FINAL

REVIEW OF DIETARY NICKEL TOXICITY AND BIOACCUMULATION: IMPLICATIONS FOR SEDIMENT PREDICTED NO EFFECT CONCENTRATIONS

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1 INTRODUCTION

The environment section of the EU Risk Assessment of Nickel performed under the Existing Substances Regulation (EEC 793/93) concluded that further sediment toxicity testing with Ni was required to support the development of risk characterization conclusions (ECB 2008). Consequently, a toxicity testing program was initiated to develop a sediment-based predicted no effect concentration (PNECsed) for Ni. Nickel toxicity in sediment is being conducted for nine benthic invertebrate species and two sediment types, which will be used to develop the PNECsed for Ni, as well as an integrated equilibrium partitioning (EqP)-based bioavailability model.

The nine benthic invertebrates being tested are the amphipods *Hyalella azteca* and *Gammarus pseudolimnaeus*, the midges *Chironomus dilutus* and *Chironomus riparius*, the oligochaetes *Lumbriculus variegatus* and *Tubifex tubifex*, the mussel *Lampsilis siliquoidea*, the mayfly *Hexagenia* spp., and the nematode *Caenorhabditis elegans*. The sediment toxicity tests are generally being conducted following standard operating procedures (e.g., ASTM, USEPA, OECD, Environment Canada), with various modifications based on preliminary studies that are intended to improve testing performance. For example, initial life stages or test water volumes are being adjusted for some species. The feeding regime for most of the species is addition of either yeast-Cerophyll®-trout chow (YCT) or TetraFin® to the overlying water, or the addition of mixed algae for the mussel *L. siliquoidea* or bacteria for the nematode *C. elegans*. Because the study designs include frequent overlying water changes to minimize Ni uptake from overlying water via leaching from sediment, there is limited opportunity for food to accumulate Ni and dietary Ni exposure (from added food) to test organisms, therefore, is likely minimal.

The importance of the diet as an exposure route for chronic metal toxicity to aquatic invertebrates was demonstrated by Hook and Fisher (2001a,b) and has since received increasing study (De Schamphelaere and Janssen 2004; De Schamphelaere et al. 2004, 2007; Bielmeyer et al. 2006; Kolts et al. 2009). As noted by Simpson and Batley (2007), the contribution of food and sediment ingestion pathways is the most contentious area with regard to metal toxicity in sediments. The USEPA, in developing equilibrium partitioning sediment benchmarks (ESBs) for sediment mixtures, noted that the ESBs “are not designed to protect aquatic systems from metals release associated, for example, with sediment suspension, or the transport of metals into the food web from either sediment ingestion or ingestion of contaminated benthos (USEPA 2005).” Methodologies for sediment quality guideline (SQG)

development that quantitatively account for combined exposures to dissolved metal in porewater and particulate metal in food/sediment are in development. For example, Simpson (2005) described an exposure-effects model (EEM) for copper (Cu) that was developed to predict the effect of sediment-water partitioning (K_d) and Cu assimilation from ingested solids on toxicity to benthos, which was then used to evaluate how these factors may influence the derivation of sediment quality guidelines (SQGs). Although the approach holds promise, the author noted that improved mechanistic models of exposure, which are influenced by organism physiology and sediment properties, are needed to predict toxic effects in sediments. Simpson and Batley (2007) also noted that for some organisms metals assimilated from dissolved and particulate uptake pathways will cause different magnitudes of toxic effects.

It has been questioned whether the Ni PNECsed ultimately derived from the sediment toxicity program will be adequately conservative for application to the field if the tests do not explicitly account for the dietary exposure pathway. As noted above, the sediment toxicity tests are all being conducted in Ni-spiked sediments with frequent changes of overlying water. Any food related Ni exposures will be incidental and the dietary exposure likely minimal. For comparison to the current sediment toxicity program, there are no sediment toxicity studies with Ni that have explicitly evaluated sediment-only exposures, or exposures to sediment and food, in which overlying water exposures are minimal. Studies on sediment Ni toxicity to *H. azteca* (Borgmann et al. 2001a) and *L. variegatus* (Vandegehuchte et al. 2007), for example, were static or static-renewal tests in which the exposure from Ni in overlying water could not be differentiated from particulate or porewater exposures.

To evaluate the potential importance of dietary Ni to aquatic invertebrates, the objective of this review was to first compile data on the relative toxicity of Ni via dietary exposures relative to water-only or sediment-only exposures. Studies that evaluated Ni toxicity in water or sediment with and without simultaneous dietary Ni exposures were targeted. An important consideration was the level of waterborne or sediment Ni concentration that could result in the dietary Ni concentration tested, and how these levels related to direct toxicity from water or sediment (for example, are dietary Ni concentrations that result in toxicity only observed at high waterborne and sediment concentrations already sufficiently high to result in toxicity?). Finally, data were also compiled from studies that evaluated the relative contribution of dietary and waterborne/sediment exposures to Ni bioaccumulation in aquatic invertebrates, as these data may provide an indication of whether dietary Ni is toxicologically important for invertebrate species.

