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Derivation of an oral toxicity reference value for nickel

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ABSTRACT

Nickel (Ni) is in the earth's crust and can be found in environmental compartments such as water, soil, and air, as well as food. This paper presents an assessment of the oral nickel toxicity data in support of non-cancer health-based oral exposure limits or toxicity reference values (TRVs). This paper derives TRVs for three populations of interest: adults, toddlers, and people who have been dermally sensitized to nickel. The adult/lifetime TRV of 20 μg Ni/kg-day is based on post-implantation loss/perinatal mortality in a 2-generation reproductive study in rats. Several recent assessments by regulatory agencies have used the same study and endpoint, but the dose-response modeling conducted here was more appropriate for the study design. Toxicokinetic data from rats and humans indicate that the applied uncertainty factors are very conservative. Because the endpoint relates to fetal exposure and is not relevant to toddlers, a toddler TRV was derived based on decreased body weight in young rats; this TRV was also 20 μg Ni/kg-day. A separate TRV of 4 μg Ni/kg in addition to Ni in food was derived for protection of nickel-sensitized populations from flare-up of dermatitis, based on studies of single exposures in humans under conditions that maximize oral absorption.

1. Introduction

Nickel (Ni) is a natural element of the earth's crust and as a consequence it can be naturally found in environmental compartments such as water, soil, and air. Ni is an essential micro-nutrient for plant growth (Brown et al., 1987), and is therefore also present in a wide range of primary crops, animals and foodstuffs (De Brouwere et al., 2012). Ni is used in many industrial applications such as the manufacturing of stainless steel (e.g., for building, food and medical applications) and high Ni alloys (e.g., for plane turbine manufacturing), as well as the production of Ni-plated consumer articles, Ni-containing batteries, and Ni in electronic products. The industrial production and use of Ni as well as the burning of plant-based fuels (i.e., petroleum products) can contribute to the levels of Ni in environmental compartments. Therefore, for the human population the sources and pathways of Ni exposure are diverse.

The combination of each chemical form of nickel and each exposure route determines the overall absorption and bioavailability of Ni(II) ion. When bioavailability of Ni(II) from a particular substance or matrix is not known, the bioaccessibility of Ni(II) in

Abbreviations: ADI, Acceptable Daily Intake; AIC, Akaike Information Criterion; ATSDR, Agency for Toxic Substances and Disease Registry; AUC, area under the plasma concentration time curve; BMD, Benchmark Dose; BMDL, Benchmark Dose lower confidence limit; BMDS, Benchmark Dose Software; BMR, Benchmark response; CBD, chronic beryllium disease; ECB, European Chemicals Bureau; EFSA, European Food Safety Authority; FSCJ, Food Safety Commission of Japan; gof, goodness-of-fit; GD, gestation day; GLP, Good Laboratory Practice; IC, intra-litter correlation; IPCS, International Programme on Chemical Safety; LOAEL, Lowest Observed Adverse Effect Level; LSC, litter-specific covariate; MLE, Maximum Likelihood Estimate; MRL, Minimal Risk Level; Ni, Nickel; NOAEL, No Observed Adverse Effect Level; OECD, Organisation for Economic Co-operation and Development; OEHHA, Office of Environmental Health Hazard Assessment; OR, Odds Ratio; PM10, Particulate matter less than 10 μm in diameter; PND, postnatal day; POD, point of departure; RfC, Reference Concentration; RfD, Reference Dose; REL, Reference Exposure Level; RIVM, National Institute for Public Health and the Environment (the Netherlands); RTI, Research Triangle Institute; RVR, Rai and van Ryzin; SD, Sprague Dawley; SLI, Springborn Laboratories, Inc.; SNAS, systemic nickel allergy syndrome; SRAC, systemically reactivated allergic contact dermatitis; TD, toxicodynamic; TDI, Tolerable Daily Intake; TI, Tolerable Intake; TK, toxicokinetic; TRV, Toxicity Reference Value; UF, uncertainty factor; U.S. EPA, United States Environmental Protection Agency; WHO, World Health Organisation.

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synthetic fluids[1] (relative to water soluble compounds) corresponding to each route of exposure can provide an indication of the relative in vivo bioavailability. Oral toxicity studies of nickel have generally involved administration of nickel in water, either via gavage or in drinking water, conditions where the nickel is 100% bioaccessible Ni(II). This maximizes the absorption of nickel compared to nickel in food, soil or dust.

Regulatory and guidance agencies throughout the world set non-cancer health-based oral exposure limits or toxicity reference values (TRVs). Although there are some variations in the specifics of the methods, these limits are generally designed based on a specific problem formulation (e.g., consideration of a specific exposure duration and population), with the goal of protecting the population of interest from adverse effects. TRVs for the general population are generally intended to protect the exposed population of interest, including sensitive subpopulations. Although TRVs are most commonly derived for chronic exposure scenarios, it is often useful to have TRVs for other durations and populations, such as for addressing intermittent exposures (Haber et al., 2016). The resulting TRVs can then be used to derive regulatory standards protecting the population of interest, such as levels of nickel in drinking water, metal migration from food contact material, etc.

When deriving an oral TRV for nickel, several key questions need to be considered. First among these are the problem formulation:

- What is the purpose of deriving the TRV?
- What is exposure scenario(s) of interest (duration, route, etc.)?
- Who are the target populations or receptor populations (e.g., toddlers, adults)?

General questions for development of any oral TRV include:

- What are the most sensitive systemic effects of concern after oral exposure (i.e., key studies, critical effects and associated points of departure)?
- What uncertainty factors should be used to develop the TRV?
- What are the main sources of uncertainty and how do they affect the calculated value?

When deriving an oral TRV for nickel, additional questions arise because nickel is prevalent in food, and because of the substantial differences in bioavailability of nickel from different matrices (e.g., food, water, soil), and in the presence of a full versus fasted stomach:

- Do point of departure values include all sources of exposure (e.g., do they include food)?
- Should bioavailability of Ni(II) be considered in either the development of the TRV or in its application in a risk characterization? Will the TRV be defined as an absorbed dose or as an external exposure? Should media-specific TRVs be developed?

This paper aims to address the questions posed above with the goal of deriving appropriate and relevant TRVs for nickel. For this assessment, the purpose of the TRV is to identify safe oral intake levels after exposure to Ni from food, water and soil, as these are the main sources of Ni exposure (section 3.1). Exposure from food and drinking water are of interest for the entire population. In addition, ingestion of soil by young children is of particular interest, since this group is identified as being the population with the highest oral intake of soil on a per kg body weight basis. Toxicokinetics related to acute exposures to nickel (e.g., from the first drink of water in the morning on an empty stomach) as well as long-term exposures in rats and humans are considered in section 3.2. The toxicity database for nickel is discussed in section 3.3. The populations of interest include adults, children, and people who have been dermally sensitized to nickel. The development of a chronic TRV for the adult population, an acute TRV for nickel hypersensitive populations, and a TRV for young children are described in section 3.4, 3.5, and 3.6, respectively. Uncertainty in the calculations and how this was addressed in our derivations are explained in section 4, together with consideration of how medium-specific estimates of bioavailability could be used to compare exposures to the TRVs.

2. Methods

2.1. General approach to TRV derivation

The general methods for deriving TRVs are well documented in a variety of publications (e.g., IPCS, 1994; 1999; Meek et al., 1994; US EPA, 2002). In brief, the process begins with a problem formulation, identifying the purpose for deriving the TRV, as well as the exposure scenario (e.g., duration(s) and route(s)) and potential exposed population. A literature search is then conducted to identify relevant studies. The studies are reviewed to characterize the effects caused by the chemical under the exposure conditions of interest. As part of the hazard characterization, the relevance to humans of effects seen in animal studies is considered (Cohen et al., 2003; Seed et al., 2005), as well as factors that may result in specific sensitive populations. This allows one to identify the most sensitive endpoint(s) for the scenarios of interest. In particular, the goal is to identify the critical effect, defined by US EPA (2011) as “the first adverse effect, or its known precursor, that occurs to the most sensitive species as the dose rate of an agent increases.” A point of departure (POD) is then identified, typically a No Observed Adverse Effect Level (NOAEL), Lowest Observed Adverse Effect Level (LOAEL), or a benchmark dose (BMD). The approach for low-dose extrapolation depends on the mode of action. For effects that do not result from interaction with DNA, a subthreshold dose is calculated by applying uncertainty factors to the POD (IPCS, 1994; 1999; Meek et al., 1994; US EPA, 2012). There are some differences across agencies in the specifics of uncertainty factor (UF) application, but all organisations include UFs for human variability, extrapolation from experimental animals to humans, and various database deficiencies, such as not having a NOAEL. For this assessment, the methods of IPCS (1994. 1999) were used.

There are a number of recent authoritative reviews for nickel (US EPA, 1991; Health Canada, 1996a; 1996b; RIVM, 2001; ATSDR, 2005; WHO, 2007; OEHHA, 2012; FSCJ, 2012; EFSA, 2015). Therefore, the literature search was conducted only for studies published since 2014, relying on the authoritative reviews to ensure that the literature on nickel toxicity has been adequately captured. The remainder of the steps in the risk assessment process were followed, as described in the previous paragraph.

2.2. Benchmark dose modeling methods

2.2.1. General methods

All BMD modeling was done using extra risk. Extra risk at dose d (ER(d)) is defined as

[1] The bioaccessible concentration of Ni(II) ion is defined as the fraction of the material (food, soil, plated item) that can be released as soluble ion in a particular solution (e.g., synthetic fluids relevant to each route of exposure). The bioaccessibility of Ni from a Ni-containing substance provides a high end estimate of its in vivo bioavailability (i.e., not all bioaccessible Ni gets absorbed).

$$ER(d) = [p(d) - p(0)]/[1 - p(0)]$$

where p(d) is the probability of response at dose d.

All modeling was done using BMDS (version 2.6, U.S. EPA, 2015), using all of the standard suite of models appropriate for the type of endpoint. Thus, incidence data were modeled using the standard dichotomous models, continuous data (such as body weight) were modeled using the continuous models, and data from developmental toxicity studies were modeled using BMD models that capture the "nested" nature of the data (i.e., that the litter, not the pup, is the experimental unit). The models were fit by maximum likelihood techniques (U.S. EPA, 2015). For quantal data, BMDs corresponding to 10% extra risk and 95% lower bounds on the BMDs (the BMDLs) were obtained using profile likelihood methods (U.S. EPA, 2015); for the developmental studies, BMDs corresponding to 5% extra risk were also calculated. For continuous endpoints, the BMD corresponding to a change in the mean of one control standard deviation was calculated. Where there was also information on the degree of change that is considered adverse (e.g., a 10% change in body weight), this was also used as the basis for the BMD. In all cases where there was an option to run with a restricted or unrestricted version of the model, the appropriate parameter was restricted; not all models offer an option for restriction.

