	0.8807	0.4875
11.0	26-Jul-04	LH	vMg	7.6	86.0	90.4	92.2	4.58	249	788	100	0.7328	0.3100
11.0	26-Jul-04	LH	vMg	7.6	86.0	90.4	92.2	4.58	249	788	67	0.9116	0.3850
11.0	26-Jul-04	LH	vMg	7.6	84.0	87.6	89.8	4.48	241	1164	50	0.5861	0.3333
11.0	26-Jul-04	LH	vMg	7.6	84.0	87.6	89.8	4.48	241	1164	67	0.9168	0.4200
11.0	26-Jul-04	LH	vMg	7.6	84.0	87.6	89.8	4.48	241	1164	83	0.8198	0.5420
11.0	26-Jul-04	LH	vMg	7.6	81.3	83.9	86.7	4.33	231	1752	67	0.5562	0.4350
11.0	26-Jul-04	LH	vMg	7.6	81.3	83.9	86.7	4.33	231	1752	67	0.6815	0.5800
11.0	26-Jul-04	LH	vMg	7.6	81.3	83.9	86.7	4.33	231	1752	117	0.9397	0.4025
12.0	26-Jul-04	MSAM	vMg	7.8	81.3	97.4	97.7	4.33	950	107	83	0.9576	0.3900
12.0	26-Jul-04	MSAM	vMg	7.8	81.3	97.4	97.7	4.33	950	107	100	1.1788	0.3700
12.0	26-Jul-04	MSAM	vMg	7.8	81.3	97.4	97.7	4.33	950	107	83	0.9885	0.2775
12.0	26-Jul-04	MSAM	vMg	7.8	80.1	94.8	95.6	4.27	924	345	100	0.8028	0.3933
12.0	26-Jul-04	MSAM	vMg	7.8	80.1	94.8	95.6	4.27	924	345	83	1.0618	0.3620
12.0	26-Jul-04	MSAM	vMg	7.8	80.1	94.8	95.6	4.27	924	345	117	0.6399	0.2571
12.0	26-Jul-04	MSAM	vMg	7.8	79.4	93.3	94.4	4.23	910	503	100	0.6725	0.3767
12.0	26-Jul-04	MSAM	vMg	7.8	79.4	93.3	94.4	4.23	910	503	100	0.7414	0.4550
12.0	26-Jul-04	MSAM	vMg	7.8	79.4	93.3	94.4	4.23	910	503	100	1.0408	0.3067
12.0	26-Jul-04	MSAM	vMg	7.8	78.4	91.3	92.8	4.18	890	762	117	0.8766	0.3743
12.0	26-Jul-04	MSAM	vMg	7.8	78.4	91.3	92.8	4.18	890	762	83	1.1493	0.4400
12.0	26-Jul-04	MSAM	vMg	7.8	78.4	91.3	92.8	4.18	890	762	100	0.9749	0.3717
12.0	26-Jul-04	MSAM	vMg	7.8	77.1	88.7	90.6	4.11	865	1200	100	0.7123	0.3980
12.0	26-Jul-04	MSAM	vMg	7.8	77.1	88.7	90.6	4.11	865	1200	100	1.2130	0.3633
12.0	26-Jul-04	MSAM	vMg	7.8	77.1	88.7	90.6	4.11	865	1200	100	0.8537	0.3167
12.0	26-Jul-04	MSAM	vMg	7.8	75.4	85.2	87.7	4.02	831	1805	100	0.7861	0.3800
12.0	27-Jul-04	MSAM	vMg	7.8	75.4	85.2	87.7	4.02	831	1794	100	0.6614	0.3950
12.0	28-Jul-04	MSAM	vMg	7.8	75.4	85.2	87.7	4.02	831	1794	100	0.8637	0.3117
13.0	9-Feb-05	LH	vMg	7.7	90.3	97.9	98.2	4.81	245	102	50	.	0.1233
13.0	9-Feb-05	LH	vMg	7.7	90.3	97.9	98.2	4.81	245	102	80	1.110198	0.1033
13.0	9-Feb-05	LH	vMg	7.7	90.3	97.9	98.2	4.81	245	102	70	1.425603	0.0843
13.0	9-Feb-05	LH	vMg	7.7	88.5	95	95.9	4.72	238	328	80	1.110198	0.1033
13.0	9-Feb-05	LH	vMg	7.7	88.5	95	95.9	4.72	238	328	90	1.425603	0.1089
13.0	9-Feb-05	LH	vMg	7.7	88.5	95	95.9	4.72	238	328	50	0.985752	0.1120
13.0	9-Feb-05	LH	vMg	7.7	87.1	92.8	94.1	4.64	232	537	80	1.150044	0.1143
13.0	9-Feb-05	LH	vMg	7.7	87.1	92.8	94.1	4.64	232	537	90	0.846389	0.0775
13.0	9-Feb-05	LH	vMg	7.7	87.1	92.8	94.1	4.64	232	537	100	1.145311	0.1029
13.0	9-Feb-05	LH	vMg	7.7	84.7	89.3	91.2	4.51	223	939	90	1.288329	0.1043
13.0	9-Feb-05	LH	vMg	7.7	84.7	89.3	91.2	4.51	223	939	90	1.211569	0.1650
13.0	9-Feb-05	LH	vMg	7.7	81.7	89.3	91.2	4.35	223	939	90	1.284380	0.0867
13.0	9-Feb-05	LH	vMg	7.7	81.4	84.7	87.4	4.34	212	1650	60	.	0.1140
13.0	9-Feb-05	LH	vMg	7.7	81.4	84.7	87.4	4.34	212	1650	100	.	0.0971
13.0	9-Feb-05	LH	vMg	7.7	81.4	84.7	87.4	4.34	212	1650	70	.	0.0829
13.0	9-Feb-05	LH	vMg	7.7	77.8	79.7	83.1	4.15	199	2760	80	0.762639	0.0700
13.0	9-Feb-05	LH	vMg	7.7	77.8	79.7	83.1	4.15	199	2760	40	0.741289	0.1425
13.0	9-Feb-05	LH	vMg	7.7	77.8	79.7	83.1	4.15	199	2760	50	0.651877	0.1380
14.0	9-Feb-05	MSAM	vMg	7.9	79.9	97.4	97.7	4.26	828	109	90	1.448204	0.0800
14.0	9-Feb-05	MSAM	vMg	7.9	79.9	97.4	97.7	4.26	828	109	100	1.884577	0.0980
14.0	9-Feb-05	MSAM	vMg	7.9	79.9	97.4	97.7	4.26	828	109	100	2.705061	0.0722
14.0	9-Feb-05	MSAM	vMg	7.9	77.6	92	93.3	4.14	782	700	80	1.831552	0.0800
14.0	9-Feb-05	MSAM	vMg	7.9	77.6	92	93.3	4.14	782	700	110	1.793853	0.0725
14.0	9-Feb-05	MSAM	vMg	7.9	77.6	92	93.3	4.14	782	700	60	1.582484	0.1475
14.0	9-Feb-05	MSAM	vMg	7.9	76.1	88.7	90.6	4.06	754	1166	120	2.587205	0.0900
14.0	9-Feb-05	MSAM	vMg	7.9	76.1	88.7	90.6	4.06	754	1166	90	2.055171	0.0800
14.0	9-Feb-05	MSAM	vMg	7.9	74.2	84.7	87.3	3.95	720	1866	60	1.525763	0.0838
14.0	9-Feb-05	MSAM	vMg	7.9	72.5	81.3	84.4	3.86	691	2659	90	1.341265	0.1044
14.0	9-Feb-05	MSAM	vMg	7.9	72.5	81.3	84.4	3.86	691	2659	100	1.349782	0.1030
15.0	18-Apr-03	MSAM	vNa	7.2	91.7	94.5	98.4	4.89	969	303	100	1.06093	0.2300

Test		Medium			% Free (MINTEQA2)			MINTEQA2 µmol/L			survival (%)	blank corr	
#	date	Base	Add	pH	Ni	Ca	Mg	Ni	Ca	Mg	7-d	NiT (µg/g)	weight/bug (mg)
15.0	18-Apr-03	MSAM	vNa	7.2	91.7	94.5	98.4	4.89	969	303	83	0.809019	0.3140
15.0	18-Apr-03	MSAM	vNa	7.2	91.7	94.5	98.4	4.89	969	303	83	1.150333	0.3180
15.0	18-Apr-03	MSAM	vNa	7.2	91.7	94.5	98.4	4.89	969	303	83	0.997704	0.2900
15.0	18-Apr-03	MSAM	vNa	7.2	89.8	90.7	99	4.79	930	305	83	1.447778	0.2800
15.0	18-Apr-03	MSAM	vNa	7.2	89.8	90.7	99	4.79	930	305	100	0.918267	0.3083
15.0	18-Apr-03	MSAM	vNa	7.2	87.8	87.8	99.8	4.68	900	308	83	0.810218	0.3380
15.0	18-Apr-03	MSAM	vNa	7.2	87.8	87.8	99.8	4.68	900	308	100	0.789246	0.2833
16.0	5-Oct-04	MSAM	vNa	7.5	90.6	96.3	97.1	4.83	939	297	90	1.094421	0.2125
16.0	5-Oct-04	MSAM	vNa	7.5	90.6	96.3	97.1	4.83	939	297	100	1.276014	0.1410
16.0	5-Oct-04	MSAM	vNa	7.5	90.6	96.3	97.1	4.83	939	297	70	1.454904	0.1483
16.0	5-Oct-04	MSAM	vNa	7.5	90.6	96.3	97.1	4.83	939	297	100	1.395728	0.1389
16.0	5-Oct-04	MSAM	vNa	7.5	90.6	96.3	97.1	4.83	939	297	90	1.500848	0.1271
16.0	5-Oct-04	MSAM	vNa	7.5	90.6	96.3	97.1	4.83	939	297	110	1.500462	0.1322
16.0	5-Oct-04	MSAM	vNa	7.5	90.6	96.3	97.1	4.83	939	297	80	1.425786	0.1900
16.0	5-Oct-04	MSAM	vNa	7.5	90.6	96.3	97.1	4.83	939	297	90	2.061455	0.1789
16.0	5-Oct-04	MSAM	vNa	7.5	90.6	96.3	97.1	4.83	939	297	70	1.201302	0.1967
16.0	5-Oct-04	MSAM	vNa	7.5	90.6	96.3	97.1	4.83	939	297	100	1.6604	0.1500
16.0	5-Oct-04	MSAM	vNa	7.5	90.6	96.3	97.1	4.83	939	297	110	1.399743	0.1670
16.0	5-Oct-04	MSAM	vNa	7.5	90.6	96.3	97.1	4.83	939	297	110	1.942615	0.1337
16.0	5-Oct-04	MSAM	vNa	7.5	90.6	96.3	98	4.83	939	300	100	1.201848	0.1644
16.0	5-Oct-04	MSAM	vNa	7.5	90.6	96.3	98	4.83	939	300	100	1.448204	0.1600
16.0	5-Oct-04	MSAM	vNa	7.5	90.6	96.3	98	4.83	939	300	80	1.168299	0.2100
17.0	15-Mar-04	LH		7.7	84	97	98	0	405	127	100	0.005679	0.9240
17.0	15-Mar-04	LH		7.7	84	97	98	0	405	127	100	.	0.6567
17.0	15-Mar-04	LH		7.7	84	97	98	0	405	127	.	.	.
17.0	15-Mar-04	LH		7.7	84	97	98	1.66	405	127	83	0.229811	0.9460
17.0	15-Mar-04	LH		7.7	84	97	98	1.66	405	127	117	0.275298	0.7586
17.0	15-Mar-04	LH		7.7	84	97	98	1.66	405	127	117	.	0.7014
17.0	15-Mar-04	LH		7.7	84	97	98	2.78	405	127	117	0.74716	0.6000
17.0	15-Mar-04	LH		7.7	84	97	98	2.78	405	127	117	1.003094	0.5029
17.0	15-Mar-04	LH		7.7	84	97	98	2.78	405	127	117	0.649598	0.4720
17.0	15-Mar-04	LH		7.7	84	97	98	4.64	405	127	117	1.083966	0.6783
17.0	15-Mar-04	LH		7.7	84	97	98	4.64	405	127	83	0.609937	0.6975
17.0	15-Mar-04	LH		7.7	84	97	98	4.64	405	127	100	0.664389	0.7033
17.0	15-Mar-04	LH		7.7	84	97	98	7.73	405	127	83	0.923213	0.6220
17.0	15-Mar-04	LH		7.7	84	97	98	7.73	405	127	100	1.067262	0.5240
17.0	15-Mar-04	LH		7.7	84	97	98	7.73	405	127	83	1.009468	0.6925
17.0	15-Mar-04	LH		7.7	84	97	98	12.9	405	127	100	.	0.7400
17.0	15-Mar-04	LH		7.7	84	97	98	12.9	405	127	83	2.228151	0.6480
17.0	15-Mar-04	LH		7.7	84	97	98	12.9	405	127	83	.	0.6820
17.0	15-Mar-04	LH		7.7	84	97	98	21.5	405	127	67	2.620516	0.5700
17.0	15-Mar-04	LH		7.7	84	97	98	21.5	405	127	83	1.468081	0.7200
17.0	15-Mar-04	LH		7.7	84	97	98	21.5	405	127	100	1.703769	0.6050
17.0	15-Mar-04	LH		7.7	84	97	98	35.8	405	127	67	1.725893	0.6700
17.0	15-Mar-04	LH		7.7	84	97	98	35.8	405	127	83	1.963528	0.5200
17.0	15-Mar-04	LH		7.7	84	97	98	35.8	405	127	67	3.915335	0.4600

SEVEN-DAY NICKEL BIOACCUMULATION TEST CHEMICAL CONFIRMATION DATA

Test	Analysis	Test Day	Nominal	Measured	Units	Meas/Nom
1	Ni	0	2500	2290	µg/L	0.92
1	Ni	0	313	290	µg/L	0.93
2	Ni	0	78	88	µg/L	1.13
2	Ni	0	625	584	µg/L	0.93
2	Ni	0	2500	2320	µg/L	0.93
2	Ni	0	10000	8760	µg/L	0.88
2	Ni	7	313	318	µg/L	1.02
3	Ni	8	2500	2510	µg/L	1.00
4	Ni	0	313	253	µg/L	0.81
4	Ni	0	313	261	µg/L	0.83
4	Ni	0	313	254	µg/L	0.81
4	Ni	0	313	267	µg/L	0.85
4	Ni	0	313	264	µg/L	0.84
4	Ni	0	313	258	µg/L	0.82
4	Ni	0	313	268	µg/L	0.86
4	Ni	7	313	245	µg/L	0.78
4	Ni	7	313	244	µg/L	0.78
4	Ni	7	313	243	µg/L	0.78
4	Ca	0	313	313	mg/L	1.00
4	Ca	0	178	175	mg/L	0.98
4	Ca	0	95	92.4	mg/L	0.97
4	Ca	0	56	53.6	mg/L	0.96
4	Ca	0	36	33.5	mg/L	0.93
4	Ca	0	27	24	mg/L	0.89
4	Ca	0	17	14.8	mg/L	0.87
5	**no chemical data - nominal Ni spikes (313 used)**					
6	Ca	0	331	325	mg/L	0.98
6	Ca	0	52	54	mg/L	1.04
6	Ca	0	25	22	mg/L	0.88
6	Ca	0	17	15	mg/L	0.88
6	Ni	0	313	318	µg/L	1.02
6	Ni	0	313	296	µg/L	0.95
6	Ni	0	313	308	µg/L	0.98
7	**no K chem data**					
7	Ni	0	313	278	µg/L	0.89
7	Ni	0	313	271	µg/L	0.87
7	Ni	0	313	273	µg/L	0.87
7	Ni	0	313	269	µg/L	0.86
7	Ni	0	313	256	µg/L	0.82
7	Ni	0	313	261	µg/L	0.83
8	Mg	0	54	53.6	mg/L	0.99
8	Mg	0	16	15.3	mg/L	0.96
8	Mg	0	3.3	3.61	mg/L	1.09
8	Ni	0	313	286	µg/L	0.91
8	Ni	0	313	336	µg/L	1.07
9	Mg	0	26.4	28.8	mg/L	1.09
9	Mg	0	14.4	15.8	mg/L	1.10
9	Mg	0	8.64	9.55	mg/L	1.11
9	Mg	0	6	6.39	mg/L	1.07

SEVEN-DAY NICKEL BIOACCUMULATION TEST CHEMICAL CONFIRMATION DATA

Test	Analysis	Test Day	Nominal	Measured	Units	Meas/Nom
9	Mg	0	2.64	3.42	mg/L	1.30
9	Ni	0	313	286	µg/L	0.91
9	Ni	0	313	284	µg/L	0.91
9	Ni	0	313	288	µg/L	0.92
9	Ni	0	313	292	µg/L	0.93
9	Ni	0	313	300	µg/L	0.96
10	Mg	0	26.4	28.6	mg/L	1.08
10	Mg	0	14.4	15.7	mg/L	1.09
10	Mg	0	8.64	9.5	mg/L	1.10
10	Mg	0	6	6.3	mg/L	1.05
10	Mg	0	2.64	3.3	mg/L	1.25
10	Ni	0	313	296	µg/L	0.95
10	Ni	0	313	302	µg/L	0.96
10	Ni	0	313	296	µg/L	0.95
10	Ni	0	313	288	µg/L	0.92
10	Ni	0	313	294	µg/L	0.94
11	Mg	0	48	48.5	mg/L	1.01
11	Mg	0	28.8	31.1	mg/L	1.08
11	Mg	0	16.8	20.5	mg/L	1.22
11	Mg	0	9.6	13.7	mg/L	1.43
11	Mg	0	6.24	9.5	mg/L	1.52
11	Ni	0	2.4	2.82	µg/L	1.18
11	Ni	0	313	332	µg/L	1.06
11	Ni	0	313	338	µg/L	1.08
11	Ni	0	313	329	µg/L	1.05
11	Ni	0	313	333	µg/L	1.06
11	Ni	0	313	349	µg/L	1.12
11	Ni	0	313	348	µg/L	1.11
12	Mg	0	48	49.1	mg/L	1.02
12	Mg	0	28.8	31.8	mg/L	1.10
12	Mg	0	16.8	19.7	mg/L	1.17
12	Mg	0	9.6	12.8	mg/L	1.33
12	Mg	0	6.24	8.67	mg/L	1.39
12	Mg	0	2.4	2.62	mg/L	1.09
12	Mg	0	313	315	mg/L	1.01
12	Mg	0	313	313	mg/L	1.00
12	Mg	0	313	314	mg/L	1.00
12	Mg	0	313	322	mg/L	1.03
12	Mg	0	313	324	mg/L	1.04
12	Mg	0	313	334	mg/L	1.07
13	Mg	0	2.5	2.49	mg/L	1.00
13	Mg	0	5.5	8.2	mg/L	1.49
13	Mg	0	11	13.7	mg/L	1.25
13	Mg	0	22.1	24.7	mg/L	1.12
13	Mg	0	44.2	45.3	mg/L	1.02
13	Mg	0	84.4	79.7	mg/L	0.94
13	Ni	0	313	306	µg/L	0.98
13	Ni	0	313	301	µg/L	0.96
13	Ni	0	313	304	µg/L	0.97

SEVEN-DAY NICKEL BIOACCUMULATION TEST CHEMICAL CONFIRMATION DATA

Test	Analysis	Test Day	Nominal	Measured	Units	Meas/Nom
13	Ni	0	313	300	µg/L	0.96
13	Ni	0	313	306	µg/L	0.98
13	Ni	0	313	303	µg/L	0.97
14	Mg	0	2.5	2.68	mg/L	1.07
14	Mg	0	6.33	18.4	mg/L	2.91
14	Mg	0	24	23.5	mg/L	0.98
14	Mg	0	29.5	30.9	mg/L	1.05
14	Mg	0	45.2	51	mg/L	1.13
14	Mg	0	84.4	75	mg/L	0.89
14	Ni	0	313	328	µg/L	1.05
14	Ni	0	313	288	µg/L	0.92
14	Ni	0	313	295	µg/L	0.94
14	Ni	0	313	294	µg/L	0.94
14	Ni	0	313	277	µg/L	0.88
14	Ni	0	313	289	µg/L	0.92
15	Na	0	92	61.6	mg/L	0.67
15	Na	0	46	37.3	mg/L	0.81
15	Na	0	23	12.9	mg/L	0.56
15	Na	0	11.5	12.5	mg/L	1.09
15	Na	0	5.8	209	mg/L	36.03
15	Ni	0	313	277	µg/L	0.88
15	Ni	0	313	276	µg/L	0.88
15	Ni	0	313	284	µg/L	0.91
15	Ni	0	313	271	µg/L	0.87
15	Ni	0	313	260	µg/L	0.83
16	Na	0	167	159	mg/L	0.95
16	**no Ni chem data**					
17	Ni	7	2500	2350	µg/L	0.94
17	Ni	7	2500	2380	µg/L	0.95