2 METHODS

The scientific literature was searched for studies on dietary Ni toxicity to aquatic invertebrates. Of particular interest were studies in which organisms were exposed to dietary Ni as well as waterborne or sediment Ni. Ideally, studies would consist of environmentally relevant combined exposures (e.g., the test organism being exposed to the same waterborne Ni concentration as its food). This would provide useful information on the relative importance of the two exposure routes. In addition to dietary Ni toxicity data, the influence of dietary Ni on bioaccumulation in the test organism was of interest. Bioaccumulation data provides useful information on exposure potential from multiple exposure routes, but not necessarily toxicity.

In considering the relative importance of dietary and waterborne metal exposures it is also important that the dietary exposure concentrations are relevant to the corresponding metal concentration in the water and/or sediment. Ideally, the dietary metal should be derived from the same waterborne and/or sediment concentration to which the test organism being exposed. This helps ensure the environmental relevance of the combined exposure pathway. This also has important implications as to whether the dietary metal concentration will be at a toxicologically significant level, which is expected to vary depending on food (i.e., prey) type. For a metal with a high bioaccumulation potential in a given food, the importance of the dietary exposure route may be higher at lower water and/or sediment levels. An essential metal that is actively taken up at low exposure levels may thus be relatively more important in the food of consumer organisms (e.g., the situation with selenium).

3 RESULTS AND DISCUSSION

3.1 Toxicity of Ni from Dietary Exposures

Studies on dietary Ni toxicity to invertebrates is limited. Meyer et al. (2005), for example, summarized the availability of data on the effects of dietary metals on the physiological responses, survival, growth, and reproduction in aquatic organisms, but no toxicity data were identified for dietary Ni. No dietary Ni toxicity studies were identified for benthic invertebrates in the current review; however, two studies were identified for zooplankton, which are summarized below.

Bielmeyer et al. (2006) exposed the diatom (*Thalassiosira pseudonana*) to Ni (and, separately, Ag, Cu, and Zn), and then fed the diatom to the marine copepod *Acartia tonsa* for 7 days. Copepod survival and reproduction were measured during the exposure period. The dietary EC20 for Ni, based on reduced reproduction, was 15.3 µg/g dry wt. (the NOEC and LOEC were 23.4 and 58.1 µg/g dry wt., respectively) (Figure 1). It should be noted that the EC20 value was extrapolated below the NOEC, the lowest Ni concentration tested (although mean reproduction declined by approximately 30% in the lowest Ni treatment relative to the control, there was sufficient variability in the response, particularly in the controls, that the reduction was statistically insignificant [$p<0.05$]). The reproductive EC20 values for copepods, based on the initial waterborne metal concentration to which diatoms were initially exposed, were 2.4, 0.64, 1.2, and 0.3 µg/L for Ni, Ag, Cu, and Zn, respectively. All of these EC20s are below their respective saltwater chronic criteria recommended by the USEPA. For Ni, the EC20 was approximately 71% lower than the chronic criterion. The Ni NOEC and LOEC, based on the waterborne Ni concentrations to which diatoms were exposed, were 3.82 and 7.60 µg/L, respectively (Figure 1). Waterborne Ni concentrations in the copepod exposure media were below the detection limit (<0.10 µg/L). Accordingly, chronic Ni criteria do not appear to be adequately protective of dietary toxicity to *A. tonsa* under this exposure scenario. The authors did note, however, that the environmental realism of the study may be uncertain due to feeding of mono-algal diets to copepods and the adequacy of essential metal levels, which may have resulted in unrealistic metal accumulation by the algae and reduced nutritional value. The authors also noted that tests were performed in water with low DOC concentrations and using soluble metal forms, which optimizes Ni bioavailability.

Evens et al. (2009) exposed algae (*Pseudokirchneriella subcapitata*) to Ni concentrations ranging from 230.5 to 3,484 µg/L and fed the algae to *D. magna* in a 21-d chronic study. The NOEC and LOEC for dietary Ni effects on growth and reproduction resulted from algae that were exposed to 462.9 and 900.4 µg/L, respectively (the dietary Ni NOEC and LOEC were 68.5 and 85.6 µg/g dry wt., respectively) (Figure 2). For comparison, Ni HC5s for representative natural waters in Europe range from 7.1-43.6 µg/L (ECB 2008). Pane et al. (2004) found that the Ni NOEC and LOEC for survival, growth, and reproduction were 42 and 85 µg/L, respectively (at a hardness of approximately 45 mg/L). Accordingly, the waterborne Ni concentration necessary to achieve a dietary Ni concentration chronically toxic to *D. magna* was above the HC5 values for developing the aquatic PNEC. This suggests that dietary Ni toxicity to *D. magna* is not significant at threshold levels for waterborne Ni toxicity.

