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Secondary Poisoning Risk Assessment of Terrestrial Birds and Mammals Exposed to Nickel

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ABSTRACT

The European Union's Existing Substances regulation (EEC 793/93) was developed to assess the ecological risks posed by chemical substances such as Ni and includes the assessment of secondary poisoning risks. The basic structure of this secondary poisoning risk assessment followed the Technical Guidance Document on Risk Assessment and thus included development of predicted exposure concentrations (PECs) and predicted no-effect concentrations (PNECs). A PEC to PNEC ratio greater than 1.0 is indicative of potential risk. The Technical Guidance Document on Risk Assessment provides a generic framework for assessing secondary poisoning risks and prescribes the following terrestrial food chain: soil → earthworm → worm-eating bird or mammal. This secondary poisoning evaluation was conducted at the regional level, and it was found that the generic approach resulted in widespread estimates of potential risk, even at ambient Ni soil concentrations. Accordingly, a tiered approach was used with increasing levels of refinement, including consideration of bioavailability, consideration of a variable diet, and development of dose-based PNEC values. Based on the refined approach, all PEC to PNEC ratios were less than 1.0, except for a ratio of 1.4 in a scenario focused on a regional clay soil, which was of natural origin. This regional-level secondary poisoning evaluation highlighted key risk assessment components that should be considered in future localized secondary poisoning assessments of Ni and other metals, including ingestion rate to body weight ratios for the test organisms used to derive PNECs versus the representative wildlife species evaluated, the appropriateness of high assessment factors for deriving PNECs for naturally occurring essential elements, representative dietary compositions, relative metal bioavailability between the dietary toxicity study and natural diets, and ground-truthing of the risk predictions versus background concentrations. Integr Environ Assess Manag 2012;8:107–119. © 2011 SETAC

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INTRODUCTION

Nickel is refined and smelted in 7 locations in Europe, with an annual production level of 1.75×10^5 tons in 2000. Nickel is used in many downstream industrial applications in Europe, including production of stainless steel and batteries, as a catalyst, and in chemical manufacturing. Although emissions from Ni production and downstream industrial uses are substantial (they account for approximately 4% and 3% of total Ni emissions to water and air, respectively), unintended, diffuse sources account for the majority of total emissions. Fossil fuel combustion, for example, accounts for 70% of total Ni emissions to air. The European Union (EU) Existing Substances regulation (EEC 793/93) was developed to assess risks of ongoing production and use of chemical substances such as Ni. For environmental exposures, risk is characterized for aquatic (freshwater and marine), sediment, and terrestrial compartments following approaches described in the Technical Guidance Document (TGD; ECB 2003). The TGD also includes an approach for assessing secondary poisoning, which is an assessment of risks from dietary chemical exposures in terrestrial and aquatic food chains.

Secondary poisoning risk assessments are performed in the EU Existing Substances framework to address concerns associated with ''toxic effects in the higher members of the food chain. . .which result from ingestion of organisms from lower trophic levels that contain accumulated substances'' (ECB 2003). The focus on ''higher members of the food chain'' implies that organisms at higher trophic levels are more sensitive than organisms at lower trophic levels and/or that the tissue concentrations of the accumulated substance may increase at higher trophic levels (i.e., the substance biomagnifies). There are insufficient toxicity data to support or refute whether higher trophic level organisms are intrinsically more sensitive to Ni than are organisms at lower trophic levels. In addition, no data exist to support that Ni biomagnifies in aquatic or terrestrial environments. Several studies (Outridge and Schuehammer 1993; Torres and Johnson 2001; Campbell et al. 2005; Muir et al. 2005) and bioaccumulation review articles (Biddinger and Gloss 1984; Suedel et al. 1994) all suggest that Ni is unlikely to biomagnify, and some studies suggest Ni exhibits biodilution, i.e., decreases in concentration as it ascends in the food chain (Campbell et al. 2005). Regardless, dietary metal exposure can be an important pathway for wildlife, regardless of their trophic position (Fairbrother et al. 2007; McLaughlin et al.

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2010). For substances that do not show evidence of biomagnification, assessing risks from secondary poisoning should be based on environmentally relevant food chains in which dietary metal exposure is relatively high and the sensitivity of the consumer organism is high. However, the sensitivities of wild species to a substance are rarely known, so ascertaining their relative sensitivities is difficult (accordingly, uncertainty factors are often applied to existing toxicity data to account for the potentially more sensitive species).

The spatial scale of the secondary poisoning risk evaluation required to comply with EEC 793/93 is at the regional level. The TGD provides an operational definition for a hypothetical European region, which is defined as an area of 40 000 km^2 and a population of 20 million individuals. The EU System for the Evaluation of Substances (EUSES) model is used to estimate exposure concentrations in this hypothetical region based on a scaled average of all Ni emissions to air and water. Estimated regional Ni concentrations as well as concentrations measured in true representative regions of Europe were used as the basis for calculating dietary exposure concentrations in this secondary poisoning assessment. The primary objectives of this article are 2-fold: 1) highlight the uncertainties in conducting regional-level risk assessments and the limitations of such assessments, using the Ni secondary poisoning risk assessment as an example, and 2) identify the lessons learned from this regional secondary poisoning assessment for Ni that can be used to guide future site-specific or localized secondary poisoning risk assessments for Ni. Secondary poisoning risk assessments were conducted for both terrestrial and aquatic environments, but this article focuses on the terrestrial evaluation with many of the same concepts applying to the aquatic environment.

METHODS

The basic structure of this risk assessment also followed the TGD (ECB 2003) and thus included the development of predicted exposure concentrations (PECs) and predicted no-effect concentrations (PNECs), which are then compared to determine whether a potential risk exists for representative bird and mammal species exposed to dietary Ni. This regional risk evaluation initially followed the TGD, but we found that the TGD approach was overly generic, being more relevant to bioaccumulative organic chemical substances than metals, for example, and that the default assumptions led to unrealistic conclusions for Ni (e.g., ambient background Ni concentrations in soil posing potential risk). Accordingly, a tiered approach was used in which the 1st tier followed the generic TGD approach, and then increasing levels of refinement were used to increase the environmental relevance of the PECs and PNECs (Figure 1).

Exposure characterization

The TGD defines dietborne Ni exposure concentrations as the PEC_{oral}, for which values were calculated for terrestrial food chains, with birds and mammals serving as the consumer organisms. The 1st step was to identify the food chains that would be evaluated in the risk assessment. The 2nd step was to then identify Ni concentrations measured in food items relevant to the food chains identified, or to identify approaches for estimating Ni concentrations in food items if empirical data were unavailable.

Food chains evaluated. Risk to top predators of the birds and mammals represented in the identified food chains was not assessed, because Ni is subject to biodilution (Campbell et al. 2005), meaning that estimated Ni exposure to the predators of birds and mammals would be lower than that experienced by the birds and mammals themselves. Following the TGD, a common terrestrial food chain was identified for birds and mammals: soil → earthworm → worm-eating bird or mammal.

Under a generic framework, this pathway is generally considered relevant and conservative in secondary poisoning assessments, because earthworms have a high exposure potential to chemicals in soil, are commonly tested in bioaccumulation studies, and are an important food organism to a variety of wildlife species. An assumption is made that when predators ingest earthworms, they also ingest the full gut content of the worms, which is composed of soil. As such, the earthworm would represent the highest soil dose among potential prey organisms, and hence the largest total dose of Ni as well, but not necessarily the highest bioavailable dose. In nature, the diet of birds and mammals is rarely expected to consist of 100% earthworms. For example, the European starling (*Sturnus vulgaris*), which consumes earthworms, also feeds on other invertebrates and fruits and grains. The common shrew (*Sorex araneus*) may also consume substantial quantities of insects, spiders, isopods, centipedes, snails, and slugs (Churchfield 1990). Accordingly, assuming a 100% earthworm diet for these species may overestimate Ni exposure if earthworms have higher Ni concentrations than other prey species. As such, as a 2nd tier to this assessment, a 2nd food chain was assumed for worm-eating birds and mammals that consume other invertebrates. As discussed later, isopods were identified as an additional representative food item for worm-eating birds and mammals, because a Ni bioaccumulation factor (BAF) is available for this taxon (a BAF is required to estimate Ni concentrations in isopods from soil Ni concentrations) and isopods can be a relatively common prey item in the shrew diet (Churchfield 1990). No Ni BAFs were identified for other potential shrew prey items.

