Responses to Major Comments on Technical Support Document

Public Health Goal

For

Nickel

In Drinking Water

Prepared by

Pesticide and Environmental Toxicology Section
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

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INTRODUCTION

The following are responses to major comments received by the Office of Environmental Health Hazard Assessment (OEHHA) on the proposed public health goal (PHG) technical support document for nickel as discussed at the PHG workshop held on November 5, 1999, or as revised following the workshop. Some commenters provided comments on both the first and second drafts. For the sake of brevity, we have selected the more important or representative comments for responses. Comments appear in quotation marks where they are directly quoted from the submission; paraphrased comments are in italics.

These comments and responses are provided in the spirit of the open dialogue among scientists that is part of the process under Health and Safety Code Section 57003. For further information about the PHG process or to obtain copies of PHG documents, visit the OEHHA Web site at www.oehha.ca.gov. OEHHA may also be contacted at:

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RESPONSES TO MAJOR COMMENTS RECEIVED

Comments from Toxicology Excellence for Risk Assessment (TERA)

Comment 1: The Office of Environmental Health Hazard Assessment's (OEHHA) non-cancer end-point is based on a two-generation rat reproductive study of Smith et al. (1993). The Toxicology Excellence for Risk Assessment (TERA) does not consider this study reliable as a basis for the reference dose (RfD) estimation. TERA stated that there was no clear dose-response trend in the Smith et al. (1993) study, making any identification of a no-observed-adverse effect-level (NOAEL) or lowest-observed-adverse-effect-level (LOAEL) equivocal.

"TERA suggests OEHHA reconsider the use of the Smith et al. (1993) study as the basis of its RfD, but that it also consider the existing uncertainties in the reproductive studies as an adequate basis for the use of an additional uncertainty factor. Both TERA and EPA have given this issue a lot of thought, and the positions of both groups have gone through independent peer review."

Response 1: The revised PHG is based on three reproduction toxicity studies in rat (Smith et al., 1993; Springborn Laboratory, 2000a, 2000b). OEHHA identified the oral dose of 1.12 mg Ni/kg-d as the appropriate NOAEL value, from the lower dose-range Springborn Laboratory (2000b) study. This NOAEL is lower than the LOAELs (based on early pup mortality) identified in the studies reported by Springborn Laboratory (2000a) and Smith et al. (1993). Together, these three studies appear to more clearly define the reproductive toxicity LOAEL and NOAEL.

Comment 2: "OEHHA did not consider nickel in the diet of Smith et al. (1993) study. Nor did EPA consider dietary nickel intake. In contrast, TERA developed its RfD as additional nickel, that is, the intake in addition to that found in the diet of the test animals or humans. Data on the levels of dietary nickel in the Vyskocil et al. (1994) study were not available, so TERA could not develop an RfD based on total nickel intake. The recognition by OEHHA that its RfD already includes nickel in the diet would affect its choice of relative source contribution (RSC). TERA has data on p. 107 of its toxicology review text on intake from various sources. These data indicate that nickel intake from food is typically 25 or more times the intake from drinking water."

Response 2: In their paper, Smith et al. (1993) only stated that female rats were housed in plastic cages. They did not report the level of nickel in the laboratory chow that was used to feed the rats.

OEHHA realizes that diet is an important source of nickel. However, the absorbed nickel dose resulted from food is not likely to be as high as 25 or more times the dose from drinking water. This is because food generally reduces the bioavailability of soluble nickel. Sunderman et al. (1989), Nielsen et al. (1999) and several other researchers have shown that when soluble nickel is ingested with water and the subject is fasting, the percentage of administered soluble nickel absorbed can be as high as 30 percent. However, when the subject is not fasting or when soluble nickel is ingested with food, the percentage of administered soluble nickel absorbed can be as low as 1 percent.

The relative source contribution (RSC) factor in the calculation of nickel PHG has been changed. At the PHG level of 12 µg/L, assuming a water consumption rate of 2 L/day, the estimated intake dose from water would be 24 µg/day. Assuming a dietary intake dose of approximately 200 µg/day (Myron et al., 1978; Nielsen and Flyvholm, 1984; Smart and Sherlock,, 1987) the

contribution of water to total intake of nickel would be about 11 percent. Actual mean level of nickel in California drinking water is about 20 μg/L, which would correspond to 40 μg/day, or 17 percent of total exposure. Considering that the bioavailability of soluble nickel in water is higher than the bioavailability of soluble nickel in food, a RSC of about 30 percent seemed to be a reasonable estimate and was used in the calculation of the PHG.

Comment 3: "OEHHA uses a factor of 10 for cancer potential. In contrast, TERA concludes that the carcinogenicity of soluble nickel compounds following oral exposure cannot be determined because there are inadequate data to perform an assessment."