The dietary NOECs and LOECs for *A. tonsa* and *D. magna* are reasonably similar, being 23.4 and 58.1 µg/g dry wt. for *A. tonsa* and 68.5 and 85.6 µg/g dry wt. for *D. magna* (the relationships between dietary Ni and reproductive impairment are compared in Figure 3a). An important distinction between the two studies is the difference in bioconcentration factors (BCFs) for Ni uptake by the algal food, with a much lower waterborne Ni concentration resulting in dietary toxicity to *A. tonsa* than required for *D. magna* (Figure 3b). This demonstrates that in the field, therefore, whether Ni concentrations reach toxic levels in the diets of invertebrates depends not only on the sensitivity of the exposed species, but also the bioaccumulation potential in food items. Evens et al. (2010) also demonstrated that the nutritional quality of the food influence dietary Ni toxicity to *D. magna*, which places the causality of the effects observed in the Evens et al. (2009) study into question. That is, Evens et al. (2010) suggest that the observed effects may be a function of nutritional quality of the food, and not of Ni dietary exposure.

3.2 Bioaccumulation of Ni from Dietary Exposures

For many aquatic invertebrates the dietary exposure pathway accounts for the a major proportion of total metal bioaccumulation (Reinfelder et al. 1998). More aquatic invertebrates studies have evaluated dietary Ni bioaccumulation than dietary Ni toxicity. These are summarized below.

- **Amphipod (*Hyalella azteca*)**—Borgmann et al. (2007) placed *H. azteca* at three sites in each of two rivers for 17 days. Organisms were fed plant and detrital material collected from the same site or other sites. Cd, Cu, and Se were the only metals with a significant relationship between metal concentration in *H. azteca* and their food, while the diet had no significant effect on the concentrations of Ag, As, Bi, Sb, U, and Zn. For remaining metals, including Ni, concentrations in food varied less than four-fold, which made it difficult to determine whether these metals in *H. azteca* were bioaccumulated from food.
- **Oligochaete (*Tubifex tubifex*)**—Gillis et al. (2004) exposed the oligochaete worm *T. tubifex* to Ni in sediment (and, separately, to Cd and Pb). *T. tubifex* were exposed to a field-collected sediment with a spiked Ni concentration of 1.24 µmol/g dry wt. (72.8 µg/g dry wt.) for six weeks. The exposure system was a beaker containing 100 mL of sediment, 150 mL of water, and 80 mg of crushed Nutrafin^{TM}. Thus, test organisms were

not explicitly exposed to dietary Ni, but the added food may have accumulated Ni from the test beaker. The Ni concentration in tissue peaked after 12 hours (whereas Cd and Pb concentrations in tissue increased throughout the exposure period). After the six-week exposure organisms were then transferred to either clean water or sediment to evaluate depuration. After nine hours in water, Ni concentrations declined significantly ($p<0.05$), suggesting that the majority of the Ni was associated with the gut content (neither Cd nor Pb concentrations declined significantly during this time period). Likewise, after 16 hours of depuration in clean sediment tissue Ni concentrations were significantly ($p<0.05$) reduced, and after one week of depuration Ni concentrations in *T. tubifex* were no longer significantly elevated above the pre-exposure concentration. The limited absorption of Ni into *T. tubifex* tissues, based on the majority of the Ni being associated with the gut contents, indicates that dietary Ni is not an important exposure pathway for Ni toxicity to this organism.

- **Cladoceran (*Daphnia magna*)—**Watras et al. (1985) exposed *D. magna* to aqueous Ni alone or to a combination of aqueous and dietary Ni (algae were exposed to the same aqueous Ni concentration) in a 13 day exposure. They found that approximately 95% of bioaccumulated Ni in *D. magna* was via the aqueous exposure route. Komjarova and Blust (2009) similarly found that after a four day combined exposure to aqueous (15.7 µg Ni^{2+}/L) and dietary Ni (~40 µg/g dry wt. total Ni or ~12 µg/g dry wt. internal algae) that 100% of the bioaccumulated Ni in *D. magna* was from the aqueous exposure route. The assimilation efficiency (AE) for Ni was essentially 0% after the 4-d exposure, compared to 6%, 32%, 61%, and 81% for Pb, Zn, Cu, and Cd, respectively. Consistent with these data, Evens et al. (2009), following 21-d exposures to dietary Ni concentrations of 33.7 and 68.5 µg/g (internal Ni concentrations in algae) similarly resulted in non-detectable Ni levels (<4.0 µg/g dry wt.) in *D. magna*.

- **Copepods (*Calanus sinicus* and *Labidocera euchaeta*)—**Wang et al. (2007) evaluated the influence of nitrate and phosphate on Ni uptake by phytoplankton (*Prorocentrum donghaiense* and *Skeletonema costatum*) and trophic transfer to the marine copepods *C. sinicus* and *L. euchaeta*. The Ni AEs varied between copepod species and phytoplankton diets, and increased with increasing nutrient addition. AEs ranged from 9.7 to 32.7% at the lowest nutrient levels and from 28.0 to 48.4% at the highest nutrient levels. Thus, reported Ni AEs for these copepod species are higher than those reported for the cladoceran *D. magna.*

- **Phantom Midge (*Chaoborus flavicans*)—**Ponton and Hare (2010) fed phantom midges (*C. flavicans*) Ni-contaminated *D. magna*. The mean (±SD) Ni AE was 14.0±12.5%. According to the authors, the variability in AEs (4-56%) was due to differences in ingestion rates (i.e., midges consuming a lot of prey had lower AEs than midges consuming less prey). It is thought that consumption of fewer prey have a slower gut passage time and a likely more efficient assimilation of Ni). Ponton and Hare (2010) estimated that the relative importance of waterborne and dietary Ni in *C. flavicans* is approximately 2/3 and 1/3, respectively, although this would presumably vary with AE. The Ni AEs measured in *C. flavicans*, therefore, are within the range of those summarized for copepods in the preceding bullet.