The common shrew was identified as the representative mammalian species in this assessment, because earthworms can constitute a large proportion of its diet, and it has a high daily food ingestion rate of up to 80% to 90% of its body weight (Churchfield 1990). In southern England, earthworms were the most frequently (64%) observed prey item in common shrew diets (Churchfield 1990), and at a site near Oxford, England, between 20% and 100% of common shrew guts contained earthworms (Pernetta 1976). However, the percentage of earthworms in a typical shrew diet was not reported; snails, slugs, isopods, spiders, and mites can also be relatively frequent components of shrew diets (Churchfield 1990). According to a study summarized in USEPA (1993), earthworms constituted 31.4% of a short-tailed shrew (*Blarina* sp.) diet by volume, with the remainder of the diet largely comprising other invertebrates. The European mole (*Talpa europaea*) could also be considered a candidate species for this assessment, because it also feeds largely on earthworms and soil invertebrates, and occasionally reptiles, birds, and mammals, and it also may daily consume its body weight in food. Thus, the results of the secondary poisoning assessment for the shrew are expected to be equally applicable to the mole. Ultimately, identifying a receptor with a high food

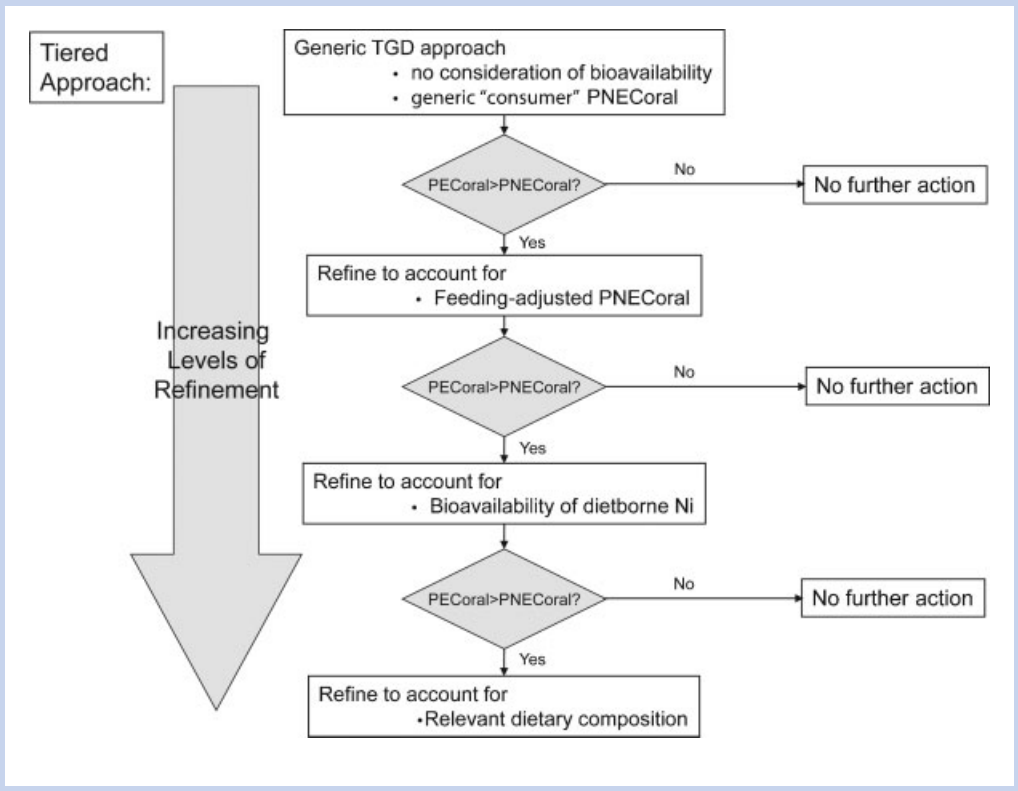

Figure 1. Flow chart for tiered risk characterization approach. PEC_{oral} = predicted exposure concentration, oral; $PNEC_{oral}$ = predicted no-effect concentration, oral; TGD = Technical Guidance Document.

ingestion rate to body weight ratio, and assuming a 100% earthworm diet, results in substantial conservatism in the exposure estimates.

The European starling (*Sturnus vulgaris*) was identified as a representative passerine bird that feeds on earthworms as well as a variety of other food items. On the basis of data compiled in Dunning (1993), the mean weight of male and female starlings is 82.3 g. Using the allometric equation for passerine birds in USEPA (1993), the estimated food ingestion rate is 16.9 g/d on a dry weight (dry wt) basis (106 g wet weight/d, assuming an earthworm water content of 84% [USEPA 1993]). Thus, on a wet weight (wet wt) basis, the starling would be estimated to consume 129% of its body weight daily. This is consistent with a food ingestion rate of 1.34 g food per gram body weight (134%) reported for the starling in Crocker et al. (2002). However, Crocker et al. (2002) also reported that starlings feeding on arthropods consume 64% of their body weight on a daily basis due to differences in energy values and water contents between different foods. Thus, ingestion rates, assuming a 100% earthworm diet, may be biased high for a natural diet, because wild birds are unlikely to feed exclusively on earthworms. Crocker et al. (2002) also predicted earthworm intake rates for other bird species, which, when included with the starling intake rate, result in a mean daily intake rate of 87% relative to body weight across species. For simplicity, it was therefore assumed in this assessment that both the common shrew and earthworm-eating birds may consume 90% of their body weight on a daily basis.

Overall, the earthworm-based food chain was deemed to be appropriately conservative in the context of a regional-scale assessment. As discussed, an alternative isopod-based food chain was evaluated as part of a sensitivity analysis in the current assessment, but isopods have lower Ni BAFs (Torres and Johnson 2001). In local-scale secondary poisoning risk assessments, site-specific data should be used to identify food chains and predators with the greatest exposure potential.

Although this evaluation focused on potential dietary Ni risks, birds and mammals may also simultaneously be exposed to surface water Ni via drinking water ingestion. However, potential Ni risks to birds and mammals via drinking water is predicted to be negligible. For example, the 90th percentile surface water Ni concentrations compiled in the Forum of European Geological Surveys (FOREGS) database (http://www.gtk.fi/foregs/geochem/index.htm) is approximately 5 μg/L. Based on the allometric equations in USEPA (1993), the drinking water ingestion rates are approximately 13% and 20% of body weight for the worm-eating bird and shrew, respectively, evaluated in this assessment. Based on these ingestion rates, the 90th percentile Ni concentration of 5 μg/L is predicted to result in Ni doses of 6.7×10^{-4} and 1.0×10^{-3} mg/kg body weight/d for the worm-eating bird and shrew, respectively. These Ni doses are <1% of the bird- and mammal-based $PNEC_{oral}$ values used in this assessment (presented below) and, therefore, the surface water Ni pathway contributes negligibly to the secondary poisoning assessment.

