Species Sensitivity Distribution Evaluation for Chronic Nickel Toxicity to Marine Organisms

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ABSTRACT

In Europe, the European Union's Existing Substances Regulation (EEC 793/93), the REACH Regulation, and Water Framework Directive all share common guidance for conducting environmental effects assessments, which can be further used to derive predicted no effect concentrations (PNECs) and environmental quality standards (EQS) for chemical substances. To meet the criteria for using a species sensitivity distribution (SSD) in the effects assessment of Ni for marine organisms, chronic toxicity data from the published scientific literature were augmented with toxicity testing of several additional marine species including: a unicellular alga (*Dunalliela tertiolecta*), a diatom (*Skeletonema costatum*), 2 macroalgae (*Champia parvula*, *Macrocystis pyrifera*), 2 mollusks (*Crassostrea gigas*, *Mytilus galloprovincialis*), 2 echinoderms (*Dendraster excentricus*, *Strongylocentrotus purpuratus*), a polychaete (*Neanthes arenaceodentata*), and a fish (*Cyprinodon variegatus*). Based on this updated database, which includes chronic Ni toxicity data for a total of 17 marine species, HC5 values (hazardous concentrations to 5% of the species) were derived using an SSD. The most sensitive species is a tropical sea urchin from the Caribbean region, *Diadema antillarum*, which has an EC10 that is approximately 6-fold less than the EC10 for the second most sensitive species tested. There is some uncertainty in the representativeness of *D. antillarum* to temperate European marine waters because 1) a European sea urchin species (*Paracentrotus lividus*) is approximately 48-fold less sensitive to Ni, and (2) ambient marine Ni concentrations in at least some European waters closely approach the *D. antillarum* EC10. The HC5 values with and without *D. antillarum* included in the SSD are 3.9 and 20.9 μg/L, respectively. Site-specific toxicity testing with local species may be warranted for locations where Ni concentrations fall between the range in HC5s of 3.9 to 20.9 μg/L. *Integr Environ Assess Manag* 2013;9999:1–10. © 2013 SETAC

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INTRODUCTION

Nickel (Ni) was the subject of a recent comprehensive risk assessment that was conducted under the European Union's (EU) Existing Substances Regulation (EEC 793/93). The overall goal of the risk assessment was to determine if the ongoing production and use of Ni in Europe posed unacceptably high risks to occupational, consumer, and environmental receptors. The general approach for determining risk to environmental receptors was the comparison of predicted no effect concentrations (PNECs) to predicted exposure concentrations (PECs), where risk characterization ratios (PEC:PNEC) greater than 1 are concluded to show unacceptable risk. Other relevant regulations in the EU are REACH and the Water Framework Directive, under which Ni is considered a priority substance, and for which a single environmental quality standard (EQS) needs to be derived. All of these regulations share common guidance.

Among the endpoints addressed in the environmental risk assessment process was chronic Ni toxicity to marine organisms. Although the risk assessment guidance supporting the Existing Substances Risk Assessment process allows for the use of species sensitivity distributions (SSDs) for determining PNECs, the guidance for chronic marine endpoints is relatively vague relative to that for chronic freshwater endpoints. For freshwater systems, the EU Technical Guidance Document (TGD) (ECB 2003) recommends that high-quality chronic toxicity data be available for 10 to 15 species from 8 different taxonomic groups. As these groups include insects, which are not represented in marine systems, and cladocerans, which are rare in marine systems, the TGD guidance cannot be directly applied to marine systems. In general, however, the goal of between 10 to 15 species that cover a broad range of representative marine taxa should be considered consistent with the intent of the more specific freshwater guidance and should, therefore, be appropriate for the determination of a marine SSD.

At the time this effort was initiated, previous studies had developed high-quality chronic Ni toxicity data for 5 marine species including a fish, *Atherinops affinis* (Hunt et al. 2002); 2 crustaceans, *Mysidopsis bahia* (Gentile et al. 1982) and *Mysidopsis intii* (Hunt et al. 2002); an echinoderm, *Paracentrotus lividus* (Novelli et al. 2003), and a mollusk, *Haliotis*

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rufescens (Hunt et al. 2002). However, developing an SSD based on just these 5 species would not meet the recommended number of 10 to 15 species in the EU risk assessment guidance. In addition, the hazardous concentration for 5% of the species (i.e., the HC5) would be highly uncertain with such a small data set. More importantly, important marine taxa such as bivalves, marine algae, and annelids would not be represented.

The primary objective of this study was to augment the chronic Ni toxicity database for marine species to satisfy the criteria for using the SSD approach to recommend a marine Ni PNEC. Toxicity data were therefore generated for an additional 10 species of marine organisms, including a unicellular alga (*Dunalliela tertiolecta*), a diatom (*Skeletonema costatum*), 2 macroalgae (*Champia parvula* and *Macrocystis pyrifera*), 2 mollusks (*Crassostrea gigas* and *Mytilus galloprovincialis*), 2 echinoderms (*Dendraster excentricus* and *Strongylocentrotus purpuratus*), a polychaete (*Neanthes arenaceodentata*), and a fish (*Cyprinodon variegatus*).

A secondary objective of this study was to determine the effects of different sources of marine water on Ni toxicity. A number of studies have indicated that chronic Ni toxicity to freshwater fish (Deleebeeck et al. 2007), cladocerans (Deleebeeck et al. 2008), and algae (Deleebeeck et al. 2009) is influenced by dissolved organic carbon (DOC), Ca^{2+}, Mg^{2+}, and pH. Marine waters from 4 different United States coastal areas were used in toxicity tests with *S. costatum* and *M. galloprovincialis* to determine the potential variability in dissolved Ni toxicity as a function of seawater chemistry. Marine waters are expected to show relatively constant concentrations of Ca^{2+} and Mg^{2+} and levels of pH. Therefore, the focus of this comparison was on the influence of DOC on toxicity to *S. costatum* and *M. galloprovincialis*. These species were chosen because of their wide use in marine toxicity tests and their relative sensitivity to metals (Arnold 2005).