- **Alderfly (*Sialis velata*)**—Dumas and Hare (2008) exposed the midge *C. riparius* and oligochaete worm *T. tubifex* to Ni-spiked sediment for two weeks and then placed the organisms in clean sediment for 24 hours to depurate their gut contents. The midges and worms were then fed to the alderfly *Sialis velata*. The sediments contained a Ni concentration of 16 µmol/g dry wt. (939 µg/g dry wt.), which resulted in steady-state Ni concentrations of approximately 0.8 and 0.3 µmol/g dry wt. in midges and worms, respectively (or 47 and 18 µg/g dry wt., respectively). The Ni biota-sediment accumulation factors (BSAFs), therefore, were approximately 0.05 and 0.02 for midges and worms, respectively. The Ni AEs for alderflies fed midges and worms were 48% and 83%, respectively. Thus, much more Ni was assimilated by alderflies compared to *D. magna*, for example, which had a Ni AE of approximately 0%, and the phantom midge, which had a mean Ni AE of 14%. The authors noted that because the alderfly assimilated a large proportion of dietary Ni, it can readily be transferred along aquatic food chains and that dietary Ni can be an important exposure route for aquatic animals. However, it should be emphasized that the sediment concentration of 939 µg/g dry wt. is high relative to existing bulk sediment guidelines. MacDonald et al. (2000), for example, derived a probable effects concentration (PEC) of 48.6 µg/g dry wt. It is uncertain whether dietary uptake of Ni would be toxicologically significant at sediment concentrations closer to sediment threshold levels.
- **Snail (*Lymnaea stagnalis*)**—Croteau and Luoma (2008) exposed lettuce to Ni (and, separately, Cd and Cu) and then fed the lettuce to the snail *L. stagnalis* for 18 hours. The AE for Ni was high (95%), but trophic transfer factors TTFs were low (approximately 0.1). Although toxicity was not explicitly evaluated in the study, the food ingestion rate declined from 0.182 g/g/d at low dietary Ni exposures to 0.105 g/g/d at high dietary Ni exposures. The authors noted that impaired feeding is a typical response reflecting dietary metals stress. Ball et al. (2006) similarly found that dietary Cd affected reproduction in *Hyalella azteca*, which corresponded with reduced feeding.

Overall, Ni bioaccumulation potential from the diet is highly variable between aquatic invertebrates. In the cladoceran *D. magna* and the oligochaete *T. tubifex*, Ni uptake via the gut is limited. However, dietary Ni AEs averaged 14% in the phantom midge, 27% in marine copepods, 48 and 83% in alderflies fed midges and worms, respectively, and 95% in a snail. Variables such as the nutrient quality of the diet and gut passage time were shown to influence Ni AEs within individual species.

3.3 Evaluation of Dietary Ni Exposures at Threshold Levels in Sediment

The primary objective of this evaluation was to evaluate whether a sediment PNEC for Ni based on sediment toxicity test exposures that did not explicitly include exposure to dietary Ni will be adequately protective of potential dietary Ni toxicity. Because the importance of dietary Ni exposure in sediment is, in part, related to the Ni concentration in sediment, it is important to understand the contribution of dietary Ni associated with Ni threshold levels in sediment. The sediment PNEC for Ni is still in development, but existing bulk sediment guidelines for Ni may provide an indication of the relative magnitude of a Ni threshold in sediment. MacDonald et al. (2000), for example, derived a consensus-based threshold effect concentration (TEC) of 22.7

µg/g dry wt. and probable effect concentration (PEC) of 48.6 µg/g dry wt. The bioavailability of Ni in sediments varies depending on site-specific factors such as the acid volatile sulfide (AVS) concentration and the fraction of organic carbon (Di Toro et al. 2005). Further, De Jonge et al. (2010) demonstrated that the bioaccumulation of Ni and other metals in benthic and epibenthic macroinvertebrates is highly dependent on species-specific feeding behavior and ecology. Accordingly, although the relationship between AVS and SEM concentrations has an important influence on certain divalent metal concentration in porewater and uptake via respiration, AVS-bound metals may still be bioavailable to benthos that feed on sediment because metal bioavailability may be modified in the gut of the invertebrate (De Jonge et al. 2010). Further, Griscom et al. (2000) demonstrated that some bivalves can assimilate metals from sulfides and Lee et al. (2000) found that certain bivalves and polychaetes bioaccumulated Ni and other metals from sediments even when SEM<<AVS, which the authors suggested was best explained if dietary uptake from ingested sediments was the primary route of exposure.