Estimating Ni concentrations in prey items. The next step was to identify Ni concentrations in European environments that could be used to develop PEC_{oral} values for the food chain pathways discussed above. The $PEC_{regional}$ soil Ni concentrations were identified on the basis of the bioavailability scenarios used within a previously developed terrestrial

Table 1. Representative soils for evaluating the terrestrial food chain secondary poisoning model

Type	Soil use	Country	pH	OM%	Clay%	CEC (meq/100 g soil)	Percent moisture[a]	Background Ni mg/kg dry wt	Background Ni mg/kg wet wt
1. Acid sandy soil	Arable land	Sweden	4.8	2.8	7	2.4	25.3	2	1.6
2. Loamy soil	Arable land	The Netherlands	7.5	2.2	26	20	34.8	19	14.1
3. Peaty soil	Grassland	The Netherlands	4.7	40	24	35	124.8	26	11.6
4. Acid sandy soil	Forest	Germany	3.0	9	7	6	45.8	1	0.69
5. Clay soil	Woodland	Greece	7.4	4.5	46	36	28.6	81	63
6. Different types	Arable + forest	Denmark	6.3	0.6	8.9	10.4	na[b]	Na[b]	8.4

[a]Percent moisture calculated as water content after soils were saturated and applied with 100 cm suction. Percent moisture is calculated as (g H_2O/g dry soil) × 100%.
[b]Information for this soil is not available. A soil with similar composition was identified as ''Leuven,'' the characteristics of which are as follows: pH = 6.7, percent organic matter (OM%) = 10.9, clay percent (Clay%) = 10, cation exchange capacity (CEC) = 7.8, background Ni = 11 mg/kg, percent moisture = 31.6.

effects assessment (Table 1) (ECB 2008). These regional soil types have varying characteristics, with pH ranging from 3.0 to 7.5, organic matter ranging from 0.6% to 40%, clay content ranging from 7% to 46%, and cation exchange capacity (CEC) ranging from 2.4 to 36 meq/100 g soil. The regional background Ni concentrations for these soil types ranged from 1 to 81 mg/kg dry wt.

Nickel concentrations in earthworms were then estimated from these soil concentrations, using Ni BAFs for earthworms. Earthworm BAFs were compiled for various soil types based on a review of the scientific literature and ranged from 0.05 to 1.86 on a dry wt basis (Figure 2). Smolders et al. (2009) showed that CEC was directly related to Ni toxicity to soil-dwelling organisms, and therefore, relationships between CEC and Ni BAFs were evaluated. The maximum BAF of 1.86 is from a soil that likely has elevated Ni bioavailability due to its combined low CEC of 5.3 meq/100 g and low pH of 4.8, although these levels are similar to some of the regional soil characteristic evaluated here (Table 1). However, some BAFs from other studies are similar in magnitude, suggesting that perhaps this maximum BAF is not unique. The overall trend is for decreasing BAFs with increasing soil Ni concentrations (Figure 3), but the 2 lowest BAFs (0.05 and 0.07; Figure 2) from Ma (1982) were derived using control soils (not shown in the relationship between BAF versus soil Ni in Figure 3, because soil Ni concentrations were not reported in Ma [1982]). The relationship between BAFs and soil CEC also shows a decreasing trend (Figure 3). However, with the exception of Beyer et al. (1982), no studies reported both the soil Ni concentration and soil CEC. Therefore, it is not possible to differentiate the degree to which soil Ni concentration and CEC influence the magnitude of the BAF. Accordingly, all BAFs were pooled (Figure 2). The BAFs were distributed lognormally, and a geometric mean BAF of 0.30,

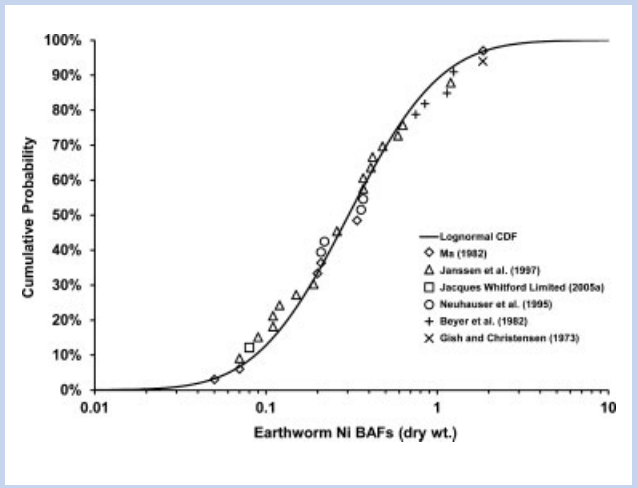

Figure 2. Distribution of earthworm bioaccumulation factors (BAFs) for Ni. Soil content was voided from the earthworm gut, at least partially, in each study. CDF = cumulative distribution function.

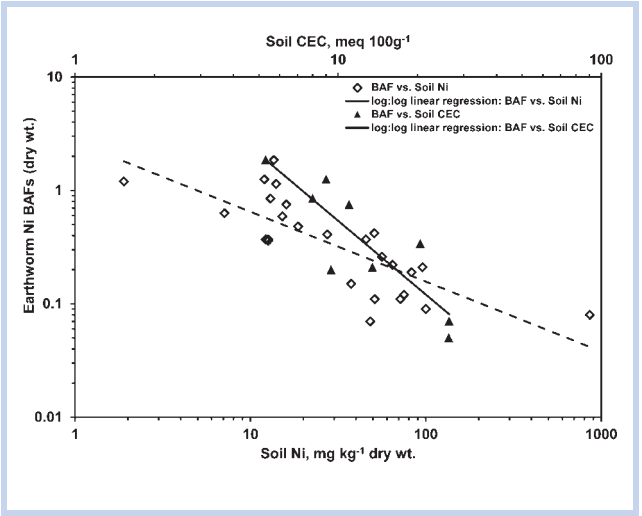

Figure 3. Inverse relationship between earthworm Ni bioaccumulation factors (BAFs) versus soil Ni concentrations and soil cation exchange capacity (CEC). Bioaccumulation factor versus soil Ni relationship based on data from Beyer et al. (1982), Gish and Christensen (1973), Neuhauser et al. (1995), Janssen et al. (1997), and Jacques Whitford Ltd. (2005a). Bioaccumulation factor versus soil CEC relationship based on data from Beyer et al. (1982) and Ma (1982). Soil Ni concentrations and soil CEC were not both reported in every study shown in Figure 2.

on a dry wt basis, was calculated and used to estimate earthworm Ni concentrations from soil Ni concentrations.

The earthworm BAFs were all derived on the basis of Ni concentrations in soil and earthworms expressed on a dry wt basis. Thus, for each regional soil in Table 1, the Ni concentration in earthworms was first estimated on a dry wt basis (Eqn. 1) and then converted to a wet wt basis (Eqn. 2). In the latter equation, it was assumed that the moisture content in earthworms is 84% (USEPA 1993).

$$C_{\text{earthworm, dry wt}} = C_{\text{soil, dry wt}} \times BAF_{\text{dry wt}} \quad (1)$$

where $C_{\text{soil, dry wt}}$ is from Table 4; $BAF_{\text{dry wt}}$ is 0.30 (see text)

$$C_{\text{earthworm, wet wt}} = C_{\text{earthworm, dry wt}} \times (1-0.84) \quad (2)$$

where 0.84 is the moisture fraction in the earthworm.

Based on the above equations, the estimated Ni concentrations in earthworm tissue range from 0.048 to 3.8 mg/kg wet wt for the regional soils (Table 2). The earthworm BAFs are all based on studies in which soil was voided (or at least partially voided) from the earthworm gut prior to chemical analysis. Thus, the BAFs reflect the amount of Ni accumulated in earthworm tissue, but they may underestimate the Ni exposure potential by a worm-eating bird or mammal that would also ingest the soil content in the worm gut. According to the TGD, gut loading of earthworms depends heavily on soil conditions and available food, and reported values range from 2% to 20% (kilogram soil in gut [dry wt] per kilogram voided worm [wet wt]), with 10% being a reasonable value. Using the gut loading value of 10%, the Ni concentration in the earthworm, including the gut contents, can be estimated from

$$C_{\text{earthworm+gut contents, wet wt}} = C_{\text{soil, dry wt}} \times 0.1 + C_{\text{earthworm, wet wt}} \quad (3)$$

Where $C_{\text{soil, dry wt}}$ is from Table 1

$C_{\text{earthworm, wet wt}}$ is from Table 2

0.1 = kilogram soil in gut (dry wt) per kilogram voided worm (wet wt)

Using the equation and taking into account the BAF of 0.30, wildlife exposure to soil-associated Ni is approximately 3 times greater than exposure to Ni in earthworm tissue. The estimated Ni concentrations in earthworms (tissue and gut contents) range from 0.15 to 12 mg/kg wet wt (Table 2).