APPENDIX A: NICKEL TIME-SERIES TEST DATA

Test	Age (weeks)	Medium	Time (hrs)	Total Ni (µmol/L)	NiT (µmol/g dw)	mg/bug
5.1	18	DC	0	5.33	0.04658	0.5960
5.1	18	DC	0	5.33	0.13884	0.5017
5.1	18	DC	4	5.33	2.03193	0.4880
5.1	18	DC	4	5.33	2.22048	0.4950
5.1	18	DC	8	5.33	2.68853	0.4567
5.1	18	DC	8	5.33	2.44379	0.4633
5.1	18	DC	18	5.33	3.32803	0.5000
5.1	18	DC	18	5.33	1.98685	0.6217
5.1	18	DC	26	5.33	2.27496	0.5700
5.1	18	DC	26	5.33	2.84326	0.5675
5.1	18	DC	34	5.33	3.10846	0.6200
5.1	18	DC	34	5.33	2.54252	0.6100
5.1	18	DC	48	5.33	4.3009	0.5800
5.1	18	DC	48	5.33	1.76766	0.6920
40.1	18	DC	0	42.59	0.04658	0.5960
40.1	18	DC	0	42.59	0.13884	0.5017
40.1	18	DC	4	42.59	4.63379	0.5520
40.1	18	DC	4	42.59	4.7701	0.5240
40.1	18	DC	8	42.59	4.89584	0.5820
40.1	18	DC	8	42.59	7.43903	0.6140
40.1	18	DC	18	42.59	4.1365	0.5720
40.1	18	DC	18	42.59	4.07349	0.5943
40.1	18	DC	26	42.59	4.45986	0.5567
40.1	18	DC	26	42.59	4.9447	0.6180
40.1	18	DC	34	42.59	3.21501	0.5250
40.1	18	DC	34	42.59	4.09162	0.5200
40.1	18	DC	48	42.59	1.89253	0.5383
40.1	18	DC	48	42.59	0.78302	0.5460
40.2	18	LHM	0	42.59	0.09047	0.5514
40.2	18	LHM	0	42.59	0.11269	0.5017
40.2	18	LHM	4	42.59	3.95659	0.5800
40.2	18	LHM	4	42.59	4.58995	0.4483
40.2	18	LHM	8	42.59	13.09371	0.6133
40.2	18	LHM	8	42.59	12.94692	0.4040
40.2	18	LHM	18	42.59	4.57251	0.5367
40.2	18	LHM	18	42.59	4.91152	0.4850
40.2	18	LHM	26	42.59	6.72722	0.4850
40.2	18	LHM	26	42.59	5.1592	0.6440
40.2	18	LHM	34	42.59	5.92526	0.4533
40.2	18	LHM	34	42.59	5.62419	0.5600
40.2	18	LHM	48	42.59	3.96345	0.4540
40.2	18	LHM	48	42.59	7.0194	0.4550
40.3	18	LH-ACC	0	42.59	0.09047	0.5514
40.3	18	LH-ACC	0	42.59	0.11269	0.5017
40.3	18	LH-ACC	4	42.59	1.46466	0.4167
40.3	18	LH-ACC	4	42.59	3.14504	0.6250
40.3	18	LH-ACC	8	42.59	3.02293	0.6650
40.3	18	LH-ACC	8	42.59	4.47001	0.5100
40.3	18	LH-ACC	18	42.59	6.25566	0.4314
40.3	18	LH-ACC	18	42.59	3.30943	0.7620
40.3	18	LH-ACC	26	42.59	6.42736	0.6525
40.3	18	LH-ACC	26	42.59	5.8993	0.8400
40.3	18	LH-ACC	34	42.59	4.9553	0.4075
40.3	18	LH-ACC	34	42.59	7.26088	0.4683
40.3	18	LH-ACC	48	42.59	5.69903	0.5700
40.3	18	LH-ACC	48	42.59	5.51535	0.4940

DC = dechlorinated tap water LHM = low hardness medium

LH-ACC = organisms preacclimated to low hardness meddium

APPENDIX B: 28-DAY NICKEL TOXICITY TEST DATA

				% Free (MINTEQA2)			Survival (%)				MINTEQA2 µmol/L					blank corr		
test	date		pH	Ni	Ca	Mg	7d	14d	21d	28d	Ni	diss	Ca	Mg	pH	NiT (µmol/g dw)	NiTB (µmol/g dw)	weight/bug (mg)
18.0	2-Apr-03	Y	6.4	83.7	94.9	95.6	100	67	40		0.00	0.00	830	353	6.4	.	.	.
18.0	2-Apr-03	Y	6.4	83.7	94.9	95.6	100	60	47		0.67	0.67	830	353	6.4	.	.	.
18.0	2-Apr-03	Y	6.4	83.7	94.9	95.6	100	60	20		1.11	1.11	830	353	6.4	.	.	.
18.0	2-Apr-03	Y	6.4	83.7	94.9	95.6	93	47	27		1.85	1.85	830	353	6.4	.	.	.
18.0	2-Apr-03	Y	6.4	83.7	94.9	95.6	80	47	7		3.08	3.08	830	353	6.4	.	.	.
18.0	2-Apr-03	Y	6.4	83.7	94.9	95.6	60	7	0		5.13	5.13	830	353	6.4	.	.	.
18.0	2-Apr-03	Y	6.4	83.7	94.9	95.6	53	13	0		8.56	8.56	830	353	6.4	.	.	.
18.0	2-Apr-03	Y	6.4	83.7	94.9	95.6	33	7	0		14.26	14.26	830	353	6.4	.	.	.
19.0	2-Apr-03	Y	7.4	80.6	94.8	95.4	80	60	40		0.00	0.00	830	353	7.4	.	.	.
19.0	2-Apr-03	Y	7.4	80.6	94.8	95.4	105	90	85		0.65	0.65	830	353	7.4	.	.	.
19.0	2-Apr-03	Y	7.4	80.6	94.8	95.4	65	55	50		1.07	1.07	830	353	7.4	.	.	.
19.0	2-Apr-03	Y	7.4	80.6	94.8	95.4	100	60	45		1.79	1.79	830	353	7.4	.	.	.
19.0	2-Apr-03	Y	7.4	80.6	94.8	95.4	80	55	30		2.97	2.97	830	353	7.4	.	.	.
19.0	2-Apr-03	Y	7.4	80.6	94.8	95.4	90	30	5		4.94	4.94	830	353	7.4	.	.	.
19.0	2-Apr-03	Y	7.4	80.6	94.8	95.4	50	15	0		8.24	8.24	830	353	7.4	.	.	.
19.0	2-Apr-03	Y	7.4	80.6	94.8	95.4	50	5	0		13.73	13.73	830	353	7.4	.	.	.
20.0	2-Apr-03	Y	8.7	46.6	92.2	93.5	100	95	65		0.00	0.00	807	346	8.7	.	.	.
20.0	2-Apr-03	Y	8.7	46.6	92.2	93.5	100	65	50		0.37	0.37	807	346	8.7	.	.	.
20.0	2-Apr-03	Y	8.7	46.6	92.2	93.5	85	30	0		0.62	0.62	807	346	8.7	.	.	.
20.0	2-Apr-03	Y	8.7	46.6	92.2	93.5	40	25	5		1.03	1.03	807	346	8.7	.	.	.
20.0	2-Apr-03	Y	8.7	46.6	92.2	93.5	50	25	0		1.71	1.71	807	346	8.7	.	.	.
20.0	2-Apr-03	Y	8.7	46.6	92.2	93.5	45	10	0		2.86	2.86	807	346	8.7	.	.	.
20.0	2-Apr-03	Y	8.7	46.6	92.2	93.5	30	5	0		4.76	4.76	807	346	8.7	.	.	.
20.0	2-Apr-03	Y	8.7	46.6	92.2	93.5	15	0	0		7.94	7.94	807	346	8.7	.	.	.
21.0	10-Apr-03	Y	8.1	71.6	94.5	95.3	87	87	60	67	0.00	0.00	234	271	8.1	.	.	.
21.0	10-Apr-03	Y	8.1	71.6	94.5	95.3	73	53	27	20	0.57	0.57	234	271	8.1	.	.	.
21.0	10-Apr-03	Y	8.1	71.6	94.5	95.3	60	13	0	0	0.95	0.95	234	271	8.1	.	.	.
21.0	10-Apr-03	Y	8.1	71.6	94.5	95.3	40	7	0	0	1.59	1.59	234	271	8.1	.	.	.
21.0	10-Apr-03	Y	8.1	71.6	94.5	95.3	33	13	7	0	2.63	2.63	234	271	8.1	.	.	.
21.0	10-Apr-03	Y	8.1	71.6	94.5	95.3	13	0	0	0	4.39	4.39	234	271	8.1	.	.	.
21.0	10-Apr-03	Y	8.1	71.6	94.5	95.3	13	0	0	0	7.32	7.32	234	271	8.1	.	.	.
21.0	10-Apr-03	Y	8.1	71.6	94.5	95.3	7	0	0	0	12.20	12.20	234	271	8.1	.	.	.
22.0	10-Apr-03	Y	8	71.8	94.3	95.1	100	93	93	87	0.00	0.00	825	351	8.0	.	.	.

				% Free (MINTEQA2)			Survival (%)				MINTEQA2 µmol/L					blank corr		
test	date		pH	Ni	Ca	Mg	7d	14d	21d	28d	Ni	diss	Ca	Mg	pH	NiT (µmol/g dw)	NiTB (µmol/g dw)	weight/bug (mg)
22.0	10-Apr-03	Y	8	71.8	94.3	95.1	93	80	53	40	0.57	0.57	825	351	8.0	.	.	.
22.0	10-Apr-03	Y	8	71.8	94.3	95.1	93	80	60	27	0.95	0.95	825	351	8.0	.	.	.
22.0	10-Apr-03	Y	8	71.8	94.3	95.1	100	60	47	40	1.59	1.59	825	351	8.0	.	.	.
22.0	10-Apr-03	Y	8	71.8	94.3	95.1	47	7	7	0	2.64	2.64	825	351	8.0	.	.	.
22.0	10-Apr-03	Y	8	71.8	94.3	95.1	67	0	0	0	4.40	4.40	825	351	8.0	.	.	.
22.0	10-Apr-03	Y	8	71.8	94.3	95.1	27	0	0	0	7.34	7.34	825	351	8.0	.	.	.
22.0	10-Apr-03	Y	8	71.8	94.3	95.1	13	0	0	0	12.23	12.23	825	351	8.0	.	.	.
23.0	10-Apr-03	Y	7.8	84	97	97	100	95	85	75	0.00	0.00	3153	279	7.8	.	.	.
23.0	10-Apr-03	Y	7.8	84	97	97	95	75	45	35	0.67	0.67	3153	279	7.8	.	.	.
23.0	10-Apr-03	Y	7.8	84	97	97	100	80	70	70	1.12	1.12	3153	279	7.8	.	.	.
23.0	10-Apr-03	Y	7.8	84	97	97	90	40	10	0	1.86	1.86	3153	279	7.8	.	.	.
23.0	10-Apr-03	Y	7.8	84	97	97	90	35	10	0	3.09	3.09	3153	279	7.8	.	.	.
23.0	10-Apr-03	Y	7.8	84	97	97	80	35	5	0	5.15	5.15	3153	279	7.8	.	.	.
23.0	10-Apr-03	Y	7.8	84	97	97	70	10	0	0	8.59	8.59	3153	279	7.8	.	.	.
23.0	10-Apr-03	Y	7.8	84	97	97	30	0	0	0	14.31	14.31	3153	279	7.8	.	.	.
24.0	24-Apr-03	Y	6.4	83.7	95	96	93	80	53	53	0.00	0.00	831	355	6.4	.	.	.
24.0	24-Apr-03	Y	6.4	83.7	95	96	93	87	73	80	0.67	0.67	831	355	6.4	.	.	.
24.0	24-Apr-03	Y	6.4	83.7	95	96	87	67	47	40	1.11	1.11	831	355	6.4	.	.	.
24.0	24-Apr-03	Y	6.4	83.7	95	96	93	80	60	53	1.85	1.85	831	355	6.4	.	.	.
24.0	24-Apr-03	Y	6.4	83.7	95	96	100	93	60	47	3.08	3.08	831	355	6.4	.	.	.
24.0	24-Apr-03	Y	6.4	83.7	95	96	87	47	0	0	5.13	5.13	831	355	6.4	.	.	.
24.0	24-Apr-03	Y	6.4	83.7	95	96	87	33	7	0	8.56	8.56	831	355	6.4	.	.	.
24.0	24-Apr-03	Y	6.4	83.7	95	96	20	0	0	0	14.26	14.26	831	355	6.4	.	.	.
25.0	24-Apr-03	Y	7.3	81.3	94.8	95.5	100	107	93	93	0.00	0.00	830	353	7.3	.	.	.
25.0	24-Apr-03	Y	7.3	81.3	94.8	95.5	100	100	93	93	0.65	0.65	830	353	7.3	.	.	.
25.0	24-Apr-03	Y	7.3	81.3	94.8	95.5	107	107	93	87	1.08	1.08	830	353	7.3	.	.	.
25.0	24-Apr-03	Y	7.3	81.3	94.8	95.5	87	87	73	60	1.80	1.80	830	353	7.3	.	.	.
25.0	24-Apr-03	Y	7.3	81.3	94.8	95.5	87	73	0	0	2.99	2.99	830	353	7.3	.	.	.
25.0	24-Apr-03	Y	7.3	81.3	94.8	95.5	80	53	7	7	4.99	4.99	830	353	7.3	.	.	.
25.0	24-Apr-03	Y	7.3	81.3	94.8	95.5	40	7	0	0	8.31	8.31	830	353	7.3	.	.	.
25.0	24-Apr-03	Y	7.3	81.3	94.8	95.5	27	0	0	0	13.85	13.85	830	353	7.3	.	.	.
26.0	24-Apr-03	Y	8.4	59.2	93.4	94.5	107				0.00	0.00	817	349	8.4	.	.	.
26.0	24-Apr-03	Y	8.4	59.2	93.4	94.5	87				0.47	0.47	817	349	8.4	.	.	.
26.0	24-Apr-03	Y	8.4	59.2	93.4	94.5	87				0.79	0.79	817	349	8.4	.	.	.
26.0	24-Apr-03	Y	8.4	59.2	93.4	94.5	47				1.31	1.31	817	349	8.4	.	.	.

				% Free (MINTEQA2)			Survival (%)				MINTEQA2 µmol/L					blank corr		
test	date		pH	Ni	Ca	Mg	7d	14d	21d	28d	Ni	diss	Ca	Mg	pH	NiT (µmol/g dw)	NiTB (µmol/g dw)	weight/bug (mg)
26.0	24-Apr-03	Y	8.4	59.2	93.4	94.5	53				2.18	2.18	817	349	8.4	.	.	.
26.0	24-Apr-03	Y	8.4	59.2	93.4	94.5	13				3.63	3.63	817	349	8.4	.	.	.
26.0	24-Apr-03	Y	8.4	59.2	93.4	94.5	33				6.05	6.05	817	349	8.4	.	.	.
26.0	24-Apr-03	Y	8.4	59.2	93.4	94.5	0				10.09	10.09	817	349	8.4	.	.	.
27.0	15-May-03	Y	8.4	59.2	93.4	94.5	113	107	100	100	0.00	0.00	817	349	8.4	.	.	.
27.0	15-May-03	Y	8.4	59.2	93.4	94.5	100	93	80	80	0.17	0.17	817	349	8.4	.	.	.
27.0	15-May-03	Y	8.4	59.2	93.4	94.5	80	67	60	60	0.28	0.28	817	349	8.4	.	.	.
27.0	15-May-03	Y	8.4	59.2	93.4	94.5	93	33	13	13	0.47	0.47	817	349	8.4	.	.	.
27.0	15-May-03	Y	8.4	59.2	93.4	94.5	107	20	7	0	0.79	0.79	817	349	8.4	.	.	.
27.0	15-May-03	Y	8.4	59.2	93.4	94.5	33	0	0	0	1.31	1.31	817	349	8.4	.	.	.
27.0	15-May-03	Y	8.4	59.2	93.4	94.5	40	0	0	0	2.18	2.18	817	349	8.4	.	.	.
27.0	15-May-03	Y	8.4	59.2	93.4	94.5	27	0	0	0	3.63	3.63	817	349	8.4	.	.	.
27.0	15-May-03	Y	8.4	59.2	93.4	94.5	13	0	0	0	6.05	6.05	817	349	8.4	.	.	.
28.0	27-Jun-03	Y	8.1	71.5	94.4	95.3	75	60	40	20	0.00	0.00	234	271	8.1	.	.	.
28.0	27-Jun-03	Y	8.1	71.5	94.4	95.3	70	60	35	10	0.12	0.12	234	271	8.1	.	.	.
28.0	27-Jun-03	Y	8.1	71.5	94.4	95.3	80	70	65	50	0.21	0.21	234	271	8.1	.	.	.
28.0	27-Jun-03	Y	8.1	71.5	94.4	95.3	70	55	65	25	0.34	0.34	234	271	8.1	.	.	.
28.0	27-Jun-03	Y	8.1	71.5	94.4	95.3	70	55	40	30	0.57	0.57	234	271	8.1	.	.	.
28.0	27-Jun-03	Y	8.1	71.5	94.4	95.3	60	35	20	5	0.95	0.95	234	271	8.1	.	.	.
28.0	27-Jun-03	Y	8.1	71.5	94.4	95.3	50	10	5	0	1.58	1.58	234	271	8.1	.	.	.
28.0	27-Jun-03	Y	8.1	71.5	94.4	95.3	35	5	0	0	2.63	2.63	234	271	8.1	.	.	.
28.0	27-Jun-03	Y	8.1	71.5	94.4	95.3	15	0	0	0	4.39	4.39	234	271	8.1	.	.	.
28.0	27-Jun-03	Y	8.1	71.5	94.4	95.3	5	0	0	0	7.31	7.31	234	271	8.1	.	.	.
29.0	27-Jun-03	Y	8.2	66.3	93.9	94.9	100	87	80	80	0.00	0.00	749	328	8.2	.	.	.
29.0	27-Jun-03	Y	8.2	66.3	93.9	94.9	127	107	107	107	0.19	0.19	749	328	8.2	.	.	.
29.0	27-Jun-03	Y	8.2	66.3	93.9	94.9	107	93	73	60	0.32	0.32	749	328	8.2	.	.	.
29.0	27-Jun-03	Y	8.2	66.3	93.9	94.9	87	73	60	40	0.53	0.53	749	328	8.2	.	.	.
29.0	27-Jun-03	Y	8.2	66.3	93.9	94.9	87	60	33	13	0.88	0.88	749	328	8.2	.	.	.

				% Free (MINTEQA2)			Survival (%)				MINTEQA2 µmol/L					blank corr		
test	date		pH	Ni	Ca	Mg	7d	14d	21d	28d	Ni	diss	Ca	Mg	pH	NiT (µmol/g dw)	NiTB (µmol/g dw)	weight/bug (mg)
29.0	27-Jun-03	Y	8.2	66.3	93.9	94.9	73	47	7	13	1.47	1.47	749	328	8.2	.	.	.
29.0	27-Jun-03	Y	8.2	66.3	93.9	94.9	73	13	7	0	2.44	2.44	749	328	8.2	.	.	.
29.0	27-Jun-03	Y	8.2	66.3	93.9	94.9	47	0	0	0	4.07	4.07	749	328	8.2	.	.	.
29.0	27-Jun-03	Y	8.2	66.3	93.9	94.9	27	0	0	0	6.78	6.78	749	328	8.2	.	.	.
30.0	4-Jul-03	Y	8	84.3	97	97.5	107	100	87	47	0.00	0.00	2837	281	8.0	.	.	.
30.0	4-Jul-03	Y	8	84.3	97	97.5	100	93	93	73	0.68	0.68	2837	281	8.0	.	.	.
30.0	4-Jul-03	Y	8	84.3	97	97.5	80	80	80	73	1.12	1.12	2837	281	8.0	.	.	.
30.0	4-Jul-03	Y	8	84.3	97	97.5	100	93	53	40	1.87	1.87	2837	281	8.0	.	.	.
30.0	4-Jul-03	Y	8	84.3	97	97.5	67	7	0	0	3.10	3.10	2837	281	8.0	.	.	.
30.0	4-Jul-03	Y	8	84.3	97	97.5	73	20	0	0	5.17	5.17	2837	281	8.0	.	.	.
30.0	4-Jul-03	Y	8	84.3	97	97.5	27	0	0	0	8.62	8.62	2837	281	8.0	.	.	.
30.0	4-Jul-03	Y	8	84.3	97	97.5	13	0	0	0	14.36	14.36	2837	281	8.0	.	.	.
31.0	9-Jul-03	Y	6.7	83.4	94.9	95.5	100	85	65	45	0.00	0.00	830	353	6.7	.	.	.
31.0	9-Jul-03	Y	6.7	83.4	94.9	95.5	95	90	90	75	0.67	0.67	830	353	6.7	.	.	.
31.0	9-Jul-03	Y	6.7	83.4	94.9	95.5	95	100	85	65	1.11	1.11	830	353	6.7	.	.	.
31.0	9-Jul-03	Y	6.7	83.4	94.9	95.5	100	90	80	65	1.85	1.85	830	353	6.7	.	.	.
31.0	9-Jul-03	Y	6.7	83.4	94.9	95.5	100	80	55	45	3.07	3.07	830	353	6.7	.	.	.
31.0	9-Jul-03	Y	6.7	83.4	94.9	95.5	95	60	20	0	5.12	5.12	830	353	6.7	.	.	.
31.0	9-Jul-03	Y	6.7	83.4	94.9	95.5	60	15	0	0	8.53	8.53	830	353	6.7	.	.	.
31.0	9-Jul-03	Y	6.7	83.4	94.9	95.5	15	0	0	0	14.21	14.21	830	353	6.7	.	.	.
32.0	12-Oct-03	Y	7.9	87.5	95.6	96.5	100	100	100		0.00	0.00	239	282	7.9	.	.	.
32.0	12-Oct-03	Y	7.9	87.5	95.6	96.5	100	93	80		0.25	0.25	239	282	7.9	.	.	.
32.0	12-Oct-03	Y	7.9	87.5	95.6	96.5	107	107	107		0.42	0.42	239	282	7.9	.	.	.
32.0	12-Oct-03	Y	7.9	87.5	95.6	96.5	107	87	87		0.70	0.70	239	282	7.9	.	.	.
32.0	12-Oct-03	Y	7.9	87.5	95.6	96.5	113	93	53		1.16	1.16	239	282	7.9	.	.	.
32.0	12-Oct-03	Y	7.9	87.5	95.6	96.5	107	53	33		1.94	1.94	239	282	7.9	.	.	.
32.0	12-Oct-03	Y	7.9	87.5	95.6	96.5	87	33	0		3.22	3.22	239	282	7.9	.	.	.
32.0	12-Oct-03	Y	7.9	87.5	95.6	96.5	67	0	0		5.37	5.37	239	282	7.9	.	.	.
32.0	12-Oct-03	Y	7.9	87.5	95.6	96.5	33	0	0		8.94	8.94	239	282	7.9	.	.	.
33.0	12-Oct-03	Y	7.8	89.3	96.3	97.1	107	100	100		0.00	0.00	963	280	7.8	.	.	.