As a cursory evaluation, Ni concentrations in potential food items were estimated from the TEC of 22.7 µg/g dry wt. recommended in MacDonald et al. (2000). Nickel concentrations in food items were estimated using BSAFs, which were derived from the Ni concentration in an organism to the Ni concentration in the sediment to which it was exposed (both concentrations on a dry weight basis). Nickel BSAFs are expected to be variable between sediment types due to differences in bioavailability and between species due to differences in feeding strategy and internal detoxification mechanisms (e.g. storage, excretion). However, from field studies, Ni BSAFs in benthic and epibenthic macroinvertebrates are generally below one. For example, ORNL (1998) compiled BSAFs for Ni from field studies with oligochaetes (e.g., *L. variegatus*, *Limnodrilus hoffmeistri*, *T. tubifex*), dragonflies (multiple species), and composites of multiple benthic invertebrate taxa, and derived mean BSAFs of 0.129 in depurated invertebrates and 1.313 in non-depurated invertebrates (thereby demonstrating that a large proportion of the sediment Ni was not assimilated by the invertebrates). Applying the mean BSAF of 0.129 (gut-depurated organisms) to the TEC of 22.7 µg/g dry wt. results in an estimated invertebrate Ni concentration of 2.9 µg/g dry wt. If this concentration is considered representative of "dietary Ni" for other benthic or epibenthic organisms, this concentration is below the dietary Ni NOEC and LOEC of 23.4 and 58.1 µg/g dry wt. for *A. tonsa* and 68.5 and 85.6 µg/g dry wt. for *D. magna*. In other words, at a "threshold" Ni concentration in sediment, the Ni concentration estimated in food using a BSAF is less than dietary Ni NOECs available for *A. tonsa* and *D. magna*. Alternatively, a critical Ni concentration in sediment can be derived based on the dietary NOEC of 23.4 µg/g dry wt. and the BSAF of 0.129, which results in 181 µg/g dry wt. (23.4 µg/g dry wt. / 0.129). This critical Ni concentration in sediment is greater than bulk sediment TEC, indicating that the TEC is protective of dietary exposure.

3.4 Characterizing Potential Ni Risks in Sediment and Surface Water

Although sediment and surface water are typically analyzed as separate lines of evidence in ecological risk assessment, it is important to consider the partitioning of Ni between sediment particles, porewater, and overlying water. Given that Ni BSAFs from field studies are generally low (i.e., mean of 0.129 in depurated organisms [ORNL 1998]), which appears to at least be in part due to poor assimilation efficiency due to BSAFs that were approximately an order of magnitude higher in non-depurated organisms, it appears that Ni is not highly bioavailable

relative to bulk sediment concentrations, regardless of whether food was included in the exposure or not (the field data would implicitly include dietary exposures). This is consistent with some of the Ni bioaccumulation studies summarized above. *T. tubifex*, for example, assimilated a small proportion of the Ni associated with sediment in the gut (Gillis et al. 2004).

The sediment toxicity studies being conducted with Ni to develop a PNECsed include frequent water changes to minimize exposures of test organisms to Ni in overlying water. Sediment studies conducted to-date with Ni have been static tests, which can result in very high Ni concentrations in overlying water. Borgmann et al. (2001a) used Imhoff settling cones with a water:sediment ratio of 67:1, which resulted in a more constant overlying water quality than in tests conducted in beakers with a water:sediment ratio of 4:1. However, although the cones resulted in more constant water quality, Ni concentrations still increased in overlying water with increasing sediment Ni concentrations. The mean water-based LC50 value for *H. azteca* exposed to Ni in cones was 610 nmol/L (or 35.8 µg/L). The authors found that Ni in overlying water was a reliable predictor of Ni toxicity. Borgmann et al. (2001b) found that metal toxicity to amphipods exposed to field-collected sediments with varying levels of metals, and in which Ni was identified as the primary cause of toxicity, was similar in amphipods exposed to overlying water in cages placed above the sediment as in amphipods exposed directly to sediment.

Vandegehuchte et al. (2007) hypothesized that Ni^{2+} in porewater determines the chronic toxicity of Ni in sediments to the oligochaete *Lumbriculus variegates*. Toxicity testing was conducted in two natural sediments. The sediments were spiked with $NiCl_2$, which resulted in measured Ni concentrations ranging from 127 to 3,847 µg/g dry wt. between both sediments. The sediments were then allowed to equilibrate for 70 days. Adult *L. variegates* were exposed to the sediments for 28 days. The exposure system was a jar containing 400 g wet sediment and 250 mL overlying water, with 60-70 percent of the overlying water renewed twice per week. Organisms were fed ground TetraMin® fish flakes at a rate of 200 µg per organism per day. Ni concentrations in overlying water increased with increasing sediment concentration and ranged from a mean of 5 µg/L in control treatments to a mean of 2,559 µg/L in the highest treatment. The authors found that $[SEM\text{-}AVS]f_{OC}$ at the surface (top 1 cm) was a good estimator of toxicity (*L. variegatus* biomass), but that overlying water Ni^{2+} was an equally good predictor. Thus, the authors were not able to determine the relative importance of the overlying water and porewater exposure routes on Ni toxicity to *L. variegatus*.