As discussed earlier, a 2nd terrestrial food chain that includes isopods was also evaluated. Based on data provided in Torres and Johnson (2001), a mean Ni BAF of 0.066 (dry wt) was derived for isopods, which is lower than the geometric mean Ni BAF for earthworms of 0.30. Consequently, the dietary Ni concentration assuming a 100% earthworm diet is approximately 4.5 times higher than a 100% isopod diet, and even higher when considering the soil Ni in the worm gut. This indicates that the 100% earthworm diet may indeed be conservative for wildlife that feed on a variety of invertebrate taxa.

Nickel concentrations in isopods were estimated from the same soil concentrations using the Ni BAF of 0.066 and assuming an isopod moisture content of 0.65 (Torres and Johnson 2001), using the same methods as for the earthworms (Eqns. 1 and 2).

Nickel bioavailability in food. The toxicity values used to derive the avian and mammalian PNECs were based on test organism exposures to highly soluble, and hence bioavailable, forms of Ni (see the *Effects Characterization* section below). In the mammalian toxicity study, rats were exposed to $NiSO_4$, a highly soluble compound, in drinking water. In the bird toxicity studies, soluble Ni compounds were mixed in the experimental diets. Thus, both the mammalian and avian PNECs are expected to overestimate the bioavailability of biologically incorporated Ni in natural diets. In addition, in the terrestrial pathway, soil-adsorbed Ni in the earthworm gut is expected to have reduced bioavailability. Differences in the bioavailability of a substance between exposure media can be described using a relative absorption factor (RAF), which is the ratio of the absorbed fraction of the substance from one exposure medium versus the absorbed fraction from another exposure medium. The following summarizes whether and how relative bioavailability between the PEC_{oral} and $PNEC_{oral}$

Table 2. Estimation of Ni concentrations in earthworms and isopods from Ni concentrations in soil

		Earthworms				Isopods		
Type	Soil Ni (mg/kg dry wt)	BAF (dry wt)	Earthworm Ni (mg/kg dry wt)	Earthworm Ni, voided gut contents (mg/kg wet wt)[a]	Earthworm Ni, with gut contents (mg/kg wet wt)[b]	BAF (dry wt)	Isopod Ni (mg/kg dry wt)	Isopod Ni (mg/kg wet wt)[c]
$PEC_{regional}$								
1. Acid sandy soil	2	0.30	0.60	0.096	0.30	0.066	0.13	0.046
2. Loamy soil	19	0.30	5.7	0.91	2.8	0.066	1.3	0.44
3. Peaty soil	26	0.30	7.8	1.2	3.8	0.066	1.7	0.60
4. Acid sandy soil	1	0.30	0.3	0.048	0.15	0.066	0.066	0.023
5. Clay soil	81	0.30	24	3.8	12	0.066	5.3	1.9
6. Different types	11	0.30	3.3	0.53	1.6	0.066	0.73	0.25

[a]Wet wt Ni concentrations estimated from dry wt Ni concentrations assuming an earthworm moisture content of 84% (USEPA 1993).
[b]Earthworm Ni concentrations with gut contents included were estimated per Equation 3.
[c]Wet wt Ni concentrations estimated from dry wt Ni concentration assuming an isopod moisture content of 65% (Torres and Johnson 2001).

values was quantified using RAFs and incorporated into the risk analysis.

Limited bioavailability data are available for Ni in mammals, although in one study, human volunteers were provided $NiSO_4$ in drinking water in a 1st experiment and $NiSO_4$ in food in a 2nd experiment (Sunderman et al. 1989). The mean (±SD) mass fraction of Ni dose absorbed from the gastrointestinal tract was 27% (±17%) for drinking water and 0.7% (±0.4%) for food. Thus, on average, $NiSO_4$ absorbed from food was approximately 2.5% of that absorbed from drinking water (i.e., an RAF of 2.5%, or in other words, the fraction of $NiSO_4$ absorbed from water was approximately 40 times that absorbed from food). For example, in the 2-generation rat study discussed below (see the *Effects Characterization* section), the no-observed-adverse-effect level (NOAEL) was based on a dose of 1.1 mg Ni/kg/d (dosing of Ni in water, administered by gavage). Using a water-based absorption factor of 27% based on Sunderman et al. (1989), the NOAEL of the 2-generation rat study is associated with an absorbed Ni dose of 0.297 mg Ni/kg/d (1.1 mg Ni/kg/d × 0.27). Thus, if a mammal was consuming dietary Ni, the total dietary dose would need to be 42.4 mg Ni/kg/d to achieve the absorbed Ni dose of 0.297 mg Ni/kg/d, i.e., (0.297 mg Ni/kg/d)/(0.007) = 42.4 mg Ni/kg/d.

The bioavailability of Ni associated with soil in the earthworm gut was evaluated using data from Jacques Whitford Ltd. (2005b). In this study, rats were administered either soluble Ni sulfate or soil Ni slurries. The absorption of Ni in the rats was determined, and an RAF between 2.1% and 3.9% was calculated, depending on the soil. In this assessment, an RAF of 3.9% for Ni in soil, at the upper end of the range, was assumed to account for differences in the bioavailability of Ni from water versus soils. Because the estimated Ni concentrations in the earthworms with non-voided guts represent a mixture of bioaccumulated and soil-adsorbed Ni, and the relative absorption factors differ between these 2 compartments, it is necessary to determine an overall RAF. Based on the earthworm Ni concentrations with and without voided guts in Table 2, 24% of the estimated total Ni concentration is bioaccumulated in earthworm tissue and 76% is adsorbed to soil. Thus, the overall weighted RAF for bioaccumulated and soil-adsorbed Ni in earthworms to mammals can be calculated as

$$\text{Weighted RAF for mammals consuming earthworms} = 0.24\ (2.5\%) + 0.76\ (3.9\%) = 3.6\% \quad (4)$$

It was assumed that an isopod would not have the same relative level of soil in its gut as an earthworm, because isopods do not ingest soil. Therefore, the Ni RAF for mammals consuming isopods was assumed to be equivalent to the food RAF of 2.5%.

Thus, the RAF of 0.036 was quantitatively included in the shrew secondary poisoning risk estimates. These RAFs are based on extremely limited data, so the degree of conservatism associated with these values are unknown. However, as shown later in the risk characterization, if RAFs of these magnitudes were not considered, elevated Ni risks would be predicted for the shrew at soil Ni concentrations well below 55 mg/kg, which is already within the range of background in some regions.

The avian PNECs were based on a study in which mallards were exposed to $NiSO_4$ added to duck starter mash in the laboratory (Cain and Pafford 1981). No studies were identified on the relative bioavailability of $NiSO_4$ added to laboratory diets versus biologically incorporated Ni. Consequently, an avian dietary RAF was not derived for birds (i.e., it was assumed that Ni in the duck starter mash and biologically incorporated Ni are equally bioavailable). Likewise, given that no data on the bioavailability of Ni in soil are available for birds, an avian soil RAF was not considered. Assuming that Ni bioavailability in the mallard study diet is the same as biologically incorporated Ni in nature, as well as the same as Ni bioavailability in soils, is likely a conservative (i.e., cautious) assumption.