Identification of the best fitting models was accomplished in a two-step process (US EPA, 2012). First, the acceptable models were identified based on the goodness-of-fit *P*-value (acceptable models had $P > 0.1$). This *P*-value is an evaluation of the overall model fit. It is calculated based on the Chi square and the number of degrees of freedom. Once the acceptable models were identified, the best fitting model(s) were evaluated by taking the following points into consideration (US EPA, 2012):

- Akaike Information Criterion (AIC). Because the fit generally improves as additional parameters are added within a family of models, the AIC is used to determine whether the improvement in fit justifies estimating additional parameters. Among models with similar fit, the AIC prefers less complex models. Specifically, the AIC is a measure of overall fit that takes into account the number of model parameters, and is calculated as $-2LL + 2p$, where *LL* is the log-likelihood at the maximum likelihood estimates [MLEs] for *p* estimated parameters. Smaller AICs (to the left on the number line) are better when comparing two models. Note that only the difference in AIC is meaningful, not the actual value. For a similar degree of fit, AIC rewards the less complex model. Because the AIC includes an adjustment of 2 times the number of parameters, a difference of 2 in the AIC (a unitless measure) is often considered meaningful, since a difference of that magnitude means that adding the parameters improved the fit more than the penalty for adding the parameters.[2]
- The scaled residuals, evaluated at the dose with a response closest to the benchmark response. The scaled residuals compare the observed and expected response at that dose and provide a measure of local fit. Smaller absolute values for the scaled residuals are better, and models with a scaled residual that has an absolute value of 2 or larger are rejected (US EPA, 2012).
- Visual fit is evaluated only subjectively, with a focus on how well the model fits the underlying data, especially at the lower-dose end of the data. Additionally, visual fit considers whether the shape of the model is biologically plausible, and other potential issues, such as whether all of the data fall on one side of the model curve.

When there is no clear choice of the best-fitting model, and more than one model has a similar fit, results from all models with similarly good fit are averaged.

2.2.2. Modeling of the nested data

Inputs to this modeling include the dam dose level, number of pups in the litter for that dam, and the number of affected pups in each litter (by dam). This approach is preferred to modeling the total number of affected pups across all litters within a dose group (i.e., using a dichotomous model), since the nested models can take into account the potential for intra-litter correlations (IC) (U.S. EPA, 2012).

The three nested models available in the U.S. EPA's BMDS software package (Nested Logistic [NLN], NCTR, and Rai and van Ryzin [RVR]) were used to analyze the nested data. The nested models allow one to specify a covariate, called a litter-specific covariate (LSC), in addition to dose, to help account for the individual response rates and the variability (within and across dose levels) seen in the litter response rate. In all analyses considered here, the total number of implantation sites was used for the LSC.

Akaike Information Criterion (AIC) values were calculated for the alternative model forms to determine if the IC and/or the LSC was a significant contributor to model fit, and to select the "best fitting" model from among the NLN, NCTR, and RVR models available in BMDS (U.S. EPA, 2015).

We used likelihood ratio test statistics to determine whether it was appropriate (on a statistical basis) to combine the different data sets (the one-generation range finding study, the first generation from the two-generation study, and the second generation from the two-generation study). Likelihood ratio test statistics yielding *P*-values >0.05 for the hypothesis that the data sets have the same dose-response imply that the data sets in question could be combined. The likelihood (or log-likelihood) is a reflection of the relative fit of the models (larger log-likelihoods indicate better fit).

To do this test, the BMDS models were fit to the three data sets separately, to each pairwise combination of the data sets, and to the combination of all three data sets. The sum of the log-likelihoods for the single-data-set analyses could be compared to the log-likelihood obtained from the combined analyses.

Therefore, a likelihood ratio test could be performed to determine if the combined-set model fit was adequately close to the fit of the pair of set-specific model fits. The likelihood ratio test statistic is given by:

$$LLR = -2(LL_c - (LL_a + LL_b))$$

where LL_c is the log-likelihood for the model fit to the combined data, LL_a is the log-likelihood for the model fit to one of the data sets, and LL_b is the log-likelihood for the model fit to the other data set.

The version of BMDS used for this analysis (2.6.0.1) has a bootstrap approach for assessing model fit, avoiding problematic issues for the approximate chi-squared-based goodness-of-fit (gof) statistics.

3. RESULTS

3.1. Sources of exposure

This section briefly reviews the sources of exposure to nickel and the chemical forms of nickel present in those matrices. Such a

[2] Note that this approach is different from that of the US EPA (2012), which recommends for consistency that the lowest AIC be chosen among acceptable models, even if the differences are small.

review is useful for the problem formulation, in order to assure that TRVs relevant to important exposure scenarios are derived. Identification of the exposure pathways and matrices helps in determining the durations and populations for which TRVs may be needed. This information is also used in the risk characterization effort, when exposures in target populations are compared to TRVs.

Food: Nickel is essential to microorganisms, plants and certain mammals (Eskew et al., 1983; Brown et al., 1987; ATSDR, 2005). Nickel is naturally present in plants as complex organic molecules. Thus, organic nickel compounds are present in food (vegetable or animal origin), yielding an average daily dietary intake of 100–300 μg Ni. Food is the highest contributor to oral exposure to nickel. Additional contributions of nickel to the diet can originate from cooking pots and pans made with nickel-containing alloys; these contributions are usually very low compared to nickel in food (ECB, 2008).

Drinking water: Nickel is present in water as the hydrated Ni(II) ion (water soluble form of nickel). Its presence in water is mostly the result of the ions being naturally leached from minerals and soils (Pyle and Couture, 2011). Oral exposure to nickel from drinking water is usually lower than from foods.

Soil: Depending on the underlying geology and soil chemistry parameters such as pH, nickel in non-industrial soils can occur in a range of mineral forms, including oxides (with Fe, Mn, Al, and other constituents) and silicates (McGrath, 1995). Exposure to soil is through the dermal and oral routes and although it is negligible for adults (even more so for the dermal route), it could be significant for toddlers (particularly for small particles that adhere to the skin and are later ingested) (US EPA, 2008).

Air: The chemical forms of nickel predominantly present in ambient air (PM10) are water soluble sulfates and oxidic nickel, including nickel monoxides and complex Ni-Mg and Ni-Fe oxides (Galbreath et al., 2003). Neither nickel metal nor sulfidic compounds are present in ambient air to any significant extent (Huggins et al., 2011).

Consumer Items: Exposure to nickel compounds in consumer items (e.g., batteries) is very limited. Nickel metal can be present (in massive forms) as a surface finishing on large items or as part of an alloy (e.g., electronics, knobs, coins). Ni-containing consumer items that are in direct contact with the skin (e.g., belt buckles, piercing items) can release Ni. However, low-Ni releasing alloys are generally used for items in prolonged skin contact, and systemic absorption via skin is very low. Oral exposure to nickel may occur in children through mouthing of metallic nickel objects or toys, or through wearing of orthodontic devices made of nickel-containing alloys, but exposure levels are low. Ni(II) ions can be released from internal medical devices (e.g., Ni-containing alloys used in prostheses). The extent of release can depend on factors such as inflammation that affect corrosion and may increase the levels of metal ions at the site. The contribution from medical devices to systemic exposure to nickel is limited to a small subset of the population. This contribution is quite variable, generally very low (compared to oral exposure), and hard to predict. Cigarette smoking can also contribute to the daily nickel exposure (nickel oxides), although results on whether this translates into significantly elevated levels of Ni in blood or urine are mixed, likely due to wide variability in dietary intake (Torjussen et al., 2003; Rosati et al., 2016; Afridi et al., 2010).

In summary, naturally occurring inorganic nickel compounds can be found in soil, water and air, but exposure to them can be vastly different depending on the matrix and the compound considered. Exposure to consumer items through oral, dermal or implantation routes generally contributes little to systemic levels of nickel.

3.2. Nickel toxicokinetics and bioavailability considerations

The main sources of exposure to nickel for the adult population are: food, water and air, while for toddlers, soil can be a major source of nickel. Information on bioavailability of Ni from these sources is provided below together with a more detailed discussion on the toxicokinetics of Ni after oral exposure to Ni in drinking water.

Estimates of Ni absorption from food are lacking. Studies of volunteers (discussed below) indicate that oral absorption of nickel from drinking water ingested with food ranges from 1 to 5%; these estimates are usually assumed (conservatively) to apply to the absorption of Ni from food. It is likely that the actual absorption of nickel from food is lower.[3]

Studies addressing the bioavailability of Ni from soil are limited but have shown that the oral absorption of nickel from soil in laboratory animals is lower than from water. There is a good correlation between the in vitro bioaccessibility of Ni from soils and in vivo bioavailability; and these parameters depend on the type of soil considered, the minerology of nickel, the weathering of the soil, and the particle size fraction (Vasiluk et al., 2011).

Lung deposition of nickel particles in air depends on the aerodynamic particle diameter. Absorption of deposited Ni depends on the specific form of nickel and the bioavailability of that form of Ni. Particles larger than about 10 μm in diameter are deposited in the upper respiratory tract, swallowed and absorbed from the gastrointestinal tract (oral exposure). Smaller particles can penetrate to the bronchoalveolar region and interact directly with lung tissue or be absorbed, depending on the bioavailability. Soluble nickel is absorbed readily by both humans and rodents. Quantitative information on the degree of absorption is not available for humans, but a good correlation between air exposure to soluble nickel compounds and urinary Ni levels has been found in workers' studies (e.g., Thomassen et al., 1999). In rats and mice, nickel sulfate deposited in the lung cleared rapidly, with most of the material clearing with a half-time of 1–3 days (Benson et al., 1995). Nickel levels in blood and urine were not measured in that study, but it is likely that most of the clearance was via absorption, because particulates of soluble nickel dissolve rapidly in the respiratory tract. In a related study, the clearance half-time in rats of nickel subsulfide was 4 days, and that of nickel oxide was about 120 days (Benson et al., 1994).

Nickel is rapidly absorbed following ingestion, with the extent of absorption varying based on the amount of food in the stomach. In studies with human volunteers the fasting state has been shown to increase nickel absorption from a water solution (e.g., Nielsen et al., 1999; Sunderman et al., 1989); oral absorption ranges from 1 to 5% (when ingested with food) to 12–27% (when ingested under fasting) (e.g., Nielsen et al., 1999). Even when Ni is 100% bioaccessible its oral absorption is never higher than ~30%.

It appears that rats absorb nickel from water solutions to an extent comparable to humans, although a direct comparison is complicated by differences in fasting status under standard dosing scenarios. In rat studies the absorption of Ni after gavage in water has been estimated at about 10% (Ishimatsu et al., 1995). Since rats are nocturnal feeders, the standard approach of dosing in the morning results in an exposure that is somewhere in between dosing with food and dosing under fasting conditions. Thus the absorption of Ni from water in rats (~10%) seems to be in the range of absorption of Ni by humans under comparable intermediate

[3] Oral bioaccessibility of nickel (II) ion from foods can range widely depending on the type of food (Olivares Arias et al., 2015), and it is always lower than that of nickel in water.

fasting exposure scenarios (7–23% in Nielsen et al., 1999).

After oral absorption, the Ni(II) ions in serum are bound to protein carriers, in particular albumin (Sarkar, 1984). The principal binding site for Ni in albumin (histidine) is the same in rats, humans and bovines (Hendel and Sunderman, 1972). In rats and mice, upon oral dosing with various soluble Ni compounds, Ni was found predominantly in the kidneys (e.g., Ambrose et al., 1976; Ishimatsu et al., 1995) with lesser amount found in the liver, heart, lung, and fat (e.g., Ambrose et al., 1976). In general, increases in tissue levels are directly proportional to the Ni intake (Cempel and Janicka, 2002). Ni has been shown to cross the placenta in pregnant animals (Hou et al., 2011). Nickel does not undergo any kind of metabolism before excretion (e.g., unlike arsenic, Ni is not methylated). Orally absorbed nickel is mainly excreted through urine with a fairly rapid excretion half-time (24–28 h) (Sunderman et al., 1989). Nickel has not been shown to accumulate in the body.

The majority of nickel in blood (and consequently nickel in target organs for adverse effects) comes from naturally occurring nickel in the diet (food and water), with <1% coming from inhaled ambient air when exposures to PM10 nickel are $\leq$ 20 ng/m^3 (De Brouwere et al., 2012). Thus, even without any exposure to nickel in the workplace or from other anthropogenic sources, nickel levels can be detected in blood and urine of the general population. Typical blood and urine levels of nickel are $\leq$2.0 μg Ni/liter (range 0.6–3.8 μg Ni/liter; Minoia et al., 1990) and 2.0 μg Ni/liter (range 0.5–6 μg Ni/liter; Sunderman et al., 1986), respectively.