Bessom (2008) hypothesized that mobilization of Ni from sediment is a major source to overlying water, and thus periphyton and macroinvertebrates. In addition, Bessom (2008) hypothesized that periphyton, rather than waterborne Ni, is the primary source of Ni to *D. magna* and *H. azteca*. *D. magna* and *H. azteca* were exposed to low and high Ni concentrations in water and periphyton using a 2×2 factorial design. Low and high waterborne Ni concentrations were 3 and 200 µg/L and low and high Ni concentrations in periphyton were 64 and 400 µg/g dry wt. The periphyton with a low Ni concentration were collected directly from a local creek, while the periphyton with a high Ni concentration were collected from the same source, but exposed to 200 µg/L for 96 hours. Ni exposures by *D. magna* and *H. azteca* were 48 hours. Ni concentrations in *D. magna* were highest in organisms exposed to high Ni in water, with Ni uptake being negligibly higher in the treatment with low Ni in water and high Ni in

periphyton. A similar patterns was observed for *H. azteca*, where Ni in periphyton insignificantly increased Ni concentrations in *H. azteca*.

It appears that, from a toxicological or risk-based perspective, sediments as a source of Ni to overlying water is the critical pathway for Ni exposures. This appears to be particularly true for epibenthic invertebrates, such as *H. azteca*. This also suggests that dietary exposures by deposit-feeding benthos, such as oligochaetes, may not be important at threshold levels in sediment. Accordingly, the water PNEC used to assess Ni in water will integrate the contribution of sediment Ni along with other sources (e.g., direct inputs from discharges).

The question of whether dietary Ni exposures is important in estimating potential risk is then equally important when considering the protectiveness of the waterborne PNEC. As summarized above, the dietary Ni toxicity data for the marine copepod *A. tonsa* suggests that chronic waterborne Ni criteria may not be protective of reproductive effects, while data for the freshwater cladoceran *D. magna* suggests that chronic waterborne Ni criteria are protective. An important factor is variability in the bioaccumulation potential of Ni from water to food items. The Ni BCFs for the diatoms used as the diet in the *A. tonsa* study (Bielmeyer et al. 2006) were more than an order of magnitude higher than the Ni BCFs for the algae used as the diet in the *D. magna* study (Evens et al. 2009). Clearly, the importance of dietary Ni exposure on toxicity to aquatic invertebrates may vary depending on site-specific Ni bioaccumulation potential.

4 REVIEW OF PRELIMINARY SEDIMENT TOXCITY DATA AND FUTURE RESEARCH CONSIDERATIONS

The sediment Ni toxicity testing completed to-date indicates that *Hexagenia* sp. biomass is the most sensitive species and endpoint to Ni in sediment and will, therefore, strongly influence the sediment PNEC for Ni. Based on Ni spiked into eight different field-collected sediments with varying levels of AVS and total organic carbon (TOC), the concentration-response relationships between total recoverable Ni in sediment and *Hexagenia* biomass substantially overlapped (EC50s ranged from 593 to 4215 µg/g dry wt. across all eight sediments, but varied by less than a factor of two in seven of the eight sediments) (Besser et al. 2010a). In contrast, relationships between *Hexagenia* biomass versus SEM(Ni)-AVS and biomass versus porewater Ni were more variable between sediments. Accordingly, it was initially hypothesized that Ni exposure to *Hexagenia* may be dominated by ingestion (Besser et al. 2010a).

Nickel toxicity in the two sediments tested in Task 2 of the project, expressed as SEM(Ni)-EC20 values for biomass, were higher (i.e., less toxic) than in the six sediments tested in Task 3. In a follow-up evaluation of the sediment toxicity data, Besser et al. (2010b) provided a hypothesis as to why this occurred, suggesting it was due to differences in the study design that influenced rates of oxidation of AVS. If the hypothesis is accepted and the Task 3 sediment toxicity results are considered separately from the Task 2 results, the SEM(Ni)-EC20 values from Task 3 are found to increase with increasing AVS concentrations. This suggests that increased AVS is indeed reducing the bioavailability of Ni to *Hexagenia*. However, the data also suggest that dietary Ni may have been a toxicologically relevant exposure pathway when AVS is in excess of SEM(Ni). For example, in five of the six sediments tested, the 20% reduction in biomass occurred when the difference in SEM(Ni)-AVS was negative, i.e., AVS was in excess. This indicates that ingestion of AVS-bound Ni may have been responsible for the observed toxicity.

However, the relative importance of water and particulate Ni exposures is still uncertain between sediments with varying conditions, including differences in SEM and AVS, as well as in other modifying factors, such as total organic carbon. It is quite likely that ingestion of particulate Ni is more relevant for lower levels of effects, while exposure to waterborne Ni results in higher levels of effects. Differences in toxicity mechanisms following these two routes of exposure may result in different levels of sensitivity, that is, it requires a lot more nickel to cause an effect via the oral route than by gill exposure. When AVS is in excess and there is little to no gill exposure, the lower level (EC20) effects of diet are evident, but when SEM(Ni) is available in porewater, gill exposure predominates and greater responses (>EC20) are seen. This is seen in Test 3 where the SEM(Ni)-AVS difference associated with a 20% reduction in biomass was negative in five of six sediments, yet higher levels of biomass reduction, (e.g., 50%) were observed when SEM(Ni) exceeded AVS.