PEC_{oral} tiers. As discussed, a tiered approach was used in this assessment, where the 1st tier followed the generic secondary poisoning approach in the TGD and subsequent tiers had increasing levels of refinement that represented a more reasonable level of conservatism for a regional assessment. Ultimately 3 tiers were identified: 1) assume RAF of 1.0 and 100% earthworm diet, 2) assume RAF of 0.036 (mammals only) and 100% earthworm diet, and 3) assume RAF of 0.036 (mammals only) and a mixed earthworm and arthropod diet (Figure 1).

Effects characterization

The $PNEC_{oral}$ values represent dietary Ni concentrations below which adverse effects are not expected for birds or mammals. $PNEC_{oral}$ values were identified in 2 tiers. In Tier 1, derivation of $PNEC_{oral}$ values followed the standard guidance in the TGD and did not account for any species-specific differences in food ingestion rates and body weights. In Tier 2, $PNEC_{oral}$ values were derived on the basis of species-specific food ingestion rate to body weight ratios for the birds and mammals considered in this evaluation (i.e., worm-eating bird, shrew).

Birds. Six subchronic and chronic feeding studies in which bird diets were spiked with a Ni salt were identified. The most sensitive (i.e., lowest) no-observed-effect concentration (NOEC), focusing on endpoints related to survival, growth, and the occurrence of tremors, was 200 mg Ni/kg based on a 90-d study with mallard ducklings. Tremors were observed in all ducklings fed a dietary Ni concentration of 800 mg/kg or greater. Although all but 2 ducklings in the 800 mg/kg dietary treatment survived throughout the 90-d exposure period, all ducklings that developed tremors also had pronounced edema in the toes and leg joints. At a dietary Ni concentration of 200 mg/kg, all ducklings survived, and none developed tremors. Overall, therefore, 200 mg/kg appears to be an appropriate NOEC for this secondary poisoning analysis. No effects on growth, survival, or reproduction in chickens or mallards have been observed at this measured dietary concentration or lower in any of the other 5 studies (Table 3). The next most sensitive study was that of a 21-d exposure of White Plymouth day-old male chickens (*Gallus domesticus*) (Ling and Leach 1979), where a LOEC based on a 14% reduction in body weight was observed. An EC10 (effective concentration for 10% of the study population) of 288 mg/kg was calculated based on an analysis of log-transformed dietary Ni data versus the percent reduction in body weight

Table 3. Summary of Ni toxicity studies with birds

Species	Exposure duration	Endpoint	Dietary Ni (mg/kg) NOEC	Dietary Ni (mg/kg) LOEC	Total or added Ni	Reference
Chicken *Gallus domesticus*	42-d	Body weight	>150	–	Added	Wilson et al. 2001
Chicken *Gallus domesticus*	21-d	Body weight	–	<300 (14% reduction)	Added	Ling and Leach 1979
Chicken *Gallus domesticus*	28-d	Body weight	>13	–	Total	Oscar et al. 1995
Chicken *Gallus domesticus*	28-d	Body weight	500	700 (31% reduction)	Added	Weber and Reid 1968
Mallard *Anas platyrhynchos*	90-d	Body weight, reproduction	>800	–	Added	Eastin and O'Shea 1981
Mallard *Anas platyrhynchos*	90-d	Tremors	200	800 (100%)	Added	Cain and Pafford 1981
		Mortality	800	1200 (71%)[a]		
		Growth	800	1200 (22%)[a]		

[a]Through 60 d of exposure.
LOEC = lowest-observed-effect concentration; NOEC = no-observed-effect concentration.

(Waegeneers and Smolders 2003). More confidence was placed in the NOEC of Cain and Pafford (1981) than the EC10 of Ling and Leach (1979), because the former was from a longer study.

A Tier 1 bird $PNEC_{oral}$ value was developed following the TGD, by which generic bird $PNEC_{oral}$ values are calculated through division of the NOEC by an assessment factor (AF) of 30 to account for interspecies variation in sensitivity and laboratory-to-field extrapolation:

$$\text{Tier 1 bird } PNEC_{oral} = (200\,\text{mg/kg})/30 = 6.7\,\text{mg/kg} \quad (5)$$

We also derived a Tier 2 bird $PNEC_{oral}$ value from the dietary NOEC of 200 mg/kg for mallards by accounting for differences in food ingestion rate to body weights ratios between mallards and worm-eating birds, as represented by European starlings. First, a dose-based NOAEL for the mallard was estimated using the mallard duckling body weights in Cain and Pafford (1981) and the allometric equation from Nagy (1987) for estimating the food ingestion rate. In Cain and Pafford (1981), tremors were observed in ducklings at approximately 28 d of age; therefore, the 28-d body weights were used to determine the relevant body weight (457 g based on the mean of males and females). Using the allometric equation for birds from Nagy (1987) resulted in an estimated food ingestion rate of 34.9 g/d on a dry wt basis (175 g/d on a wet wt basis, assuming a water content of 80% in food). Thus, the estimated body weight (bw) to daily food intake (dfi) ratio for mallard ducklings was estimated to be 2.6 (457 g bw divided by 175 g/d wet wt). The resulting estimated NOAEL for mallard ducklings was 77 mg Ni/kg bw/d (mg/kg/d). As discussed previously, a daily food consumption rate of 90% of body weight per day was assumed for worm-eating birds. Based on this food consumption rate, the bw to dfi conversion factor is 1.1 (1 kg wet bw divided by 0.9 kg wet food per kilogram wet bw per day). Applying the bw to dfi conversion factor of 1.1 to the duckling-based NOAEL of 77 mg/kg/d results in a dietary NOEC of 85 mg/kg for the earthworm-eating bird.

Following the TGD, as was done in developing the Tier 1 avian $PNEC_{oral}$ values above, the chronic NOEC is typically divided by an AF of 30 to account for interspecies variation in sensitivity and laboratory-to-field extrapolation. In developing the Tier 2 avian $PNEC_{oral}$ values, the NOECs used reflect species-specific differences in food ingestion rate to body weight ratios. The 30-fold AF may be appropriate when applying ecotoxicity data among unrelated, taxonomically distinct organisms, e.g., from invertebrate ecotoxicity data to algae or fish. In this assessment, toxicity data are being related within narrower, more closely related groups, e.g., from one bird to another. Consequently, an AF of 10 was used to account for interspecies variation in sensitivity and laboratory-to-field extrapolation. The magnitude of this AF is consistent with TGD guidance for extrapolating chronic aquatic toxicity data, where an AF of 10 is used to extrapolate data among aquatic invertebrates, algae, and fish (i.e., across multiple kingdoms and phyla) (ECB 2003). Using an AF of 10, the following Tier 2 avian $PNEC_{oral}$ value was developed

$$\text{Earthworm-eating bird } PNEC_{oral} = (85\,\text{mg/kg})/10 = 8.5\,\text{mg/kg} \quad (6)$$

Mammals. The NOAEL used to derive the mammalian PNEC value was taken from the previously completed human health Ni risk assessment (ECB 2008), which was identified as 1.1 mg Ni/kg bw/d (mg/kg/d). This was the most sensitive and reliable mammalian NOAEL of 8 repeated oral dose (chronic) toxicity studies with Ni. This NOAEL was based on a reproductive study of rats exposed to $NiSO_4$ via drinking water. The following describes how this NOAEL was used to derive Tier 1 and Tier 2 mammalian $PNEC_{oral}$ values.