				% Free (MINTEQA2)			Survival (%)				MINTEQA2 µmol/L					blank corr		
test	date		pH	Ni	Ca	Mg	7d	14d	21d	28d	Ni	diss	Ca	Mg	pH	NiT (µmol/g dw)	NiTB (µmol/g dw)	weight/bug (mg)
33.0	12-Oct-03	Y	7.8	89.3	96.3	97.1	120	93	80		0.26	0.26	963	280	7.8	.	.	.
33.0	12-Oct-03	Y	7.8	89.3	96.3	97.1	107	107	107		0.43	0.43	963	280	7.8	.	.	.
33.0	12-Oct-03	Y	7.8	89.3	96.3	97.1	107	87	87		0.72	0.72	963	280	7.8	.	.	.
33.0	12-Oct-03	Y	7.8	89.3	96.3	97.1	107	93	53		1.19	1.19	963	280	7.8	.	.	.
33.0	12-Oct-03	Y	7.8	89.3	96.3	97.1	100	53	33		1.98	1.98	963	280	7.8	.	.	.
33.0	12-Oct-03	Y	7.8	89.3	96.3	97.1	100	33	0		3.29	3.29	963	280	7.8	.	.	.
33.0	12-Oct-03	Y	7.8	89.3	96.3	97.1	87	0	0		5.48	5.48	963	280	7.8	.	.	.
33.0	12-Oct-03	Y	7.8	89.3	96.3	97.1	47	0	0		9.13	9.13	963	280	7.8	.	.	.
33.0	12-Oct-03	Y	7.8	89.3	96.3	97.1	27	0	0		15.21	15.21	963	280	7.8	.	.	.
34.0	12-Oct-03	Y	8.5	44	92.3	93.5	107	100	100		0.00	0.00	692	269	8.5	.	.	.
34.0	12-Oct-03	Y	8.5	44	92.3	93.5	100	107	93		0.13	0.13	692	269	8.5	.	.	.
34.0	12-Oct-03	Y	8.5	44	92.3	93.5	107	107	93		0.21	0.21	692	269	8.5	.	.	.
34.0	12-Oct-03	Y	8.5	44	92.3	93.5	87	60	60		0.35	0.35	692	269	8.5	.	.	.
34.0	12-Oct-03	Y	8.5	44	92.3	93.5	107	93	47		0.58	0.58	692	269	8.5	.	.	.
34.0	12-Oct-03	Y	8.5	44	92.3	93.5	100	13	0		0.97	0.97	692	269	8.5	.	.	.
34.0	12-Oct-03	Y	8.5	44	92.3	93.5	73	0	0		1.62	1.62	692	269	8.5	.	.	.
34.0	12-Oct-03	Y	8.5	44	92.3	93.5	67	0	0		2.70	2.70	692	269	8.5	.	.	.
34.0	12-Oct-03	Y	8.5	44	92.3	93.5	47	0	0		4.50	4.50	692	269	8.5	.	.	.
35.0	25-Mar-04	Y	8.1	81.8	96.6	97.1	105	105	95	90	0.00	0.00	3792	400	8.1	0.0510	0.0113	0.2671
35.0	25-Mar-04	Y	8.1	81.8	96.6	97.1	100	75	55	35	0.47	0.47	3792	400	8.1	0.4070	0.1562	0.1200
35.0	25-Mar-04	Y	8.1	81.8	96.6	97.1	85	50	45	35	0.78	0.78	3792	400	8.1	.	.	0.0700
35.0	25-Mar-04	Y	8.1	81.8	96.6	97.1	100	55	35	30	1.30	1.30	3792	400	8.1	1.2352	0.7837	0.0667
35.0	25-Mar-04	Y	8.1	81.8	96.6	97.1	95	45	30	30	2.17	2.17	3792	400	8.1	2.8571	1.4679	0.0433
35.0	25-Mar-04	Y	8.1	81.8	96.6	97.1	100	25	5	15	3.62	3.62	3792	400	8.1	.	.	
35.0	25-Mar-04	Y	8.1	81.8	96.6	97.1	95	10	5	5	6.02	6.02	3792	400	8.1	.	.	
35.0	25-Mar-04	Y	8.1	81.8	96.6	97.1	60	5	0	0	10.03	10.03	3792	400	8.1	.	.	
35.0	25-Mar-04	Y	8.1	81.8	96.6	97.1	40	0	0	0	16.72	16.72	3792	400	8.1	.	.	
36.0	28-Mar-04	Y	8.9	34.5	89.6	91.8	100	100	95	95	0.00	0.00	784	363	8.9	0.0636	0.0285	0.2705
36.0	28-Mar-04	Y	8.9	34.5	89.6	91.8	100	95	90	85	0.08	0.08	784	363	8.9	0.8987	0.8351	0.1495
36.0	28-Mar-04	Y	8.9	34.5	89.6	91.8	90	70	50	40	0.14	0.14	784	363	8.9	0.6736	0.4636	0.1075
36.0	28-Mar-04	Y	8.9	34.5	89.6	91.8	95	70	60	50	0.23	0.23	784	363	8.9	1.5727	1.2949	0.0812

			% Free (MINTEQA2)				Survival (%)				MINTEQA2 µmol/L					blank corr		
test	date		pH	Ni	Ca	Mg	7d	14d	21d	28d	Ni	diss	Ca	Mg	pH	NiT (µmol/g dw)	NiTB (µmol/g dw)	weight/bug (mg)
36.0	28-Mar-04	Y	8.9	34.5	89.6	91.8	95	30	20	15	0.38	0.38	784	363	8.9	.	.	
36.0	28-Mar-04	Y	8.9	34.5	89.6	91.8	65	25	15	0	0.63	0.63	784	363	8.9	.	.	
36.0	28-Mar-04	Y	8.9	34.5	89.6	91.8	55	15	0	0	1.06	1.06	784	363	8.9	.	.	
36.0	28-Mar-04	Y	8.9	34.5	89.6	91.8	45	0	0	0	1.76	1.76	784	363	8.9	.	.	
37.0	12-Apr-04	Y	8.1	78.6	93.1	94.1	100	100	100	94	0.00	0.00	3677	1018	8.1	0.0643	0.0169	0.1927
37.0	12-Apr-04	Y	8.1	78.6	93.1	94.1	100	100	100	100	0.20	0.20	3677	1018	8.1	0.2644	0.2256	0.1439
37.0	12-Apr-04	Y	8.1	78.6	93.1	94.1	106	106	100	100	0.42	0.42	3677	1018	8.1	0.3728	0.3378	0.1794
37.0	12-Apr-04	Y	8.1	78.6	93.1	94.1	94	94	76	76	0.84	0.84	3677	1018	8.1	0.5984	0.4758	0.0745
37.0	12-Apr-04	Y	8.1	78.6	93.1	94.1	71	24	6	6	1.67	1.67	3677	1018	8.1	.	.	
37.0	12-Apr-04	Y	8.1	78.6	93.1	94.1	65	0	0	0	3.35	3.35	3677	1018	8.1	.	.	
37.0	12-Apr-04	Y	8.1	78.6	93.1	94.1	41	6	0	0	6.70	6.70	3677	1018	8.1	.	.	
37.0	12-Apr-04	Y	8.1	78.6	93.1	94.1	29	0	0	0	13.39	13.39	3677	1018	8.1	.	.	
37.0	12-Apr-04	Y	8.1	78.6	93.1	94.1	6	0	0	0	26.78	26.78	3677	1018	8.1	.	.	
38.0	19-Apr-04	Y	8.2	69.5	91	92.6	100	100	100	95	0.00	0.00	953	823	8.2	.	.	0.2900
38.0	19-Apr-04	Y	8.2	69.5	91	92.6	95	95	90	90	0.18	0.18	953	823	8.2	.	.	0.1307
38.0	19-Apr-04	Y	8.2	69.5	91	92.6	95	95	95	80	0.37	0.37	953	823	8.2	.	.	0.0400
38.0	19-Apr-04	Y	8.2	69.5	91	92.6	100	90	70	60	0.74	0.74	953	823	8.2	.	.	0.0383
38.0	19-Apr-04	Y	8.2	69.5	91	92.6	100	70	50	40	1.48	1.48	953	823	8.2	.	.	
38.0	19-Apr-04	Y	8.2	69.5	91	92.6	100	35	5	5	2.96	2.96	953	823	8.2	.	.	
38.0	19-Apr-04	Y	8.2	69.5	91	92.6	60	0	0	0	5.92	5.92	953	823	8.2	.	.	
38.0	19-Apr-04	Y	8.2	69.5	91	92.6	25	5	0	0	11.84	11.84	953	823	8.2	.	.	
38.0	19-Apr-04	Y	8.2	69.5	91	92.6	25	0	0	0	29.60	29.60	953	823	8.2	.	.	
39.0	27-Apr-04	Y	8.2	54.2	90.2	91.7	100	100	95	95	0.00	0.00	4285	1355	8.2	0.0287	-0.0418	0.1676
39.0	27-Apr-04	Y	8.2	54.2	90.2	91.7	100	100	90	85	0.14	0.14	4285	1355	8.2	0.2988	0.2191	0.1575
39.0	27-Apr-04	Y	8.2	54.2	90.2	91.7	105	95	60	45	0.29	0.29	4285	1355	8.2	0.4326	0.2067	0.1113
39.0	27-Apr-04	Y	8.2	54.2	90.2	91.7	95	85	60	40	0.58	0.58	4285	1355	8.2	0.6011	0.3218	0.1029
39.0	27-Apr-04	Y	8.2	54.2	90.2	91.7	95	85	30	20	1.15	1.15	4285	1355	8.2	6.0484	4.7919	0.0400
39.0	27-Apr-04	Y	8.2	54.2	90.2	91.7	95	20	0	0	2.31	2.31	4285	1355	8.2	.	.	
39.0	27-Apr-04	Y	8.2	54.2	90.2	91.7	70	20	0	0	4.62	4.62	4285	1355	8.2	.	.	
39.0	27-Apr-04	Y	8.2	54.2	90.2	91.7	20	0	0	0	9.23	9.23	4285	1355	8.2	.	.	
39.0	27-Apr-04	Y	8.2	54.2	90.2	91.7	30	5	0	0	23.09	23.09	4285	1355	8.2	.	.	
40.0	11-Jun-04	Y	7.7	88.5	97	97.5	100	85	70	60	0.00	0.00	340	108	7.7	0.0003	-0.0001	0.2010
40.0	11-Jun-04	Y	7.7	88.5	97	97.5	100	95	95	95	0.05	0.05	340	108	7.7	0.1577	0.0952	0.1810

			% Free (MINTEQA2)				Survival (%)				MINTEQA2 µmol/L					blank corr		
test	date		pH	Ni	Ca	Mg	7d	14d	21d	28d	Ni	diss	Ca	Mg	pH	NiT (µmol/g dw)	NiTB (µmol/g dw)	weight/bug (mg)
40.0	11-Jun-04	Y	7.7	88.5	97	97.5	90	90	90	70	0.12	0.12	340	108	7.7	0.1677	0.0885	0.1850
40.0	11-Jun-04	Y	7.7	88.5	97	97.5	100	95	90	85	0.24	0.24	340	108	7.7	0.3992	0.3353	0.1060
40.0	11-Jun-04	Y	7.7	88.5	97	97.5	115	115	115	110	0.47	0.47	340	108	7.7	0.5455	0.4592	0.0640
40.0	11-Jun-04	Y	7.7	88.5	97	97.5	95	80	80	50	0.94	0.94	340	108	7.7	1.4218	1.0751	0.0600
40.0	11-Jun-04	Y	7.7	88.5	97	97.5	85	25	5	5	1.88	1.88	340	108	7.7	.	.	
40.0	11-Jun-04	Y	7.7	88.5	97	97.5	35	5	0	0	3.77	3.77	340	108	7.7	.	.	
40.0	11-Jun-04	Y	7.7	88.5	97	97.5	45	0	0	0	7.54	7.54	340	108	7.7	.	.	
41.0	20-Jun-04	Y	7.9	80	91.8	93.2	105	105	90	85	0.00	0.00	6839	1791	7.9	0.0388	-0.0061	0.2635
41.0	20-Jun-04	Y	7.9	80	91.8	93.2	100	100	95	75	0.27	0.27	6839	1791	7.9	0.1952	0.1271	0.1735
41.0	20-Jun-04	Y	7.9	80	91.8	93.2	100	100	95	85	0.53	0.53	6839	1791	7.9	0.2111	0.1479	0.1674
41.0	20-Jun-04	Y	7.9	80	91.8	93.2	100	85	85	85	1.06	1.06	6839	1791	7.9	0.3240	0.2007	0.0906
41.0	20-Jun-04	Y	7.9	80	91.8	93.2	100	80	65	40	2.13	2.13	6839	1791	7.9	1.6236	1.0323	0.0486
41.0	20-Jun-04	Y	7.9	80	91.8	93.2	105	75	20	10	4.27	4.27	6839	1791	7.9	3.5779	.	0.0200
41.0	20-Jun-04	Y	7.9	80	91.8	93.2	70	30	5	0	8.52	8.52	6839	1791	7.9	.	.	
41.0	20-Jun-04	Y	7.9	80	91.8	93.2	55	0	0	0	17.04	17.04	6839	1791	7.9	.	.	
41.0	20-Jun-04	Y	7.9	80	91.8	93.2	35	0	0	0	34.08	34.08	6839	1791	7.9	.	.	
41.0	20-Jun-04	Y	7.9	80	91.8	93.2	35	0	0	0	68.15	68.15	6839	1791	7.9	.	.	
42.0	24-Jun-04	Y	8.1	71.2	85.4	87.9	100	95	95	90	0.00	0.00	798	1704	8.1	0.0716	0.0336	0.3662
42.0	24-Jun-04	Y	8.1	71.2	85.4	87.9	90	95	80	80	0.24	0.24	798	1704	8.1	0.4771	0.4018	0.1846
42.0	24-Jun-04	Y	8.1	71.2	85.4	87.9	100	95	75	65	0.47	0.47	798	1704	8.1	1.2666	1.0962	0.0883
42.0	24-Jun-04	Y	8.1	71.2	85.4	87.9	110	85	70	60	0.95	0.95	798	1704	8.1	.	.	0.0567
42.0	24-Jun-04	Y	8.1	71.2	85.4	87.9	100	70	30	15	1.89	1.89	798	1704	8.1	.	.	
42.0	24-Jun-04	Y	8.1	71.2	85.4	87.9	95	45	0	0	3.80	3.80	798	1704	8.1	.	.	
42.0	24-Jun-04	Y	8.1	71.2	85.4	87.9	80	15	0	0	7.58	7.58	798	1704	8.1	.	.	
42.0	24-Jun-04	Y	8.1	71.2	85.4	87.9	60	0	0	0	15.16	15.16	798	1704	8.1	.	.	
42.0	24-Jun-04	Y	8.1	71.2	85.4	87.9	25	0	0	0	30.33	30.33	798	1704	8.1	.	.	
42.0	24-Jun-04	Y	8.1	71.2	85.4	87.9	5	0	0	0	60.65	60.65	798	1704	8.1	.	.	
43.0	9-Jul-04	Y	8.8	12.2	72.1	79.6	100	100	100	100	0.00	0.00	685	229	8.8	0.1563	0.0948	0.1663
43.0	9-Jul-04	Y	8.8	12.2	72.1	79.6	100	95	90	85	0.02	0.02	685	229	8.8	0.2370	0.2015	0.3067
43.0	9-Jul-04	Y	8.8	12.2	72.1	79.6	100	90	65	55	0.03	0.03	685	229	8.8	0.4543	0.1817	0.0667
43.0	9-Jul-04	Y	8.8	12.2	72.1	79.6	95	90	65	45	0.07	0.07	685	229	8.8	.		0.0240
43.0	9-Jul-04	Y	8.8	12.2	72.1	79.6	100	55	30	10	0.13	0.13	685	229	8.8	1.2267	0.6247	0.0500
43.0	9-Jul-04	Y	8.8	12.2	72.1	79.6	50	20	0	0	0.26	0.26	685	229	8.8	.	.	
43.0	9-Jul-04	Y	8.8	12.2	72.1	79.6	65	5	0	0	0.52	0.52	685	229	8.8	.	.	

				% Free (MINTEQA2)			Survival (%)				MINTEQA2 µmol/L					blank corr		
test	date		pH	Ni	Ca	Mg	7d	14d	21d	28d	Ni	diss	Ca	Mg	pH	NiT (µmol/g dw)	NiTB (µmol/g dw)	weight/bug (mg)
43.0	9-Jul-04	Y	8.8	12.2	72.1	79.6	30	0	0	0	1.04	1.04	685	229	8.8	.	.	
43.0	9-Jul-04	Y	8.8	12.2	72.1	79.6	40	0	0	0	2.08	2.08	685	229	8.8	.	.	
43.0	9-Jul-04	Y	8.8	12.2	72.1	79.6	10	0	0	0	4.16	4.16	685	229	8.8	.	.	
44.0	22-Jul-04	Y	8	78	95.6	96.3	95	95	90	90	0.00	0.00	803	275	8.0	0.0221	-0.0051	0.3683
44.0	22-Jul-04	Y	8	78	95.6	96.3	100	100	95	85	0.21	0.21	803	275	8.0	0.2934	0.2604	0.3218
44.0	22-Jul-04	Y	8	78	95.6	96.3	100	100	85	80	0.42	0.42	803	275	8.0	0.4299	0.3087	0.0931
44.0	22-Jul-04	Y	8	78	95.6	96.3	100	85	60	50	0.83	0.83	803	275	8.0	0.3408	-0.2065	0.0412
44.0	22-Jul-04	Y	8	78	95.6	96.3	100	50	25	10	1.66	1.66	803	275	8.0	1.5091	-1.0709	0.0350
44.0	22-Jul-04	Y	8	78	95.6	96.3	70	25	0	0	3.32	3.32	803	275	8.0	.	.	
44.0	22-Jul-04	Y	8	78	95.6	96.3	50	0	0	0	6.64	6.64	803	275	8.0	.	.	
44.0	22-Jul-04	Y	8	78	95.6	96.3	15	0	0	0	13.29	13.29	803	275	8.0	.	.	
45.0	2-Aug-04	Y	7.7	88	97	98.2	100	100	90	80	0.00	0.00	267	101	7.7	0.1321	0.0525	0.1419
45.0	2-Aug-04	Y	7.7	88	97	98.2	105	105	100	95	0.12	0.09	267	101	7.7	0.0904	0.0210	0.1368
45.0	2-Aug-04	Y	7.7	88	97	98.2	100	100	90	90	0.23	0.18	267	101	7.7	0.1982	0.1263	0.1394
45.0	2-Aug-04	Y	7.7	88	97	98.2	100	100	95	85	0.47	0.37	267	101	7.7	.	.	
45.0	2-Aug-04	Y	7.7	88	97	98.2	90	80	55	50	0.94	0.74	267	101	7.7	0.5702	0.2017	0.0613
45.0	2-Aug-04	Y	7.7	88	97	98.2	90	90	30	10	1.87	1.47	267	101	7.7	.	.	0.0250
45.0	2-Aug-04	Y	7.7	88	97	98.2	90	25	5	0	3.75	2.94	267	101	7.7	.	.	
45.0	2-Aug-04	Y	7.7	88	97	98.2	75	0	0	0	7.50	5.88	267	101	7.7	.	.	
45.0	2-Aug-04	Y	7.7	88	97	98.2	35	0	0	0	14.99	11.77	267	101	7.7	.	.	
45.0	2-Aug-04	Y	7.7	88	97	98.2	0	0	0	0	29.99	23.54	267	101	7.7	.	.	
46.0	5-Aug-04	Y	8.2	70.4	95.3	96.1	100	100	100	95	0.00	0.00	958	282	8.2	0.0693	-0.0042	0.1367
46.0	5-Aug-04	Y	8.2	70.4	95.3	96.1	100	100	95	80	0.09	0.08	958	282	8.2	0.1068	0.0466	0.1875
46.0	5-Aug-04	Y	8.2	70.4	95.3	96.1	100	100	100	95	0.19	0.16	958	282	8.2	0.1697	0.0983	0.1406
46.0	5-Aug-04	Y	8.2	70.4	95.3	96.1	95	95	95	95	0.37	0.32	958	282	8.2	0.5645	0.4859	0.1278
46.0	5-Aug-04	Y	8.2	70.4	95.3	96.1	100	90	70	55	0.75	0.64	958	282	8.2	.	.	
46.0	5-Aug-04	Y	8.2	70.4	95.3	96.1	85	55	50	15	1.50	1.27	958	282	8.2	2.4484	1.7795	0.0450
46.0	5-Aug-04	Y	8.2	70.4	95.3	96.1	65	15	5	0	3.00	2.55	958	282	8.2	.	.	
46.0	5-Aug-04	Y	8.2	70.4	95.3	96.1	50	0	0	0	6.00	5.10	958	282	8.2	.	.	
46.0	5-Aug-04	Y	8.2	70.4	95.3	96.1	40	0	0	0	11.99	10.20	958	282	8.2	.	.	
46.0	5-Aug-04	Y	8.2	70.4	95.3	96.1	10	0	0	0	23.99	20.39	958	282	8.2	.	.	
47.0	26-Aug-04	Y	7.7	87	96	98.1	105	100	100	90	0.00	0.00	228	97	7.7	0.0189	-0.1065	0.1029
47.0	26-Aug-04	Y	7.7	87	96	98.1	100	90	90	80	0.23	0.13	228	97	7.7	0.1469	0.0388	0.1044
47.0	26-Aug-04	Y	7.7	87	96	98.1	100	100	100	100	0.46	0.26	228	97	7.7	0.1617	0.0474	0.0832
47.0	26-Aug-04	Y	7.7	87	96	98.1	100	90	70	55	0.93	0.52	228	97	7.7	0.8054	-0.8364	0.0110