The final objective of this study was to aggregate the newly developed Ni toxicity data with the historical data, as well as recently published data from other studies, to determine a Ni HC5 for marine waters as the basis for determining a chronic Ni PNEC. This PNEC was compared with ambient Ni exposure data available for coastal European waters as an initial risk characterization.

MATERIALS AND METHODS

Published studies

Nickel toxicity studies with marine organisms were initially identified based on a review of the scientific literature, including the EU risk assessment report for Ni (ECB 2008). Acceptable Ni toxicity studies needed to meet minimum reliability requirements, including analytical verification of Ni concentrations in the test waters and use of standard approved test methods. Nickel EC10 values were used in the present evaluation, which were not reported by the original study authors (use of EC10 values for determining an HC5 value is consistent with the TGD (ECB 2003), but we note that exceedance of an EC10 does not in itself necessarily constitute "hazard"). For those studies cited in the ECB (2008), we used the EC10 values calculated for that report, which were based on raw data provided by the original study authors (i.e., Hunt et al. 2002; Novelli et al. 2003; Bielmyer et al. 2005). There were 2 additional studies with a mysid (*Mysidopsis bahia*, now reclassified under the genus *Americamysis* [Price et al. 1994; Lussier et al. 1999]) and a mussel (*Mytilus trossolus*), for which we calculated EC10 values using the US Environmental Protection Agency's (USEPA) Toxicity Relationship Analysis Program (TRAP, Version 1.21). The results of the Ni toxicity study with *A. bahia* were reported in both Gentile et al. (1982) and Lussier et al. (1985). The concentration-response data reported in Lussier et al. (1985) were used to calculate the Ni EC10. The Ni toxicity data for *M. trossolus* were published by Nadella et al. (2009), who provided the raw data for calculating the EC10. The concentration-response data and calculated Ni EC10s using TRAP are provided in Figures S1 and S2 in Supplemental Information.

Toxicity test methods for the present study

Chronic Ni toxicity studies with marine organisms were conducted to augment those identified in the scientific literature. All chronic Ni toxicity studies, both those previously published and those summarized below, were conducted using either $NiCl_2$ or $NiSO_4$, both of which are soluble Ni salts. The basic test methods and exposure conditions for these studies are summarized here.

Algae

Dunaliella tertiolecta. The Ni toxicity test with the green alga *D. tertiolecta* was conducted at the Parametrix Environmental Research Laboratory (PERL, Corvallis, OR). Algae were exposed to Ni (as $NiCl_2 \times 6H_2O$) in a static 72-h toxicity test following OECD Test Guideline 201 (OECD 2006). The test water used was Yaquina Bay (Newport, OR) natural seawater (29‰ salinity). The Ni EC10 was derived based on the average specific growth rate of the algae. Dissolved Ni concentrations were measured at PERL using atomic absorption spectrophotometry (AAS). DOC analyses were conducted by Columbia Analytical Services (Kelso, WA) using USEPA Method 415.1.

Skeletonema costatum. Nickel toxicity tests with the diatom *S. costatum* were conducted at PERL. Algae were exposed to Ni (as $NiCl_2 \times 6H_2O$) in static 72-h toxicity tests following OECD Test Guideline 201 (OECD 2006). Four tests were conducted with separate sources of natural seawater collected from coastal US waters (Yaquina Bay, OR; Shannon Point, WA; Vallejo, CA; Cape Fear, NC) amended with growth media (without EDTA). DOC concentrations varied from 1.2 to 2.7 mg/L in the natural waters. One additional test was conducted using synthetic seawater (without EDTA) with a DOC concentration of 0.2 mg/L. The Ni EC10 was derived based on the average specific growth rate of the diatoms. Dissolved Ni concentrations were measured at PERL using AAS and DOC concentrations were measured by Columbia Analytical Services using USEPA Method 415.1.

Champia parvula. The Ni toxicity test with the red macroalga (*C. parvula*) was conducted at New England Bioassay (Manchester, CT). Algae were exposed to Ni (as $NiCl_2 \times 6H_2O$) in a chronic static reproduction test following procedures described in USEPA (2002). One male and 5 female branches of the macroalga were added to exposure chambers containing artificial seawater (salinity of 30‰) amended with GP2/ASW growth media. Macroalgae were exposed to Ni for 48 h and then transferred to clean exposure chambers containing only GP2/ASW growth media for a 5-d recovery period, after which the numbers of cystocarps per chamber were determined. Dissolved Ni concentrations were measured at PERL

using AAS and DOC concentrations were measured by Columbia Analytical Services using USEPA Method 415.1.

Macrocystis pyrifera. The Ni toxicity test with the giant kelp (*M. pyrifera*) was conducted at the Golder Associates North Vancouver Laboratory (British Columbia, Canada). Zoospores and embryonic gametophytes were exposed to Ni (as $NiSO_4 \times 6H_2O$) in a static 48-h spore germination and germ tube growth toxicity test according to procedures described in USEPA (1995). The dilution water was natural seawater, typically with a 28‰ salinity adjusted to 34‰ with hypersaline brine. Sublethal Ni toxicity was assessed by measuring the percentage of zoospore germination and the germination tube length at the end of the 48-h exposure period. Dissolved Ni concentrations were measured by CanTest Laboratories (Burnaby, British Columbia, Canada) using inductively coupled plasma mass spectroscopy (ICP-MS), with special extraction and/or dilution to minimize interference caused by the sample matrix.

Polychaete

Neanthes arenaceodentata. The Ni toxicity test with the polychaete (*N. arenaceodentata*) was conducted at PERL. Test organisms were exposed to Ni (as $NiCl_2 \times 6H_2O$) in a 127-d chronic life cycle test following procedures described in ASTM E1562 (ASTM 2000). The dilution water was reconstituted seawater with a salinity of 30‰. The Ni EC10 was derived based on the number of emergent juveniles. Dissolved Ni concentrations were measured at PERL using AAS and DOC concentrations were measured by Columbia Analytical Services using USEPA Method 415.1.