Based on the available lines of evidence, it does appear that *Hexagenia* were exposed to Ni via ingestion of particles (possibly including AVS-bound Ni), and that increasing particulate Ni concentrations resulted in decreasing *Hexagenia* biomass (although the relative importance of particulate versus water Ni exposures to the observed toxicity is uncertain). In addition to the weak associations between toxicity and SEM(Ni)-AVS and porewater Ni, the reductions in *Hexagenia* biomass that were observed in some treatments with negative SEM(Ni)-AVS indicate that ingestion of particulate Ni was a toxicologically relevant exposure route. As summarized above, De Jonge et al. (2010) found that bioaccumulation of metals in benthic taxa was primarily influenced by total metal concentrations in the sediment (regardless of AVS), while metal bioaccumulation in epibenthic taxa was mostly explained by the more bioavailable metal fractions in both the sediment and the water. The results for *Hexagenia*, a benthic taxon, are consistent with the overall observations of De Jonge et al. (2010).

The objective of this review was to evaluate whether a Ni PNECsed derived from the sediment testing program was adequately protective of dietary Ni exposures. As stated above, the importance of dietary Ni exposure on toxicity to aquatic invertebrates may vary depending on site-specific Ni bioaccumulation potential into food items. It may also depend on uptake from incidentally ingested sediment particles. Therefore, if ingestion is indeed a relevant exposure pathway contributing to Ni toxicity in *Hexagenia*, the important questions are: (1) how does the toxicity of Ni vary between ingested sediment particles and potential food items (detritus, diatoms, algae) for the deposit-feeding *Hexagenia*; and (2) what is the rate and mass of Ni uptake from sediment to potential food items? In order to address these questions, we propose the following for consideration in developing a research program.

Empirical toxicity testing is necessary to evaluate whether dietary Ni would increase sediment Ni toxicity to *Hexagenia*. The dietary Ni concentration evaluated in each sediment treatment needs to be environmentally relevant (i.e., the dietary Ni concentrations should not be unrealistically high or unrealistically low relative to the Ni concentration in sediment). To achieve this, food items (e.g., diatoms, algae) would be grown on sediment spiked with a range of Ni concentrations and then allowed to equilibrate. The sediment could be from one or more of the field sources previously used. Toxicity testing would then be conducted using a 2×4 factorial design:

Sediment	Food
Clean	Clean
Clean	Nickel
Nickel	Clean
Nickel	Nickel

In the “clean” food experiments, *Hexagenia* would be fed as in the Task 2 and 3 experiments. The “nickel” treatments would include a series of Ni concentrations, selected based on the Task 2 and 3 test results. The toxicity data from each sediment/food combination would be statistically evaluated to determine whether dietary Ni resulted in significantly higher toxicity and, therefore, whether the PNECsed needs to be adjusted to account for dietary toxicity. Based on the above discussion regarding the Task 3 toxicity data, the basic study design proposed here should probably be expanded to include a range of SEM and AVS conditions.

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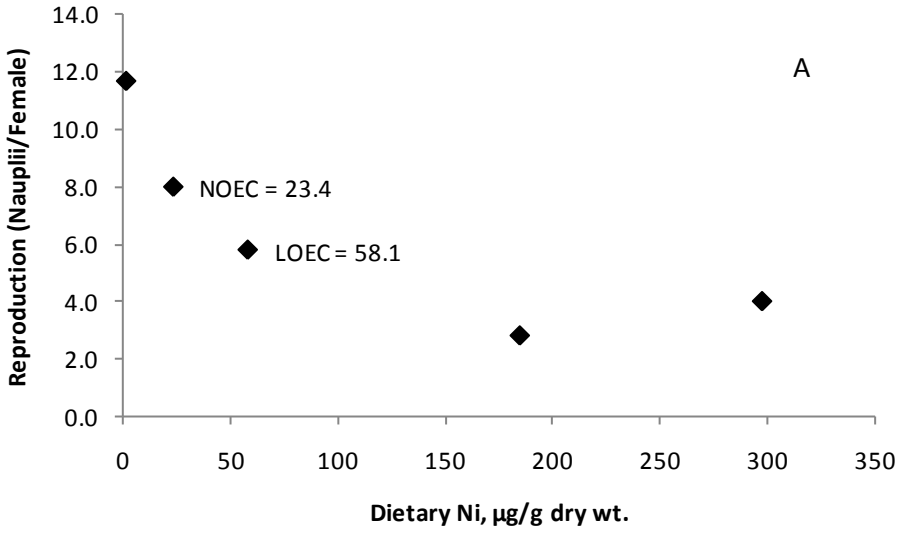
FIGURES

Figure 1. Relationship between (a) dietary Ni and (b) waterborne Ni (for algae exposure) and reproductive impairment in the copepod *Acartia tonsa*. Data from Bielmeyer et al. (2006).