				% Free (MINTEQA2)			Survival (%)				MINTEQA2 µmol/L					blank corr		
test	date		pH	Ni	Ca	Mg	7d	14d	21d	28d	Ni	diss	Ca	Mg	pH	NiT (µmol/g dw)	NiTB (µmol/g dw)	weight/bug (mg)
47.0	26-Aug-04	Y	7.7	87	96	98.1	95	75	20	15	1.85	1.04	228	97	7.7	0.8178	0.0954	0.0625
47.0	26-Aug-04	Y	7.7	87	96	98.1	90	5	0	0	3.71	2.08	228	97	7.7	.	.	
47.0	26-Aug-04	Y	7.7	87	96	98.1	65	0	0	0	7.41	4.15	228	97	7.7	.	.	
47.0	26-Aug-04	Y	7.7	87	96	98.1	35	0	0	0	14.82	8.30	228	97	7.7	.	.	
47.0	26-Aug-04	Y	7.7	87	96	98.1	15	0	0	0	29.65	16.60	228	97	7.7	.	.	
48.0	30-Aug-04	Y	8	83.2	96	97.9	100	105	100	100	0.00	0.00	336	101	8.0	0.0042	-0.1073	0.0853
48.0	30-Aug-04	Y	8	83.2	96	97.9	85	85	85	85	0.22	0.15	336	101	8.0	0.0910	-0.0116	0.1467
48.0	30-Aug-04	Y	8	83.2	96	97.9	95	95	90	90	0.44	0.31	336	101	8.0	0.0771	-0.0304	0.0933
48.0	30-Aug-04	Y	8	83.2	96	97.9	100	70	65	65	0.89	0.61	336	101	8.0	0.6770	.	0.0844
48.0	30-Aug-04	Y	8	83.2	96	97.9	85	65	60	40	1.77	1.22	336	101	8.0	0.8140	-0.1893	0.0257
48.0	30-Aug-04	Y	8	83.2	96	97.9	80	10	0	0	3.54	2.45	336	101	8.0	.	.	
48.0	30-Aug-04	Y	8	83.2	96	97.9	50	0	0	0	7.09	4.89	336	101	8.0	.	.	
48.0	30-Aug-04	Y	8	83.2	96	97.9	40	0	0	0	14.18	9.78	336	101	8.0	.	.	
48.0	30-Aug-04	Y	8	83.2	96	97.9	10	0	0	0	28.35	19.56	336	101	8.0	.	.	
49.0	16-Sep-04	Y	7.5	91	97	98	95	95	.	.	0.00	0.00	267	109	7.5	.	.	0.1047
49.0	16-Sep-04	Y	7.5	91	97	98	95	95	95	95	0.24	0.11	267	109	7.5	.	.	
49.0	16-Sep-04	Y	7.5	91	97	98	100	95	90	85	0.48	0.21	267	109	7.5	0.1974	0.0541	0.1050
49.0	16-Sep-04	Y	7.5	91	97	98	95	90	85	75	0.97	0.42	267	109	7.5	0.1704	0.0450	0.0960
49.0	16-Sep-04	Y	7.5	91	97	98	95	85	55	40	1.94	0.85	267	109	7.5	3.4695	1.8277	0.0367
49.0	16-Sep-04	Y	7.5	91	97	98	85	30	5	5	3.88	1.70	267	109	7.5	.	.	
49.0	16-Sep-04	Y	7.5	91	97	98	70	0	0	0	7.75	3.40	267	109	7.5	.	.	
49.0	16-Sep-04	Y	7.5	91	97	98	55	0	0	0	15.50	6.79	267	109	7.5	.	.	
49.0	16-Sep-04	Y	7.5	91	97	98	10	0	0	0	31.01	13.58	267	109	7.5	.	.	
50.0	9-Feb-05	Y	7.7	90.4	97.9	98.2	100	95	85	80	0.00	0.00	247	105	7.7	.	0.1523	0.0522
50.0	9-Feb-05	Y	7.7	90.4	97.9	98.2	95	90	90	90	0.12	0.12	247	105	7.7		-0.2071	0.0392
50.0	9-Feb-05	Y	7.7	90.4	97.9	98.2	95	90	85	55	0.24	0.24	247	105	7.7	1.7038	1.1722	0.0278
50.0	9-Feb-05	Y	7.7	90.4	97.9	98.2	90	65	65	30	0.48	0.48	247	105	7.7	3.1349	2.4705	0.0667
50.0	9-Feb-05	Y	7.7	90.4	97.9	98.2	80	10	5	5	0.96	0.96	247	105	7.7	.	.	
50.0	9-Feb-05	Y	7.7	90.4	97.9	98.2	80	10	5	0	1.93	1.93	247	105	7.7	.	.	
50.0	9-Feb-05	Y	7.7	90.4	97.9	98.2	60	0	0	0	3.85	3.85	247	105	7.7	.	.	
50.0	9-Feb-05	Y	7.7	90.4	97.9	98.2	35	0	0	0	7.70	7.70	247	105	7.7	.	.	
51.0	9-Feb-05	Y	8.1	75.2	95.4	96.1	105	95	90	90	0.00	0.00	902	286	8.1	0.1056	0.0198	0.0816
51.0	9-Feb-05	Y	8.1	75.2	95.4	96.1	95	95	95	95	0.20	0.20	902	286	8.1	0.4332	0.2535	0.0493

				% Free (MINTEQA2)			Survival (%)				MINTEQA2 µmol/L					blank corr		
test	date		pH	Ni	Ca	Mg	7d	14d	21d	28d	Ni	diss	Ca	Mg	pH	NiT (µmol/g dw)	NiTB (µmol/g dw)	weight/bug (mg)
51.0	9-Feb-05	Y	8.1	75.2	95.4	96.1	95	85	85	80	0.40	0.40	902	286	8.1	0.8242	0.4547	0.0327
51.0	9-Feb-05	Y	8.1	75.2	95.4	96.1	105	80	60	30	0.80	0.80	902	286	8.1	1.5103	-0.3898	0.0233
51.0	9-Feb-05	Y	8.1	75.2	95.4	96.1	95	70	10	5	1.60	1.60	902	286	8.1	.	.	
51.0	9-Feb-05	Y	8.1	75.2	95.4	96.1	80	20	0	0	3.20	3.20	902	286	8.1	.	.	
51.0	9-Feb-05	Y	8.1	75.2	95.4	96.1	85	10	0	0	6.41	6.41	902	286	8.1	.	.	
51.0	9-Feb-05	Y	8.1	75.2	95.4	96.1	65	0	0	0	12.81	12.81	902	286	8.1	.	.	
52.0	16-Feb-05	Y	7.7	90.2	97.9	98.2	95	90	85	80	0.00	0.00	3843	113	7.7	.	.	0.0950
52.0	16-Feb-05	Y	7.7	90.2	97.9	98.2	80	85	80	70	0.24	0.24	3843	113	7.7	0.1387	-0.0158	0.0782
52.0	16-Feb-05	Y	7.7	90.2	97.9	98.2	85	50	45	50	0.48	0.48	3843	113	7.7	0.3029	-0.1893	0.0675
52.0	16-Feb-05	Y	7.7	90.2	97.9	98.2	90	70	75	70	0.96	0.96	3843	113	7.7	2.9064	2.6459	0.0729
52.0	16-Feb-05	Y	7.7	90.2	97.9	98.2	70	75	60	40	1.92	1.92	3843	113	7.7	.	.	.
52.0	16-Feb-05	Y	7.7	90.2	97.9	98.2	70	20	0	0	3.84	3.84	3843	113	7.7	.	.	.
52.0	16-Feb-05	Y	7.7	90.2	97.9	98.2	50	0	0	0	7.68	7.68	3843	113	7.7	.	.	.
52.0	16-Feb-05	Y	7.7	90.2	97.9	98.2	25	0	0	0	15.37	15.37	3843	113	7.7	.	.	.

28-DAY NICKEL TOXICITY TEST CHEMICAL CONFIRMATION DATA

		Solution Preparation						Solution Exchange					
		Total Ni (µg/L)			Dissolved Ni			Total Ni (µg/L)			Dissolved Ni		
Test	day	nom	meas	m/n	nom	meas	m/n	nom	meas	m/n	nom	meas	m/n
18	**no chem data**												
19	**no chem data**												
20	**no chem data**												
21	0	1000	853	0.85									
21	7	1000	822	0.82									
21	14	1000	890	0.89									
21	21	1000	855	0.86									
22	0	1000	791	0.79									
22	7	1000	805	0.81									
22	14	1000	881	0.88									
22	21	1000	826	0.83									
23	0	1000	775	0.78									
23	7	1000	825	0.83									
23	14	1000	848	0.85									
24	**no chem data**												
25	**no chem data**												
26	**no chem data**												
27	**no chem data**												
28	0	600	495	0.83									
29	0	600	507	0.85									
30	0	1000	768	0.77									
31	**no chem data**												
32	0	600	587	0.98									
32	7	600	586	0.98									
32	14	600	544	0.91									
33	0	600	539	0.90									
33	7	600	584	0.97									
33	14	600	540	0.90									
34	0	1000	954	0.95									
34	7	600	606	1.01									
34	14	600	567	0.95									
35	0	1200	1210	1.01									
35	7	1200	1160	0.97									
35	14	1200	946	0.79									
35	21	720	682	0.95									
36	7							300	171	0.57			
36	14	108	94.4	0.87				180	178	0.99			
36	21							108	83.1	0.77			
36	28							39	38	0.97			
37	0	2000	1990	1.00									
37	0	250	261	1.04									
37	7	2000	1850	0.93									
37	7	125	122	0.98									
37	14	1000	941	0.94									
37	14	250	214	0.86									
37	14	31.3	32.1	1.03									
37	21	125	107	0.86									

		Solution Preparation						Solution Exchange					
		Total Ni (µg/L)			Dissolved Ni			Total Ni (µg/L)			Dissolved Ni		
Test	day	nom	meas	m/n	nom	meas	m/n	nom	meas	m/n	nom	meas	m/n
38	0	2000	1900	0.95									
38	0	15.6	16.7	1.07									
38	7	62.5	60.3	0.96									
38	7	2000	1750	0.88									
38	7	500	449	0.90									
38	14	2000	1640	0.82									
38	21	250	210	0.84									
39	0	2000	1650	0.83									
39	7	2000	1680	0.84									
39	14	2000	1580	0.79									
39	21	500	423	0.85									
40	0	500	543	1.09									
40	7	500	502	1.00									
40	14	500	471	0.94									
40	21	250	227	0.91									
41	0	5000	4810	0.96									
41	7	2500	2720	1.09				5000	4550	0.91			
41	7	39	36.2	0.93									
41	14	5000	4600	0.92				2500	2360	0.94			
41	21	625	551	0.88				313	288	0.92			
41	28							313	270	0.86			
42	0	5000	4810	0.96									
42	7	5000	4780	0.96				1250	1100	0.88			
42	14	2500	2570	1.03									
42	21	625	560	0.90									
43	0	broken sample vessel											
43	7	2000	1820	0.91									
43	14	no sample											
43	21	250	253	1.01									
43	28							62.5	57.5	0.92	62.5	52.6	0.84
44	0	missing											
44	7	1000	1040	1.04									
44	14	1000	1010	1.01									
44	21	250	247	0.99									
44	28							125	115	0.92	125	114	0.91
45	0	2000	2200	1.10									
45	7	2000	2140	1.07									
45	14	1000	1060	1.06									
45	21	250	260	1.04									
45	28	250	251	1.00				250	251	1.00	250	197	0.79
46	0	2000	2070	1.04									
46	7	2000	2120	1.06									
46	14	2000	1970	0.99									
46	21	250	238	0.95									
46	28							250	234	0.94	250	200	0.8
47	0	2000	2030	1.02									
47	7	2000	1920	0.96	2000	763	0.38						
47	14				2000	743	0.37						
47	21	250	297	1.19	250	105	0.42	125	130	1.04	125	72.1	0.58

		Solution Preparation						Solution Exchange					
		Total Ni (µg/L)			Dissolved Ni			Total Ni (µg/L)			Dissolved Ni		
Test	day	nom	meas	m/n	nom	meas	m/n	nom	meas	m/n	nom	meas	m/n
47	28							125	137	1.10	125	78.8	0.63
48	0	2000	2000	1.00									
48	7	2000	1900	0.95	2000	860	0.43						
48	14	2000	1950	0.98	2000	1130	0.57	250	254	1.02	250	159	0.64
48	21												
48	28							125	117	0.94	125	88.6	0.71
49	0	2000	1830	0.92	2000	638	0.32						
49	7	2000	1770	0.89	2000	1510	0.76	2000	1840	0.92	2000	806	0.40
49	14	2000	1890	0.95	2000	705	0.35						
49	21	250	221	0.88									
50	0	500	489	0.98									
50	7	500	483	0.97									
50	14	500	475	0.95									
50	21	500	481	0.96									
51	0	1000	934	0.93									
51	7	1000	955	0.96									
51	14	1000	954	0.95									
51	21	500	461	0.92									
52	0	1000	900	0.90									
52	7	1000	907	0.91									
52	14	500	442	0.88									
52	21	250	229	0.92									

shading indicates tests with added DOC

Comparison of Slopes - LC50 vs Calcium

Test	*Ca*	LC50s (Ni2+, µmol/L)			
	mg/L	*7-d*	*14-d*	*21-d*	*28-d*
21.0	9.9	1.31			
22.0	35	4.50	1.53		
23.0	130	11.09	1.83		
28.0	9.9	2.20	0.75	0.64	.
30.0	117	6.10	2.30	1.63	
32.0	10	6.84	2.27	1.31	
33.0	40	8.73	3.41	1.84	
35.0	157	13.40	1.23	0.68	
37.0	158	5.09	1.35	1.05	1.05
40.0	14	**4.59**	**1.45**	**1.09**	**0.99**
41.0	298	25.87	4.81	2.41	1.39
42.0	37.4	16.81	2.71	1.06	0.83
44.0	33.6	6.11	1.78	0.91	0.68
50.0	10.1	1.61	0.57	0.47	0.29
51.0	37.8	9.46	1.99	1.17	0.82
SE		0.000589291	0.00012699	6.36672E-05	3.4847E-05
Slope		0.002332272	0.000285707	0.000143898	0.00010064
DF		14	13	11	6
SE^2		3.47264E-07	1.61265E-08	4.05351E-09	1.21432E-09

	7 vs 14	7 vs 21	7 vs 28	14 vs 21	14 vs 28
A = sqrt(SE^2 (1) + SE^2 (2))	0.000602819	0.00059272	0.00059032	0.000142056	0.000131685
B = slope1-slope2	0.002046565	0.002188374	0.002231632	0.000141809	0.000185067
B/A = t value	3.39	3.69	3.78	1.00	1.41
DF	25	23	18	22	17

SE= standard error
slope = slope of the regression line (LC50 vs cation)
DF = degrees of freedom
sqrt = square root

Comparison of Slopes - LC50 vs pH

	pH	LC50s (Ni2+, µmol/L)			
		7-d	*14-d*	*21-d*	*28-d*
	6.4	8.27			
	7.4	11.47			
	8.7	1.9	0.5		
	8	4.43	1.51		
	6.4	10.71	5.69		
	7.3	7.64	4.13	1.87	1.63
	8.4	1.81	0.38	0.29	0.28
	8.2	3.56	1.03	0.6	0.46
	6.7	9.29	4.81	2.67	
	8.9	1.31	0.3	0.21	0.16
SE		0.675076159	0.192672615	0.136342709	0.205357826
Slope		-3.68636826	-2.345079233	-1.207324841	-0.969402985
DF		9	7	4	3
SE^2		0.455727821	0.037122737	0.018589334	0.042171837

	7 vs 14	**7 vs 21**	**7 vs 28**	**14 vs 21**	**14 vs 28**
A = sqrt(SE^2 (1) + SE^2 (2))	3.081077915	3.078068829	3.081897179	1.41844956	1.426738118
B = slope1-slope2	8.458202684	7.320448292	7.082526437	2.988104281	2.750182425
B/A = t value	2.75	2.38	2.30	2.11	1.93
DF	14	11	10	9	8

SE= standard error
slope = slope of the regression line (LC50 vs cation)
DF = degrees of freedom
sqrt = square root

Comparison of Slopes - LC50 vs Magnesium

Mg	LC50s (Ni2+, µmol/L)			
mg/L	***7d***	***14d***	***21d***	***28d***
351	4.43	1.51		
328	3.56	1.03	0.6	0.46
823	8.68	1.83	1.07	0.76
1704	16.53	2.66	1.05	0.82
275	6.16	1.8	0.91	0.68
286	9.3	1.96	1.15	0.81

SE	0.001907241	0.000296352	0.000191798	0.000120938
Slope	0.007379078	0.000740488	0.000119079	0.000119079
DF	5	5	4	4
SE^2	3.63757E-06	8.78246E-08	3.67863E-08	1.46259E-08

	7 vs 14	**7 vs 21**	**7 vs 28**	**14 vs 21**	**14 vs 28**
A = sqrt(SE^2 (1) + SE^2 (2))	0.000659612	0.000619718	0.000601573	0.000230028	0.000175364
B = slope1-slope2	0.001591784	0.002213193	0.002213193	0.000166628	0.000166628
B/A = t value	2.41	3.57	3.68	0.72	0.95
DF	8	7	7	7	7

SE= standard error
slope = slope of the regression line (LC50 vs cation)
DF = degrees of freedom
sqrt = square root

Comparison of Slopes - Plots of Ni (µmol/L)/Nit (µmol/g dw) vs Ni (µmol/L)

test 1			test 2			test 3			test 17		
Ni	NiT	Ni/NiT	Ni	NiT	Ni/NiT	Ni	NiT	Ni/NiT	Ni	NiT	Ni/NiT
0.254	0.45	0.56	1.1	0.93	1.18	1.7	0.34	4.993	1.66	0.23	7.22
0.254	0.63	0.40	1.1	1.18	0.93	1.7	0.24	7.077	1.66	0.28	6.03
0.254	0.29	0.87	1.1	1.11	0.99	1.7	0.40	4.278	2.78	0.75	3.72
0.522	0.66	0.80	2.2	2.19	1.01	2.9	0.34	8.475	2.78	1.00	2.77
0.522	0.41	1.28	2.2	1.58	1.39	2.9	0.33	8.743	2.78	0.65	4.27
1.043	0.97	1.07	2.2	0.92	2.40	2.9	0.36	8.123	4.64	1.08	4.28
1.043	1.31	0.79	4.4	1.61	2.74	4.9	0.43	11.492	4.64	0.61	7.60
2.09	1.66	1.26	4.4	2.43	1.81	4.9	0.82	5.952	4.64	0.66	6.98
2.09	1.70	1.23	4.4	1.89	2.32	4.9	0.63	7.830	7.73	0.92	8.37
4.19	2.12	1.97	8.8	2.16	4.08	8.1	0.60	13.496	7.73	1.07	7.24
4.19	1.94	2.16	8.8	3.21	2.74	8.1	0.73	11.126	7.73	1.01	7.66
4.19	1.53	2.74	17.7	2.90	6.10	8.1	0.50	16.206	12.88	2.23	5.78
8.36	2.67	3.13	17.7	2.73	6.47	13.5	1.61	8.398	21.47	2.62	8.19
8.36	2.16	3.87	35.4	3.10	11.40	13.5	1.47	9.169	21.47	1.47	14.62
16.7	2.16	7.73	35.4	2.03	17.47	13.5	0.77	17.494	21.47	1.70	12.60
16.7	1.87	8.92				22.5	1.36	16.522	35.78	1.73	20.73
16.7	1.99	8.39				22.5	1.28	17.597	35.78	1.96	18.22
33.4	2.56	13.02				22.5	1.09	20.655	35.78	3.92	9.14
33.4	1.64	20.33				37.6	1.98	19.010			
33.4	3.44	9.71				37.6	0.77	49.131			
						37.6	0.81	46.552			

	test 1	test 2	test 3	test 17
SE	0.036620018	0.030782777	0.11843432	0.0555879
Slope	0.420990948	0.379852726	0.822670959	0.3260835
DF	19	14	20	17
SE^2	0.001341026	0.000947579	0.014026688	0.00309

	1 vs 2	1 vs 3	1 vs 17	2 vs 3	2 vs 17	3 vs 17
A = sqrt(SE^2 (1) + SE^2 (2))	0.047839368	0.123966584	0.066566037	0.1223694	0.06354204	0.130830806
B = slope1-slope2	0.041138222	-0.401680011	0.094907463	-0.442818	0.053769241	0.496587474
B/A	0.86	-3.24	1.43	-3.62	0.85	3.80
DF	31	37	34	32	35	15

SE= standard error
slope = slope of the regression line (LC50 vs cation)
DF = degrees of freedom
sqrt = square root

APPENDIX C

Derivation of L_{T0} for the Non-competitive Model:

Assume that the formation of a biotic ligand (L_T) depends on an enzyme (E), and that the change in the L_T concentration is a function of the rate of formation of the ligand minus the rate of destruction of the ligand so that:

$$\frac{dL_T}{dt} = k_s*E - k_d*L_T \qquad \text{(equation 1)}$$

where,

dL_T = change in L_T concentration
dt = change in time
k_s = rate of synthesis of L_T by the enzyme (E)
E = concentration of the enzyme required for L_T synthesis (µmol/g)
k_d = rate of degradation of L_T

At equilibrium, $\frac{dL_T}{dt} = 0$ and, therefore, $k_s*E = k_d*L_T$ or

$$L_T = \frac{k_s*E}{k_d} \qquad \text{(equation 2)}$$

Also, assume that calcium may bind to the enzyme, rendering it inactive, and that the reaction is represented by:

E + Ca <==> ECa

where,

Ca = concentration of calcium (µmol/L)
ECa = enzyme-calcium complex, which renders E inactive (µmol/g)

At equilibrium, $$K_{CaE} = \frac{ECa}{E*Ca} \qquad \text{(equation 3)}$$

where K_{CaE} = conditional binding constant for ECa (L/µmol)

The total amount of E (E_T) = $E + ECa = E + K_{CaE}*E*Ca = E*(1 + K_{CaE}*Ca)$ or

$$E = E_T /(1 + K_{CaE}*Ca) \qquad \text{(equation 4)}$$

E can then be replaced in equation 2, so that,

$$L_T = \frac{k_s * E_T}{k_d\ (1 + K_{CaE} * Ca)} \qquad \text{(equation 5)}$$

The term $k_s * E$ can be replaced by L_{T0}
k_d

$$L_T = \frac{L_{T0}}{(1 + K_{CaE} * Ca)} \qquad \text{(equation 6)}$$

Where,

L_{T0} = total concentration of ligand, as influenced by Ca and
K_{CaE} = *b2* in the non-competitive models described in Sections 6.0 and 9.0.