Echinoderms

Dendraster excentricus and Strongylocentrotus purpuratus. The Ni toxicity tests with the sand dollar (*D. excentricus*) and the purple sea urchin (*S. purpuratus*) were conducted at Northwestern Aquatic Sciences (Newport, OR). Sand dollar and sea urchin embryos were exposed to Ni (as $NiCl_2 \times 6H_2$) in static 48-hr toxicity tests following procedures described in ASTM E1563 (ASTM 1995). The tests were conducted with natural seawater from Yaquina Bay (Newport, OR), which had a salinity of 30‰. The Ni EC10s were derived based on normal shell development. Dissolved Ni concentrations were measured at PERL using AAS and DOC concentrations were measured by Columbia Analytical Services using USEPA Method 415.1.

Paracentrotus lividus. The Ni toxicity test with the Mediterranean sea urchin (*P. lividus*) was conducted at Aegean University (Mytilene, Greece). Sea urchin embryos were exposed to Ni (as $NiCl_2 \times 6H_2$) in a static 72-h toxicity test following procedures described in ASTM E1563-98 (ASTM 2004a). The test was conducted with natural seawater from Mytilene, which had a salinity of 38‰ and DOC concentration of 1.0 mg/L. The Ni EC10 was derived based on normal shell development. Dissolved Ni concentrations were measured using Zeeman-corrected graphite furnace atomic absorption spectrophotometry (GF-AAS) and DOC concentrations were measured using a Tekmar Apollo 9000 analyzer.

Mollusks

Mytilus galloprovincialis. The Ni toxicity tests with the blue mussel (*M. galloprovincialis*) were conducted at Northwestern Aquatic Sciences. Blue mussel embryos were exposed to Ni in static 48-h toxicity tests following procedures described in ASTM E724 (ASTM 1998). Four tests were conducted with separate sources of natural seawater that had collected from coastal US waters (Yaquina Bay, OR; Shannon Point, WA; Vallejo, CA; Cape Fear, NC). The DOC concentrations in the natural seawater varied from 1.2 to 2.7 mg/L and the salinities ranged from 29.5 to 30.1‰. The Ni EC10s were derived based on normal shell development.

C*rassostrea gigas*. The Ni toxicity test with the Pacific oyster (C. *gigas*) was conducted at Northwestern Aquatic Sciences. Pacific oyster embryos were exposed to Ni (as $NiCl_2 \times 6H_2$) in a static 48-h toxicity test following procedures described in ASTM E724 (ASTM 1998). The test was conducted with natural seawater from Yaquina Bay (Newport, OR), which had a salinity of 30‰ salinity and a DOC concentration of 1.2 mg/L. The Ni EC10 was derived based on normal shell development. Dissolved Ni concentrations were measured at PERL using AAS and DOC concentrations were measured by Columbia Analytical Services using USEPA Method 415.1.

Fish

Cyprinodon variegatus. The Ni toxicity test with sheepshead minnow (C. *variegatus*) was conducted at the Golder Associates North Vancouver Laboratory. Sheepshead minnow larvae (<48-h old at test initiation) were exposed to Ni (as $NiSO_4 \times 6H_2O$) in a 28-day survival and growth toxicity test following procedures described in ASTM E1241-98 (ASTM 2004b), using a flow-through exposure system. The flow-through system provided continuous delivery of fresh test solutions at a flow rate of 6 mL/min. Natural seawater (28‰) was used as the dilution water. The Ni EC10 was calculated based on growth (dry weight). Dissolved Ni concentrations were measured by CanTest Laboratories using ICP-MS, with special extraction and/or dilution to minimize interference caused by the sample matrix.

Species sensitivity distribution analysis

Once all of the marine Ni toxicity data were compiled, an SSD analysis was conducted by identifying the best-fitting distributions to the log-transformed Ni EC10s using the Decision Tools software program (Palisade Corporation 2008). This software program considers several distribution types, including lognormal, Weibull, inverse Gaussian, exponential, γ, log-logistic, and several others. Three goodness-of-fit statistics were used to describe each distribution's fit to the raw toxicity data: 1) χ^2, 2) Komolgorov–Smirnov, and 3) Anderson–Darling. Each goodness-of-fit statistic provides a measure of the deviation of the fitted distribution from, in this case, the raw EC10 data. The 3 best-fitting distributions, based on consideration of all 3 goodness-of-fit tests, were selected to evaluate whether the SSD, and its associated 5th percentile (i.e., HC5), was sensitive to the statistical distribution-type selected. The 90% confidence intervals on the HC5s were derived using a bootstrap method in which either 16 or 17 random samples (the number of values used to derive the SSDs in this evaluation) were collected 10 000 times. For each of the 10 000 iterations, the HC5 estimate for each of the 16 or 17 random samples was calculated and the 90% confidence interval was calculated as the 5th and 95th percentiles of the 10 000 HC5 estimates.

Ambient Ni concentrations in European marine waters

As a point of comparison for the marine Ni HC5 values derived in the current study, ambient Ni concentration data for European marine waters were identified. Heijerick and Van Sprang (2008) compiled ambient Ni concentration data for estuarine and estuarine-influenced coastal waters, as well as for open marine waters and the Baltic Sea (given its semi-enclosed conditions and brackish properties). Data from estuaries with anthropogenic point sources were excluded. Following guidance from the TGD, Heijerick and Van Sprang (2008) derived reasonable worst-case (RWC) ambient Ni concentrations of 3.34, 0.30, and 0.79 μg/L for estuarine and estuarine-coastal waters, open marine waters, and the Baltic Sea, respectively, which were calculated as the 90th percentile of the ambient Ni data compiled for each of these categories of water bodies.