Measures of internal dose, such as AUC (the area under the plasma concentration x time curve) or clearance, can be useful in refining the interspecies extrapolation in developing the TRV (see Section 3.4.3) (IPCS, 2005; U.S. EPA, 2012). Evaluating the relationship between applied dose and AUC in humans ingesting nickel is complicated by the impact of fasting on nickel *absorption*. However, *clearance* is independent of fasting state. Nielsen et al. (1999) estimated the mean nickel clearance in a group of eight male volunteers as 8.15–8.40 mL/min.

Unfortunately, no published studies in rats calculated AUC or clearance, or provided data that can be used to calculate such a measure following oral dosing of rats. However, unpublished data in SD rats receiving a single oral (gavage) dose of 1.1 mg Ni/kg as Ni sulfate hexahydrate indicate that clearance of nickel following oral exposure is comparable to or slower than that in humans (AO personal communication). Since the internal dose of nickel is determined primarily by the percent absorption, the similarity of the clearance (which is a surrogate measure of internal dose) is consistent with the observation that absorption is comparable in rats and humans under comparable fasting conditions. Furthermore, kinetics appear to be linear in SD rats, for both single and repeated exposures to 1.1–28 mg Ni/kg-day as Ni sulfate hexahydrate (Heim et al., 2007; Oller and Erexson, 2007; AO personal communication), which includes the dose range of the PODs derived in this paper. Blood/plasma levels of Ni (ng Ni/mL) measured 24 h after the last dose in a carcinogenicity study and in a single dose study are linearly related to the oral dose (Heim et al., 2007; AO personal communication). Furthermore, blood and urinary Ni levels were linearly correlated in an oral carcinogenicity study for the range of doses tested (2.1–11 mg Ni/kg-day as Ni sulfate hexahydrate) (Heim et al., 2007).

3.3. Evaluation of toxicity data

The toxicity database related to ingested nickel has been summarized by many authoritative reviews in support of the development of TRVs. Table 1 summarizes in reverse chronological order the chronic TRVs developed by a variety of different organisations, including by one of the authors of the current assessment (LH). Critical effects identified by these various organisations included increased post-implantation loss/perinatal lethality and perinatal mortality in rat studies[4]; decreased body weight, decreased liver and heart weight, and kidney effects (albuminuria) in rat studies; and eczema and other dermal responses in nickel-sensitized volunteers.

The TRVs published prior to 2002 were based on a variety of studies and endpoints, but tended to focus on critical effects of decreased body and organ weights in adult animals (Ambrose et al., 1976). Health Canada (1996a, 1996b) developed separate values for nickel sulfate and nickel chloride, with the former based on the Ambrose et al. (1976) study and the latter based on a reproductive effect (dead pups in Smith et al., 1993). Haber et al. (2000) based their RfD on a study (Vyskočil et al., 1994) that was either published after the earlier assessments or not cited by them, and which had a lower effect level (based on albuminuria) than that in the Ambrose et al. (1976) study. However, as noted by more recent reviews (OEHHA, 2012; EFSA, 2015), there are several limitations to the Vyskočil et al. (1994) study, leading to substantial uncertainty in the effect level. In addition, kidney effects were not noted as a sensitive endpoint in other repeated dose studies (EFSA, 2015), including in a Good Laboratory Practice (GLP) compliant chronic study (Heim et al., 2007).

Recent assessments have focused on post-implantation loss/perinatal lethality observed in rat studies and dermal reactions after single (acute) oral exposure in sensitized people as the basis for TRVs. Responses in nickel-sensitized people will be addressed in Section 3.5. The post-implantation loss/perinatal lethality data have been strengthened by the recent availability of a GLP-compliant and guideline-compliant 2-generation study and its associated range-finding study (SLI, 2000a; 2000b). These studies are discussed in detail in the remainder of this section. However, prior to discussing these studies, it is worth noting one additional recent study. Heim et al. (2007) published a GLP-compliant and guideline-compliant 2-year bioassay in rats. Although the effects observed in this study did not result in the lowest (most sensitive) points of departure, the study provides important support for the critical effect level derived from the 2-generation reproductive study, particularly since the Heim study is of much higher quality than the only other available 2-year study (Ambrose et al., 1976).

Thus, in light of the many recent reviews of nickel, our assessment of potential critical effects focuses on the recent 2-year rat study (Heim et al., 2007), the 2-generation rat reproduction studies (SLI, 2000a; 2000b), and studies of systemically reactivated allergic contact dermatitis (SRAC). In addition, studies relevant for a child-specific TRV are discussed in Section 3.6. A search of the recent literature did not identify any other recent publications that might be the basis for an updated TRV.

3.4. Development of an adult TRV

3.4.1. Identification of the point of departure

Based on previous toxicity reviews and our review of the literature, consideration of the TRV applicable to the adult population focused on the effects observed after repeated exposures in the two-year bioassay of Heim et al. (2007) and the two-generation reproductive toxicity study of SLI (2000b).

Heim et al. (2007) conducted an oral carcinogenicity study in which groups of 60 F344 rats/sex/dose were administered nickel sulfate hexahydrate once daily by gavage in water at 0, 10, 30, or 50 mg/kg-day (0, 2.2, 6.6, or 11 mg Ni/kg-day) for 105 weeks. The study was GLP compliant and was conducted in general compliance

[4] The exact terminology used for the critical effect varied across the agencies.

Table 1
Overview of chronic oral nickel TRVs.

Author, Year	Nickel Compound	Critical Effect	Risk Value (μg Ni/kg-day)	Study	Comments
EFSA[a], 2015	Soluble nickel compounds	Post-implantation loss	TDI, 2.8	SLI, 2000a, 2000b	Based on $BMDL_{10}$ of 0.28 mg Ni/kg-day from combined data from both studies and a UF of 100 (10A, 10H[b,c])
FSCJ, 2012	Presumably soluble nickel	Flare-up of existing eczema in fasted subjects	TDI, 4	Nielsen et al., 1990 (*sic*)	Based on a LOAEL of 0.012 mg/kg (single exposure) and a UF of 3, for using a LOAEL close to a NOAEL (and presumably recognizing that the study was conducted with a sensitive population). As reported by EFSA, 2015
OEHHA, 2012	Soluble nickel	Perinatal mortality	REL, 11	SLI, 2000a, 2000b, s upported by Smith et al. 1993	Based on NOAEL of 1.12 mg/kg-day and a UF of 100 (10A, 10H).
WHO, 2007	Soluble nickel	Post-implantation/ perinatal lethality	TDI, 11	SLI, 2000b	Based on NOAEL of 1.1 mg/kg-day and a UF of 100 (10A 10H). The authors derived the same drinking water guideline value from this study and from the Nielsen et al. (1999) study, based on a LOAEL of 0.012 mg/kg for nickel provocation in sensitized and fasted subjects and a UF of 1, since the study was conducted with a sensitive population.
ATSDR, 2005[c]	Nickel chloride, Nickel sulfate	N/A	MRL, N/A	N/A	No value derived based on the conclusion that the available data are insufficient to establish a threshold for developmental effects, due to the NOAELs and LOAELs ranging over more than an order of magnitude. The data were considered to be stronger for developmental toxicity (effects on the fetus) than reproductive toxicity (fertility).
RIVM, 2001	Soluble, Nickel chloride, Nickel sulfate, Nickel oxide, Nickel subsulfide	Decreased body and organ weight	TDI, 50	Ambrose et al., 1976	Based on NOAEL of 5 mg/kg-day and a UF of 100 (10A, 10H). The SLI study was not cited in the report, and it is likely that the assessment authors were unaware of its existence.
Haber et al., 2000	Soluble	Albuminuria in 6 month drinking water study	RfD, 8	Vyskočil et al., 1994	Based on LOAEL of 7.6 mg/kg-day and UF of 1000 (10A, 10H, combined 10 for S, minimal LOAEL and D). RfD based on dose *in addition to* amount in food. Published before the SLI data were available.
Health Canada, 1996a, 1996b	Nickel chloride	Reproductive effects, increased dead pups/litter	TI, 1.3	Smith et al., 1993	Based on LOAEL of 1.3 mg/kg-day and UF of 1000 (10A, 10H, 10L)
Health Canada, 1996a, 1996b	Nickel sulfate	Decreased liver and heart relative weights	TI, 50	Ambrose et al., 1976	Based on NOAEL of 5 mg/kg-day and a UF of 100 (10A, 10H)
US EPA, 1991	Soluble, Nickel chloride, Nickel sulfate	Decreased body and organ weight	RfD, 20	Ambrose et al., 1976	Based on NOAEL of 5 mg/kg-day and a UF of 300 (10A, 10H, 3D). The additional UF of 3 for database deficiencies is applied for inadequacies in reproductive studies.

[a] Abbreviations: ATSDR – Agency for Toxic Substances and Disease Registry; EFSA – European Food Safety Authority; FSCJ – Food Safety Committee of Japan; MRL – minimal risk level; N/A – not applicable; OEHHA – Office of Environmental Health Hazard Assessment, California EPA; REL – reference exposure level; RfD – reference dose; RIVM - National Institute for Public Health and the Environment (the Netherlands); TDI – tolerable daily intake; TI – tolerable intake; WHO – World Health Organisation.
[b] Abbreviations for uncertainty factors: A = interspecies factor; H = human variability; L = LOAEL to NOAEL; S = subchronic to chronic; D = database deficiencies.
[c] Considers both intermediate duration (subchronic) and chronic MRLs.

with EPA and OECD carcinogenicity test guidelines. Mortality was high, with overall survival of 40–52% in males and 55–77% in females. However, at least 24–25 animals/group survived to study termination, due to the inclusion of 60 animals/group at study initiation. Unexpected early deaths were attributed to pulmonary toxicity resulting from partial aspiration of the gavage solution, and this was hypothesized to be due to gastric back pressure. To avoid back pressure from gavage administered on a full stomach early in the morning, the gavage time was delayed until later in the morning, resulting in increased survival. Since absorption is higher on an empty stomach, this modification would have resulted in an increased internal dose for the same applied dose. The low overall survival was not, however, due to the gavage issue, since this issue resulted in only a few deaths.

Dose-related decreases in terminal body weight were noted in both males and females, and were not attributable to decreased food intake. The decrease reached 10% (generally considered an adverse level of body weight decrease) in females at the high dose and reached 11% and 12% in males at the mid- and high doses, respectively. There was no evidence of a chemical-related effect on hematology parameters. Histopathology evaluation of a guideline set of tissues found no chemical-related increases in any neoplastic

or non-neoplastic lesions. Based on decreased body weight gain in males, the NOAEL is 10 mg/kg-day (2.2 mg Ni/kg-day), and the LOAEL is 30 mg/kg-day (6.7 mg Ni/kg-day). Based on a BMR (Benchmark Response) of a 10% decrease in body weight in males (the more sensitive sex), the BMDL is 2.3 mg Ni/kg-day. Additional details on the BMD modeling for the Heim et al. (2007) study are provided in the supplemental materials.