APPENDIX D

SEVEN-DAY CADMIUM BIOACCUMULATION TEST DATA

	Test	Medium		minteq (% free)				umol/L as free ion			% surv	weight/	CdT
#	date	Base	Add	Ca	Mg	Cd	pH	Ca	Mg	Cd	7-d	bug (mg)	µmol/g
1	1-Dec-03	DC		93.2	94.3	71.4	8.3	746	322	0.00000	90	0.2567	0.0250
1	1-Dec-03	DC		93.2	94.3	71.4	8.3	746	322	0.00000	100	0.3140	0.0204
1	1-Dec-03	DC		93.2	94.3	71.4	8.3	746	322	0.00089	90	0.2478	0.0657
1	1-Dec-03	DC		93.2	94.3	71.4	8.3	746	322	0.00089	70	0.2629	0.0565
1	1-Dec-03	DC		93.2	94.3	71.4	8.3	746	322	0.00146	100	0.2300	0.0846
1	1-Dec-03	DC		93.2	94.3	71.4	8.3	746	322	0.00146	100	0.2310	0.0735
1	1-Dec-03	DC		93.2	94.3	71.4	8.3	746	322	0.00248	90	0.2344	0.1285
1	1-Dec-03	DC		93.2	94.3	71.4	8.3	746	322	0.00248	90	0.2956	0.0986
1	1-Dec-03	DC		93.2	94.3	71.4	8.3	746	322	0.00413	80	0.2925	0.1531
1	1-Dec-03	DC		93.2	94.3	71.4	8.3	746	322	0.00413	100	0.2470	0.1753
1	1-Dec-03	DC		93.2	94.3	71.4	8.3	746	322	0.00699	100	0.2630	0.2350
1	1-Dec-03	DC		93.2	94.3	71.4	8.3	746	322	0.00699	100	0.2270	0.3052
1	1-Dec-03	DC		93.2	94.3	71.4	8.3	746	322	0.01143	80	0.1975	0.3304
1	1-Dec-03	DC		93.2	94.3	71.4	8.3	746	322	0.01143	70	0.2214	0.3793
1	1-Dec-03	DC		93.2	94.3	71.4	8.3	746	322	0.01906	90	0.2267	
1	1-Dec-03	DC		93.2	94.3	71.4	8.3	746	322	0.01906	90	0.2700	0.5795
1	1-Dec-03	DC		93.2	94.3	71.4	8.3	746	322	0.03176	100	0.2230	0.7767
1	1-Dec-03	DC		93.2	94.3	71.4	8.3	746	322	0.03176	90	0.2111	0.37685
2	1-Dec-03	LH		97.5	97.9	92.6	7.8	334	117	0.00000	100	0.2320	0.0365
2	1-Dec-03	LH		97.5	97.9	92.6	7.8	334	117	0.00000	90	0.2067	0.0443
2	1-Dec-03	LH		97.5	97.9	92.6	7.8	334	117	0.00115	90	0.2356	0.0921
2	1-Dec-03	LH		97.5	97.9	92.6	7.8	334	117	0.00115	80	0.2388	0.0925
2	1-Dec-03	LH		97.5	97.9	92.6	7.8	334	117	0.00189	80	0.2300	0.1529
2	1-Dec-03	LH		97.5	97.9	92.6	7.8	334	117	0.00189	80	0.1275	0.0591
2	1-Dec-03	LH		97.5	97.9	92.6	7.8	334	117	0.00321	60	0.1283	0.0833
2	1-Dec-03	LH		97.5	97.9	92.6	7.8	334	117	0.00321	90	0.2967	0.1464
2	1-Dec-03	LH		97.5	97.9	92.6	7.8	334	117	0.00535	100	0.2300	0.2671
2	1-Dec-03	LH		97.5	97.9	92.6	7.8	334	117	0.00535	100	0.2620	0.2505
2	1-Dec-03	LH		97.5	97.9	92.6	7.8	334	117	0.00906	70	0.2029	0.4827
2	1-Dec-03	LH		97.5	97.9	92.6	7.8	334	117	0.00906	100	0.2450	
2	1-Dec-03	LH		97.5	97.9	92.6	7.8	334	117	0.01483	80	0.2175	0.4716
2	1-Dec-03	LH		97.5	97.9	92.6	7.8	334	117	0.01483	100	0.2240	0.8652
2	1-Dec-03	LH		97.5	97.9	92.6	7.8	334	117	0.02472	90	0.2189	0.6505
2	1-Dec-03	LH		97.5	97.9	92.6	7.8	334	117	0.02472	80	0.2138	0.6880
2	1-Dec-03	LH		97.5	97.9	92.6	7.8	334	117	0.04119	60	0.1200	0.8128
2	1-Dec-03	LH		97.5	97.9	92.6	7.8	334	117	0.04119	60	0.1933	1.3563
5	4-Dec-03	LH	Ca4	98.8	99	66.2	7.5	3705	110	0.00000	70	0.3086	0.0220
5	4-Dec-03	LH	Ca4	98.8	99	66.2	7.5	3705	110	0.00135	40	0.3050	0.0315
5	4-Dec-03	LH	Ca4	98.8	99	66.2	7.5	3705	110	0.00230	50	0.3680	0.0435
5	4-Dec-03	LH	Ca4	98.8	99	66.2	7.5	3705	110	0.00383	60	0.3325	0.0602
5	4-Dec-03	LH	Ca4	98.8	99	66.2	7.5	3705	110	0.00648	40	0.3425	0.0951
5	4-Dec-03	LH	Ca4	98.8	99	66.2	7.5	3705	110	0.01060	50	0.4200	0.1217

Test		Medium		minteq (% free)				umol/L as free ion			% surv	weight/	CdT
#	date	Base	Add	Ca	Mg	Cd	pH	Ca	Mg	Cd	7-d	bug (mg)	µmol/g
5	4-Dec-03	LH	Ca4	98.8	99	66.2	7.5	3705	110	0.01767	70	0.3100	0.1850
5	4-Dec-03	LH	Ca4	98.8	99	66.2	7.5	3705	110	0.02945	40	0.3625	0.2570
6	4-Dec-03	LH	Mg1	89	91	86.3	7.6	245	936	0.00000	80	0.2700	
6	4-Dec-03	LH	Mg1	89	91	86.3	7.6	245	936	0.00000	80	0.3086	
6	4-Dec-03	LH	Mg1	89	91	86.3	7.6	245	936	0.00177	80	0.2875	
6	4-Dec-03	LH	Mg1	89	91	86.3	7.6	245	936	0.00177	80	0.3443	
6	4-Dec-03	LH	Mg1	89	91	86.3	7.6	245	936	0.00299	90	0.1522	
6	4-Dec-03	LH	Mg1	89	91	86.3	7.6	245	936	0.00299	80	0.3600	
6	4-Dec-03	LH	Mg1	89	91	86.3	7.6	245	936	0.00499	70	0.2571	
6	4-Dec-03	LH	Mg1	89	91	86.3	7.6	245	936	0.00499	80	0.3560	
6	4-Dec-03	LH	Mg1	89	91	86.3	7.6	245	936	0.00845	70	0.2429	
6	4-Dec-03	LH	Mg1	89	91	86.3	7.6	245	936	0.00845	80	0.3550	
6	4-Dec-03	LH	Mg1	89	91	86.3	7.6	245	936	0.01382	60	0.2214	
6	4-Dec-03	LH	Mg1	89	91	86.3	7.6	245	936	0.01382	60	0.3517	
6	4-Dec-03	LH	Mg1	89	91	86.3	7.6	245	936	0.02303	60	0.2880	
6	4-Dec-03	LH	Mg1	89	91	86.3	7.6	245	936	0.02303	80	0.3000	
6	4-Dec-03	LH	Mg1	89	91	86.3	7.6	245	936	0.03839	50	0.1860	
6	4-Dec-03	LH	Mg1	89	91	86.3	7.6	245	936	0.03839	40	0.3225	
7	24-Dec-03	LH	K0.2	97.9	98.2	93.5	7.5	269	105	0.00000	100	0.3120	0.0228
7	24-Dec-03	LH	K0.2	97.9	98.2	93.5	7.5	269	105	0.00324	90	0.3856	0.2146
7	24-Dec-03	LH	K0.2	97.9	98.2	93.5	7.5	269	105	0.00541	90	0.3629	0.1302
7	24-Dec-03	LH	K0.2	97.9	98.2	93.5	7.5	269	105	0.00915	100	0.3800	0.5927
7	24-Dec-03	LH	K0.2	97.9	98.2	93.5	7.5	269	105	0.01497	90	0.2988	0.4914
7	24-Dec-03	LH	K0.2	97.9	98.2	93.5	7.5	269	105	0.02496	100	0.4275	2.1384
7	24-Dec-03	LH	K0.2	97.9	98.2	93.5	7.5	269	105	0.04159	30	0.3967	1.3596
7	24-Dec-03	LH	K0.2	97.9	98.2	93.5	7.5	269	105	0.06988	0		
8	24-Dec-03	LH	Na3.5	97.5	98.6	94.8	7.5	268	105	0.00000	80	0.3675	0.0484
8	24-Dec-03	LH	Na3.5	97.5	98.6	94.8	7.5	268	105	0.00329	90	0.1943	0.3462
8	24-Dec-03	LH	Na3.5	97.5	98.6	94.8	7.5	268	105	0.00548	100	0.5420	0.1832
8	24-Dec-03	LH	Na3.5	97.5	98.6	94.8	7.5	268	105	0.00928	90	0.2638	0.3385
8	24-Dec-03	LH	Na3.5	97.5	98.6	94.8	7.5	268	105	0.01518	70		
8	24-Dec-03	LH	Na3.5	97.5	98.6	94.8	7.5	268	105	0.02530	70	0.4329	0.6859
8	24-Dec-03	LH	Na3.5	97.5	98.6	94.8	7.5	268	105	0.04217	30	0.2933	1.3224
8	24-Dec-03	LH	Na3.5	97.5	98.6	94.8	7.5	268	105	0.07085	0		
10	30-Jan-04	LH	pH7	98	98.3	96	7	270	105	0.00000	100	0.2440	0.0400
10	30-Jan-04	LH	pH7	98	98.3	96	7	270	105	0.00000	100	0.2850	0.0881
10	30-Jan-04	LH	pH7	98	98.3	96	7	270	105	0.00333	100	0.2925	
10	30-Jan-04	LH	pH7	98	98.3	96	7	270	105	0.00333	100	0.1980	0.0874
10	30-Jan-04	LH	pH7	98	98.3	96	7	270	105	0.00555	100	0.2225	0.1475
10	30-Jan-04	LH	pH7	98	98.3	96	7	270	105	0.00555	100	0.2400	0.1568
10	30-Jan-04	LH	pH7	98	98.3	96	7	270	105	0.00940	100	0.2525	0.1395
10	30-Jan-04	LH	pH7	98	98.3	96	7	270	105	0.00940	80	0.2250	0.2242
10	30-Jan-04	LH	pH7	98	98.3	96	7	270	105	0.01537	100	0.2680	0.2557
10	30-Jan-04	LH	pH7	98	98.3	96	7	270	105	0.01537	100	0.1825	0.3378

Test		Medium		minteq (% free)				umol/L as free ion			% surv	weight/	CdT
#	date	Base	Add	Ca	Mg	Cd	pH	Ca	Mg	Cd	7-d	bug (mg)	µmol/g
10	30-Jan-04	LH	pH7	98	98.3	96	7	270	105	0.02562	100	0.2600	0.3252
10	30-Jan-04	LH	pH7	98	98.3	96	7	270	105	0.02562	80	0.2300	0.4073
10	30-Jan-04	LH	pH7	98	98.3	96	7	270	105	0.04270	100	0.1920	0.4938
10	30-Jan-04	LH	pH7	98	98.3	96	7	270	105	0.04270	80	0.1900	0.5563
11	30-Jan-04	DC	pH7	94.6	95.3	88.5	7	757	326	0.00000	100	0.2050	0.0142
11	30-Jan-04	DC	pH7	94.6	95.3	88.5	7	757	326	0.00000	100	0.1900	0.0195
11	30-Jan-04	DC	pH7	94.6	95.3	88.5	7	757	326	0.00307	100	0.1880	0.0613
11	30-Jan-04	DC	pH7	94.6	95.3	88.5	7	757	326	0.00307	100	0.2867	0.0596
11	30-Jan-04	DC	pH7	94.6	95.3	88.5	7	757	326	0.00512	100	0.2500	0.0705
11	30-Jan-04	DC	pH7	94.6	95.3	88.5	7	757	326	0.00512	100	0.2520	0.0813
11	30-Jan-04	DC	pH7	94.6	95.3	88.5	7	757	326	0.00866	100	0.2475	0.1229
11	30-Jan-04	DC	pH7	94.6	95.3	88.5	7	757	326	0.00866	100	0.1920	0.1401
11	30-Jan-04	DC	pH7	94.6	95.3	88.5	7	757	326	0.01417	100		
11	30-Jan-04	DC	pH7	94.6	95.3	88.5	7	757	326	0.01417	100	0.3200	0.1526
11	30-Jan-04	DC	pH7	94.6	95.3	88.5	7	757	326	0.02362	100	0.2020	0.3108
11	30-Jan-04	DC	pH7	94.6	95.3	88.5	7	757	326	0.02362	100	0.2320	0.2927
11	30-Jan-04	DC	pH7	94.6	95.3	88.5	7	757	326	0.03937	80	0.1867	0.4919
11	30-Jan-04	DC	pH7	94.6	95.3	88.5	7	757	326	0.03937	100	0.2260	0.3770
12	2-Feb-04	LH	Ca2.5	98.7	98.8	75.5	7.4	2391	118	0.00000	100	0.2340	0.0586
12	2-Feb-04	LH	Ca2.5	98.7	98.8	75.5	7.4	2391	118	0.00000	100	0.1750	0.0759
12	2-Feb-04	LH	Ca2.5	98.7	98.8	75.5	7.4	2391	118	0.00262	100	0.2210	0.0765
12	2-Feb-04	LH	Ca2.5	98.7	98.8	75.5	7.4	2391	118	0.00262	80	0.2175	0.0890
12	2-Feb-04	LH	Ca2.5	98.7	98.8	75.5	7.4	2391	118	0.00437	80	0.2786	0.0985
12	2-Feb-04	LH	Ca2.5	98.7	98.8	75.5	7.4	2391	118	0.00437	100	0.2130	0.0911
12	2-Feb-04	LH	Ca2.5	98.7	98.8	75.5	7.4	2391	118	0.00739	90	0.2044	0.1151
12	2-Feb-04	LH	Ca2.5	98.7	98.8	75.5	7.4	2391	118	0.00739	90	0.1833	0.1326
12	2-Feb-04	LH	Ca2.5	98.7	98.8	75.5	7.4	2391	118	0.01209	70	0.2186	0.1593
12	2-Feb-04	LH	Ca2.5	98.7	98.8	75.5	7.4	2391	118	0.01209	70	0.2114	0.1611
12	2-Feb-04	LH	Ca2.5	98.7	98.8	75.5	7.4	2391	118	0.02015	100	0.2478	0.2426
12	2-Feb-04	LH	Ca2.5	98.7	98.8	75.5	7.4	2391	118	0.02015	100	0.1911	0.2359
12	2-Feb-04	LH	Ca2.5	98.7	98.8	75.5	7.4	2391	118	0.03359	90	0.2263	0.3421
12	2-Feb-04	LH	Ca2.5	98.7	98.8	75.5	7.4	2391	118	0.03359	100	0.2750	0.3559
13	9-Mar-04	LH	Ca1.4	98.1	98.4	82.5	7.6	1373	117	0.00000	80	0.7825	0.0400
13	9-Mar-04	LH	Ca1.4	98.1	98.4	82.5	7.6	1373	117	0.00000	100	0.7940	0.0255
13	9-Mar-04	LH	Ca1.4	98.1	98.4	82.5	7.6	1373	117	0.00000	80	0.8250	0.0205
13	9-Mar-04	LH	Ca1.4	98.1	98.4	82.5	7.6	1373	117	0.00477	80	0.7400	0.1231
13	9-Mar-04	LH	Ca1.4	98.1	98.4	82.5	7.6	1373	117	0.00477	100	0.5425	0.1076
13	9-Mar-04	LH	Ca1.4	98.1	98.4	82.5	7.6	1373	117	0.00477	100	0.6780	0.1460
13	9-Mar-04	LH	Ca1.4	98.1	98.4	82.5	7.6	1373	117	0.00807	100	0.7800	0.1461
13	9-Mar-04	LH	Ca1.4	98.1	98.4	82.5	7.6	1373	117	0.00807	80	0.8025	0.1449
13	9-Mar-04	LH	Ca1.4	98.1	98.4	82.5	7.6	1373	117	0.00807	100	0.5267	0.1791
13	9-Mar-04	LH	Ca1.4	98.1	98.4	82.5	7.6	1373	117	0.01321	80	0.7150	0.2228
13	9-Mar-04	LH	Ca1.4	98.1	98.4	82.5	7.6	1373	117	0.01321	80	0.7775	
13	9-Mar-04	LH	Ca1.4	98.1	98.4	82.5	7.6	1373	117	0.01321	100	0.6220	0.1976
13	9-Mar-04	LH	Ca1.4	98.1	98.4	82.5	7.6	1373	117	0.02202	80	0.7925	0.3082

Test		Medium		minteq (% free)				umol/L as free ion			% surv	weight/	CdT
#	date	Base	Add	Ca	Mg	Cd	pH	Ca	Mg	Cd	7-d	bug (mg)	µmol/g
13	9-Mar-04	LH	Ca1.4	98.1	98.4	82.5	7.6	1373	117	0.02202	80	0.8000	0.2890
13	9-Mar-04	LH	Ca1.4	98.1	98.4	82.5	7.6	1373	117	0.02202	100	0.8540	0.3111
13	9-Mar-04	LH	Ca1.4	98.1	98.4	82.5	7.6	1373	117	0.03670	80	0.8667	0.3701
13	9-Mar-04	LH	Ca1.4	98.1	98.4	82.5	7.6	1373	117	0.03670	100	0.7320	0.3466
13	9-Mar-04	LH	Ca1.4	98.1	98.4	82.5	7.6	1373	117	0.03670	100	0.7620	0.4173
13	9-Mar-04	LH	Ca1.4	98.1	98.4	82.5	7.6	1373	117	0.06165	60	0.6733	0.69276
13	9-Mar-04	LH	Ca1.4	98.1	98.4	82.5	7.6	1373	117	0.06165	80	0.6550	0.61541
13	9-Mar-04	LH	Ca1.4	98.1	98.4	82.5	7.6	1373	117	0.06165	80	0.6175	0.46217
13	9-Mar-04	LH	Ca1.4	98.1	98.4	82.5	7.6	1373	117	0.10276	40	0.3350	1.43053
13	9-Mar-04	LH	Ca1.4	98.1	98.4	82.5	7.6	1373	117	0.10276	40	0.4950	1.9457
13	9-Mar-04	LH	Ca1.4	98.1	98.4	82.5	7.6	1373	117	0.10276	40	0.5300	1.18621
14	9-Mar-04	LH		97.7	98	93.7	7.6	318	109	0.00000	100	0.7240	0.02446
14	9-Mar-04	LH		97.7	98	93.7	7.6	318	109	0.00000	100	0.7360	0.023
14	9-Mar-04	LH		97.7	98	93.7	7.6	318	109	0.00000	100	0.6540	0.02834
14	9-Mar-04	LH		97.7	98	93.7	7.6	318	109	0.00542	100	0.6720	0.18066
14	9-Mar-04	LH		97.7	98	93.7	7.6	318	109	0.00542	100	0.5420	0.17389
14	9-Mar-04	LH		97.7	98	93.7	7.6	318	109	0.00542	100	0.7080	
14	9-Mar-04	LH		97.7	98	93.7	7.6	318	109	0.00917	100	0.6940	0.28005
14	9-Mar-04	LH		97.7	98	93.7	7.6	318	109	0.00917	100	0.5840	0.26532
14	9-Mar-04	LH		97.7	98	93.7	7.6	318	109	0.00917	100	0.6860	0.19503
14	9-Mar-04	LH		97.7	98	93.7	7.6	318	109	0.01501	100	0.4175	
14	9-Mar-04	LH		97.7	98	93.7	7.6	318	109	0.01501	80	0.5725	
14	9-Mar-04	LH		97.7	98	93.7	7.6	318	109	0.01501	100	0.6160	
14	9-Mar-04	LH		97.7	98	93.7	7.6	318	109	0.02501	80	0.6075	0.47208
14	9-Mar-04	LH		97.7	98	93.7	7.6	318	109	0.02501	60	0.8267	0.52434
14	9-Mar-04	LH		97.7	98	93.7	7.6	318	109	0.02501	60	0.6633	0.56331
14	9-Mar-04	LH		97.7	98	93.7	7.6	318	109	0.04168	60	0.6150	0.72303
14	9-Mar-04	LH		97.7	98	93.7	7.6	318	109	0.04168	20	0.5400	0.60202
14	9-Mar-04	LH		97.7	98	93.7	7.6	318	109	0.04168	60	0.3900	0.95812
14	9-Mar-04	LH		97.7	98	93.7	7.6	318	109	0.07002	0		
14	9-Mar-04	LH		97.7	98	93.7	7.6	318	109	0.07002	0		
14	9-Mar-04	LH		97.7	98	93.7	7.6	318	109	0.07002	0		
14	9-Mar-04	LH		97.7	98	93.7	7.6	318	109	0.11671	0		
14	9-Mar-04	LH		97.7	98	93.7	7.6	318	109	0.11671	0		
14	9-Mar-04	LH		97.7	98	93.7	7.6	318	109	0.11671	0		
41	30-Sep-04	DC	pH7	94.6	95.3	88.5	7	757	326	0.00000	90	0.1833	0.0000
41	30-Sep-04	DC	pH7	94.6	95.3	88.5	7	757	326	0.00000	90	0.2370	0.0000
41	30-Sep-04	DC	pH7	94.6	95.3	88.5	7	757	326	0.00472	100	0.2070	0.0756
41	30-Sep-04	DC	pH7	94.6	95.3	88.5	7	757	326	0.00472	100	0.2010	0.0779
41	30-Sep-04	DC	pH7	94.6	95.3	88.5	7	757	326	0.00984	80	0.1950	0.1198
41	30-Sep-04	DC	pH7	94.6	95.3	88.5	7	757	326	0.00984	100	0.2380	0.1727
41	30-Sep-04	DC	pH7	94.6	95.3	88.5	7	757	326	0.01968	100	0.1800	0.2768
41	30-Sep-04	DC	pH7	94.6	95.3	88.5	7	757	326	0.01968	90	0.1944	0.3630
41	30-Sep-04	DC	pH7	94.6	95.3	88.5	7	757	326	0.03937	70	0.1943	0.5770
41	30-Sep-04	DC	pH7	94.6	95.3	88.5	7	757	326	0.03937	90	0.2050	0.4375