RESULTS

Toxicity of Ni to marine organisms

Acceptable chronic Ni toxicity data were identified in the scientific literature for 7 species and chronic Ni toxicity studies were completed for an additional 10 species (Table 1). The most sensitive marine species tested to date is the tropical long-spined sea urchin (*Diadema antillarum*), with a Ni EC10 of 2.9 μg/L. Overall, the sensitivity of sea urchin species varies over 2 orders of magnitude, with species mean Ni EC10s for other sea urchin species being 139 and 335 μg/L for *P. lividus* and *S. purpuratus*, respectively. The second most sensitive species, a mysid (*A. bahia*), has a Ni EC10 that is approximately 6 times greater (17 μg/L) than the lowest EC10 of (2.9 μg/L). Finally, marine fish (sheepshead minnow, topsmelt silverside) are among the least sensitive organisms to Ni, along with the green alga *D. tertiolecta*.

The influence of varying water chemistry on the bioavailability and toxicity of dissolved Ni to marine organisms has not been heavily studied. Almost all of the studies evaluated marine Ni toxicity in full-strength seawater with a salinity of 28.5‰ to 38‰ and a pH near 8. In addition, DOC concentrations were either low (often ≤1.2 mg/L) or not reported (but likely low given the sources of test waters). The influence of DOC concentrations ranging from 1.2 mg/L to 2.7 mg/L and from 0.22 mg/L to 2.7 mg/L was evaluated for the mussel *M. galloprovincialis* and the diatom *S. costatum*, respectively, but there was no clear influence of DOC on dissolved Ni toxicity over these ranges (Table 1). However, it is possible that DOC would be found to modify Ni toxicity to sensitive marine species over a broader range of DOC concentrations (see Discussion).

Species sensitivity distribution analysis

Based on all 17 species mean EC10s, the overall best fitting distributions based on the χ^2, Komolgorov–Smirnov, and Anderson–Darling goodness-of-fit tests were the logistic, inverse Gaussian, and γ distributions. The logistic distribution provides a better fit to the *D. antillarum* EC10 in the lower tail of the distribution, whereas both the inverse Gaussian and γ distributions underestimate the *D. antillarum* EC10 (Figure 1). The 5th percentile (90% confidence interval) of the SSD is 3.9 (0.22–21.5) μg Ni/L based on the logistic distribution, 6.7 (2.0–22.7) μg Ni/L based on the inverse Gaussian distribution, and 6.8 (2.0–22.4) μg Ni/L based on the γ distribution.

Diadema antillarum appears to be uniquely sensitive to Ni based on the Ni toxicity data available to date, and it may not be relevant to European waters because it is a tropical species from outside of European waters (see *Discussion*). For these reasons, SSDs were also developed with this species excluded from the data set. The overall best-fitting distributions were the log-logistic, Pearson V, and lognormal, all of which provided very similar fits to the EC10 data (Figure 2). The 5th percentile (90% confidence interval) of the SSD with *D. antillarum* excluded is 20.9 (11.7–42.7) μg Ni/L based on the log-logistic model, which was the best fitting based on all 3 goodness-of-fit tests. The 5th percentile is slightly overestimated, at least based on the percentiles of the ranked EC10s, as the lowest EC10 of 17 μg Ni/L is at approximately the 6th percentile of the SSD.

Comparison of SSDs to ambient Ni concentrations in European marine waters

The highest ambient dissolved Ni concentration reported in Heijerick and Van Sprang (2008) for European marine waters was 3.75 μg/L, which is just below the marine Ni HC5 of 3.9 μg/L when *D. antillarum* is included in the SSD and well below the marine Ni HC5 of 20.9 μg/L when *D. antillarum* is excluded from the SSD (Figure 3).

DISCUSSION

Chronic Ni toxicity data, meeting certain acceptability requirements (e.g., analytical verification of Ni concentrations in the test waters and use of standard approved test methods), are available for 17 species, including 4 protist species, 11 invertebrate species (including echinoderms, mollusks, arthropods, and a polychaete), and 2 fish species. Accordingly, chronic Ni toxicity data are available for a relatively diverse set of marine organisms. The Ni EC10s span several orders of magnitude, ranging from 2.9 μg/L for the long-spined sea urchin (*D. antillarum*) to 20 760 μg/L for the sheepshead minnow (*C. variegatus*). Because of Ni EC10s being available for fewer than 20 marine species, the 5th percentile of the SSD will necessarily be driven by the EC10 for the most sensitive species, *D. antillarum*. The Ni HC5 of 3.9 μg/L (with *D. antillarum* included in the SSD) is greater than the Ni EC10 of 2.9 μg/L for *D. antillarum*, but this is associated with an effect level of just 13% based on the concentration–response curve provided in Bielmyer et al. (2005).

Species relevance to temperate European marine waters

Diadema antillarum is a tropical sea urchin species found in the coral reef systems of the Caribbean (Bielmyer et al. 2005), so the relevance of its sensitivity to Ni and other substances compared to species in European marine waters may be considered uncertain. However, because toxicity data are only available for a small fraction of the species that may be exposed to a substance in nature, a basic principle behind the SSD approach is that the sensitivities of untested species could be represented by other species in the SSD (assuming that the SSD was developed based on a sufficiently diverse set of species). For comparison to *D. antillarum*, Ni EC10s of 89 and 217 μg/L are available for a European sea urchin species, *P. lividus* (Table 1) where the test conditions (e.g., temperature, salinity, test duration) were basically similar. This could perhaps provide some evidence that sea urchin species found in European waters are less sensitive than the tropical *D. antillarum*, but even