The Tier 1 mammalian $PNEC_{oral}$ value was derived following the TGD, in which the dose-based Ni NOAEL of 1.1 mg/kg/d was converted to a dietary NOEC, expressed as milligrams of Ni per kilogram, using the bw to dfi conversion factor of 20 cited in the TGD for rats older than 6 weeks. This resulted in an estimated dietary NOEC of 22 mg/kg for rats. Based on the TGD, the generic mammalian $PNEC_{oral}$ value was calculated through division of the NOEC by an AF of 30 to account for interspecies variation in sensitivity and

laboratory-to-field extrapolation

$$\text{Tier 1 mammalian PNEC}_{oral} = (22\,mg/kg)/30 = 0.73\,mg/kg \quad (7)$$

To calculate the Tier 2 mammalian $PNEC_{oral}$ value, the rat-based NOAEL of 1.1 mg/kg/d was adjusted based on the bw to dfi ratio for shrews. Based on a food consumption rate of 90% of its bw per day, the bw to dfi conversion factor for the common shrew was calculated to be 1.1 (1 kg bw wet wt divided by 0.9 kg food wet wt/kg bw/d). Applying the bw to dfi conversion factor of 1.1 to the NOAEL of 1.1 mg/kg/d resulted in a dietary NOEC of 1.2 mg Ni/kg for the common shrew.

Consistent with the reasoning behind the development of the Tier 2 avian $PNEC_{oral}$ values, an AF of 10 was applied to the shrew dietary NOEC to estimate its $PNEC_{oral}$ value

$$\text{Common shrew PNEC}_{oral} = (1.2\,mg/kg)/10 = 0.12\,mg/kg \quad (8)$$

The mammalian PNECs are based on a toxicity study in which rats were exposed to $NiSO_4$ in drinking water. Nickel bioavailability via this form and exposure route is expected to be conservatively high relative to the dietary exposure route in nature. The RAFs discussed previously were derived to account for the differences in bioavailability between the PEC_{oral} and $PNEC_{oral}$ values.

Risk characterization

The risk characterization combined the results of the PEC_{oral}, relative bioavailability, and $PNEC_{oral}$ evaluations. The risk analysis was conducted in tiers, with the 1st tier being the most simplified and subsequent tiers being based on increasing levels of complexity (Figure 1). For each tier, a PEC_{oral} to $PNEC_{oral}$ ratio was calculated as follows:

$$\text{PEC}_{oral} \text{ to PNEC}_{oral} \text{ ratio} = (\text{PEC}_{oral} \times \text{relative absorption factor})/\text{PNEC}_{oral} \quad (9)$$

The intent of the tiered approach was to more clearly show the influence of the PNEC, PEC, and bioavailability assumptions on secondary poisoning risk estimates.

RESULTS

Birds

In Tier 1, regional PEC_{oral} values were compared to the generic $PNEC_{oral}$ value of 6.7 mg/kg. All regional PEC_{oral} ratios were <1, except for a ratio of 1.8 in the clay soil based on the $PNEC_{oral}$ of 6.7 mg/kg (Table 4). In Tier 2 of the risk characterization, using the $PNEC_{oral}$ adjusted for the

Table 4. PEC_{oral} to $PNEC_{oral}$ ratios for a worm-eating bird

Tier	$PNEC_{oral}$ (mg/kg)	RAF	Type	PEC_{oral} (mg/kg)	PEC_{oral} to $PNEC_{oral}$ ratio
1	Generic = 6.7	1	1. Acid sandy soil	0.30	0.04
	(100% earthworm diet)		2. Loamy soil	2.8	0.4
			3. Peaty soil	3.8	0.6
			4. Acid sandy soil	0.15	0.02
			5. Clay soil	12	1.8
			6. Different types	1.6	0.2
2a	Worm-eating bird = 8.5	1	1. Acid sandy soil	0.30	0.04
	(100% earthworm diet)		2. Loamy soil	2.8	0.3
			3. Peaty soil	3.8	0.4
			4. Acid sandy soil	0.15	0.02
			5. Clay soil	12	1.4
			6. Different types	1.6	0.2
2b	Worm-eating bird = 8.5	1	1. Acid sandy soil	0.17	0.02
	(50% earthworm diet;		2. Loamy soil	1.6	0.2
	50% isopod diet)		3. Peaty soil	2.2	0.3
			4. Acid sandy soil	0.087	0.01
			5. Clay soil	7.0	0.8
			6. Different types	0.93	0.1

PEC_{oral} to $PNEC_{oral}$ ratio = (PEC_{oral}/$PNEC_{oral}$) × RAF; RAF = relative absorption factor.

worm-eating bird bw to dfi ratio slightly lowers the regional PEC_{oral} to $PNEC_{oral}$ ratios to 1.4, assuming a 100% earthworm diet, and to 0.8, assuming a 50%:50% earthworm and isopod diet (Tiers 2a and 2b in Table 4).

Mammals

In Tier 1, regional PEC_{oral} values were compared to the generic $PNEC_{oral}$ of 0.73 mg/kg assuming an RAF of 1. Several regional PEC_{oral} ratios were >1 (Table 5). In Tier 2, the $PNEC_{oral}$ value of 0.12 mg/kg, adjusted for the shrew bw to dfi ratio, was used, which resulted in higher ratios because the shrew-adjusted $PNEC_{oral}$ value was lower than the generic $PNEC_{oral}$ value (Table 5). Tier 3 included incorporation of the RAF to account for difference in Ni bioavailability between the PEC_{oral} and $PNEC_{oral}$ values. For Tier 3a, in which a 100% earthworm diet was assumed, 2 of the regional scenarios resulted in PEC to PNEC ratios >1. The Ni concentrations in these soils were 26 mg/kg (peaty soil) and 81 mg/kg (clay soil). It should be noted that the ambient Ni concentration in European soils, 27.8 mg Ni/kg, would yield a PEC to PNEC ratio of 1.2. This indicates that the use of the shrew food chain to evaluate potential secondary poisoning risks from Ni is very conservative. In Tier 3b, it was assumed that the shrew diet contains 30% earthworms and 70% other invertebrates (as represented by isopods). The PEC to PNEC ratios were 1.4 or less when the RAFs of 0.036 and 0.025 for the earthworm and isopod, respectively, were included (Table 5). In just a single regional scenario, the clay soil with a Ni concentration of 81 mg/kg (clay soil) resulted in a PEC to PNEC ratio >1. These PEC to PNEC ratios for Tier 3 of the shrew evaluation were similar to that observed for the terrestrial worm-eating bird. The 2 soils with the highest Ni concentrations, 26 and 81 mg/kg, are also characterized by high CEC (35 and 36 meq/100 g, respectively). Based on the relationships between earthworm Ni BAFs versus soil Ni

Table 5. PEC_{oral} to $PNEC_{oral}$ ratios for common shrews

Tier	$PNEC_{oral}$ (mg/kg)	RAF	Type	PEC_{oral} (mg/kg)	PEC_{oral} to $PNEC_{oral}$ ratio
1	Generic = 0.73	1	1. Acid sandy soil	0.30	0.4
			2. Loamy soil	2.8	3.8
			3. Peaty soil	3.8	5.2
			4. Acid sandy soil	0.15	0.2
			5. Clay soil	12	16
			6. Different types	1.6	2.2
2	Shrew = 0.12	1	1. Acid sandy soil	0.30	2.5
			2. Loamy soil	2.8	23
			3. Peaty soil	3.8	32
			4. Acid sandy soil	0.15	1.3
			5. Clay soil	12	100
			6. Different types	1.6	13
3a	Shrew = 0.12	0.036	1. Acid sandy soil	0.30	0.09
	(100% earthworm diet)		2. Loamy soil	2.8	0.8
			3. Peaty soil	3.8	1.1
			4. Acid sandy soil	0.15	0.05
			5. Clay soil	12	3.6
			6. Different types	1.6	0.5
3b	Shrew = 0.12	0.036[a] 0.025[b]	1. Acid sandy soil	0.12	0.03
	(30% earthworm diet; 70% isopod diet)		2. Loamy soil	1.1	0.3
			3. Peaty soil	1.6	0.4
			4. Acid sandy soil	0.061	0.02
			5. Clay soil	4.9	1.4
			6. Different types	0.66	0.2

[a]Earthworm diet.
[b]Isopod diet.
PEC_{oral} to $PNEC_{oral}$ ratios = (PEC_{oral}/$PNEC_{oral}$) × RAF; RAF = relative absorption factor.

concentration and soil CEC shown in Figure 3, the earthworm BAFs for these soils are likely <0.1, suggesting that the Tier 3 PEC to PNEC ratios for these soils would be <1.