	Test	Medium		minteq (% free)				umol/L as free ion			% surv	weight/	CdT
#	date	Base	Add	Ca	Mg	Cd	pH	Ca	Mg	Cd	7-d	bug (mg)	µmol/g
41	30-Sep-04	DC	pH7	94.6	95.3	88.5	7	757	326	0.07874	80	0.1813	0.9559
41	30-Sep-04	DC	pH7	94.6	95.3	88.5	7	757	326	0.07874	80	0.1914	1.3066
42	30-Sep-04	DC	pH9	87.7	90.4	36.7	9.1	702	309	0.00000	100	0.2100	0.0000
42	30-Sep-04	DC	pH9	87.7	90.4	36.7	9.1	702	309	0.00000	100	0.1856	0.0000
42	30-Sep-04	DC	pH9	87.7	90.4	36.7	9.1	702	309	0.00196	100	0.2100	0.0000
42	30-Sep-04	DC	pH9	87.7	90.4	36.7	9.1	702	309	0.00196	100	0.2171	0.0000
42	30-Sep-04	DC	pH9	87.7	90.4	36.7	9.1	702	309	0.00408	80	0.2578	0.0322
42	30-Sep-04	DC	pH9	87.7	90.4	36.7	9.1	702	309	0.00408	80	0.2238	0.0000
42	30-Sep-04	DC	pH9	87.7	90.4	36.7	9.1	702	309	0.00816	90	0.2225	0.0630
42	30-Sep-04	DC	pH9	87.7	90.4	36.7	9.1	702	309	0.00816	100	0.1760	0.0425
42	30-Sep-04	DC	pH9	87.7	90.4	36.7	9.1	702	309	0.01633	100	0.2140	0.1048
42	30-Sep-04	DC	pH9	87.7	90.4	36.7	9.1	702	309	0.01633	90	0.2078	0.1199
42	30-Sep-04	DC	pH9	87.7	90.4	36.7	9.1	702	309	0.03265	100	0.1744	0.3253
42	30-Sep-04	DC	pH9	87.7	90.4	36.7	9.1	702	309	0.03265	100	0.2020	0.2528
46	20-Feb-05	SAM	Mg1	89.8	91.6	74.9	8.1	822	954	0.00000	100	0.2467	0.0048
46	20-Feb-05	SAM	Mg1	89.8	91.6	74.9	8.1	822	954	0.00000	100	0.2650	0.0067
46	20-Feb-05	SAM	Mg1	89.8	91.6	74.9	8.1	822	954	0.00833	100	0.2180	0.1306
46	20-Feb-05	SAM	Mg1	89.8	91.6	74.9	8.1	822	954	0.00833	100	0.1943	0.1570
46	20-Feb-05	SAM	Mg1	89.8	91.6	74.9	8.1	822	954	0.01666	100	0.2700	0.2416
46	20-Feb-05	SAM	Mg1	89.8	91.6	74.9	8.1	822	954	0.01666	100	0.2517	0.2121
46	20-Feb-05	SAM	Mg1	89.8	91.6	74.9	8.1	822	954	0.03332	100	0.2157	0.4714
46	20-Feb-05	SAM	Mg1	89.8	91.6	74.9	8.1	822	954	0.03332	100	0.2160	0.5602
46	20-Feb-05	SAM	Mg1	89.8	91.6	74.9	8.1	822	954	0.06664	57	0.2175	1.0226
46	20-Feb-05	SAM	Mg1	89.8	91.6	74.9	8.1	822	954	0.06664	57	0.2900	0.7056
46	20-Feb-05	SAM	Mg1	89.8	91.6	74.9	8.1	822	954	0.13327	14	0.2600	1.3687
46	20-Feb-05	SAM	Mg1	89.8	91.6	74.9	8.1	822	954	0.13327	0		
49	6-Mar-05	SAM		95.7	96.3	81.7	7.8	904	286	0.00000	71	0.4260	0.0050
49	6-Mar-05	SAM		95.7	96.3	81.7	7.8	904	286	0.00000	100	0.3014	0.0067
49	6-Mar-05	SAM		95.7	96.3	81.7	7.8	904	286	0.00581	86	0.3717	0.0718
49	6-Mar-05	SAM		95.7	96.3	81.7	7.8	904	286	0.00581	100	0.3671	0.0692
49	6-Mar-05	SAM		95.7	96.3	81.7	7.8	904	286	0.01134	100	0.3617	0.1148
49	6-Mar-05	SAM		95.7	96.3	81.7	7.8	904	286	0.01134	100	0.3571	0.1139
49	6-Mar-05	SAM		95.7	96.3	81.7	7.8	904	286	0.02275	100	0.3643	0.2233
49	6-Mar-05	SAM		95.7	96.3	81.7	7.8	904	286	0.02275	100	0.3129	0.2925
49	6-Mar-05	SAM		95.7	96.3	81.7	7.8	904	286	0.04543	100	0.4138	0.4731
49	6-Mar-05	SAM		95.7	96.3	81.7	7.8	904	286	0.04543	57	0.3625	0.4909
49	6-Mar-05	SAM		95.7	96.3	81.7	7.8	904	286	0.09086	71	0.2260	1.0078
49	6-Mar-05	SAM		95.7	96.3	81.7	7.8	904	286	0.09086	71	0.2840	1.1528
50	13-Mar-05	SAM-MG	Ca4	98.8	99	67.6	7.3	3804	122	0.00000	71	0.3560	0.0060
50	13-Mar-05	SAM-MG	Ca4	98.8	99	67.6	7.3	3804	122	0.00000	71	0.3960	0.0054
50	13-Mar-05	SAM-MG	Ca4	98.8	99	67.6	7.3	3804	122	0.00962	100	0.3760	0.0568
50	13-Mar-05	SAM-MG	Ca4	98.8	99	67.6	7.3	3804	122	0.00962	71	0.4040	0.0529
50	13-Mar-05	SAM-MG	Ca4	98.8	99	67.6	7.3	3804	122	0.01882	86	0.3500	0.1017

	Test	Medium		minteq (% free)				umol/L as free ion			% surv	weight/	CdT
#	date	Base	Add	Ca	Mg	Cd	pH	Ca	Mg	Cd	7-d	bug (mg)	µmol/g
50	13-Mar-05	SAM-MG	Ca4	98.8	99	67.6	7.3	3804	122	0.01882	57	0.3375	0.1318
50	13-Mar-05	SAM-MG	Ca4	98.8	99	67.6	7.3	3804	122	0.03759	71	0.5300	0.1477
50	13-Mar-05	SAM-MG	Ca4	98.8	99	67.6	7.3	3804	122	0.03759	71	0.3940	0.1987
50	13-Mar-05	SAM-MG	Ca4	98.8	99	67.6	7.3	3804	122	0.07518	86	0.4020	0.3187
50	13-Mar-05	SAM-MG	Ca4	98.8	99	67.6	7.3	3804	122	0.07518	86	0.3950	0.3604
50	13-Mar-05	SAM-MG	Ca4	98.8	99	67.6	7.3	3804	122	0.15036	57	0.4825	0.5532
50	13-Mar-05	SAM-MG	Ca4	98.8	99	67.6	7.3	3804	122	0.15036	71	0.3400	0.6280
50	13-Mar-05	SAM-MG	Ca4	98.8	99	67.6	7.3	3804	122	0.30071	43	0.4400	0.8627

CADMIUM BIOACCUMULATION TEST CHEMISTRY DATA

		Total Cd (µg/L)		
Test	day	nom	meas	meas/nom
1	0	5	4.52	0.90
2	0	5	3.9	0.78
5	0	5	5	1.00
6	0	5	5.37	1.07
7	missing			
8	0	8.3	7.11	0.86
10	0	5	4.56	0.91
11	0	5	4.04	0.81
12	no Cd data			
13	7	5	5.29	1.06
13	7	8.4	7.58	0.90
13	7	14	12.8	0.91
14	0	14	13.6	0.97
14	0	8.4	7.68	0.91
41	7	10	7.49	0.75
42	0	10	8.57	0.86
42	7	10	2.28	0.23
46	0	20	16.8	0.84
49	0	12.5	11	0.88
50	0	50	43.1	0.86

APPENDIX D: CADMIUM TIME-SERIES TEST DATA

Test	Age (weeks)	Base	time (hr)	Cd2+ (µmol/L)	Cd (µmol/g dw)	weight/bug (mg)
9	11w	LH	0	0.0411	0.017477902	0.194
9	11w	LH	3	0.0411	0.018683274	0.175
9	11w	LH	3	0.0411	0.031471825	0.208
9	11w	LH	6	0.0411		0.173
9	11w	LH	6	0.0411	0.03770936	0.273
9	11w	LH	21	0.0411	0.197570254	0.218
9	11w	LH	14	0.0411	0.113851201	0.183
9	11w	LH	17	0.0411	0.146436472	0.278
9	11w	LH	24	0.0411	0.244103078	0.284
9	11w	LH	24	0.0411	0.375876519	0.211
9	11w	LH	49	0.0411	0.351979537	0.224
9	11w	LH	49	0.0411	0.354793486	0.198
9	11w	LH	49	0.0411	0.57553074	0.218
9	11w	LH	72	0.0411	0.517835626	0.212
9	11w	LH	72	0.0411	0.583852313	0.220
9	11w	LH	**72**	0.0411	0.462948383	0.226
9	11w	LH	96	0.0411	0.747330961	0.163
9	11w	LH	96	0.0411	0.506604161	0.149
9	11w	LH	96	0.0411	0.683070018	0.157
9	11w	LH	192	0.0411	1.108199678	0.243
9	11w	LH	192	0.0411	1.100798307	0.185
9	11w	LH	192	0.0411	1.072257466	0.173
26	5w	DC	0	0.0141	0.012330987	0.337
26	5w	DC	0	0.0141	0.009987451	0.543
26	5w	DC	24	0.0141	0.093553406	0.426
26	5w	DC	24	0.0141	0.09904743	0.485
26	5w	DC	72	0.0141		0.345
26	5w	DC	72	0.0141	0.158282038	0.427
26	5w	DC	144	0.0141		
26	5w	DC	144	0.0141	0.310730956	0.355
26	5w	DC	192	0.0141	0.268074558	0.446
26	5w	DC	192	0.0141	0.3944708	0.583
26	5w	DC	240	0.0141	0.394802691	0.605
26	5w	DC	240	0.0141	0.45015532	0.623
26	5w	DC	336	0.0141	0.773804405	0.473
26	5w	DC	336	0.0141	0.350984739	0.440
27	5w	LH	0	0.0164	0.012330987	0.337
27	5w	LH	0	0.0164	0.009987451	0.543
27	5w	LH	24	0.0164	0.104657959	0.595
27	5w	LH	24	0.0164	0.098963803	0.548
27	5w	LH	72	0.0164	0.324680822	0.543
27	5w	LH	72	0.0164	0.377502418	0.452
27	5w	LH	144	0.0164		
27	5w	LH	144	0.0164	0.491394038	0.462
27	5w	LH	192	0.0164	0.731210277	0.510
27	5w	LH	192	0.0164	0.59657621	0.675
27	5w	LH	240	0.0164	0.682021036	0.617
27	5w	LH	240	0.0164	0.819252771	0.602
27	5w	LH	336	0.0164	0.768336077	0.628
27	5w	LH	336	0.0164	1.129748848	0.526
34	8w	LH	0	0.0066		
34	8w	LH	0	0.0066	0.0667194	
34	8w	LH	72	0.0066	0.1250641	

Test	Age (weeks)	Base	time (hr)	Cd2+ (µmol/L)	Cd (µmol/g dw)	weight/bug (mg)
34	8w	LH	72	0.0066	0.0999980	
34	8w	LH	168	0.0066	0.2728751	0.356
34	8w	LH	168	0.0066	0.2788275	0.335
34	8w	LH	168	0.0066	0.3041166	0.430
34	8w	LH	240	0.0066	0.3271405	0.434
34	8w	LH	240	0.0066	0.4494784	0.333
34	8w	LH	240	0.0066	0.2854110	0.480
34	8w	LH	336	0.0066	0.3495943	0.342
34	8w	LH	336	0.0066	0.4764396	0.298
34	8w	LH	336	0.0066	0.4143705	0.614
34	8w	LH	336	0.0066	0.3267712	0.673
35	8w	SAM	0	0.0057		
35	8w	SAM	0	0.0057	0.059306177	
35	8w	SAM	72	0.0057	0.16346265	
35	8w	SAM	72	0.0057	0.116240107	
35	8w	SAM	168	0.0057	0.158317337	0.393
35	8w	SAM	168	0.0057	0.148708026	0.402
35	8w	SAM	168	0.0057	0.146521143	0.408
35	8w	SAM	240	0.0057	0.147242922	0.508
35	8w	SAM	240	0.0057	0.193256336	0.387
35	8w	SAM	240	0.0057	0.17869209	0.460
35	8w	SAM	336	0.0057	0.204328313	0.640
35	8w	SAM	336	0.0057	0.200158347	0.448
35	8w	SAM	336	0.0057	0.255715607	0.526
35	8w	SAM	336	0.0057	0.180788185	0.620
39	9w	LH	0	0.0164	0.008742517	0.494
39	9w	LH	0	0.0164	0.008593497	0.440
39	9w	LH	72	0.0164	0.209491086	0.406
39	9w	LH	72	0.0164	0.267743235	0.530
39	9w	LH	168	0.0164	0.345875485	0.534
39	9w	LH	168	0.0164	0.386396462	0.478
39	9w	LH	168	0.0164	0.343572167	0.543
39	9w	LH	336	0.0164	0.664524525	0.618
39	9w	LH	336	0.0164	0.543297746	0.643
39	9w	LH	336	0.0164	0.690325557	0.648
39	9w	LH	336	0.0164	0.610643805	0.733
39	9w	LH	504	0.0164	0.866560428	0.616
39	9w	LH	504	0.0164	1.044362666	0.587
39	9w	LH	504	0.0164	0.86399045	0.673
39	9w	LH	504	0.0164	0.987054109	0.635
40	9w	DC	0	0.0150	0.008742517	0.494
40	9w	DC	0	0.0150	0.008593497	0.440
40	9w	DC	72	0.0150	0.084870762	0.380
40	9w	DC	72	0.0150	0.096557656	0.528
40	9w	DC	168	0.0150	0.19306655	0.490
40	9w	DC	168	0.0150	0.199163389	0.570
40	9w	DC	168	0.0150	0.15611361	0.636
40	9w	DC	336	0.0150	0.272415205	0.678
40	9w	DC	336	0.0150	0.308858711	0.748
40	9w	DC	336	0.0150	0.512703799	0.717
40	9w	DC	336	0.0150	0.229780588	0.695
40	9w	DC	504	0.0150	0.33442425	0.840
40	9w	DC	504	0.0150	0.412840478	0.918
40	9w	DC	504	0.0150	0.320562722	1.067

APPENDIX E

28-DAY CADMIUM TOXICITY TEST DATA

Test		Medium		minteq (% free)				µmol/L as free ion						Percentage Survival				weight/	CdT	CdT
#	date	Base	Add	Ca	Mg	Cd	pH	Ca	Mg	Na	K	Cd	Cd adj DOC	7-d	14-d	21-d	28-d	bug (mg)	µg/g	µmol/g
3	1-Dec-03	DC		93.9	94.8	79.4	8	751	324	1217	48.7	0.00000	0.00000	100	100	100	100	0.2276	0.0000	0.0000
3	1-Dec-03	DC		93.9	94.8	79.4	8	751	324	1217	48.7	0.00099	0.00099	85	85	85	85	0.1682	11.4895	0.1022
3	1-Dec-03	DC		93.9	94.8	79.4	8	751	324	1217	48.7	0.00162	0.00162	90	75	75	75	0.1713	16.7372	0.1489
3	1-Dec-03	DC		93.9	94.8	79.4	8	751	324	1217	48.7	0.00275	0.00275	100	95	90	85	0.2575	16.0340	0.1427
3	1-Dec-03	DC		93.9	94.8	79.4	8	751	324	1217	48.7	0.00459	0.00459	85	70	55	45	0.0344	34.3871	0.3059
3	1-Dec-03	DC		93.9	94.8	79.4	8	751	324	1217	48.7	0.00777	0.00777	100	85	75	65	0.3378	25.6118	0.2279
3	1-Dec-03	DC		93.9	94.8	79.4	8	751	324	1217	48.7	0.01272	0.01272	90	85	70	55	0.2227	62.1469	0.5529
3	1-Dec-03	DC		93.9	94.8	79.4	8	751	324	1217	48.7	0.02119	0.02119	100	70	50	45	0.1822	108.5732	0.9660
3	1-Dec-03	DC		93.9	94.8	79.4	8	751	324	1217	48.7	0.03532	0.03532	90	30	15	0			
4	1-Dec-03	LH		97.9	98.2	94.1	7.7	269	105	174	12.8	0.00000	0.00000	100	100	100	100	0.3013	0.0000	0.0000
4	1-Dec-03	LH		97.9	98.2	94.1	7.7	269	105	174	12.8	0.00117	0.00117	100	100	100	90	0.2500		
4	1-Dec-03	LH		97.9	98.2	94.1	7.7	269	105	174	12.8	0.00193	0.00193	90	90	90	90	0.2479		
4	1-Dec-03	LH		97.9	98.2	94.1	7.7	269	105	174	12.8	0.00327	0.00327	95	95	90	75	0.2920		
4	1-Dec-03	LH		97.9	98.2	94.1	7.7	269	105	174	12.8	0.00544	0.00544	100	90	70	70	0.1118		
4	1-Dec-03	LH		97.9	98.2	94.1	7.7	269	105	174	12.8	0.00921	0.00921	95	80	60	50	0.2140	126.5888	1.1262
4	1-Dec-03	LH		97.9	98.2	94.1	7.7	269	105	174	12.8	0.01507	0.01507	95	80	35	25	0.2400	240.6250	2.1408
4	1-Dec-03	LH		97.9	98.2	94.1	7.7	269	105	174	12.8	0.02512	0.02512	80	50	25	5	0.5400	401.1111	3.5686
4	1-Dec-03	LH		97.9	98.2	94.1	7.7	269	105	174	12.8	0.04186	0.04186	75	20	0	0			
15	18-Mar-04	LH		97.8	98.1	92.4	7.8	301	97	257	12.8	0.00000	0.00000	100	100	100	95	0.2399	17.6481	0.1570
15	18-Mar-04	LH		97.8	98.1	92.4	7.8	301	97	257	12.8	0.00460	0.00460	90	90	90	85	0.2326	38.3606	0.3413
15	18-Mar-04	LH		97.8	98.1	92.4	7.8	301	97	257	12.8	0.00765	0.00765	100	100	95	95	0.2229	84.2336	0.7494
15	18-Mar-04	LH		97.8	98.1	92.4	7.8	301	97	257	12.8	0.01315	0.01315	100	100	90	80	0.2500	123.8198	1.1016
15	18-Mar-04	LH		97.8	98.1	92.4	7.8	301	97	257	12.8	0.02137	0.02137	70	65	60	60	0.2950	181.8305	1.6177
15	18-Mar-04	LH		97.8	98.1	92.4	7.8	301	97	257	12.8	0.03535	0.03535	35	20	5	5	0.0500		
15	18-Mar-04	LH		97.8	98.1	92.4	7.8	301	97	257	12.8	0.05919	0.05919	0	0	0	0			
15	18-Mar-04	LH		97.8	98.1	92.4	7.8	301	97	257	12.8	0.09865	0.09865	0	0	0	0			