Springborn Laboratories, Inc. (SLI) conducted a range-finding 1-generation study (SLI, 2000a), followed by a definitive two-generation reproductive toxicity study (SLI, 2000b), both conducted according to Good Laboratory Practice (GLP) principles, under OECD guidelines. In both studies, groups of male and female Sprague-Dawley rats were gavaged daily with nickel sulfate hexahydrate. In the range-finding study, the rats received doses of 0, 2.2, 4.4, 6.6, 11, or 17 mg Ni/kg-day as nickel sulfate hexahydrate, beginning two weeks prior to mating, through the day of scheduled sacrifice (males on PND 0 and dams on PND 21). In the two-generation study, daily doses of 0, 0.22, 0.56, 1.12 or 2.23 mg Ni/kg-day were administered beginning 70 days prior to mating, and continuing through mating and parturition. Litters were culled to eight pups/litter on postnatal day (PND) 4, and dosing of the F1 rats started on PND 21, at the same dose of nickel sulfate as their parents. F1 rats were mated after a minimum of 70 days of treatment. The male rats were sacrificed after 16–18 weeks of treatment, and the dams were sacrificed on PND 21. All the F1 pups not selected for breeding and all the F2 pups were sacrificed on PND 21. In addition to evaluation of pup viability and growth, reproductive measures included estrous cyclicity and sperm parameters, as well as histopathology focusing on the male and female reproductive tracts.

In the range-finding study, mean post-implantation loss (gestation day 6 [GD6]-PND 0) was significantly increased at 6.6 mg Ni/kg-day and above (Table 2). The ratio of dead:live pups was also increased at all doses except 11 mg Ni/kg-day, but there was no dose-response except at the high dose. There was some indication of an increase in post-implantation loss at the lowest dose tested (2.2 mg Ni/kg-day), based on visual analysis of the data. The increase was not statistically significant, although it is recognized that the statistical power was low (only 8 litters/group as appropriate for a range-finding study), and so a marginal effect could have been missed.

In the 2-generation study, there was no statistically significant effect on post-implantation loss (GD6-PND 0) at any dose, but there was a statistically significant increase at 2.2 mg Ni/kg-day in "mean post-implantation loss and postnatal loss[5] on day 4" in the first mating (F0/F1 generation), but not the second mating (F1/F2 generation) (Table 2). In our chi-square analysis of the data, there was also a borderline statistically significant increase ($p = 0.05$ in a two-sided test) at the high dose in the number of dams with 4 or more losses per litter, with 8/28 falling into that category at the high dose, but none of the controls. In addition, 7/28 high-dose animals had 5 or more postimplantation losses. Postimplantation losses of a few animals per litter is not uncommon, but post-implantation losses of 5 or 6 are unusual. There was no effect on the number of dams with high losses in the F1/F2 generation. The litters with high loss were not associated with higher numbers of implantations or higher numbers of dead pups, indicating that the loss was not related to over-burdening the mother. No effects on fertility endpoints were observed.[6]

[5] EFSA (2015) referred to this endpoint as postimplantation loss + perinatal lethality day 4.

[6] It should be noted that the developmental effects of Ni sulfate and chloride are specific and limited to perinatal mortality as a prenatal development toxicity study (RTI, 1988) did not detect increases in fetal death, malformations or variations.

Based on this study, it is clear that nickel can cause post-implantation loss in rats at a sufficiently high dose, but it is less clear whether the high dose in the 2-generation study was an adverse effect level, in light of the inconsistency between the F0 and F1 generations. The observation of a clear effect in the F0 dams, but no effect on post-implantation loss in the F1 dams is puzzling, since some of the purposes of the second generation are to (1) replicate the results of the first generation, (2) to amplify a possible weak effect in the first generation, and (3) identify effects on the eggs in the developing F1 embryo. However, it appears that the increase in the F0 generation was not due to chance, in light of the statistically significant increase in "mean post-implantation loss and postnatal loss on day 4" and the large number of dams with losses of 4, 5, and 6 pups. Based on these considerations, the high dose of the two-generation study, 2.2 mg Ni/kg-day, was judged to be an equivocal LOAEL (reflecting the minimal exceedance of the control response, although the effect is quite weak). The second-highest dose of 1.1 mg Ni/kg-day was a clear NOAEL. This determination is consistent with assessments of international regulatory agencies (WHO, 2007; ECB, 2008; OEHHA, 2012; EFSA, 2015).

3.4.2. BMD modeling

BMD modeling was conducted on the incidence of post-implantation loss (day 0) observed in the one generation range finding study (SLI, 2000a) and for the post-implantation loss data in both the first and second generation of the two-generation study (SLI, 2000b). Both studies were conducted in the same laboratory with the same strain of rats treated with nickel sulfate hexahydrate. Modeling was conducted using the three models for nested data available in BMDS 2.6.0.1 (NLN, NCTR, RVR). Because there was no difference in postnatal loss on day 4 across the dose groups in either generation, we modeled the primary endpoint of post-implantation loss, rather than combining the data with postnatal loss. The modeled data are presented in Supplemental Tables S-8 and S-9. Note that the modeling is done based on the post-implantation loss in each litter.

We considered inclusion of both a litter-specific covariate (LSC) (used to control for pretreatment conditions related to the dam or unrelated to treatment) and a parameter for intralitter correlation (IC) in the modeling. Based on comparison of the fit of the different combinations, as measured by the log-likelihood and the fit adjusted for the number of parameters (the Akaike Information Criterion, AIC), we determined that considering model forms with the IC parameters but without the LSC parameters resulted in the best fit across models and across data sets. (See Supplemental.) Hence, the remainder of the investigation used only the models that had IC but did not include LSC. Because the NCTR and RVR models are the same when LSC is excluded from those models, subsequent results are presented for the NLN and NCTR models only. Using this model parameterization (IC, no LSC) fit to all individual data sets was adequate, based on the goodness of fit (gof) p values (Supplemental Table S-4).

Analysis of the log-likelihoods indicated that all of the combinations of the three datasets (range-finding, and first and second generations of the two-generation study) appeared to be reasonable (Supplemental, Table S-5). However, the bootstrap goodness of fit p-values (calculated using a different statistical test, based on scaled residuals) were relatively poor for combinations that included both the 2-gen-1 (first generation of the 2-generation study) and 2-gen-2 (second generation of the 2-generation study) data sets (Supplemental, Table S-6). Nevertheless, graphical depictions of model fits to the various combinations (Supplemental, Figs. 1–4) show no great discrepancies between the predictions for post-implantation loss (the curves in those plots) and the range of observed probabilities. We attempted to improve the gof p-

Table 2
Summary of results from SLI (2000a, 2000b).

One-Generation (SLI, 2000a)					
Dose (mg Ni/kg-day)	Mean Post-Implantation Loss on Day 0	Litters with Post-Implantation Loss (%)[a]	Litters with ≥3 Post-Implantation Losses (%)[b]	Litters with ≥4 Post-Implantation Losses (%)[b]	Mean Post-Implantation Loss and Postnatal Loss on Day 4 (%)
Historical Control	1.5 (mean) 0.88–2.31 (range)	Not available	Not available		Not available
0	0.4	2/8 (25)	0/8 (0)	0/8 (0)	0.6 (3.6)
2.2	2.6	5/8 (63)	1/8 (13)	1/8 (13)	2.6 (16.2)
4.4	1.5	6/8 (75)	1/8 (13)	1/8 (13)	1.9 (13.2)
6.6	2.3*	6/7 (86)	2/7 (29)	2/7 (29)	2.5 (15.9)
11	2.7**	7/7 (100)	3/7 (43)	3/7 (43)	2.9 (18.6)
17	4.8**	8/8 (100)	7/8 (88)	5/8 (63)	5.9 (50.3)
Two-Generation (SLI, 2000b) F0/F1 Generation					
Dose (mg Ni/kg-day)	Mean Post-Implantation Loss on Day 0	Litters with Post-Implantation Loss (%)	Litters with ≥ 3 Post-Implantation Losses (%)	Litters with ≥ 4 Post-Implantation Losses (%)	Mean Post-Implantation Loss and Postnatal Loss on Day 4 (%)
0	0.9	13/25 (52)	3/25 (12)	0/25 (0)	1.0 (7.1)
0.2	1.5	18/26 (69)	3/26 (12)	3/26 (12)	1.6 (12)
0.6	1.2	15/25 (60)	5/25 (20)	3/25 (12)	1.4 (9.6)
1.1	1.3	19/26 (73)	5/26 (19)	1/26 (3.8)	1.4 (11)
2.2	2.1	19/28 (68)	9/28 (32)	8/28† (29)	2.3**(16*)
Two-Generation (SLI, 2000b) F1/F2 Generation					
Dose (mg Ni/kg-day)	Post-Implantation Loss on Day 0 (Mean)	Litters with Post-Implantation Loss (%)	Litters with ≥ 3 Post-Implantation Losses (%)	Litters with ≥ 4 Post-Implantation Losses (%)	Mean Post-Implantation Loss and Postnatal Loss on Day 4 (%)
0	0.9	13/24 (54)	0/24 (0)	0/24 (0)	1.2 (7.8)
0.2	1.9	18/26 (69)	4/26 (15)	1/26 (3.8)	1.3 (10.1)
0.6	1.3	16/25 (64)	3/25 (12)	2/25 (8.0)	1.6 (14.5)
1.1	1.3	18/23 (78)	3/23 (13)	1/23 (4.3)	1.4 (9.5)
2.2	1.2	14/24 (58)	4/24 (17)	1/24 (4.2)	1.5 (15.8)

*P < 0.05; **P < 0.01; †P = 0.05; N/A = not assessed.
[a] Related to the endpoint used for modeling. Actual data used for modeling are in Supplemental Tables S-8 and S-9.
[b] Included for comparison and perspective, but not used in the benchmark dose modeling.

values by allowing each group to have its own IC value (even when those two groups had the same dose) but that did not improve the fit of the models to the data (results not shown). The reason for the difference in inferences from the likelihood ratio and from the gof evaluations is not known, but the gof results suggest that combinations that include both 2-gen-1 and 2-gen-2 should be given less credence than the other combinations when it comes to BMD and BMDL estimates.

Table 3 presents the BMD/BMDLs by model and combination. The BMDs/BMDLs are very consistent across model and across combination. The combination of the two data sets from the two-generation study (by themselves, without the 1-gen data set; data set C) yielded slightly lower BMD and BMDL estimates than the other combinations (this was a combination that has less credence based on gof). The inclusion of the 1-gen data set yielded consistent (and slightly greater) BMD/BMDL estimates regardless of the combination that included that data set. Apparently the higher doses in that data set decreased the slope of the dose-response curve, so as not to over-estimate the probability of response at those higher doses.

U.S. EPA has used a benchmark response (BMR) of 5% for calculation of the BMDL for developmental toxicity endpoints, based on analyses of the comparability of NOAELs and BMD(Ls) for a large data base of developmental toxicity studies using models for nested data, as done here (Allen et al., 1994). The standard EPA practice is also to report the results for a BMR of 10% for all endpoints. Thus, Table 3 presents the results for BMRs of 5% and 10%.

For a BMR of 5% extra risk, BMDs of about 3.1 mg Ni/kg-day were calculated based on the two combinations (A and B) having the greatest credence (2.3–3.2 mg Ni/kg-day considering all combinations of data sets), and the BMDL was about 1.8 mg Ni/kg-day (1.2–2.0 mg Ni/kg-day considering all combinations of data sets). Focusing on the models and combinations with the greatest credence (A and B) and using both models, the average BMDL is 1.8 mg Ni/kg-day.

Thus, the BMDL of 1.8 mg Ni/kg-day for increased post-implantation loss in SLI (2000a, 2000b) is slightly lower than, but consistent with, the BMDL of 2.3 mg Ni/kg-day identified from the Heim et al. (2007) study based on decreased body weight in a two-year bioassay. Therefore, the point of departure for calculation of the adult TRV is 1.8 mg Ni/kg-day.