Table 1. Chronic Ni toxicity data for marine organisms

Species	Test organism age or size	Test conditions/ chemical analysis	Duration	Test water	Temp (°C)	pH	Salinity (‰)	DOC (mg/L)	Ni_{cb} (μg/L)	Endpoint	EC10 (μg/L)	Species mean EC10 (μg/L)	Reference
Americamysis bahia (mysid) [a]	Juvenile	R,M	36 d	Natural	21	—	34	—	—	Reproduction	17	17	Gentile et al. 1982; Lussier et al. 1985
Atherinops affinis (topsmelt silverside)	Larva	FT,M	40 d	Natural	20	—	34	—	—	Survival	3599	3,599	Hunt et al. 2002
Champia parvula (red alga)	Adult	R,M	10 d	Natural	23	8.0	30	1.2	<1	Reproduction	144	144	Parametrix 2007a
Crassostrea gigas (Pacific oyster)	Embryo	S,M	48 h	Natural	20.7	7.4	30	1.2	<1	Development	430.8	430.8	Parametrix 2007b
Cyprinodon variegatus (sheepshead minnow)	Larva	FT,M	28 d	Natural	25	—	30	—	6	Growth	20760	20,760	Golder 2007
Dendraster excentricus (sand dollar)	Embryo	S,M	48 h	Natural	15.4	8.1	30	1.2	<1	Development	191	191	Parametrix 2007c
Diadema antillarum (long-spined sea urchin)	Embryo	S,M	40 h	Synthetic	20	—	33	—	—	Development	2.9	2.9	Bielmyer et al. 2005
Dunaliella tertiolecta (green alga)	—	S,M	72 h	Natural	20	7.8	29.4	1.2	<1	Specific growth rate	17891	17,891	Parametrix 2007d
Haliotis rufescens (red abalone)	Embryo	R,M	22 d	Natural	15	—	34	—	—	Metamorphosis	36.4	36.4	Hunt et al. 2002
Macrocystis pyrifera (brown alga)	Zoospores	S,M	48 h	Natural	15	8.0	34	—	3	Growth	96.7	96.7	Golder 2007
Macrocystis pyrifera (brown alga)	Zoospores	S,M	48 h	Natural	15	8.0	34	—	3	Germination	494 [b]		Golder 2007
Mysidopsis intii (mysid)	Juvenile	FT,M	28 d	Natural	20	—	34	—	—	Growth	45.2	45	Hunt et al. 2002
Mytilus galloprovincialis (mussel)	Embryo	S,M	48 h	Natural	15.8	8.1	30	1.2	<1	Development	259	270	Parametrix 2007e
Mytilus galloprovincialis (mussel)	Embryo	S,M	48 h	Natural	16.1	7.9	30	1.6	<1	Development	228		Parametrix 2007e
Mytilus galloprovincialis (mussel)	Embryo	S,M	48 h	Natural	16.0	8.1	30	2.5	<1	Development	256		Parametrix 2007e
Mytilus galloprovincialis (mussel)	Embryo	S,M	48 h	Natural	16.1	8.1	30	2.7	<1	Development	350		Parametrix 2007e
Mytilus trossolus (mussel)	Embryo	S,M	48 h	Natural	20	7.9	–	—	—	Development	61.1	61.1	Nadella et al. 2009
Neanthes arenaceodentata (polychaete)	Juvenile	R,M	90 d	Natural	20	7.9	29.5	<0.5	<1	Reproduction	22.5	22.5	Parametrix 2007f
Paracentrotus lividus (sea urchin)	Embryo	S,M	72 h	Synthetic	18	8.0	35	—	—	Development	89	139	Novelli et al. 2003
Paracentrotus lividus (sea urchin)	Embryo	S,M	72 h	Natural	16.3	—	38	1.0	0.4	Development	217		Pagano 2007
Skeletonema costatum (diatom)	—	S,M	72 h	Synthetic	20	8.4	28.5	0.22	<1	Specific growth rate	265.4	316.5	Parametrix 2007g
Skeletonema costatum (diatom)	—	S,M	72 h	Natural	20	8.3	30.2	1.2	<1	Specific growth rate	190.6		Parametrix 2007g
Skeletonema costatum (diatom)	—	S,M	72 h	Natural	20	8.2	29.4	1.6	<1	Specific growth rate	773.4		Parametrix 2007g

(*Continued*)

Table 1. (*Continued*)

Species	Test organism age or size	Test conditions/ chemical analysis	Duration	Test water	Temp (°C)	pH	Salinity (‰)	DOC (mg/L)	Ni_{cb} (μg/L)	Endpoint	EC10 (μg/L)	Species mean EC10 (μg/L)	Reference
Skeletonema costatum (diatom)	—	S,M	72 h	Natural	20	8.3	29.2	2.5	<1	Specific growth rate	122.7		Parametrix 2007g
Skeletonema costatum (diatom)	—	S,M	72 h	Natural	20	8.3	29.4	2.7	<1	Specific growth rate	661.3		Parametrix 2007g
Strongylocentrotus purpuratus (sea urchin)	Embryo	S,M	48 h	Natural	15.6	8.1	30	1.2	<1	Development	335	335	Parametrix 2007c

DOC = dissolved organic carbon; EC10 = 10th percentile effect concentration; FT = flow-through; M = measured; Ni_{cb} = background Ni concentration in test dilution water; R = renewal; S = static; Temp = temperature.
[a]Formerly *Mysidopsis bahia* (Price et al. 1994; Lussier et al. 1999).
[b]Excluded from species mean EC10 because data were available for a more sensitive endpoint for this species.

aquatic species within the same genus have been shown to have highly variable sensitivity to some substances. Because Ni is a naturally occurring element, a final point to consider is how the Ni EC10 of 2.9 μg/L for *D. antillarum* compares to Ni concentrations in natural seawaters that are minimally influenced by anthropogenic activities. As summarized above, Heijerick and Van Sprang (2008) identified ambient Ni concentrations in European marine waters ranging up to 3.75 μg/L for a European estuary (Figure 3). This indicates that ambient Ni concentrations in at least some locations may exceed the Ni EC10 of 2.9 μg/L for *D. antillarum* and approach the HC5 of 3.9 μg/L derived when *D. antillarum* is included in the SSD. Given the above lines of evidence, we currently recommend that *D. antillarum* be excluded from the SSD when considering temperate European waters. The greatly increased sensitivity of *D. antillarum* relative to temperate species strongly suggests that additional Ni toxicity testing with tropical species is warranted and such testing is currently being planned.