	Test	Medium		minteq (% free)				µmol/L as free ion						Percentage Survival				weight/	CdT	CdT
#	date	Base	Add	Ca	Mg	Cd	pH	Ca	Mg	Na	K	Cd	Cd adj DOC	7-d	14-d	21-d	28-d	bug (mg)	µg/g	µmol/g
15	18-Mar-04	LH		97.8	98.1	92.4	7.8	301	97	257	12.8	0.16441	0.16441	0	0	0	0			
16	18-Mar-04	LH	Ca3	98.3	98.5	69.4	7.8	2949	113	270	12.8	0.00000	0.00000	100	90	85	75	0.2625	12.6000	0.1120996
16	18-Mar-04	LH	Ca3	98.3	98.5	69.4	7.8	2949	113	270	12.8	0.00346	0.00346	100	95	85	80	0.2349	20.4348	0.1818041
16	18-Mar-04	LH	Ca3	98.3	98.5	69.4	7.8	2949	113	270	12.8	0.00574	0.00574	105	105	105	100	0.1925	36.8571	0.3279105
16	18-Mar-04	LH	Ca3	98.3	98.5	69.4	7.8	2949	113	270	12.8	0.00988	0.00988	95	95	95	95	0.3000	61.5909	0.5479618
16	18-Mar-04	LH	Ca3	98.3	98.5	69.4	7.8	2949	113	270	12.8	0.01605	0.01605	100	95	95	80	0.2732	67.3325	0.5990439
16	18-Mar-04	LH	Ca3	98.3	98.5	69.4	7.8	2949	113	270	12.8	0.02655	0.02655	100	100	85	80	0.2500	92.4398	0.8224182
16	18-Mar-04	LH	Ca3	98.3	98.5	69.4	7.8	2949	113	270	12.8	0.04446	0.04446	85	85	80	60	0.2214	160.1941	1.4252146
16	18-Mar-04	LH	Ca3	98.3	98.5	69.4	7.8	2949	113	270	12.8	0.07409	0.07409	90	80	70	70	0.2306	179.6800	1.5985765
16	18-Mar-04	LH	Ca3	98.3	98.5	69.4	7.8	2949	113	270	12.8	0.12349	0.12349	50	15	10	10	0.2000	224.6000	1.9982206
18	25-Mar-04	SAM	Ca4	96.8	97.3	64.5	7.9	3799	400	1391	71.0	0.00000	0.00000	100	100	95	80	0.2464	2.8986	0.0258
18	25-Mar-04	SAM	Ca4	96.8	97.3	64.5	7.9	3799	400	1391	71.0	0.00321	0.00321	100	100	100	90	0.2106	18.9385	0.1685
18	25-Mar-04	SAM	Ca4	96.8	97.3	64.5	7.9	3799	400	1391	71.0	0.00534	0.00534	105	105	95	85	0.2450	20.7580	0.1847
18	25-Mar-04	SAM	Ca4	96.8	97.3	64.5	7.9	3799	400	1391	71.0	0.00918	0.00918	95	95	85	75	0.2800	27.0330	0.2405
18	25-Mar-04	SAM	Ca4	96.8	97.3	64.5	7.9	3799	400	1391	71.0	0.01492	0.01492	100	100	100	85	0.3267	45.0510	0.4008
18	25-Mar-04	SAM	Ca4	96.8	97.3	64.5	7.9	3799	400	1391	71.0	0.02468	0.02468	100	90	85	70	0.2936	80.5511	0.7166
18	25-Mar-04	SAM	Ca4	96.8	97.3	64.5	7.9	3799	400	1391	71.0	0.04132	0.04132	100	100	95	85	0.2806	108.7973	0.9679
18	25-Mar-04	SAM	Ca4	96.8	97.3	64.5	7.9	3799	400	1391	71.0	0.06886	0.06886	100	50	50	45	0.3186	126.5291	1.1257
18	25-Mar-04	SAM	Ca4	96.8	97.3	64.5	7.9	3799	400	1391	71.0	0.11477	0.11477	40	5	0	0			
19	28-Mar-04	DC	pH9	89.7	91.8	45.9	8.9	695	363	1348	48.7	0.00000	0.00000	100	100	100	95	0.2574	1.9632	0.0175
19	28-Mar-04	DC	pH9	89.7	91.8	45.9	8.9	695	363	1348	48.7	0.00380	0.00190	100	100	110	85	0.3075	44.8780	0.3993
19	28-Mar-04	DC	pH9	89.7	91.8	45.9	8.9	695	363	1348	48.7	0.00653	0.00327	95	90	90	85	0.3008	70.3836	0.6262
19	28-Mar-04	DC	pH9	89.7	91.8	45.9	8.9	695	363	1348	48.7	0.01062	0.00531	110	110	110	105	0.2950	107.5330	0.9567
19	28-Mar-04	DC	pH9	89.7	91.8	45.9	8.9	695	363	1348	48.7	0.01756	0.00878	95	95	95	85	0.2138	174.6784	1.5541
19	28-Mar-04	DC	pH9	89.7	91.8	45.9	8.9	695	363	1348	48.7	0.02940	0.01470	100	80	70	70	0.2993	194.4630	1.7301
19	28-Mar-04	DC	pH9	89.7	91.8	45.9	8.9	695	363	1348	48.7	0.04900	0.02450	65	50	40	40	0.3475	304.8201	2.7119
19	28-Mar-04	DC	pH9	89.7	91.8	45.9	8.9	695	363	1348	48.7	0.08167	0.04084	5	0	0	0			
21	19-Apr-04	SAM	Mg1	91	92.6	73.1	8.2	953	823	1022	46.2	0.00000	0.00000	100	100	100	90	0.1631	2.5287	0.0225
21	19-Apr-04	SAM	Mg1	91	92.6	73.1	8.2	953	823	1022	46.2	0.02536	0.02536	95	95	90	85	0.1607	78.0166	0.6941

	Test	Medium		minteq (% free)				µmol/L as free ion						Percentage Survival				weight/	CdT	CdT
#	date	Base	Add	Ca	Mg	Cd	pH	Ca	Mg	Na	K	Cd	Cd adj DOC	7-d	14-d	21-d	28-d	bug (mg)	µg/g	µmol/g
21	19-Apr-04	SAM	Mg1	91	92.6	73.1	8.2	953	823	1022	46.2	0.04227	0.04227	95	95	90	70	0.1685	129.4064	1.1513
21	19-Apr-04	SAM	Mg1	91	92.6	73.1	8.2	953	823	1022	46.2	0.07024	0.07024	70	35	30	25	0.1940	157.3196	1.3996
21	19-Apr-04	SAM	Mg1	91	92.6	73.1	8.2	953	823	1022	46.2	0.11706	0.11706	0	0	0	0			
21	19-Apr-04	SAM	Mg1	91	92.6	73.1	8.2	953	823	1022	46.2	0.19511	0.19511	0	0	0	0			
21	19-Apr-04	SAM	Mg1	91	92.6	73.1	8.2	953	823	1022	46.2	0.32518	0.32518	0	0	0	0			
22	27-Apr-04	SAM	SAM4	90.2	91.7	49	8.2	4285	1355	5783	261.5	0.00000	0.00000	100	95	95	90	0.2129	7.6243	0.0678
22	27-Apr-04	SAM	SAM4	90.2	91.7	49	8.2	4285	1355	5783	261.5	0.02049	0.02049	90	90	90	90	0.1877	49.1803	0.4375
22	27-Apr-04	SAM	SAM4	90.2	91.7	49	8.2	4285	1355	5783	261.5	0.03392	0.03392	90	90	85	85	0.1870	108.9519	0.9693
22	27-Apr-04	SAM	SAM4	90.2	91.7	49	8.2	4285	1355	5783	261.5	0.05667	0.05667	100	110	105	105	0.1889	133.7047	1.1895
22	27-Apr-04	SAM	SAM4	90.2	91.7	49	8.2	4285	1355	5783	261.5	0.09416	0.09416	100	95	75	55	0.2600	173.9231	1.5474
22	27-Apr-04	SAM	SAM4	90.2	91.7	49	8.2	4285	1355	5783	261.5	0.15694	0.15694	100	90	45	40	0.2813	297.0933	2.6432
22	27-Apr-04	SAM	SAM4	90.2	91.7	49	8.2	4285	1355	5783	261.5	0.26157	0.26157	95	20	0	0			
22	27-Apr-04	SAM	SAM4	90.2	91.7	49	8.2	4285	1355	5783	261.5	0.43594	0.43594	100	0	0	0			
23	11-Jun-04	LH		96.9	97.4	93	7.7	339	108	174	12.8	0.00000	0.00000	95	95	95	95	0.1331	10.2535	0.0912
23	11-Jun-04	LH		96.9	97.4	93	7.7	339	108	174	12.8	0.00256	0.00256	110	105	95	95	0.1094	53.4409	0.4755
23	11-Jun-04	LH		96.9	97.4	93	7.7	339	108	174	12.8	0.00513	0.00513	100	100	50	35	0.1225	95.9184	0.8534
23	11-Jun-04	LH		96.9	97.4	93	7.7	339	108	174	12.8	0.01034	0.01034	100	75	55	35	0.0900		
23	11-Jun-04	LH		96.9	97.4	93	7.7	339	108	174	12.8	0.02069	0.02069	70	10	0	0			
23	11-Jun-04	LH		96.9	97.4	93	7.7	339	108	174	12.8	0.04137	0.04137	5	0	0	0			
23	11-Jun-04	LH		96.9	97.4	93	7.7	339	108	174	12.8	0.08274	0.08274	0	0	0	0			
23	11-Jun-04	LH		96.9	97.4	93	7.7	339	108	174	12.8	0.16548	0.16548	0	0	0	0			
24	20-Jun-04	SAM	Ca7, Mg2	91.8	93.2	50.8	7.9	6839	1791	939	47.2	0.00000	0.00000	100	100		stock error after d14			
24	20-Jun-04	SAM	Ca7, Mg2	91.8	93.2	50.8	7.9	6839	1791	939	47.2	0.00705	0.00705	95	95					
24	20-Jun-04	SAM	Ca7, Mg2	91.8	93.2	50.8	7.9	6839	1791	939	47.2	0.01415	0.01415	115	100					
24	20-Jun-04	SAM	Ca7, Mg2	91.8	93.2	50.8	7.9	6839	1791	939	47.2	0.02825	0.02825	100	100					
24	20-Jun-04	SAM	Ca7, Mg2	91.8	93.2	50.8	7.9	6839	1791	939	47.2	0.05649	0.05649	95	90					
24	20-Jun-04	SAM	Ca7, Mg2	91.8	93.2	50.8	7.9	6839	1791	939	47.2	0.11299	0.11299	95	90					
24	20-Jun-04	SAM	Ca7, Mg2	91.8	93.2	50.8	7.9	6839	1791	939	47.2	0.22598	0.22598	45	50					
24	20-Jun-04	SAM	Ca7, Mg2	91.8	93.2	50.8	7.9	6839	1791	939	47.2	0.45196	0.45196	15	0					

	Test	Medium		minteq (% free)				µmol/L as free ion						Percentage Survival				weight/	CdT	CdT
#	date	Base	Add	Ca	Mg	Cd	pH	Ca	Mg	Na	K	Cd	Cd adj DOC	7-d	14-d	21-d	28-d	bug (mg)	µg/g	µmol/g
25	24-Jun-04	SAM	Mg1	85.4	87.9	73.4	8	798	1704	952	49.7	0.00000	0.00000	100						
25	24-Jun-04	SAM	Mg1	85.4	87.9	73.4	8	798	1704	952	49.7	0.00509	0.00509	105						
25	24-Jun-04	SAM	Mg1	85.4	87.9	73.4	8	798	1704	952	49.7	0.01019	0.01019	100						
25	24-Jun-04	SAM	Mg1	85.4	87.9	73.4	8	798	1704	952	49.7	0.02044	0.02044	105						
25	24-Jun-04	SAM	Mg1	85.4	87.9	73.4	8	798	1704	952	49.7	0.04081	0.04081	100						
25	24-Jun-04	SAM	Mg1	85.4	87.9	73.4	8	798	1704	952	49.7	0.08163	0.08163	30						
25	24-Jun-04	SAM	Mg1	85.4	87.9	73.4	8	798	1704	952	49.7	0.16326	0.16326	0						
25	24-Jun-04	SAM	Mg1	85.4	87.9	73.4	8	798	1704	952	49.7	0.32651	0.32651	0						
25	24-Jun-04	SAM	Mg1	85.4	87.9	73.4	8	798	1704	952	49.7	0.65302	0.65302	0						
28	9-Jul-04	SAM	Alk5	83.1	86.4	26.2	8.8	748	231	8000	51.3	0.00000	0.00000	100	100	95	80	0.1221	1.2865	0.0114
28	9-Jul-04	SAM	Alk5	83.1	86.4	26.2	8.8	748	231	8000	51.3	0.00145	0.00073	95	95	90	85	0.1393	38.9474	0.3465
28	9-Jul-04	SAM	Alk5	83.1	86.4	26.2	8.8	748	231	8000	51.3	0.00292	0.00146	95	90	95	75	0.2054	58.0225	0.5162
28	9-Jul-04	SAM	Alk5	83.1	86.4	26.2	8.8	748	231	8000	51.3	0.00583	0.00291	100	100	100	85	0.2527	94.5066	0.8408
28	9-Jul-04	SAM	Alk5	83.1	86.4	26.2	8.8	748	231	8000	51.3	0.01165	0.00583	100	100	55	50	0.1010	161.0891	1.4332
28	9-Jul-04	SAM	Alk5	83.1	86.4	26.2	8.8	748	231	8000	51.3	0.02331	0.01165	35	5	0	0			
28	9-Jul-04	SAM	Alk5	83.1	86.4	26.2	8.8	748	231	8000	51.3	0.04662	0.02331	5	0	0	0			
29	22-Jul-04	SAM		95.6	96.2	81.1	7.9	803	275	904	48.2	0.00000	0.00000	95	90	85	80	0.3850	10.0325	0.0893
29	22-Jul-04	SAM		95.6	96.2	81.1	7.9	803	275	904	48.2	0.00225	0.00225	100	95	90	85	0.2535	29.0023	0.2580
29	22-Jul-04	SAM		95.6	96.2	81.1	7.9	803	275	904	48.2	0.00450	0.00450	100	100	100	100	0.3215	32.5039	0.2892
29	22-Jul-04	SAM		95.6	96.2	81.1	7.9	803	275	904	48.2	0.00903	0.00903	100	95	95	95	0.3711	58.6043	0.5214
29	22-Jul-04	SAM		95.6	96.2	81.1	7.9	803	275	904	48.2	0.01804	0.01804	100	100	90	85	0.3376	158.0488	1.4061
29	22-Jul-04	SAM		95.6	96.2	81.1	7.9	803	275	904	48.2	0.03608	0.03608	90	70	60	60	0.3833	232.7391	2.0706
29	22-Jul-04	SAM		95.6	96.2	81.1	7.9	803	275	904	48.2	0.07215	0.07215	50	5	0	0			
29	22-Jul-04	SAM		95.6	96.2	81.1	7.9	803	275	904	48.2	0.14431	0.14431	5	0	0	0			
30	2-Aug-04	LH	SR5	96.9	98.2	91.9	7.7	266	101	170	12.8	0.00000	0.00000	95	95	90	90	0.1521	47.4178	0.4219
30	2-Aug-04	LH	SR5	96.9	98.2	91.9	7.7	266	101	170	12.8	0.00510	0.00255	95	100	95	95	0.1572	70.3180	0.6256
30	2-Aug-04	LH	SR5	96.9	98.2	91.9	7.7	266	101	170	12.8	0.01024	0.00512	100	90	75	75	0.1353	113.6946	1.0115
30	2-Aug-04	LH	SR5	96.9	98.2	91.9	7.7	266	101	170	12.8	0.02044	0.01022	95	70	60	60	0.1150	170.9565	1.5210
30	2-Aug-04	LH	SR5	96.9	98.2	91.9	7.7	266	101	170	12.8	0.04088	0.02044	10	5	5	5	0.2000		

	Test	Medium		minteq (% free)				µmol/L as free ion						Percentage Survival				weight/	CdT	CdT
#	date	Base	Add	Ca	Mg	Cd	pH	Ca	Mg	Na	K	Cd	Cd adj DOC	7-d	14-d	21-d	28-d	bug (mg)	µg/g	µmol/g
30	2-Aug-04	LH	SR5	96.9	98.2	91.9	7.7	266	101	170	12.8	0.08176	0.04088	0	0	0	0			
30	2-Aug-04	LH	SR5	96.9	98.2	91.9	7.7	266	101	170	12.8	0.16352	0.08176	0	0	0	0			
30	2-Aug-04	LH	SR5	96.9	98.2	91.9	7.7	266	101	170	12.8	0.32705	0.16352	0	0	0	0			
31	5-Aug-04	SAM	SR5	95.3	96.1	77.2	8.1	958	282	974	54.4	0.00000	0.00000	100	100	95	95	0.1160	3.5345	0.0314
31	5-Aug-04	SAM	SR5	95.3	96.1	77.2	8.1	958	282	974	54.4	0.00429	0.00171	100	100	95	90	0.1389	20.1920	0.1796
31	5-Aug-04	SAM	SR5	95.3	96.1	77.2	8.1	958	282	974	54.4	0.00860	0.00344	100	95	95	85	0.1350	40.5079	0.3604
31	5-Aug-04	SAM	SR5	95.3	96.1	77.2	8.1	958	282	974	54.4	0.01717	0.00687	100	100	100	100	0.1306	61.9369	0.5510
31	5-Aug-04	SAM	SR5	95.3	96.1	77.2	8.1	958	282	974	54.4	0.03434	0.01374	95	75	70	65	0.1177	126.0784	1.1217
31	5-Aug-04	SAM	SR5	95.3	96.1	77.2	8.1	958	282	974	54.4	0.06868	0.02747	60	20	20	20	0.1675	172.1493	1.5316
31	5-Aug-04	SAM	SR5	95.3	96.1	77.2	8.1	958	282	974	54.4	0.13737	0.05495	10	0	0	0			
31	5-Aug-04	SAM	SR5	95.3	96.1	77.2	8.1	958	282	974	54.4	0.27473	0.10989	0	0	0	0			
32	26-Aug-04	LH	SR10	95.8	98	89.6	7.7	228	97	174	12.8	0.00000	0.00000	100	100	100	100	0.0815	12.8834	0.1146
32	26-Aug-04	LH	SR10	95.8	98	89.6	7.7	228	97	174	12.8	0.00497	0.00298	100	80	75	55	0.0422	113.1579	1.0067
32	26-Aug-04	LH	SR10	95.8	98	89.6	7.7	228	97	174	12.8	0.00998	0.00599	100	90	40	20	0.0500	300.0000	2.6690
32	26-Aug-04	LH	SR10	95.8	98	89.6	7.7	228	97	174	12.8	0.01993	0.01196	95	100	100	95	0.0973		
32	26-Aug-04	LH	SR10	95.8	98	89.6	7.7	228	97	174	12.8	0.03986	0.02391	60	50	30	25	0.0675	517.0370	4.6000
32	26-Aug-04	LH	SR10	95.8	98	89.6	7.7	228	97	174	12.8	0.07972	0.04783	15	15	0	0			
32	26-Aug-04	LH	SR10	95.8	98	89.6	7.7	228	97	174	12.8	0.15943	0.09566	0	0	0	0			
32	26-Aug-04	LH	SR10	95.8	98	89.6	7.7	228	97	174	12.8	0.31886	0.19132	0	0	0	0			
33	30-Aug-04	LH	LM10	95.3	97.9	87.6	7.9	225	101	174	12.8	0.00000	0.00000	85	85	80	60	0.0967	25.5172	0.2270
33	30-Aug-04	LH	LM10	95.3	97.9	87.6	7.9	225	101	174	12.8	0.00486	0.00195	100	90	90	90	0.1047	51.3483	0.4568
33	30-Aug-04	LH	LM10	95.3	97.9	87.6	7.9	225	101	174	12.8	0.00976	0.00390	100	95	100	100	0.0989	73.1915	0.6512
33	30-Aug-04	LH	LM10	95.3	97.9	87.6	7.9	225	101	174	12.8	0.01948	0.00779	100	95	90	70	0.1414	111.9192	0.9957
33	30-Aug-04	LH	LM10	95.3	97.9	87.6	7.9	225	101	174	12.8	0.03897	0.01559	80	70	60	50	0.1090	232.8807	2.0719
33	30-Aug-04	LH	LM10	95.3	97.9	87.6	7.9	225	101	174	12.8	0.07794	0.03117	35	15	15	10	0.0500	433.2000	3.8541
33	30-Aug-04	LH	LM10	95.3	97.9	87.6	7.9	225	101	174	12.8	0.15587	0.06235	0	0	0	0			
33	30-Aug-04	LH	LM10	95.3	97.9	87.6	7.9	225	101	174	12.8	0.31174	0.12470	0	0	0	0			
36	13-Sep-04	SAM	Mg1	85.5	88	74.5	7.9	812	1702	957	53.8	0.00000	0.00000	100	95	80	70	0.0411	54.5946	0.4857
36	13-Sep-04	SAM	Mg1	85.5	88	74.5	7.9	812	1702	957	53.8	0.01034	0.01034	95	95	95	90	0.0947	91.6667	0.8155