3.4.3. *Identification of uncertainty factors*

Overall, the database for toxic effects after ingestion of water-soluble nickel compounds is robust, including chronic oral studies in rats (Heim et al., 2007; Ambrose et al., 1976) and dogs (Ambrose et al., 1976), subchronic studies in rats (Obone et al., 1999; Vyskočil et al., 1994; American Biogenics Corporation, 1988) and mice (Dieter et al., 1988), multiple reproductive toxicity studies, including two-generation studies in rats (SLI, 2000b; RTI, 1988; Ambrose et al., 1976) and a one-generation study involving multiple breedings (Smith et al., 1993), as well as screening developmental toxicity studies in rats (Käkelä et al., 1999) and mice (Berman and Rehnberg, 1983). There was no evidence of nickel teratogenicity in any of the developmental or reproductive toxicity studies, and one of the studies (RTI, 1988) included detailed evaluation of the F2 pups for structural developmental effects. The critical studies (SLI, 2000a; 2000b) were well-conducted according to OECD (Organisation for Economic Cooperation and Development) and GLP guidelines, and the point of departure is closely

Table 3
BMD and BMDL estimates for combined data sets (mg Ni/kg-day).

BMR (Extra Risk)	Model	Data Set Combinations							
		1-gen + 2-gen-1 (A)		1-gen + 2-gen-2 (B)		2-gen-1 + 2-gen-2[a] (C)		All 3[a] (D)	
		BMD	BMDL	BMD	BMDL	BMD	BMDL	BMD	BMDL
10%	NLN	6.34	3.89	6.43	4.24	4.90	2.32	6.15	4.05
	NCTR	6.66	3.33	6.63	3.32	4.76	2.38	6.41	3.20
5%	NLN	3.00	**1.84**	3.05	**2.01**	2.32	1.31	2.91	1.92
	NCTR	3.24	**1.62**	3.23	**1.61**	2.32	1.16	3.12	1.56

BMDL values represent 95% lower bounds on the corresponding BMD.
[a] Models including both 2-gen-1 and 2-gen-2 (whether or not the 1-gen are included – i.e., data sets C and D) are given lower credence based on gof p-values of 0.03 and 0.02 respectively, Table S6. Model results used for the final calculation of the point of departure are bolded.

supported by a guideline-compliant 2-year bioassay. Thus, no database uncertainty factor is needed. No subchronic to chronic uncertainty factor is needed, because chronic studies were considered in identification of the point of departure. Similarly, no NOAEL to LOAEL uncertainty factor is needed because the point of departure is a $BMDL_{05}$.

The human variability (intraspecies) uncertainty factor addresses whether the existing data account for sensitive individuals. The default factor of 10 can be further broken down into toxicodynamic and toxicokinetic subcomponents, either of which can be replaced by data of sufficient quality (IPCS, 2005; U.S. EPA, 2014). Based on the critical effect, pregnant women and their fetuses are the target population, but it is not known what physiological factors (i.e., contributors to toxicodynamic variability) would make some women more sensitive than others to this endpoint. Possible contributors to higher internal doses (i.e., reflecting toxicokinetic variability) for a given ingested amount include physiological factors that would increase nickel absorption or decrease excretion (e.g., altered kidney function).[7] However, insufficient quantitative information is available on toxicokinetic or toxicodynamic variability to modify the default uncertainty factor of 10.

The interspecies uncertainty factor similarly has a default value of 10 and can be broken down into toxicodynamic and toxicokinetic subcomponents, either of which can be replaced by data of sufficient quality (IPCS, 2005; U.S. EPA, 2014). For interspecies kinetics, replacement of the default requires data for both the test species and humans on the relationship between applied dose and an internal dose measure, such as clearance or area under the plasma concentration x time curve (AUC). Although the available data are not adequate to calculate a rigorous uncertainty factor based on interspecies differences, the available data indicate that the default interspecies kinetic factor of 4 (IPCS, 2005) is quite conservative (health protective). As discussed in Section 3.2, absorption of nickel appears to be similar under comparable fasting conditions; binding and excretion is also expected to be similar. Furthermore, comparison of clearance calculated for SD rats from unpublished data and clearance in humans (Nielsen et al., 1999) indicates that clearance in rats is comparable to, or slower than, that in humans. This means that, from the kinetic perspective, rats are expected to be similar to humans, or *more sensitive*, rather than the usual presumption that humans may be more sensitive. Because adequate rigorous data are not available, no adjustment is made to the interspecies kinetic subfactor, but this analysis indicates that the default of 10 for interspecies extrapolation is quite conservative, based on the highly conservative value for the kinetic subfactor.

[7] Behavioral factors that increase nickel absorption, such as drinking large amounts of water on an empty stomach, would typically be accounted for in the exposure assessment and resulting risk characterization, rather than in the TRV.

Similarly, the interspecies toxicodynamic subfactor addresses differences in the internal dose that results in a specified response. In the absence of sufficient data on internal dose, replacement of the toxicodynamic subfactor was not appropriate.

Although the data are insufficient to use chemical-specific data to rigorously replace either the toxicokinetic or toxicodynamic interspecies subfactors, some perspective on the appropriate magnitude of the overall interspecies factor can be gained by consideration of relevant epidemiology data. Vaktskjold et al. (2008) conducted two case-control studies of spontaneous abortion in workers exposed to nickel at a nickel refinery. The Birth Registry study was based on medical records of spontaneous abortions, and included 5045 outcomes, yielding 4571 controls and 474 cases. A separate questionnaire-based study included 1875 outcomes, with 1691 controls and 184 cases. For the questionnaire study, spontaneous abortion was not defined in the interviews with the women. For the Birth Registry portion of the study, spontaneous abortion was defined as delivery prior to 28 weeks of pregnancy (with the fetus < 1000 g and <35 cm), where the fetus is dead or survives less than 168 h. A separate exposure study (Thomassen et al., 1999) had been conducted with about 500 workers based on air and urine measurements, and each delivery for the women in the abortion studies was assigned to one of three categories based on occupation and workplace: unexposed, low exposure, and high nickel exposure (>70 μg/L in urine, corresponding to about 160 μg/m^3 of the water-soluble inhalable subfraction). Previous pregnancies were included in the analysis, but additional pregnancies after an abortion were not included. No overall average exposure was provided for the high-exposure group, but another related paper (Vaktskjold et al., 2006) reported urinary levels by department and how the women were assigned to the three categories of exposure. The highest average urinary level was 179 μg/L, in the "old" electrorefinery department. Overall, 366 of the outcomes (cases plus controls) were in the high exposure group, the majority (276) of which were in the electrorefinery department.

The odds ratio (OR) was calculated using multiple logistic regression, with exposure to water-soluble nickel considered a categorical variable. The adjusted OR was calculated after accounting for factors chosen a priori based on the literature: maternal age> 34 years, previous history of induced abortion, previous delivery, regular heavy lifting at work, and exposure to paints or solvents at work. For the questionnaire study, the authors reported an adjusted OR for the high-exposure group of 1.27 (95% CI 0.87–1.86). For the Birth Registry study, the adjusted OR for the high-exposure group was 0.80 (95% CI 0.53–1.23). The adjusted ORs for the low-exposure group were similarly not statistically different from 1. These results would suggest that the high exposure group was a NOAEL for spontaneous abortions and no LOAEL was identified. Although the findings are consistent with the highest exposure corresponding to a NOAEL for spontaneous abortions, the

authors do not exclude the possibility that a weak excess risk may have been present. This is consistent with our conclusion that the exposure in the study appears to identify a NOAEL, but the confidence in the NOAEL is not sufficiently high to be the basis for the TRV.

Exposure in the Vaktskjold et al. (2008) study was primarily via the inhalation route, and so no oral TRV can be calculated directly from that study. However, some information can be gleaned by a comparison of urinary levels. As noted, the highest exposed group in the Vaktskjold et al. (2008) study had a urinary nickel concentration of 179 μg/L. By comparison, rats exposed to the LOAEL for reproductive effects (2.2 mg Ni/kg-day) for all of their lifetime had urinary levels of 2300 μg Ni/L (Heim et al., 2007). Based on the direct proportionality of urinary nickel levels and intake in rats (see Section 3.2), extrapolating from the Heim et al. (2007) study suggests a steady state urine concentration of approximately 1850 μg Ni/L at the BMDL for the SLI (2000b) study. The lack of effects in the Vaktskjold et al. (2008) study indicate that humans are ≤10-fold more sensitive than rats, but these data are insufficient to demonstrate a smaller difference, in the absence of data in humans at higher exposure levels. Such higher exposures in humans are extremely unlikely to occur under environmental or modern occupational exposure conditions, since the exposure of the most highly exposed human population (studied by Vaktskjold and colleagues) was only 1/10th of the systemic exposure corresponding to the BMDL. If the epidemiology study were sufficient for developing a TRV, one might suggest a reduced uncertainty factor of 3 for human variability, since many aspects of human variability are already reflected in the study sample. However, in light of the uncertainties regarding study results, an intermediate uncertainty factor of 5 appears prudent.

Taken together, these data indicate that a total uncertainty factor of 100 (based on factors of 10 each for inter- and intraspecies variability) is very conservative for the oral TRV for the adult population, so a high-confidence TRV would be 1.8 mg Ni/kg-day divided by 100, or 18 μg Ni/kg-day, rounded to 20 μg Ni/kg-day. Based on the epidemiology data as well as the kinetic similarities between rats and humans, a total uncertainty factor of 50 also appears to be health-protective. The resulting TRV would be 1.8 mg Ni/kg-day divided by 50, or 36 μg Ni/kg-day (rounded to 40 μg Ni/kg-day). Thus, the overall range in the TRV would be 20–40 μg Ni/kg-day, taking into account the precision of the TRV to one significant figure.

3.5. Development of a TRV for nickel sensitized subpopulations

One of the more challenging issues in developing a nickel TRV is addressing nickel sensitization. Oral exposure to nickel does not cause sensitization, but dermal exposure in sufficient amounts can cause dermal nickel sensitization. Regarding the inhalation route of exposure, there is no strong evidence that dermal sensitization can be caused by inhalation exposure to nickel. For example, Mann et al. (2010) found that patch test positive children living in two German cities had higher urinary nickel levels than nonsensitized children. Since nickel in urine correlated with nickel in air, the authors concluded that nickel in air could be a contributing factor to dermal sensitization by nickel. Smith-Sivertsen and colleagues, on the other hand, found either no association with sensitization (1999) or a protective effect (2002) of nickel in the air in studies of two Norwegian and Russian populations, with low and high Ni exposures, respectively. In all of the studies, comparator cities or regions had a similar prevalence of ear piercing.

A dermal reaction may be elicited by oral nickel ingestion in some people who have already been dermally sensitized to nickel. This reaction is termed systemic contact dermatitis (also known as systemic nickel allergy syndrome or systemically reactivated allergic contact dermatitis, SNAS or SRAC, respectively). This potential for eliciting a response in sensitized people is of concern, in light of the relatively large percentage of the population that is sensitized. EFSA (2015) reported that the prevalence of nickel allergy in the U.S. is 14.3%. A higher percentage of women than men is sensitized to nickel, due to women's exposure to nickel-releasing jewelry. For example, in a study of 9334 subjects, Warshaw et al. (2014) found that the prevalence of nickel sensitivity in North America was 23% in females and 7% in males; the prevalence of nickel sensitivity was significantly increased in individuals with at least one piercing compared to those with no piercings and increased with the number of piercings.

3.5.1. Identification of a point of departure

More than 17 controlled clinical trials have been conducted to evaluate the response to ingested nickel in nickel-sensitized subjects. Despite the relatively large number of studies, the dose response for triggering systemic contact dermatitis is not well characterized. Many of the studies tested only one dose, and sample sizes were generally small. A further complication is the nature of the response being evaluated, which varies over time for an individual and exhibits significant inter-individual variability. In addition, some studies looked at exacerbation of existing dermatitis rather than initiation of a dermatitis reaction. Several of the studies had a positive response in the placebo (unexposed) group (Jensen et al., 2006). This response may reflect a contribution of factors other than nickel exposure in triggering a dermal response, thus contributing to the high intra- and inter-individual variability. Finally, all of the studies used water soluble nickel compounds, which are representative of nickel in drinking water, but not in foods.