DISCUSSION

This study estimated secondary poisoning risks for birds and mammals exposed to Ni in the terrestrial environment, which was required under the EU Existing Substances regulation (EEC 793/93). This was a regional-scale analysis of Ni in representative soils with varying background levels of Ni. The analysis initially followed the generic approach in the TGD, but we found this approach to be unrealistically conservative and, therefore, used a tiered approach that refined the risk estimates. The following initially summarizes the key variables that were adjusted in PEC and PNEC development and then considers additional points of comparison, or lines of evidence, to support use of the tiered approach. These adjustments will be relevant to forthcoming localized secondary poisoning risk assessments for Ni and would likely be relevant to secondary poisoning assessments of other metals as well.

In the 1st tier of this secondary poisoning analysis, which followed the TGD, potential risks were estimated for an earthworm-eating bird (PEC to PNEC ratio of 1.8 in the regional clay soil scenario) and for the shrew (PEC to PNEC ratios of 2.2 to 6.0 in 4 of the 6 regional soil types). Accounting for differences in ingestion rate to body weight ratios between the test organisms used to derive the PNECs (mallards, rats) and the representative worm-eating bird and mammals in Tier 2 resulted in PEC to PNEC ratios that were approximately 21% lower for birds and 508% higher for mammals. The latter is due to the much higher food ingestion rate to body weight ratio assumed for the shrew compared to the rat. Ultimately, in Tier 3, where a mixed diet of earthworms and arthropods was assumed, and relative bioavailability (i.e., RAFs) were assumed for mammals, all bird and mammal PEC to PNEC ratios were <1.0, except for a ratio of 1.4 for the regional clay soil.

All ecological risk assessments are built on numerous assumptions in order to quantify potential risks, and each assumption has an inherent level of associated uncertainty. The TGD provides a generic methodology for assessing potential risks to dietary chemical exposures, which is based on conservative assumptions to reduce the likelihood of concluding there is no risk when in fact there is, or to identify data gaps that could be filled to reduce uncertainty. We found the secondary poisoning risks for Ni, based on PEC to PNEC ratios following the TGD, were unrealistically high for the regional soils evaluated in this assessment. The most important variables influencing this conservatism were the AF of 30 applied to NOECs when deriving the PNECs, the assumption of equivalent Ni bioavailability in natural dietary doses versus the doses used to derive the NOECs, and the assumption of a 100% earthworm diet, which maximized the estimated Ni dose, because soil in the earthworm gut contributed more to the Ni dose than did Ni in earthworm tissue. The default AF of 30, in particular, is a somewhat arbitrarily defined variable in the TGD that is generically applicable to any chemical. As in this risk assessment, this variable should be critically assessed, and modified as necessary, based on chemical-specific information, such as inter- and intraspecies variability in sensitivity based on available toxicity data. In addition, for naturally occurring elements, it should also be noted that large AFs may result in unrealistically conservative PNECs. The spatial scale of this secondary poisoning risk evaluation was at the regional level, with representative background soil Ni concentrations identified for regions with varying soil types (e.g., sandy, loamy, peaty) and conditions (pH, CEC, percent organic matter). Following the TGD approach, identified as Tier 1 in this assessment, would suggest that dietary Ni exposure is a widespread risk to consumers of earthworms across large areas of Europe. Given the lack of empirical evidence to suggest that this is the case, the use of the refinements (bioavailability, species-specific modifications, dietary composition) was critical to reach reasonable conclusions. Using the most refined tiers of the assessment lead to the conclusion that background dietary Ni exposures to terrestrial mammals and birds poses a negligible risk at the regional scale.

To evaluate this regional risk conclusion further, a ''threshold'' Ni concentration in soil was back-calculated using the higher tier assumptions in this risk analysis. This threshold Ni concentration can then be compared to background soil concentrations from other locations or to existing soil quality guidelines for Ni as a means of verifying that the risk-based assumptions were still appropriately conservative for a regional-scale risk assessment. The threshold Ni concentration in soil can also be used to identify and prioritize locations that may warrant site-specific risk evaluations, or otherwise be used to determine whether direct toxicity PNECs are sufficiently protective of the potential for secondary poisoning. Threshold Ni concentrations protective of secondary poisoning can be calculated by setting the PEC to PNEC ratio to 1 and then solving for the Ni concentration in soil. The threshold Ni concentration in soil was derived for the shrew, because higher PEC to PNEC ratios were calculated for the shrew than for a worm-eating bird. Equations 1 to 3 can be combined to relate the Ni concentration in soil to the Ni concentration in earthworms (tissue and soil content in gut) and isopods on a wet wt basis, and then weighted based on a 30% earthworm diet and 70% arthropod diet, which is then equivalent to the PEC_{oral} (Eqn. 11)

$$PEC_{oral} = C_{soil,\ dry\ wt} \times [0.3 \times (BAF_{earthworm,\ dry\ wt} \times 0.16 + 0.1) + 0.7 \times BAF_{isopod,\ dry\ wt} \times 0.35] \quad (11)$$

The resulting PEC_{oral} is 3.3 mg Ni/kg wet wt. The PEC_{oral} to $PNEC_{oral}$ ratio, adjusted for the RAF, is calculated as follows:

$$\text{RAF-adjusted PEC/PNEC Ratio} = \frac{PEC_{oral} \times RAF}{PNEC_{oral}} \quad (12)$$

Finally, by rearranging and combining Equations 11 and 12, the critical PEC for Ni in soil (PEC_{soil}^*) can be back-calculated as follows:

$$PEC_{soil}^* = \frac{1 \times PNEC}{[0.3 \times (BAF_{earthworm,\ dry\ wt} \times 0.16 + 0.1) + (0.7 \times BAF_{isopod,\ dry\ wt} \times 0.35)] \times RAF} \quad (13)$$

Where PEC_{soil}^{*} = critical predicted exposure concentration of Ni in soil that results in an RAF-adjusted PEC to PNEC ratio of 1 (mg Ni/kg $soil_{dry\ wt}$); PNEC = predicted no-effect concentration for Ni in shrew diet, adjusted for species bw to dfi ratio (0.12 mg Ni/kg $food_{wet\ wt}$); BAF = bioaccumulation factor (0.30 and 0.066 for earthworms and isopods, respectively); and RAF = relative absorption factor (0.036).

The resulting PEC_{soil}^{*} is 55 mg Ni/kg dry wt. For comparison, in the terrestrial effects assessment for Ni (ECB 2008), soil HC5s (i.e., estimated hazardous concentrations to 5% of the tested species) ranged from 8.6 to 194.3 mg/kg dry wt. Based on an AF of 2, this corresponds to a PNEC range of 4.3 to 97.2 mg/kg dry wt. These PNEC values are based on the sensitivity of plants (Rooney et al. 2007), invertebrates (ECB 2008), and microbial processes (Oorts et al. 2007) to Ni in soil. The range in PNECs reflects different soil types, with CEC as the key variable influencing site-to-site differences. The critical PEC_{soil}^{*} calculated here of 55 mg/kg for the shrew is within this range of direct toxicity PNECs, suggesting that levels of Ni in soil protective of direct toxicity may not always be protective of the potential for secondary Ni poisoning in mammals. However, this comparison is somewhat simplistic, because the range in direct toxicity PNECs reflects different Ni bioavailabilities in different soil types. The food chain analysis in this secondary poisoning assessment does not account for differences in Ni bioavailability (i.e., constant earthworm and isopod BAFs were applied to different soil types). Although the earthworm BAF data from the literature reflect an inverse relationship between the BAF and CEC levels of approximately 5 to 11 meq/100 g, the data defining this relationship were determined to be too limited for estimating separate BAFs for each soil evaluated in this assessment. Other factors can influence the magnitude of BAF values, such as the soil Ni concentration, which were not consistently reported in the earthworm BAF studies.