Influence of water chemistry on Ni toxicity

The influence of water chemistry, such as DOC, pH, and various cations and anions, on Ni toxicity to freshwater organisms has been well-studied, resulting in the development of freshwater biotic ligand models (BLMs) for predicting Ni toxicity as a function of water chemistry. In contrast, the influence of water chemistry parameters, such as DOC, on Ni toxicity to saltwater organisms has not been well studied. All of the Ni toxicity tests compiled in this evaluation were conducted at a DOC concentration of less than or equal to 2.7 mg/L. However, the influence of DOC on Ni toxicity to freshwater species has been clearly documented (Deleebeeck et al. 2007, 2008, 2009; Kozlova et al. 2009). We expect that the influence of DOC on Ni toxicity in sensitive saltwater species would also be observed if testing is conducted over a wider range of DOC concentrations relevant for coastal marine waters. Arnold (2005), for example, demonstrated that Cu toxicity to *Mytilus* was significantly related to DOC concentrations when the DOC range in natural waters was 0.3 mg/L to 10 mg/L.

Comparison of HC5 values to other regulatory guidelines

As a point of comparison, the HC5 values of 3.9 and 20.9 μg/L with *D. antillarum* included and excluded from the SSD, respectively, were compared to guidelines and criteria from other jurisdictions (or guidelines developed using the approaches from other jurisdictions). The USEPA chronic ambient water quality criterion (AWQC) for dissolved Ni in saltwater, which was last updated in 1986 (USEPA 1986), is 8.2 μg/L (USEPA 2009). This criterion was calculated using an acute-chronic ratio (ACR) because there were insufficient chronic Ni toxicity data to meet the minimum phylogenetic diversity requirements. Hunt et al. (2002) later conducted acute and chronic Ni toxicity testing with 3 additional saltwater species and, following USEPA guidelines for AWQC development, derived alternative chronic saltwater Ni criteria that ranged between 11.7 μg/L and 22.4 μg/L. These alternative criteria varied depending on which ACR was used and which taxonomic name was used for certain species (during the 1990s some species in the genus *Mysidopsis* were moved to a new genus, *Americamysis*). Because the USEPA uses genus mean values, rather than species mean values, in deriving SSDs, changes to the genus could influence which toxicity values are

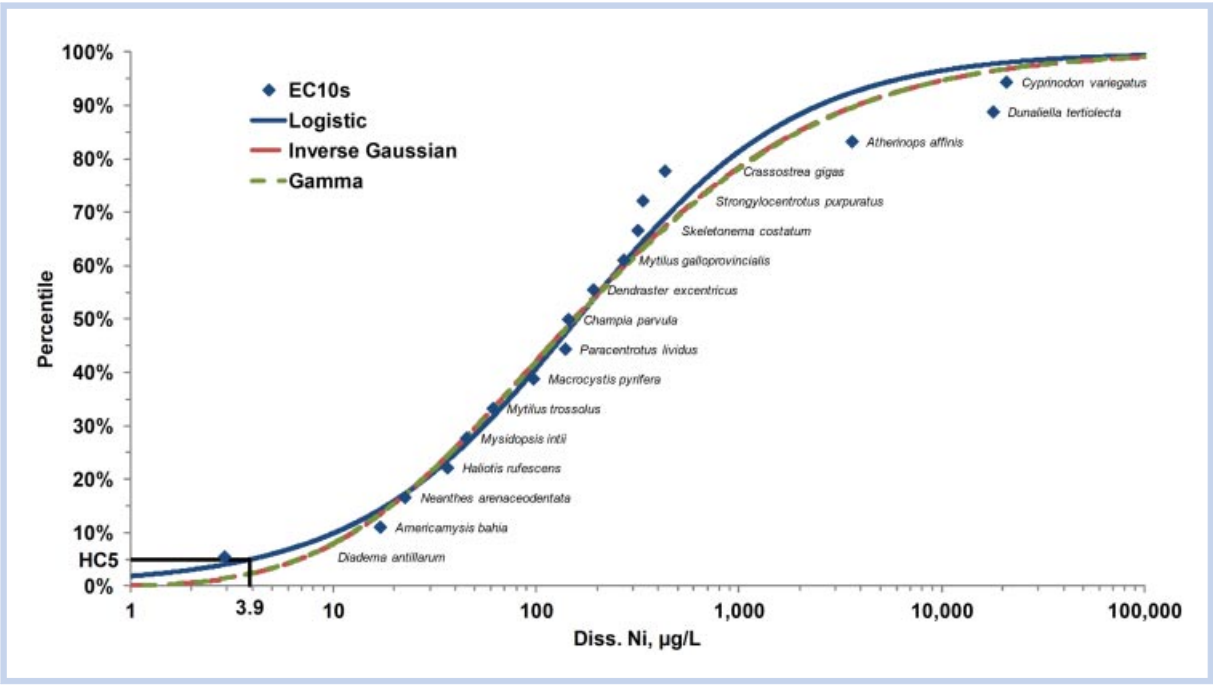

Figure 1. Nickel species sensitivity distribution (SSD) for marine organisms based on all data. Note that the inverse Gaussian and γ distributions are indistinguishable.

averaged. In the present evaluation, chronic Ni toxicity data are now available to meet the USEPA minimum diversity requirements and the use of an ACR to develop a chronic criterion would no longer be necessary. Based on EC10 values, updated chronic criteria following USEPA guidelines are 2.9 and 14.5 µg/L when *D. antillarum* is included and excluded from the SSD, respectively (and slightly lower criteria would be derived if the 4 protist species were excluded from the SSD, as the USEPA does not include these data in the SSD) (Table 2). The updated chronic criteria, with *D. antillarum* excluded, fall within the range of updated criteria proposed by Hunt et al. (2002), which were derived using an ACR.