Jensen et al. (2006) reviewed 17 studies of nickel systemic contact dermatitis, and conducted a “modified meta-analysis” with 15 studies on 401 subjects that involved only a single exposure to any nickel dose. The authors grouped the 15 studies into high, medium and low response rate, but this was only an empirical grouping, not reflecting any difference in study design or population, and there was substantial statistical overlap among the dose-response for the three groups. Focusing on nine studies that had no response in the placebo group, the authors still grouped the studies into three groups. For the three groups, they found that the exposure associated with a 10% response rate among sensitized individuals was 0.55–0.87 mg Ni (0.008–0.01 mg Ni/kg bw).

Of the studies reviewed by Jensen et al. (2006), only five studies tested at least three doses (including controls), thereby allowing for an evaluation of dose-response (summarized in Table 4). In order to evaluate the dose-response for systemic contact dermatitis, we focused on studies that were double-blinded, were placebo-controlled, and tested at least two different doses of nickel. Thus, Cronin et al. (1980) was excluded, because there was no placebo control. In addition, the Kaaber et al. (1979) study was not well documented, resulting in a number of uncertainties, including the response incidence and whether the subjects were fasted prior to nickel exposure. This resulted in three studies with dose-response data – Gawkrodger et al. (1986), Hindsén et al. (2001), and Jensen et al. (2003). All three studies confirmed nickel sensitivity in the subjects using a patch test prior to commencement of the study, and administered nickel sulfate in a capsule (or placebo) to fasted subjects. Doses were reported in mg and none of the studies reported information on the body weight of the subjects, so doses in mg/kg can only be estimated based on defaults. None of the studies measured or controlled for dietary nickel; for the low exposure levels, the contribution of Ni from the diet could have been a significant percentage of total exposure.

Table 4
Dose-response studies for systemic contact dermatitis.

Study	Study Design	Number of Days of Dosing	Number of Subjects	Fasting?	Challenge Dose (mg) [a]	Response
Cronin et al. (1980)	No placebo control, no blinding. Incidence based on the worst of the incidence of erythema, worsening of hand eczema, or flare of patch test site. 15 subjects with positive nickel patch test.	1	15	Yes	0.6 1.2 2.5	3/5 4/5 5/5
Gawkrodger et al. (1986)	Double-blind crossover study, with nickel sulfate in lactose capsules. Doses of 0.4 and 2.5 mg were given to fasting subjects on two successive days; subjects received only a single dose of 5.6 mg. 26 subjects (24 women and 2 men). All subjects had previous positive nickel patch test. Positive response defined as an accentuation of previously noted physical signs (usually worsening of microvesicular hand eczema.)	1–2	26	Yes	0 0.4 (0.8)[b] 2.5 (5.0)[b] 5.6	10/26 5/10 5/10 6/6
Hindsén et al. (2001)	Double-blind, single exposure. Thirty nickel-sensitive women with positive nickel patch tests. One month following the final patch test, patients were given a placebo or capsule containing nickel sulfate after fasting from midnight to 1 h post-challenge.	1	30	Yes	0 1.0 3.0	0/10 2/10 9/9
Jensen et al. (2003)	Double-blind, placebo-controlled. 40 nickel-sensitive volunteers (39 women and 1 man) with a history of nickel contact dermatitis with previous positive patch tests to nickel. Nickel sulfate was administered in a capsule after fasting for 12 h, 1 month after patch testing. An additional 20 non-nickel sensitive subjects were tested and none exhibited a reaction to 4.0 mg Ni or placebo.	1	40	Yes	0 0.3 1.0 4.0	1/10 4/10 4/10 7/10
Kaaber et al. (1979)	Double-blind, single-exposure. 14 nickel-hypersensitive female patients with chronic hand dermatitis. Placebo was given first to all patients. Positive response defined as increased itching or increased vesicles.	1	14	Not reported	0 0.6 1.2 2.5	0/14[c] 1/14[c] 1/14[c] 9/14[c]

[a] Doses provided in this Table do not represent the total exposure to nickel, because the contributions from the diet were not considered in any of these studies. Individual subject doses (mg/kg body weight) are not known as body weights were not recorded.
[b] Cumulative doses are shown in parenthesis.
[c] Methods are not clear as to whether each subject was exposed at each dose level, but we assume that all 14 subjects were exposed to the placebo and all three doses due to the double-blind study design. There is additional uncertainty regarding the number of positive responses per dose. Kaaber et al. (1979) only report the lowest dose at which each of the 11 responders presented with a reaction. One person first responded at 0.6 mg, one at 1.2 mg and nine at 2.5 mg; what is unclear from the report is whether the person who responded at 0.6 mg also responded at 1.2 mg or higher concentrations. The responses may, instead, be 0/14, 1/14, 2/14, and 11/14 for 0, 0.6, 1.2, and 2.5 mg, respectively.

Unlike Hindsén et al. (2001) and Jensen et al. (2003), Gawkrodger et al. (1986) tested the same people in the placebo and exposed groups. This violated the assumption of independence between dose groups, and so it was not appropriate to conduct benchmark dose modeling for that study. In light of the small sample sizes, we tried to use benchmark dose modeling to combine the results from the other two studies, but the fit was not adequate, particularly in the low-dose region. Therefore, we separately modeled the Hindsén et al. (2001) and Jensen et al. (2003) studies.

Adequate modeling results were obtained from the Hindsén et al. (2001) study (Supplemental Table S-12), with $BMDL_{10}$ values ranging from 0.22 to 0.55 mg Ni for the multistage, log-logistic and gamma models, which were the best fitting models. The average of the $BMDL_{10}$ values for the three best fitting models is 0.41 mg. These three models are clearly preferred over the quantal-linear model chosen by EFSA (2015), due to much better fit (based on the AIC and visual fit; see also Supplemental Figs. 5a–d). Although the BMDL chosen by EFSA is the lowest from all the models applied (0.11 mg Ni), this model clearly has a poor fit, based on the goodness-of-fit P-value, and the substantial scaled residual at the lowest dose. In addition, the AIC for the quantal-linear model is substantially higher (worse) than the AIC for the three better-fitting models.

Consistent with the conclusions of EFSA (2015), we identified the Jensen et al. (2003) study as the most sensitive for the identification of oral nickel exposures associated with systemic contact dermatitis. It was noted that the response was identical at the two lower doses (see Table 4), but the confidence limits on each data point were very wide due to the small sample size. Together, this meant that no model went through or close to all of the data points (see Supplemental Figs. 5a–5d), but all of the models had acceptable goodness of fit P-values and scaled residuals; comparative visual fit was difficult to evaluate. Because there was no clear reason to prefer one model over another (Supplemental Table S-12), model results were averaged to determine the final BMDL. The $BMDL_{10}$ values ranged from 0.078 to 0.45 mg Ni, and the mean $BMDL_{10}$ (after accounting for duplicates of the same mathematical equation) was 0.30 mg Ni. EFSA (2015) identified a $BMDL_{10}$ for this dataset of 0.08 mg Ni, because it resulted in the lowest $BMDL_{10}$.

Based on our analyses, the best estimate of the point of departure ($BMDL_{10}$) for development of a TRV based on systemic contact dermatitis in nickel sensitized individuals is 0.30 mg Ni, based on the average $BMDL_{10}$ from the most sensitive appropriate study. This corresponds to 4.3 μg Ni/kg, based on a 70 kg body weight, rounded to 4 μg Ni/kg. As the administered doses were not adjusted by body weight and the vast majority of the volunteers were women, it is likely that using a body weight of 70 kg may result in underestimating the exposure in terms of mg/kg body weight. *Note that these doses are on top of the dietary intake, since none of the studies controlled or eliminated dietary nickel exposure.*

3.5.2. Identification of uncertainty factor

The IPCS (2012) immunotoxicity risk assessment guidelines state that either sensitization (i.e. induction) or elicitation can be considered as the basis for a point of departure (POD) for a risk assessment, although different uncertainty factors are used for sensitization and elicitation. Specifically, while an intraspecies factor (for human variability) of 10 might be appropriate for induction of sensitization, the guidance notes that the elicitation response is already based on effects in the most susceptible individuals. Therefore, the guidance suggests that a reduced intraspecies factor, such as 1, is appropriate for intraspecies variability.

A factor of 1 for intraspecies variability is consistent with the intraspecies factor of 1 used by the U.S. EPA for the beryllium RfC (available at http://www.epa.gov/iris/subst/0012.htm), which is

based on sensitization and progression to chronic beryllium disease (CBD).[8] The factor of 1 was used because the data came from the sensitive population. The number of affected individuals in the beryllium studies (5 in one study and 11 in another) was comparable to those in the nickel systemic contact dermatitis studies.

Based on these considerations, a factor of 1 was used for the intraspecies variability uncertainty factor for the TRV. Although the sample sizes in the studies were fairly small (~10/dose), indicating that the range of variability in the sensitive subpopulation may not have been fully captured, the dosing protocol maximized the internal dose. Subjects were fasted prior to dosing, meaning that absorption was as much as 10-fold higher than in a fed state. In addition, the subjects were provided a bolus (capsule) dose. This means that peak serum levels would have been much higher than if the same dose were administered over the course of the day as part of drinking water. Finally, subjects were patients of dermatology clinics and so were likely to be more sensitive to systemic nickel dermatitis than individuals who did not seek treatment at a dermatology clinic.

Other uncertainty factors are not needed. No LOAEL to NOAEL uncertainty factor is needed, because the POD was a $BMDL_{10}$. The dose-response data are from humans, using accepted test methods. IPCS (2012) suggests that a time extrapolation factor may be appropriate for sensitization, but does not address whether such a factor is needed for elicitation. In this case, such a factor does not appear necessary, in light of the other conservative aspects of the testing. The absence of such a factor is also consistent with the EFSA (2015) assessment.

Thus, based on a POD of 4.3 μg Ni/kg and a total uncertainty factor of 1, the TRV for the nickel-sensitized population is 4 μg Ni/kg *in addition to the* normal dietary intake.

3.6. Development of a TRV for young children

In certain applications where oral TRVs are needed, for example in site-specific soil remediation, the target population is young children (e.g., 1-<6 years old). The reason for this is that the intake of soil by this age group is one of the highest on a per kg body weight basis and they also can have additional exposures to soil through pica behavior. According to the U.S. EPA data, the subgroup of children 3–6 years of age can experience ingestion of soil and dust up to 10.8 mg/kg body weight.

In sections 3.4 and 3.5 above we have discussed the derivation of oral TRVs based on toxicity effects that are relevant to exposures in adult populations and to nickel-sensitized individuals. One of these effects, perinatal mortality in pregnant females, is not relevant for populations of non-reproductive age, such as pre-pubescent children. In addition, because the toxicity occurs as a direct effect on the fetus and nickel does not accumulate in the body, exposure of children would not result in effects on the fetus when the children reach sexual maturity and become pregnant.

The second effect, acute exacerbation of dermatitis after oral exposure to nickel may be less relevant for children than adults, since it applies only to people who have already been sensitized to nickel. Young children and toddlers would thus be expected to have a lower prevalence of sensitization, since opportunities for sensitization increase with age. There is some general suggestion that allergic contact dermatitis in children for a variety of agents increases with age, but the data are very mixed (Rodrigues and Goulart, 2016; Militello et al., 2006). Some data are available on the prevalence of nickel sensitization in children, but no data specific to children <6 years old were located, and it is expected that nickel dermatitis would increase in the teenage years, when ear piercing becomes more common. Furthermore, there are no studies that have reported or investigated the oral elicitation of dermatitis in nickel-sensitive children. One study even suggests that low oral exposure to nickel prior to piercing could reduce the frequency of nickel sensitization later in life (van Hoogstraten et al., 1991). Based on all these considerations, the TRV for young children focuses on the general population of children, recognizing that the prevalence of sensitization of children is likely lower than that of adults, but may still be of concern.