Response 3: OEHHA agrees with TERA that the oral data are inadequate to perform a quantitative cancer risk assessment for nickel. However, the genotoxicity of nickel ion and the positive cancer study results by other routes introduce a significant concern regarding potential carcinogenicity by the oral route, and OEHHA believes that nickel has not been adequately tested by the oral route. IARC (1990) and NTP (1998) recently reviewed the toxicological data of nickel and nickel compounds. As stated in the PHG document, "In the overall evaluation, IARC (1990) identified nickel compounds as Group 1 carcinogens." "In a draft cancer identification document on nickel compounds, NTP (1998) recommended upgrading nickel compounds to (a) known human carcinogen(s). It was suggested that the ionic form of nickel is the ultimate carcinogenic species, and biokinetic factors may dictate the carcinogenic potential of the various soluble or insoluble nickel compounds."

For this reason and other reasons presented in the PHG document, OEHHA believes there is a reasonable possibility that nickel compounds are carcinogenic via the oral route and an uncertainty factor is used for this consideration. No changes were made to the PHG as a result of this comment.

Comment 4: TERA's (1999) RfD was based on the Vyskocil et al. (1994) study, where increased urinary albumin levels were observed at the only dose tested, 6.9 mg Ni/kg/day in males and 7.6 mg Ni/kg/day" in females. Applying an overall uncertainty factor of 1,000, a RfD of 8 μg Ni/kg/day was estimated. It is suggested that OEHHA consider using the RfD developed by TERA.

Response 4: In the development of the PHG, OEHHA reviewed the drinking water study reported by Vyskocil et al. (1994) and found several limitations:

- only one dosed group, 100 ppm;
- limited histopathological investigation and reporting;
- small sample size and short exposure duration (only ten male and ten female rats completed the six-month exposure period); and
- considerable variability in response (changes of glomerular permeability) was observed in both males and females.

It is because of these shortcomings that OEHHA did not use the study results reported by Vyskocil et al. (1994) as the basis for PHG development.

In Section 6.1.2 "Major Conclusions in the Characterization of Hazard and Dose Response: Noncancer Effects" of the "Toxicological Review of Soluble Nickel Salts", TERA (1999) stated that "The RfD is designed to protect people from sensitization, but may not necessarily be protective for sensitized individuals." In contrast, OEHHA is required by the California Safe

Drinking Water Act of 1996 to protect the general population as well as sensitive sub-populations: "OEHHA shall consider the existence of groups in the population that are more susceptible to adverse effects of the contaminants than a normal healthy adult" (see the Preface of the PHG). We believe that the PHG meets this requirement.

Comments from Nickel Producers Environmental Research Association

Comment 1: "It should be clarified in this section, that the ICNCM working group concluded that increased exposure to soluble nickel compounds increased the SMR for lung cancer only if exposures occurred in conjunction with high exposures to oxidic nickel."

Response 1: The commenter is correct in pointing out that the International Committee on Nickel Carcinogenesis (ICNCM, 1990) analyzed the available occupational data and noted that the higher risks of lung cancer observed in workers of the Kristiansand refinery compared to those who worked at Clydach may indicate the importance of oxidic nickel in increasing the potency of soluble nickel. ICNCM (1990) also noted that the interaction between soluble and insoluble nickel may explain the absence of increased lung cancer risks in the main group of Port Colborne electrolysis workers. Workers at Port Colborne were estimated to have been exposed to lower levels of insoluble nickel than workers in the electrolysis department at Kristiansand.

ICNCM (1990) found evidence to indicate soluble nickel exposure results in increased nasal cancer risk, based on the data collected from Kristiansand, Clydach, and INCO. All workers in these studies were exposed to soluble nickel and, in addition, also to low-level oxidic and sulfidic nickel. However, ICNCM (1990) stated that "Attribution of the nasal cancer risk to exposure to these [insoluble] forms of nickel instead of to the soluble nickel is difficult given the absence of increased nasal cancer risk among the men with similar low-level exposures to insoluble forms of nickel and much lower levels of soluble nickel exposure (e.g., Sudbury nonsinter workers and Port Colborne nickel anode workers)."

Lung and nasal cancers associated with inhalation exposure to nickel compounds were not evaluated in detail in the PHG development because the exposure route of concern is ingestion of drinking water. References to several comprehensive reviews on the subject were provided in the PHG document. No changes were made to the PHG as a result of this comment.

Comment 2: "It should be further noted that under the conditions used in the NTP studies, none of the rodent species showed evidence of nasal tumors after inhalation exposure to any one of the nickel compounds tested."