	Test	Medium		minteq (% free)				µmol/L as free ion						Percentage Survival				weight/	CdT	CdT
#	date	Base	Add	Ca	Mg	Cd	pH	Ca	Mg	Na	K	Cd	Cd adj DOC	7-d	14-d	21-d	28-d	bug (mg)	µg/g	µmol/g
36	13-Sep-04	SAM	Mg1	85.5	88	74.5	7.9	812	1702	957	53.8	0.02075	0.02075	95	45	25	10	0.0500		
36	13-Sep-04	SAM	Mg1	85.5	88	74.5	7.9	812	1702	957	53.8	0.04143	0.04143	65	5	0	0			
36	13-Sep-04	SAM	Mg1	85.5	88	74.5	7.9	812	1702	957	53.8	0.08285	0.08285	10	0	0	0			
36	13-Sep-04	SAM	Mg1	85.5	88	74.5	7.9	812	1702	957	53.8	0.16570	0.16570	0	0	0	0			
36	13-Sep-04	SAM	Mg1	85.5	88	74.5	7.9	812	1702	957	53.8	0.33141	0.33141	0	0	0	0			
37	16-Sep-04	LH		98	98.3	95	7.5	270	105	174	12.8	0.00000	0.00000	100	95	90	85	0.0727		
37	16-Sep-04	LH		98	98.3	95	7.5	270	105	174	12.8	0.00524	0.00524	90	85	45	15			
37	16-Sep-04	LH		98	98.3	95	7.5	270	105	174	12.8	0.01056	0.01056	95	75	20	30			
37	16-Sep-04	LH		98	98.3	95	7.5	270	105	174	12.8	0.02113	0.02113	95	55	5	5			
37	16-Sep-04	LH		98	98.3	95	7.5	270	105	174	12.8	0.04226	0.04226	10	5	0	0			
37	16-Sep-04	LH		98	98.3	95	7.5	270	105	174	12.8	0.08452	0.08452	0	0	0	0			
37	16-Sep-04	LH		98	98.3	95	7.5	270	105	174	12.8	0.16904	0.16904	0	0	0	0			
38	16-Sep-04	LH	SR20	94.5	98	87.3	7.4	260	109	187	12.8	0.00000	0.00000	100	100	100	100	0.1095	7.7626	0.0691
38	16-Sep-04	LH	SR20	94.5	98	87.3	7.4	260	109	187	12.8	0.01212	0.00242	100	100	100	100	0.0900	80.1170	0.7128
38	16-Sep-04	LH	SR20	94.5	98	87.3	7.4	260	109	187	12.8	0.02431	0.00486	100	100	95	90	0.1163	176.8817	1.5737
38	16-Sep-04	LH	SR20	94.5	98	87.3	7.4	260	109	187	12.8	0.04854	0.00971	45	20	15	10	0.0500	370.0000	3.2918
38	16-Sep-04	LH	SR20	94.5	98	87.3	7.4	260	109	187	12.8	0.09709	0.01942	0	0	0	0			
38	16-Sep-04	LH	SR20	94.5	98	87.3	7.4	260	109	187	12.8	0.19417	0.03883	0	0	0	0			
38	16-Sep-04	LH	SR20	94.5	98	87.3	7.4	260	109	187	12.8	0.38835	0.07767	0	0	0	0			
38	16-Sep-04	LH	SR20	94.5	98	87.3	7.4	260	109	187	12.8	0.77669	0.15534	0	0	0	0			
43	30-Sep-04	SAM	SR20	95.1	96.3	79.9	7.9	937	288	957	50.3	0.00000	0.00000	100	55	40	40	0.0150	33.3333	0.2966
43	30-Sep-04	SAM	SR20	95.1	96.3	79.9	7.9	937	288	957	50.3	0.01109	0.01109	100	90	20	20	0.0325	23.0769	0.2053
43	30-Sep-04	SAM	SR20	95.1	96.3	79.9	7.9	937	288	957	50.3	0.02225	0.02225	100	50	25	20	0.0500	166.6667	1.4828
43	30-Sep-04	SAM	SR20	95.1	96.3	79.9	7.9	937	288	957	50.3	0.04443	0.04443	95	5	0	0			
43	30-Sep-04	SAM	SR20	95.1	96.3	79.9	7.9	937	288	957	50.3	0.08886	0.08886	30	10	0	0			
43	30-Sep-04	SAM	SR20	95.1	96.3	79.9	7.9	937	288	957	50.3	0.17771	0.17771	0	0	0	0			
43	30-Sep-04	SAM	SR20	95.1	96.3	79.9	7.9	937	288	957	50.3	0.35543	0.35543	0	0	0	0			
43	30-Sep-04	SAM	SR20	95.1	96.3	79.9	7.9	937	288	957	50.3	0.71085	0.71085	0	0	0	0		1.8 CONC OMITTED	
44	4-Oct-04	SAM	HCA7	97.7	98	55.6	7.9	6253	282	913	51.3	0.00000	0.00000	100	45					

	Test	Medium		minteq (% free)				µmol/L as free ion						Percentage Survival				weight/	CdT	CdT
#	date	Base	Add	Ca	Mg	Cd	pH	Ca	Mg	Na	K	Cd	Cd adj DOC	7-d	14-d	21-d	28-d	bug (mg)	µg/g	µmol/g
44	4-Oct-04	SAM	HCA7	97.7	98	55.6	7.9	6253	282	913	51.3	0.00772	0.00772	95	20					
44	4-Oct-04	SAM	HCA7	97.7	98	55.6	7.9	6253	282	913	51.3	0.01548	0.01548	95	20					
44	4-Oct-04	SAM	HCA7	97.7	98	55.6	7.9	6253	282	913	51.3	0.03092	0.03092	100	10					
44	4-Oct-04	SAM	HCA7	97.7	98	55.6	7.9	6253	282	913	51.3	0.06183	0.06183	95	30					
44	4-Oct-04	SAM	HCA7	97.7	98	55.6	7.9	6253	282	913	51.3	0.12367	0.12367	90	15					
44	4-Oct-04	SAM	HCA7	97.7	98	55.6	7.9	6253	282	913	51.3	0.24733	0.24733	35	5					
44	4-Oct-04	SAM	HCA7	97.7	98	55.6	7.9	6253	282	913	51.3	0.49466	0.49466	0	0					
45	16-Feb-05	LH	Ca4	98.8	99	66.1	7.7	3878	114	237	15.4	0.00000	0.00000	65	40	45	40	0.0700	4.0816	0.0363
45	16-Feb-05	LH	Ca4	98.8	99	66.1	7.7	3878	114	237	15.4	0.00470	0.00470	65	65	60	60	0.0960	16.6667	0.1483
45	16-Feb-05	LH	Ca4	98.8	99	66.1	7.7	3878	114	237	15.4	0.00941	0.00941	70	55	40	40	0.0686	29.1667	0.2595
45	16-Feb-05	LH	Ca4	98.8	99	66.1	7.7	3878	114	237	15.4	0.01841	0.01841	55	55	50	50	0.0567	82.3529	0.7327
45	16-Feb-05	LH	Ca4	98.8	99	66.1	7.7	3878	114	237	15.4	0.03675	0.03675	70	60	50	50	0.0800	110.0000	0.9786
45	16-Feb-05	LH	Ca4	98.8	99	66.1	7.7	3878	114	237	15.4	0.07351	0.07351	65	40	35	30	0.0875	114.2857	1.0168
45	16-Feb-05	LH	Ca4	98.8	99	66.1	7.7	3878	114	237	15.4	0.14702	0.14702	60	30	20	15	0.1000	146.6667	1.3049
45	16-Feb-05	LH	Ca4	98.8	99	66.1	7.7	3878	114	237	15.4	0.29404	0.29404	0	0	0	0	0.0000		
48	20-Feb-05	LH	Ca4, Mg1	93.6	94.7	63.9	7.6	3791	1037	238	15.4	0.00000	0.00000	85	69.2	62	38.5	0.1060	2.2642	0.0201
48	20-Feb-05	LH	Ca4, Mg1	93.6	94.7	63.9	7.6	3791	1037	238	15.4	0.00455	0.00455	65	55	45	35	0.0583	17.1429	0.1525
48	20-Feb-05	LH	Ca4, Mg1	93.6	94.7	63.9	7.6	3791	1037	238	15.4	0.00910	0.00910	90	70	65	65	0.1310	16.7939	0.1494
48	20-Feb-05	LH	Ca4, Mg1	93.6	94.7	63.9	7.6	3791	1037	238	15.4	0.01779	0.01779	45	30	20	20	0.1667	28.0000	0.2491
48	20-Feb-05	LH	Ca4, Mg1	93.6	94.7	63.9	7.6	3791	1037	238	15.4	0.03553	0.03553	60	20	5	0			
48	20-Feb-05	LH	Ca4, Mg1	93.6	94.7	63.9	7.6	3791	1037	238	15.4	0.07106	0.07106	45	5	0	0			
48	20-Feb-05	LH	Ca4, Mg1	93.6	94.7	63.9	7.6	3791	1037	238	15.4	0.14213	0.14213	60	5	0	0			
48	20-Feb-05	LH	Ca4, Mg1	93.6	94.7	63.9	7.6	3791	1037	238	15.4	0.28425	0.28425	0	0	0	0			
48	20-Feb-05	LH	Ca4, Mg1	93.6	94.7	63.9	7.6	3791	1037	238	15.4	0.56851	0.56851	0	0	0	0			
17	29-Mar-04	LH	Ca4, Mg1	93.5	94.6	61.8	7.8	3670	1012	383	17.9	0.00000	0.00000	95						
17	29-Mar-04	LH	Ca4, Mg1	93.5	94.6	61.8	7.8	3670	1012	383	17.9	0.03574	0.03574	100						
17	29-Mar-04	LH	Ca4, Mg1	93.5	94.6	61.8	7.8	3670	1012	383	17.9	0.06048	0.06048	100						
17	29-Mar-04	LH	Ca4, Mg1	93.5	94.6	61.8	7.8	3670	1012	383	17.9	0.09897	0.09897	100						
17	29-Mar-04	LH	Ca4, Mg1	93.5	94.6	61.8	7.8	3670	1012	383	17.9	0.16495	0.16495	70						

	Test	Medium		minteq (% free)				µmol/L as free ion						Percentage Survival				weight/	CdT	CdT
#	date	Base	Add	Ca	Mg	Cd	pH	Ca	Mg	Na	K	Cd	Cd adj DOC	7-d	14-d	21-d	28-d	bug (mg)	µg/g	µmol/g
17	29-Mar-04	LH	Ca4, Mg1	93.5	94.6	61.8	7.8	3670	1012	383	17.9	0.27491	0.27491	20						
47	20-Feb-05	DC	pH7	94.6	95.3	88.5	7.1	757	326	1217	48.7	0.00000	0.00000	90						
47	20-Feb-05	DC	pH7	94.6	95.3	88.5	7.1	757	326	1217	48.7	0.00984	0.00984	80						
47	20-Feb-05	DC	pH7	94.6	95.3	88.5	7.1	757	326	1217	48.7	0.01968	0.01968	85						
47	20-Feb-05	DC	pH7	94.6	95.3	88.5	7.1	757	326	1217	48.7	0.03937	0.03937	90						
47	20-Feb-05	DC	pH7	94.6	95.3	88.5	7.1	757	326	1217	48.7	0.07874	0.07874	30						
47	20-Feb-05	DC	pH7	94.6	95.3	88.5	7.1	757	326	1217	48.7	0.15747	0.15747	10						

CADMIUM 28-DAY TOXICITY TESTS CHEMICAL CONFIRMATION DATA

		Measured at Solution Preparation						Measured at Solution Exchange					
		Total Cd			Filtered Cd			Total Cd			Filtered Cd		
Test	day	nom	meas	meas/nom	nom	meas	meas/nom	nom	meas	meas/nom	nom	meas	meas/nom
3	0	5	4.52	0.90									
4	0	5	3.98	0.80									
15	0	20	19	0.95									
15	14	4.3	3.54	0.82									
15	21 or 7	4.3	4.37	1.02									
16	0	20	21.6	1.08									
16	7	12	12.4	1.03									
16	14	20	18.4	0.92									
18	0	20	19.8	0.99									
18	7	20	18.9	0.95									
19	7							12	2.42	0.20			
19	14							7.2	4.37	0.61			
19	14							12	3.63	0.30			
19	21							7.2	2.27	0.32			
19	28							7.2	6.4	0.89	0.46		
22	0	100	79.4	0.79									
22	7	100	76.2	0.76									
22	14	100	77.5	0.78									
22	21	60	44.3	0.74									

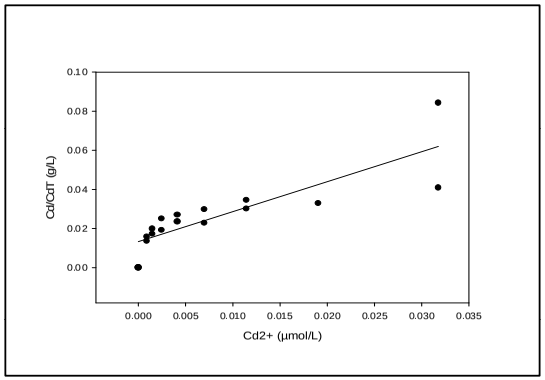
		Measured at Solution Preparation						Measured at Solution Exchange					
		Total Cd			Filtered Cd			Total Cd			Filtered Cd		
Test	day	nom	meas	meas/nom	nom	meas	meas/nom	nom	meas	meas/nom	nom	meas	meas/nom
23	0	20	15	0.75									
23	7	20	17.5	0.88									
23	14	5	3.58	0.72									
23	21	2.5	1.38	0.55									
28	0	20	16.6	0.83									
28	21	10	9.42	0.94									
28	28							5	2.64	0.53	5	1.48	0.30
29	7	20	28.4	1.42									
29	21	10	11.6	1.16									
29	28							5	3.58	0.72	5	1.95	0.39
36	0	50	45	0.90	50	24.2	0.48						
36	7	50	39.9	0.80									
36	14	12.5	12.1	0.97									
36	21	6.25	4.6	0.74									
30	0	40	38.2	0.96									
30	7	40	38.4	0.96									
30	14	10	10.1	1.01									
30	21	5	5.69	1.14									
30	28							5	2.71	0.54	5	2.33	0.47

		Measured at Solution Preparation						Measured at Solution Exchange					
		Total Cd			Filtered Cd			Total Cd			Filtered Cd		
Test	day	nom	meas	meas/nom	nom	meas	meas/nom	nom	meas	meas/nom	nom	meas	meas/nom
31	0	40	38.5	0.96									
31	7	40	40.3	1.01									
31	14	20	18.3	0.92									
31	21	10	9.43	0.94									
31	28							10	10.5	1.05	10	4.25	0.43
32	0	40	37.9	0.95									
32	7	40	33.8	0.85	40	6.74	0.17						
32	14	10	9.42	0.94	10	1.66	0.17						
32	21	10	10.6	1.06	10	1.69	0.17	10	7.21	0.72	10	5.29	0.53
32	28							5	4.49	0.90	5	3.33	0.67
33	0	40	36.2	0.91									
33	7	40	35.7	0.89	40	10.8	0.27						
33	14	40	82.3	2.06	40	34.3	0.86	10	8.81	0.88	10	4.25	0.43
33	28							10	9.53	0.95	10	3.37	0.34
38	0	100	86.8	0.87	100	25.6	0.26						
38	7	100	87	0.87	100	60.6	0.61	6.25	6.17	0.99	6.25	1.05	0.17
38	14	6.25	6.4	1.02									

APPENDIX F: 28-DAYTOXICITY TESTS PRELIMINARY ESTIMATES OF LT
Regressions of Cd/CdT vs Cd WITH R^2>0.5

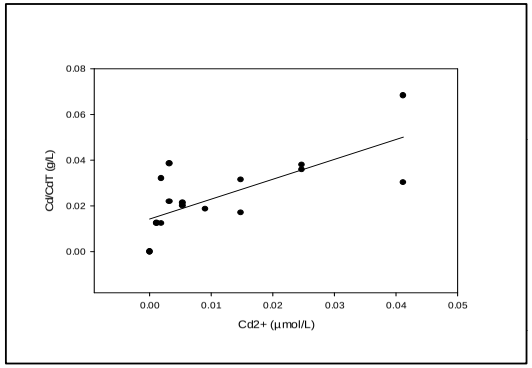
Test 1

cd	cd/cdt:	
Coefficients:		
b[0]	0.013303	LT
b[1]	1.533799	0.651975758
r ²	0.712154	

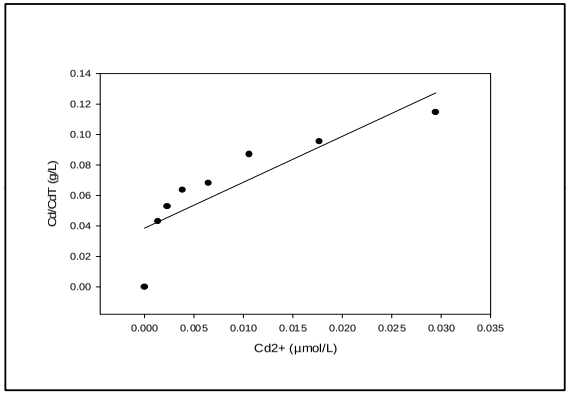


Test 2

cd	cd/cdt:	
Coefficients:		
b[0]	0.014284931	LT
b[1]	0.869620529	1.149926855
r ²	0.527234694	

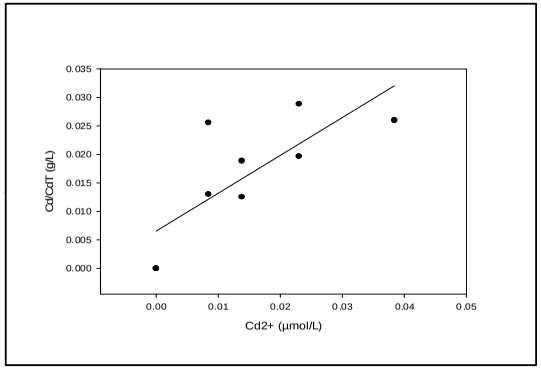


cd	cd/cdt:	
Coefficients:		
b[0]	0.038607	LT
b[1]	3.011708	0.332037459
r ²	0.73988	



Test 6

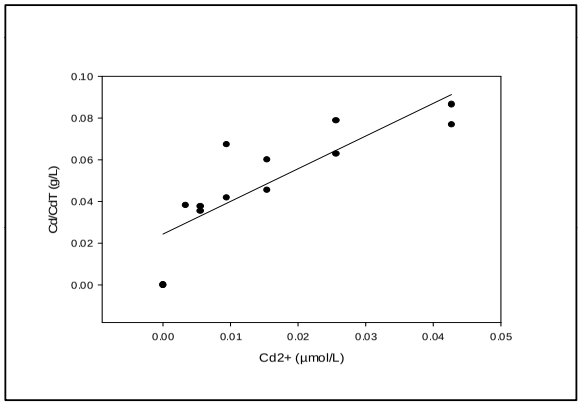
cd	cd/cdt:	
Coefficients:		
b[0]	6.53E-03	LT
b[1]	0.66399621	1.506032692
r ²	0.585996252	



Test 10

Coefficients:

b[0]	0.024385	LT
b[1]	1.564937	0.639003256
r ²	0.702904	

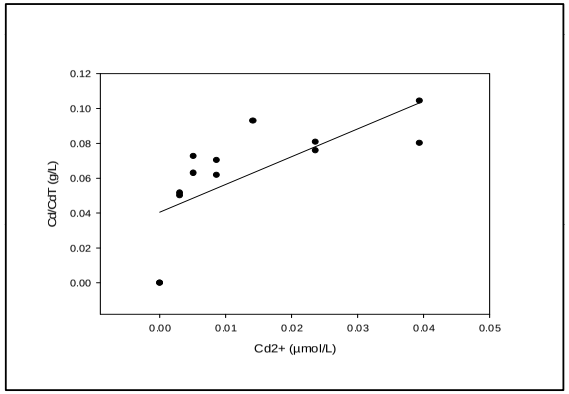


Test 11

cd cd/cdt:

Coefficients:

b[0]	0.040494219	LT
b[1]	1.593678133	0.627479275
r ²	0.500013613	

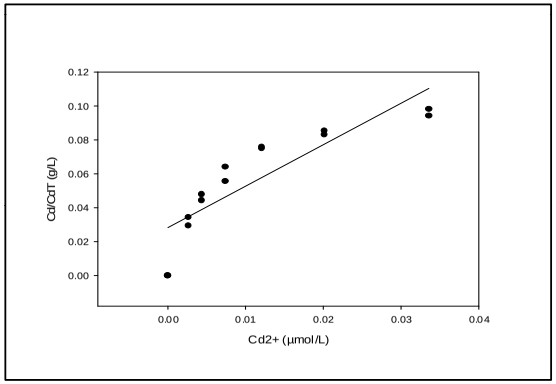


Test 12

cd cd/cdt:

Coefficients:

b[0]	0.028264	LT
b[1]	2.444669	0.409053389
r ²	0.755072	

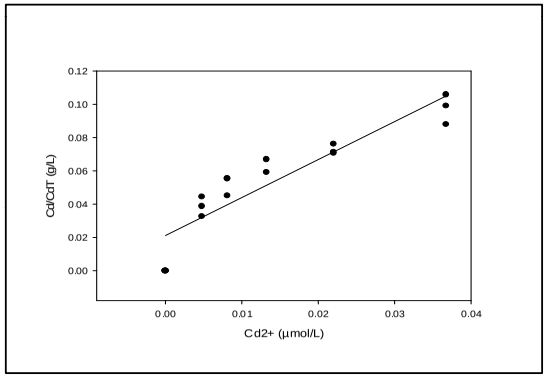


Test 13

cd cd/cdt:

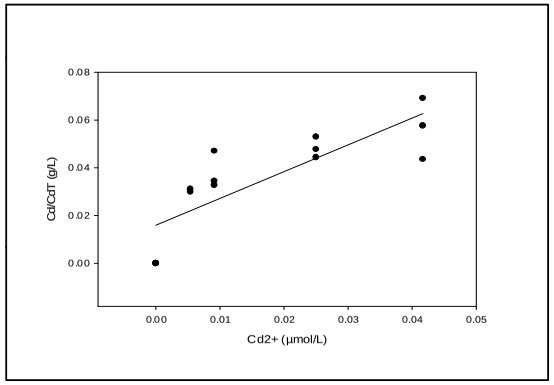
Coefficients:

b[0]	0.021084709	LT
b[1]	2.285065012	0.43762431
r ²	0.837340742	



Test 14

cd	cd/cdt:	
Coefficients:		
b[0]	0.015946	LT
b[1]	1.122841	0.890597952
r ²	0.679565	



Test 50

cd	cd/cdt:	
Coefficients:		
b[0]	0.057866432	LT
b[1]	4.997130744	0.200114836
r ²	0.647262331