Thus, for the TRV for children, it is of interest to identify the sensitive adverse effects in studies specifically designed to assess repeated exposures in young children aged >1–6 years old, the target population. Studies of greatest interest to assess such effects would evaluate effects in young animals exposed for a comparable portion of their lifetime – between weaning and sexual maturity. The test that most commonly evaluates this age range in rodents is the two-generation reproduction study. The exposure period for the F1 animals in this study type not only encompasses the period of interest in humans, but also begins earlier (during gestation) and continues past childhood into puberty and early adulthood. Thus, systemic health effects in F1 animals are particularly useful for evaluating effects in children. In addition, based on the duration of interest (5 years, or slightly less than 1/10th of the lifespan), subchronic toxicity studies also provide useful information on potential effects and effect levels, in particular since guideline subchronic studies include evaluation of a number of sensitive toxic endpoints that are not typically evaluated in reproductive studies. Unlike the study in F1 animals, the subchronic study will not, however, identify endpoints where children are more sensitive than adults. In addition, it is important to evaluate effects identified in subchronic studies to ensure that they are relevant to children.

Based on these considerations, the rest of this section focuses on the oral two-generation reproductive toxicity studies and subchronic studies available for nickel. As discussed further below, effect levels in reliable subchronic studies were all above the effect levels in the F1 generation of all 2-generation studies, and so the subchronic studies do not need to be addressed in detail here. A review of four two-generation reproductive studies (Ambrose et al., 1976; RTI, 1988; Smith et al., 1993; SLI, 2000b), focusing on general toxicity observed in the F1 rats, indicated that decreased body weight appears to be the most sensitive systemic adverse effect. Ambrose et al. (1976) measured body weights in the F1 generation only twice (at weaning and mating) and no toxicity was noted in F1 animals exposed to up to ~64 mg Ni/kg-day (1000 ppm in feed).[9] Smith et al. (1993) only measured body weights at postnatal day 21 and observed no body weight changes at the highest exposure level of 31.6 mg Ni/kg-day (administered in drinking water). The RTI (1988) and the SLI (2000b) studies contain the more detailed information on body weights for the F1 generation. The RTI (1988) and the SLI (2000b) studies are described below and are summarized in Table 5.

RTI (1988). In this study the F1b generation animals (males and females) were exposed to average levels of 0, 6.0, 25.0, and 42.0 mg Ni/kg-day (as nickel chloride hexahydrate) calculated based on drinking water consumption and levels of nickel in water. These

[8] Although the beryllium RfC is based on sensitization (i.e. induction), rather than the response in sensitized people, the endpoint can be considered analogous to nickel sensitization, because the effect on the beryllium sensitized population was considered together with the sensitization.

[9] Ambrose et al. (1976) does not report the exact amount of food consumed by the animals in the study. The U.S. EPA (1988) estimates of food consumption were used in conjunction with the known ppm amounts of nickel in feed to estimate the daily intake of nickel.

exposures took place first *in utero* (from mothers exposed to those nickel levels in drinking water), then through lactation up to postnatal week 3 (again from exposed mothers), and finally from drinking water from the end of postnatal week 2 to approximately week 26 (females) or week 23 (males). Male and female body weights in the F1 generation were measured on postnatal day 1, 4, 7 and 21 and then weekly from weaning until mating. In the last week of the lactation period, reductions in the F1b generation body weights were noticed for animals in the 25.0 and 42.0 mg Ni/kg-day exposure groups. When observed at 26 weeks (females) and 23 weeks (males), biologically equivalent to 25–35 years in humans, changes in body weights persisted only in the 42.0 mg Ni/kg-day exposure groups. Clinical observations for parameters other than body weights did not reveal additional toxicities. The authors noted that there were problems with the temperature control of the animal rooms during this study. The US EPA (1991) has refrained from using this study in the derivation of a reference dose for reproductive effects because elevated temperatures may cause reproductive effects. For the body weight effects observed in F1 rats it is less likely that the difference in room temperature (+5 °C), that applied to all the groups including the controls, could have influenced the body weights of the pups and mask a real body weight change effect. Nevertheless, with these limitations in mind, a NOAEL of 6 mg Ni/kg-day for body weight effects in F1 animals could be derived from this study.

SLI (2000b). In this study the F1b generation rats (referred to as 2-gen-2 above) (males and females) were exposed to levels of 0, 0.2, 0.6, 1.1, and 2.2 mg Ni/kg-day (as nickel sulfate hexahydrate). These exposures took place first *in utero* (from mothers exposed to those nickel levels through gavage), then through lactation up to weaning on postnatal week 3; and finally by gavage from weaning until adulthood and through the mating period (for 24 weeks). Body weights were measured on postnatal day 1, 4, 7 and 21 and then weekly until mating. No adverse effects on F1 pups' body weights were observed at any of the time points and exposure levels included in this study and the NOAEL in this study is the highest external dose of 2.2 mg Ni/kg-day.

3.6.1. Identification of a point of departure

Table 5 describes two reference studies under consideration for comparison to the oral exposure scenario for children >1–6 years old. The NOAEL of 2.2 mg Ni/kg-day for body weight effects in the F1 generation from the SLI (2000b) study is supported by body weight data from the three other reproductive studies, although this value is significantly lower than the overall NOAELs identified in the other studies. In the RTI (1988) drinking water study, a LOAEL for body weight changes of 25 mg Ni/kg-day was reported. This value appears to be in conflict with two other studies. The Smith et al. (1993) drinking water study using nickel chloride hexahydrate reported no treatment related effect on body weights or weight gains for pups exposed to average levels of nickel as high as 31.6 mg Ni/kg-day. However, body weights were measured only at postnatal day 21 (*i.e.*, the end of the lactation period) in this study, limiting its value in identifying a Point of Departure. The Ambrose et al. (1976) feeding study with nickel sulfate hexahydrate indicated that rats exposed for 12–27 weeks after birth to levels as high as ~64 mg Ni/kg-day in feed did not show significant reductions in body weight through that period. In this case, however the absorption of nickel from feed (soluble Ni compound mixed with feed) may have been lower than the absorption of nickel from water in the other 3 studies.

It is also noted that the NOAEL of 2.2 mg Ni/kg-day for decreased body weight in the SLI (2000b) study is the same as the NOAEL in the chronic study (Heim et al., 2007), as discussed in Section 3.4. This means that additional comparisons with effect levels in the subchronic studies are not needed. If the POD for children were higher than the POD in the chronic study, one would then need to compare the POD from the SLI (2000b) study with PODs from subchronic studies, to determine which is the critical effect. But in this case, the POD is the same as that from the chronic study, which has already been determined to have a lower POD than the subchronic studies.

In summary, a protective point of departure of 2.2 mg Ni/kg-day for body weight effects in children >1–6 years old is supported by the SLI (2000a,b) NOAEL. This is a very conservative point of departure, given that it is from a NOAEL in the absence of a LOAEL, and that NOAELs for body weight changes range from somewhat higher (RTI, 1988) to much higher (Ambrose et al., 1976).

3.6.2. Identification of uncertainty factors

Uncertainty factors are not needed for LOAEL to NOAEL extrapolation, since the POD is a NOAEL, or for duration of exposure extrapolation, since the exposure was for the duration of interest. Furthermore, as described for the adult TRV, the critical effect has been identified, and it is unlikely that a new study would identify a different critical effect.

The interspecies uncertainty factor of 10 is intended to account for species differences in toxicokinetics and toxicodynamics. As discussed for the adult TRV, there are insufficient data to separately characterize differences in either of these components, and thus there are insufficient data to characterize differences specific to children. Thus, the default factor of 10 is appropriate for interspecies differences.

The intraspecies uncertainty factor of 10 is intended to account for variability in response within the human population. Since we are concerned only with children, the population is likely to be less variable than if babies, adults and the elderly were also included (and a factor of 10 would be more than sufficient to cover variability in response for the >1–6 year old group).

Therefore, applying an overall uncertainty factor of 100 (10×10) to calculate the TRV for children would be appropriate and protective, based on the data available. The TRV is calculated by dividing the NOAEL of 2.2 mg Ni/kg-day based on decreased body weight by a total uncertainty factor of 100. The final TRV is 22 μg Ni/kg-day, rounded to 20 μg Ni/kg-day.

4. Discussion

4.1. Addressing uncertainty in TRV derivations

Depending on the problem formulation (i.e., the intended application of the oral TRV), and the combination of receptor and effect, chronic or acute TRVs can be derived (i.e., a "fit for purpose" approach). In general, acute toxicity TRVs are higher than chronic TRVs, but this may not necessarily be so if different receptors are considered for deriving acute and chronic TRVs. This paper has derived TRVs for three separate problem formulations and these results are summarized in Table 6.

Uncertainties in the TRV derivations arise from a combination of factors related to (1) the strength of the database; (2) the sensitivity of the endpoint examined and its relevance to the receptor (target population); (3) the dose-response and selection of a point of departure; and (4) the selection of uncertainty to account for all the sources of uncertainty and variability. Below we discuss these elements and how we address them in our TRV derivations.

4.1.1. TRV for adults – lifetime exposure

The critical decision points (critical study, critical effect, uncertainty factors) in the derivation of this TRV were similar to those used by other groups (WHO, 2007; OEHHA, 2012; EFSA, 2015).

Table 5
Animal (rat) studies investigating systemic toxicity effects in two-generation studies.[a]

Reference Studies: Reproductive Studies – F1 Generation	Exposure	Exposure Levels in mg Ni/kg-day (ppm Ni in water)[b]	Period of Exposure (start and duration)[c]	Systemic Adverse Effects	Systemic Toxicity NOAEL (mg Ni/kg-day)
SLI (2000b) (28 males & 28 females Sprague Dawley /group)	Gavage (Placenta & milk)	0, 0.2, 0.6, 1.1, 2.2	From conception, through birth (3 weeks) and lactation for 24 weeks or more post birth	Lower body weight	2.2 (highest exposure level in study)
RTI (1988) (~30 males & 30 females Sprague Dawley /group)	Drinking water (Placenta & milk)	0, 6.0 (50), 25.0 (250), 42.0 (500)			6.0 (lowest exposure level in study)

[a] Reproductive effects are not considered here since they are not relevant for the 1–6 year old group.
[b] For the RTI study, these are the average exposure levels based on water consumption and Ni content in water.
[c] Parental gametes exposed 10 weeks before pregnancy.

Nickel effects such as post-implantation loss/perinatal mortality in rats are considered the most sensitive toxicity effects observed in adult animals. Therefore, although the critical effect was observed in pregnant rats, the derived values will be protective for other toxicities in pregnant and non-pregnant females and in males. The POD ($BMDL_{5\%}$) was selected based on the best fitting models applied to the best combination of datasets from the most sensitive and robust studies; the POD value is supported by data from other existing reproductive studies. The selected UF-interspecies of 10 to account for differences in TK and TD between rats and humans is conservative as there is reasonably robust evidence that TK differences between rats and humans are small. In addition, reproductive studies with highly exposed workers have not shown associations between increased urinary nickel levels and spontaneous abortions or other reproductive endpoints at a systemic exposure level 1/10th of the systemic exposure corresponding to the BMDL in the rat study. Similarly, the default factor of 10 is appropriate for UF-intraspecies, in part based on the absence of an effect in a reasonably large epidemiology study (Vaktskjold et al., 2008). Thus, the composite uncertainty factor of 100 used here is very conservative (health-protective); a smaller uncertainty factor of 50 could even be supported, based on data indicating that toxicokinetics in humans is comparable to that in rats, and supported by the absence of effects under extremely high occupational exposures up to 1/10th the systemic dose received by the rats in the critical study.