As another point of comparison, an EQS of 8.6 µg/L was derived under the Water Framework Directive. This EQS is

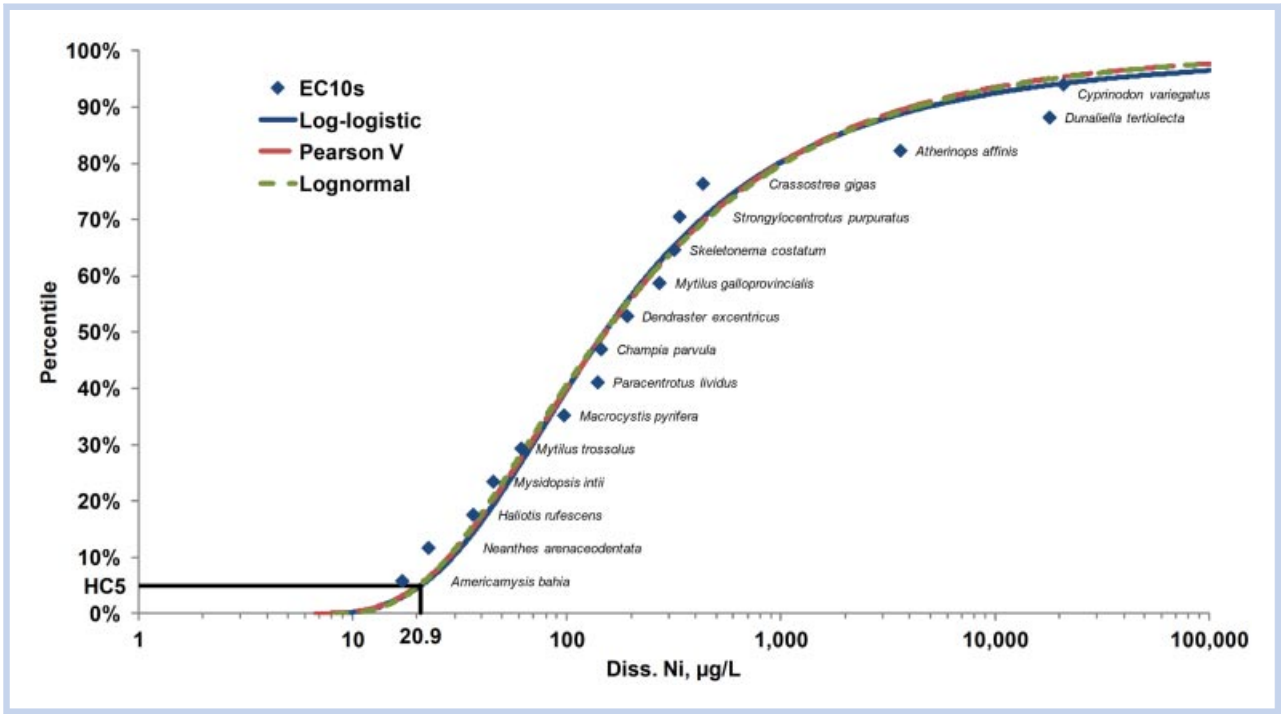

Figure 2. Nickel species sensitivity distribution (SSD) for marine organisms with the EC10 for the long-spined sea urchin (*Diadema antillarum*) excluded. Note that the Pearson V and lognormal distributions are indistinguishable.

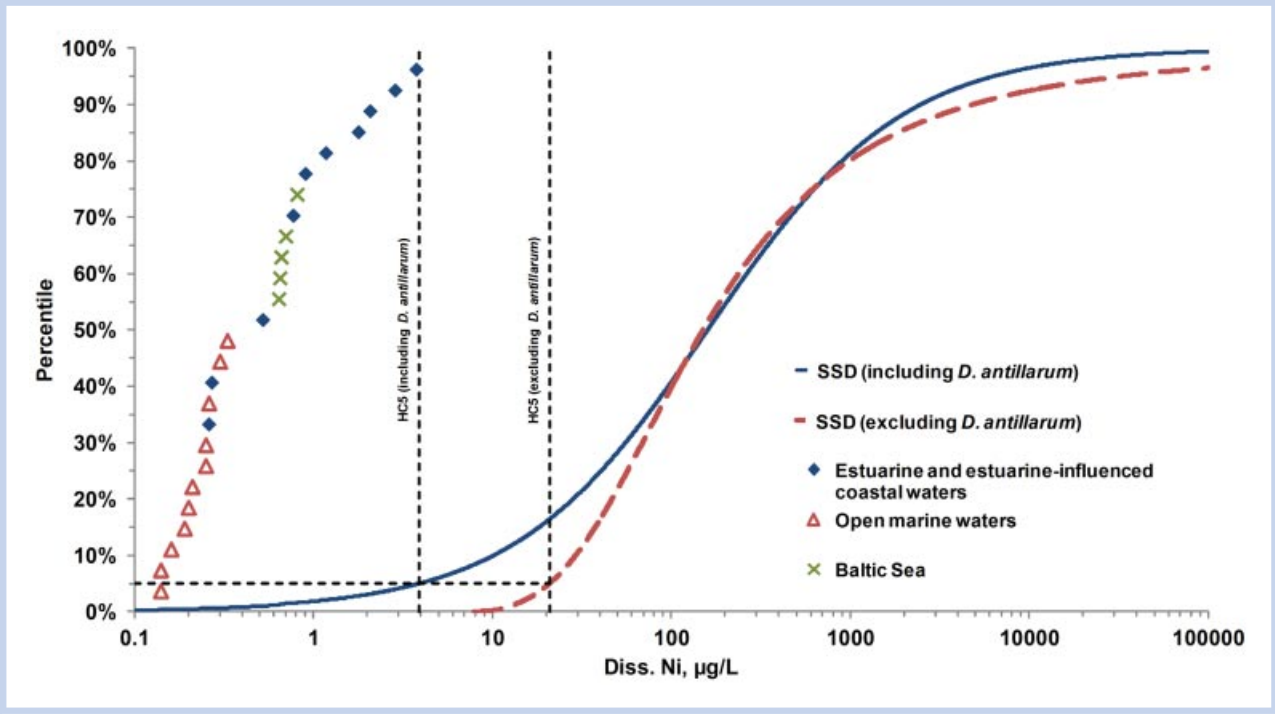

Figure 3. Comparison of Ni species sensitivity distributions (SSDs) with and without *Diadema antillarum* included to ambient Ni concentrations in estuarine and estuarine coastal waters, open marine waters, and the Baltic Sea. Ambient Ni data from Heijerick and Van Sprang (2008).