As another point of comparison for the critical PEC_{soil}^{*} of 55 mg/kg, the US Environmental Protection Agency (USEPA) has derived ecological soil screening values (Eco-SSLs) for Ni based on direct soil exposures by plants and invertebrates and food chain exposures by birds and mammals (USEPA 2007). The Ni Eco-SSLs for plants, invertebrates, birds, and mammals are 38, 280, 210, and 130 mg/kg, respectively. The avian and mammalian Eco-SSLs for Ni are both higher than the maximum direct toxicity Ni PNEC of 97.2 mg/kg dry wt obtained from ECB (2008). Accordingly, the threshold soil PEC of 55 mg/kg derived above for the shrew is approximately 2.5 times more conservative than the Eco-SSL derived for mammals. The additional conservatism in the threshold soil PEC of 55 mg/kg compared to the USEPA Eco-SSL for mammals is in large part due to differences in the dose-based Ni toxicity thresholds used, as well as in different bw to dfi ratios used for the representative mammals (shrew in this evaluation versus a weasel in the Eco-SSL). The mammalian NOAEL in this evaluation was 1.1 mg/kg/d (ECB 2008), whereas the mammalian NOAEL used to derive the Eco-SSL for mammals was 1.70 mg/kg/d. This latter NOAEL was the highest bounded NOAEL (i.e., NOAEL with a paired lowest-observed-adverse-effects level [LOAEL]) below the lowest bounded LOAEL (i.e., LOAEL with a paired NOAEL) for reproduction, survival, or growth (USEPA 2007).

Finally, ambient background Ni concentrations in soils are important indicators for determining whether a threshold PEC is reasonable. In the EU soil effects assessment for Ni (ECB 2008), ambient background Ni concentrations between 26.2 mg/kg dry wt (for agricultural soils) and 35.8 mg/kg dry wt (for grassland soils) were considered. These concentrations are the median of the 90th percentile ambient background Ni concentration for topsoils in Europe from national monitoring databases. In the toxicity tests with plants, invertebrates, and microbial processes, median (10th to 90th percentiles in parentheses) background Ni concentrations in control soils were 14 (2–40), 11 (1–60), and 18 (2–39) mg/kg, respectively (ECB 2008). As such, the threshold soil PEC of 55 mg/kg for the shrew was near or below the 90th percentile Ni concentration from the control soils in these studies. In addition, the threshold soil PEC of 55 mg/kg for the shrew is below the background Ni concentration of 81 mg/kg for a natural woodland soil in Greece (this is the scenario that resulted in an PEC to PNEC ratio of 1.4 in this secondary poisoning analysis). The above comparisons support the assumption that the food chain pathway for the shrew evaluated in this assessment used a conservative approach and that the exposure assumptions, such as the RAF, appear to be reasonable despite the uncertainties associated with the limited availability of data available for mammals.

When the tiered approach for assessing secondary poisoning risk for Ni was evaluated by the Technical Committee for New and Existing Substances, it resulted in a ''conclusion (ii),'' i.e., ''there is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already'' (ECB 2008). This conclusion was reached for the following reasons. First, when the tiered approach was followed, only 2 instances in the terrestrial food chains arose where the PEC to PNEC ratio was >1 (and, although not presented in this article, none of the aquatic food chains resulted in PEC to PNEC ratios >1). These instances were observed for the clay soil, for which the estimated PEC_{oral} was greater than the $PNEC_{oral}$ for the shrew and for the worm-eating bird. Considering the cautious assumptions about interspecies sensitivity, bioavailability of Ni, and diet composition that were made in the assessment, and that the Ni contained in the clay soils (the highest PEC to PNEC ratio) is of natural origin, a conclusion of no risk can be appropriately made for these soils as well.

Regional-scale risk assessments are inherently more uncertain than localized risk assessment due to data limitations over broad scales and averaging of site-specific factors that influence the likelihood of exposure and effects. This regional secondary poisoning risk assessment for Ni highlights several of the key risk assessment components that should be considered in future localized secondary poisoning assessments of Ni or other metals.

Key uncertainties in this secondary poisoning risk assessment include the sensitivity to Ni of the bird and mammals that were evaluated, the bioavailability of Ni to birds and mammals in natural diets relative to the bioavailability of Ni in the laboratory diets used to determine the $PNEC_{oral}$ values, and the dietary assumptions for the bird and mammal species evaluated. In each case, the methods used attempted to err toward conservatism. For example, it was assumed that the birds and mammals evaluated could be 10 times more sensitive than the organisms for which the effects concentrations are based (10-fold interspecies assessment factor). A $PNEC_{oral}$ value of 8.5 mg/kg was identified in this assessment for birds, yet in some bird studies, a dietary Ni concentration

of 25 to 50 mg/kg has been shown to have a beneficial effect on bone strength and weight of hatchlings (Eastin and O'Shae 1981; Wilson et al. 2001). It was also assumed that Ni in natural bird diets and ingested soil had the same bioavailability as in the toxicity study used to develop the PNEC, in which a Ni salt was spiked into a commercial diet (i.e., an RAF of 1 was assumed). This assumption was made due to a lack of Ni bioavailability data for birds, even though the limited data available for mammals suggest that the bioavailability of Ni in natural diets and soils is quite low (on the order of 1%–4%). Therefore, localized assessments should focus on the sensitivities of key species that are relevant to the site in question.

Several factors still render this analysis conservative, and these issues should be addressed, if possible, in site-specific local Ni risk assessments. For example, we estimated Ni concentrations in earthworms using BAFs from a variety of studies and soil types. A geometric mean of 0.30 was calculated, but the individual BAF values ranged from 0.05 to 1.86. The factors contributing to this 37-fold difference are not clear based on the data available from the different BAF studies. An inverse relationship was observed (Figure 3) between earthworm BAFs and both soil Ni concentrations and soil CEC. However, with the exception of one study (Beyer et al. 1982), none of the studies reported both soil Ni concentrations and soil CEC. Therefore, it is not possible to evaluate the relative importance of each of these factors on the magnitude of the earthworm Ni BAF (the Ni concentrations and CEC in the soils tested in Beyer et al. [1982] were similar and thus do not provide any information on relative importance of these factors on the BAF). Developing a predictive model to estimate Ni concentrations in food items based on mechanistic principles could reduce this uncertainty. In lieu of such a model, site-specific empirical relationships could still decrease uncertainty. Uptake factors may be used to estimate Ni concentrations in food items, but consideration should be given to soil properties that influence Ni bioavailability in soils (e.g., pH, CEC). It also should be noted that the relative bioavailability of Ni in various foods for different species significantly affects estimation of risk, but little is known about this for Ni and common wildlife receptors. Use of a dose-based PNEC that considers species-specific ingestion rates is less uncertain than simply using dietary concentrations. Ultimately, uncertainty in the exposure–response relationship is reduced even further when results are based on the concentration in the target organ, an area requiring further research for Ni sequestration.

Overall, it appears that small mammals with a high ingestion rate to body weight ratio and a diet that includes substantial soil ingestion, such as a diet high in earthworms, show the greatest risk for dietary Ni toxicity at soil Ni concentrations that are directly toxic to invertebrates and plants. For sites where earthworm-to-small mammal food chains are not relevant, plant- or invertebrate-based toxicity thresholds are likely to be protective of secondary poisoning to birds and mammals.

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