The TRV value of 18 μg Ni/kg body weight (rounded to 20 μg Ni/kg) is comparable to the 11 μg Ni/kg body weight derived by WHO (2007) and OEHHA (2012) based on the same SLI studies. Our value is 6-fold higher than EFSA's (2015) 2.8 μg Ni/kg body weight, even though both derivations were based on the same studies (the combination of the 1-generation study (SLI, 2000a) and first generation of the two-generation study of SLI (2000b)) and used the same uncertainty factors. In fact, we used a lower response level ($BMDL_{05}$) than the $BMDL_{10}$ used by EFSA. There are two reasons for the difference in our results. The first is that EFSA based its TRV on the incidence of litters with post-implantation loss, supported by the incidence of litters in the F1 generation with 3 or more post-implantation losses, both as simple quantal endpoints. We used the BMDS models specifically designed to address nested data from developmental toxicity studies, which accounts for the number of affected pups within each litter. Thus, the modeling conducted here is appropriately based on the litter as the unit of analysis, but takes into account the litters with more affected pups. Our nested approach retains and uses information about the probability per implantation, while recognizing that the outcomes for implantations within the same litter may be correlated; the EFSA approach loses information provided by the multiplicity of the response within litter. Where the data are available to support such modeling, use of the models for nested data is preferred for developmental toxicity data. The second reason for the difference in results is that the analysis here focused on the best-fitting model, based on the US EPA (2012) guidance, while EFSA chose the model with the lowest BMDL. EFSA has recently updated its BMD guidance (EFSA, 2017) to include AIC among the criteria for choosing the best model (as was used here, along with other criteria) to compare goodness of fit of different models.

4.1.2. TRV for sensitized population – acute exposure

Although the TRV for lifetime exposure is also designed to protect against short-term exposures, special attention needs to be paid to nickel sensitized individuals who may develop flare-ups in reaction to oral nickel from a single acute exposure; diets low in nickel are often recommended to the most sensitive patients. Therefore, this paper also presents a TRV for sensitized individuals, based on studies of nickel-sensitized individuals (with or without current dermatitis or eczema) who were exposed orally to a solution of Ni ions and observed for dermatitis or exacerbation of dermatitis. This endpoint is not relevant for non Ni-sensitive individuals who will not react dermally to Ni in the diet and for whom the higher TRV for adults should apply instead. The POD ($BMDL_{10}$) was selected based on the mean value from all models with acceptable fit that were applied to the most sensitive of the datasets, recognizing that the dose-response for this immune endpoint is highly variable. In addition, variations in concurrent

Table 6
Summary of TRVs.

	Adult Population -Lifetime Exposure	Sensitized Population- Acute Exposure	Toddler Population- Repeated exposure
TRV	20 μg Ni/kg-day	4 μg Ni/kg -*in addition to Ni in food*	20 μg Ni/kg-day
Endpoint (species)	Post-implantation loss/perinatal mortality (adult rats)	Flare-up of dermatitis (Ni sensitive humans)	Decrease in body weights (young rats)
Exposure	Repeated (water soluble Ni compound, gavage)-*Ni in food was small fraction of dose*	Single (water soluble Ni compound)-*Ni in food contributed to overall Ni exposure*	Repeated (water soluble Ni compound, gavage)- *Ni in food was small fraction of dose*
POD	$BMDL_{5\%}$ - 1.8 mg Ni/kg-day	$BMDL_{10\%}$ - 0.30 mg Ni, or 4.3 μg Ni/kg	NOAEL – 2.2 mg Ni/kg-day
UF- interspecies	10	Not relevant	10
UF-intraspecies	10	1 (already most sensitive population)	10

dietary nickel and lack of information on volunteers' body weights could have increased the observed variability within and across studies. Finally, the absence of a consistent observed increase in response to dose at the low dose end of the range in the critical study increased the uncertainty in the BMD. Furthermore, it was not possible to combine the data from multiple studies (in a single BMDS run) to improve confidence in the modeling, due to wide variability across studies. We assumed a body weight of 70 kg; this assumption may have overestimated female body weight and resulted in an underestimate of exposure and a lower POD. Considering that the selected POD (30 mg) is lower than all of the doses eliciting a response in the 16 other studies examined by Jensen et al. (2006), a review that included more than 400 nickel-sensitive volunteers, no additional uncertainty factor was applied. It is also noted that the factor of 1 is consistent with the POD being in a sensitized population tested under conditions (fasting) that maximize absorption. The TRV of 4 μg Ni/kg body weight is protective of effects that occur after a single acute exposure.

The TRV value of 4 μg Ni/kg (rounded from 4.3 μg Ni/kg) is similar to the value derived by Japan's FSCJ (2012) and ~3-fold lower than the one derived for this endpoint by WHO (2007). The difference is the choice of the study on which the POD is based and the approach for extrapolating to lower doses. FSCJ and WHO used the LOAEL in a study that tested only one dose level (Nielsen et al., 1990 [*sic*], 1999). FSCJ then applied an uncertainty factor to extrapolate to a NOAEL, while WHO said no uncertainty factor was needed, in light of the testing of nickel-sensitive individuals under fasting conditions. The much higher absorption of nickel from drinking water than from food was also noted. In contrast, the current assessment considered only studies that tested multiple dose levels and used a BMD approach based on the most sensitive of those studies. The $BMDL_{10}$ calculated here was comparable to the POD from Nielsen divided by the LOAEL to NOAEL uncertainty factor, lending additional support to the final value, since similar results were obtained from two different methods. The TRV presented here is 4-fold higher than EFSA's (2015) 1.1 μg Ni/kg body weight, even though both derivations were based on the exact same dataset and used a BMD approach. EFSA used the lowest model output, while this assessment averaged all unique models, because all of the models had acceptable fit and no single model was clearly better than the others. One important difference is that EFSA considered its TRV value as reflective of the whole diet, without recognizing that the human volunteers challenged with the nickel in solution were also being exposed to nickel from the diet. WHO (2007) used both the SLI (2000b) study and Nielsen et al. (1999), based on provocation of sensitized patients, to separately derive the same drinking water guideline value of 70 μg/L. WHO noted that LOAEL from the Nielsen et al. (1999) study was a worst-case scenario, due to the higher absorption from drinking water than from food, and in light of testing under fasting conditions.

4.1.3. TRV for toddlers

The derivation of a TRV specific for toddlers is not commonly done. Yet, for site-specific risk assessment, when the most critical population are toddlers, it is important to have a value that is based on health effects relevant to this population (e.g., not use a value based on reproductive effects).

The health effect of body weight changes observed in studies of young rats exposed to nickel was selected as the most sensitive endpoint for this target population. In this case the POD was the lowest NOAEL from all relevant studies. An UF-interspecies of 10 was applied and as discussed above, this is considered to be a conservative value. The selected UF-intraspecies of 10 to account for differences within the young human population (excluding adults and elderly) seems appropriate.

The TRV value of 20 μg Ni/kg body weight (rounded from 22 μg Ni/kg body weight) ended up being the same as the TRV for the adult population; both values are protective of effects that occur after repeated exposures.

4.2. Relationship between TRV values and exposures

When comparing the TRV values to nickel exposures other than water, it is important to consider the bioavailability of nickel from these exposure matrices in relationship to the bioavailability of Ni from water under the conditions of the study on which the TRVs are based. As indicated in Table 6, in all the studies considered here, exposures were to water soluble forms of Ni(II); i.e. 100% bioaccessible Ni(II) in gastric fluids. Even though fully bioaccessible, not all Ni(II) is absorbed after oral exposure, with the absorbed fraction dependent on the presence or absence of food in the stomach. In general, as discussed in Section 3.2, both rats and humans seem to absorb Ni to similar extents, with similar binding in serum and rapid excretion via urine. Absorption values in human studies range from 1 to 5% with food to 25–30% with 12 h fasting; a value of 10% is representative of intermediate fasting stages.

The relative bioavailability of nickel from other matrices compared to water is lower and it can be estimated by considering the relative bioaccessibility of Ni (II) in synthetic gastric fluid. Gastric fluid can be considered as worst case for gastrointestinal fluids since studies have shown that the bioaccessibility of nickel from different types of compounds is always higher at acidic pH (e.g., Henderson et al., 2012). Comprehensive studies of bioavailability and/or bioaccessibility of nickel from various types of food are currently lacking. However, there are several studies addressing the bioavailability and bioaccessibility of Ni from soils, a nickel source of particular interest for the toddler TRV.

In general, the gastric and/or gastrointestinal bioaccessibility of metals from soils has been shown to correlate well with their relative in vivo bioavailability (e.g., Diamond et al., 2016). Vasiluk et al. (2011) measured the in vitro gastro-intestinal bioaccessibility of nickel from two types of soils contaminated with nickel. A clay soil from Port Colborne (Canada) showed 11% bioaccessible nickel for two different particle size fractions (<70 μm and 150–250 μm). Soil particles less than ~50 μm in diameter are of interest as they tend to preferentially adhere to dry skin, regardless of soil type (Sheppard and Evenden, 1994; Choate et al., 2006; Yamamoto et al., 2006). A sandy soil from Sudbury (Canada) showed 4% and 19% bioaccessible nickel for the <70 μm and 150–250 μm particles, respectively. Based on their results, Vasiluk et al. (2011) concluded that relative bioaccessibility of nickel from soils can be considered as a reasonable estimate of its relative bioavailability, although more data are required to confirm these results. Based on a follow-up rat study of 20 soils, Dutton et al. (2016) concluded that the bioavailability of nickel from soils is about 1.3% of its in vitro bioaccessibility and it is strongly dependent on the speciation of the nickel. Bioavailability may have been reduced in these studies by the high level of soil ingested and it may be expected to be higher at exposure levels relevant to toddlers.

Therefore, data on relative bioaccessibility of nickel from soils (especially particles < 70 μm, the size fraction of soils that is more likely to adhere to children's hands and subsequently be ingested) can be used to adjust soil exposures before comparing them to the TRV.

Another aspect of interest is to consider what could be a worst case scenario for oral absorption of Ni and whether the current TRV is still protective of this scenario. Perhaps the best comparison for the effects of a bolus dose of nickel is the first drink of water one has in the morning. Using the standard conversions of 70 kg body weight, 2 L/day for an adult, and 20% relative source contribution,

the TRV of 20 μg Ni/kg-day for the adult receptor would result in a lifetime health advisory (i.e., recommended maximum drinking water concentration) of 0.14 mg Ni/L. A plausible upper bound for the amount consumed on an empty stomach would be drinking 0.5 L of water (absorbed under fasting). This would result in a bolus dose of 70 μg Ni or 1 μg Ni/kg, with the exact same conditions of absorption for bolus dose and TRV. This bolus dose represents ≤30% of the TRV for adult nickel-sensitive individuals, indicating that a drinking water guideline based on the general population TRV would not create problems for the nickel-sensitized population from ingestion of water on an empty stomach upon waking.

4.3. Conclusions

This paper addressed the derivation of oral nickel TRVs for 3 relevant populations (adults, children, and nickel sensitized people). The most sensitive health endpoints for identifying safe oral intake levels of nickel after exposure to food, water and soil were considered. The TRV values of 20 μg Ni/kg-day for adults and children, and 4 μg Ni/kg in addition to food, for nickel sensitized individuals, resulted from applying the most appropriate methods of data analysis and conservative uncertainty factors. When feasible, comparisons of animal and human data, as well as estimates of relative bioavailability, were used to assess the robustness of our calculations.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.yrtph.2017.03.011.

Transparency document

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