Table 2. Comparison of chronic saltwater nickel HC5 values from the present evaluation to other existing or updated HC5 values and criteria

Source	General methodology for 5th percentile derivation	Chronic saltwater Ni HC5 or AWQC (µg/L)	Comment
Present study	EU	20.9	Based on chronic SSD with *Diadema antillarum* excluded
		3.9	Based on chronic SSD with *Diadema antillarum* included
ECB (2008) [a]	EU	17.2	Based on mean 5th percentile of several statistically significant chronic SSDs (the HC5 was divided by an assessment factor of 2 to derive a SW Ni EQS of 8.6 µg/L)
USEPA (1986, 2009)	USEPA	8.2	Based on acute SSD and applying a combined SW and FW ACR of 17.99
Hunt et al. (2002)	USEPA	11.7	Based on updated acute SSD, *Americamysis* as genus for *bahia*, and applying a combined SW and FW ACR of 10.50
		20.5	Based on updated acute SSD, *Americamysis* as genus for *bahia*, and applying a SW ACR of 5.960
Present study	USEPA	14.5	Based on chronic SSD using genus mean EC10s and with *Diadema antillarum* excluded [b]
		2.9	Based on chronic SSD using genus mean EC10s and with *Diadema antillarum* included [b]
		12.8	Based on chronic SSD using genus mean EC10s and with *Diadema antillarum* and protists excluded [b,c]
		2.2	Based on chronic SSD using genus mean EC10s and with *Diadema antillarum* and protists excluded [b,c]

ACR = acute to chronic ratio; AWQC = ambient water quality criteria (USEPA [1986, 2009] and Hunt et al. [2002]); EQS = environmental quality standard; FW = freshwater; HC5 = hazardous concentration for 5% of the species (present study and ECB [2008]); SSD = species sensitivity distribution; SW = saltwater.
[a]The outcome of the Existing Substances Risk Assessment of Ni (ECB 2008) also serves as the basis for the draft Ni Environmental Quality Standard for pelagic marine organisms under the European Union's Water Framework Directive (http://ec.europa.eu/environment/water/water-framework/).
[b]When using regression-based effect levels to describe chronic toxicity data, the USEPA has generally used EC20 values rather than EC10 values (USEPA 2009). EC10 values were used here for a more direct comparison to the EU-based assessment.
[c]The USEPA does not include protists or macrophytes in the SSD for AWQC development.

based on the mean 5th percentile of several different statistically significant SSDs, which was then divided by an assessment factor of 2 due to the absence of field or mesocosm data for comparison to with the laboratory-based HC5. The mean HC5 was therefore 17.2 μg/L, or slightly lower than the HC5 of 20.9 μg/L derived in the present evaluation, when *D. antillarum* was excluded.

Species sensitivity: Comparison of saltwater and freshwater

With the exception of the tropical urchin *D. antillarum*, the 1st and 4th most sensitive species in the chronic saltwater Ni SSD are mysid crustaceans (Figure 2). The polychaete *N. arenaceodentata* is the 2nd most chronically sensitive saltwater species, whereas the sensitivity of mollusks and echinoderms are rather variable as their sensitivities fall between approximately the 17th to 77th percentiles and 41st to 71st percentiles on the SSD, respectively. Saltwater algae and diatoms also have a variable sensitivity to Ni, with the EC10s for the 4 species tested falling between approximately the 35th to 88th percentiles on the SSD. Fish clearly appear to be chronically insensitive to Ni.

The above patterns in species sensitivity are generally consistent with what is observed in freshwater, where the small crustacean *Ceriodaphnia dubia* and other cladocerans are among the most sensitive species to Ni, whereas fish are relatively insensitive (ECB 2008). The most sensitive freshwater species to Ni is currently the snail *Lymnaea stagnalis*, which appears to be slightly more sensitive than C. *dubia*. The only saltwater gastropod species tested to-date is red abalone (*Haliotis rufescens*), which is the 3rd most sensitive species in the saltwater Ni SSD. Similar to the variable sensitivity of saltwater algae and diatoms, freshwater algae and macrophytes also have a highly variable sensitivity to Ni. Some primary producers are relatively sensitive to Ni, with duckweed (*Lemna gibba*) being the 4th most sensitive species in the freshwater SSD and the alga *Scenedesmus accuminatus* being the 8th most sensitive species. Based on the above observations for freshwater, testing of sensitive species for saltwater habitats with little data should focus first on invertebrates, especially crustaceans, gastropods, and algae. As noted previously, additional information on the sensitivity of tropical sea urchins should also be collected.

Summary and Conclusions

In summary, based on data reported in the published literature and new data generated in laboratory-based toxicity studies, we derived marine Ni HC5 values of 3.9 μg/L (when *D. antillarum* was included in the SSD) and 20.9 μg/L (when *D. antillarum* was excluded from the SSD). There is some uncertainty as to whether the sensitivity of *D. antillarum* to Ni is relevant to European marine waters given that: 1) it is a tropical species, 2) a European sea urchin species is much less sensitive to Ni, and 3) ambient Ni concentrations in at least some European marine waters can approach the *D. antillarum* EC10 of 2.9 μg/L. Accordingly, we suggest that 3.9 μg/L would be a conservative threshold for Ni in temperate European marine waters and recommend a more broadly applicable threshold of 20.9 μg/L. Site-specific toxicity testing with local species may be warranted for locations where Ni concentrations fall between the range in HC5s of 3.9 μg/L to 20.9 μg/L.

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SUPPORTING INFORMATION

All Supplemental Data may be found in the online version of this article.

Figure S1. Calculated Ni EC10 for *Mysidopsis bahia* (now *Americamysis bahia*) using the USEPA's TRAP program.

Figure S2. Calculated Ni EC10 for *Mytilus trossolus* using the USEPA's TRAP program.