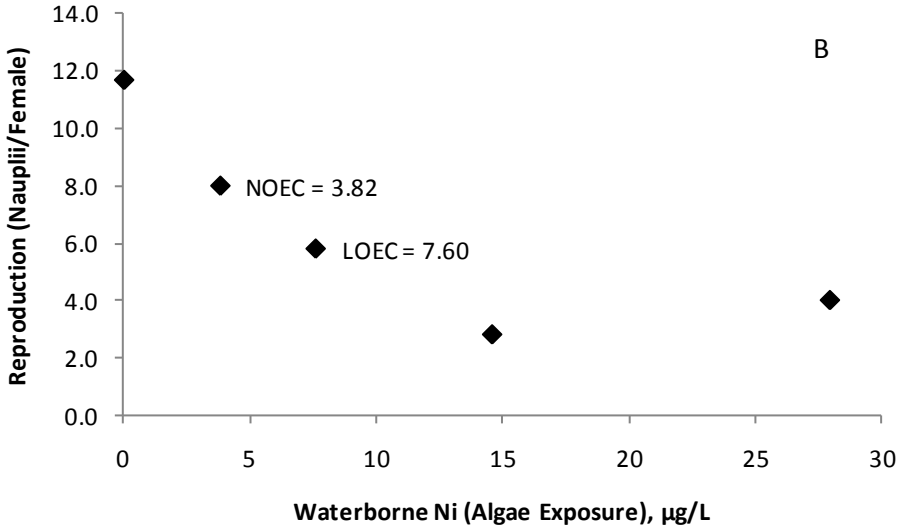
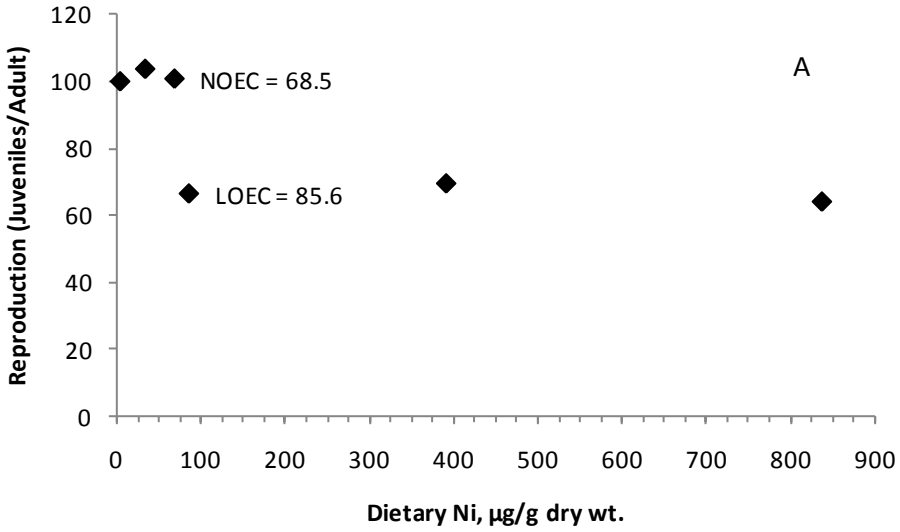


Figure 2. Relationship between (a) dietary Ni and (b) waterborne Ni (for algae exposure) and reproductive impairment in the cladoceran *Daphnia magna*. Data from Evens et al. (2009).



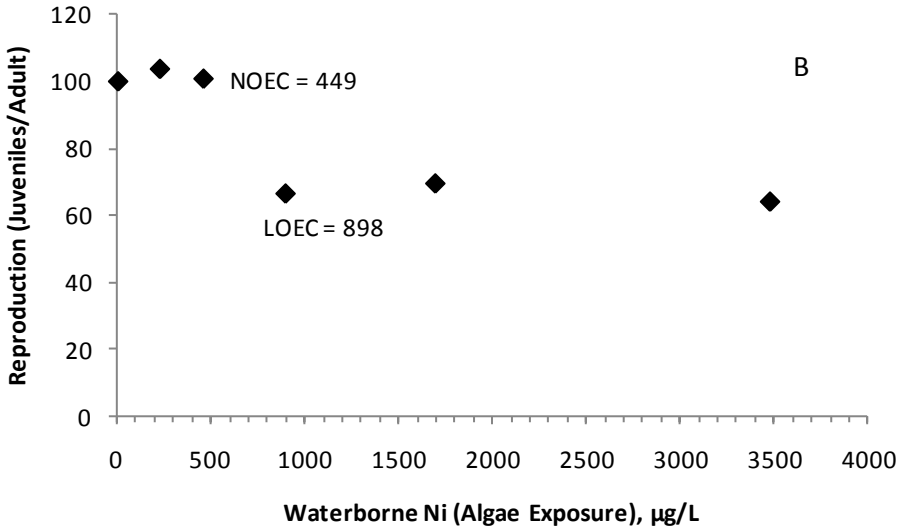


Figure 3. Comparison of (a) dietary Ni and (b) waterborne Ni (for algae exposure) versus reproductive impairment in the copepod *Acartia tonsa* (Bielmeyer et al. 2006) and the cladoceran *Daphnia magna* (Evens et al. 2009).