Response 2: The negative cancer data (i.e., nasal tumor) are not discussed in detail in the PHG because the evaluation focused on the increased incidences of adenomas or carcinomas of the lung and pheochromocytoma of the adrenal medulla in the exposed rats. No changes were made to the PHG document as a result of this comment.

Comment 3: "Because of clear problems in design that introduce difficulties in interpreting the study results – notably, the lack of a dose-response for the endpoint designated as the LOAEL and the potential complication of the study interpretation by effects on maternal prolactin levels - the Smith et al. (1993) study should not be used exclusively to derive a reference value for nickel."

Response 3: The revised PHG is based on three reproduction toxicity studies in rat (Smith et al., 1993; Springborn Laboratory, 2000a, 2000b). OEHHA identified the oral dose of 1.12 mg Ni/kg-d as the appropriate NOAEL value, from the lower dose-range Springborn Laboratory (2000b) study. This NOAEL is lower than the doses at which early pup mortality was observed (a LOAEL of 2.23 mg/kg-d was identified in the preliminary study reported by Springborn Laboratory (2000a) and a LOAEL of 1.3 mg/kg-d was identified in the study reported by Smith et al. (1993)).

Comment 4: "The basis for inferring a nickel-related effect at the lowest dose (10 ppm, or 1.3 mg/kg/day) was an increased number of pup deaths per litter in the second breeding of the animals in the study. But this effect was not seen in the litters from the first breeding; nor was there any evidence of reproductive toxicity with regard to other endpoints such as fertility, litter size, or pup weight."

Response 4: The reproductive outcomes of the first and second breedings reported by Smith et al. (1993) are different. In the first breeding, a statistically significant increase of number of pup deaths per litter was observed in the highest dosed group (250 ppm) compared with the control group. In the second breeding, significant increases of pup deaths per litter were observed in two of the three dosed groups (10 and 250 ppm). The exact reason for the differences is not known. One explanation offered by the author is that there might be an interaction between nickel toxicity and the endocrine changes associated with normal aging, and the interaction had an adverse impact on the reproductive process (Smith et al., 1993).

There are many possible reproductive and developmental adverse effects; some of the effects are related and many are not. The lack of changes in fertility, litter size, or pup weight in the Smith et al. (1993) study does not diminish the significance of their findings. The endpoint of increased number of pup deaths per litter, and the effective threshold for this effect have been clarified by the two Springborn Laboratory studies.

Comment 5: "There was evidence of a dose related negative trend in litters with dead pups across the 10 ppm (1.3 mg/kg/day) and 50 ppm (6.8 mg/kg/day) doses in the first breeding."

Response 5: The dose-response relationship is positive when the result of the highest dose group (250 ppm) is included in the trend analysis. A similar dose-response relationship has been reported by Springborn Laboratory (2000a).

Comment 6: "The fact that the 50 ppm exposure group did not demonstrate the effect upon which OEHHA's choice of a LOAEL is based is inconsistent with the concept of dose-response. Specifically, there was no evidence of a positive "trend" in proportion of litters with dead pups across the low and mid doses of the second breeding."

Response 6: There are positive trends in both the first and second breedings. As stated by Smith et al. (1993): "There was a dose-related increase in both the number and proportion per litter of pups either born dead or dying shortly thereafter (trend analysis: G1, $p<0.001$, 0.04; G2, $p<0.03$, 0.02)."

The revised PHG no longer relies exclusively on the study results of Smith et al. (1993). It is now based on three reproduction toxicity studies in rat (Smith et al., 1993; Springborn Laboratory, 2000a, 2000b). OEHHA identified the oral dose of 1.12 mg Ni/kg-d as the appropriate NOAEL value, from the lower dose-range Springborn Laboratory (2000b) study.

This NOAEL is lower than the doses at which early pup mortality was observed (a LOAEL of 2.23 mg/kg-d was identified in the preliminary study reported by Springborn Laboratory (2000a) and a LOAEL of 1.3 mg/kg-d was identified in the study reported by Smith et al. (1993)).

Comment 7: "[Consider] …the variability in the number of litters with dead pups across the two breedings of the control group (5/25 in the first breeding and 2/23 in the second)…. If the second breeding of controls had produced one more litter with a single pup death, the statistical evidence for increases in the proportion of litters with dead pups in the 10 and 50 ppm groups would be extremely weak ($p = 0.124$ and $p = 0.253$, respectively)."

Response 7: Yes, the proportion of litters with dead pups was low in controls in the second breeding, and this low value is related to the statistical significance of the results. Other interpretations of these data are possible. However, the observation that if the outcome had differed by one death, then there would be only nine chances out of ten that the effect is real, should not negate the presumption that the effect is dose-related. We have to work with the data we have.

Comment 8: "…[C]omparisons of numbers of pup deaths in treated groups to those which occurred in a control group with demonstrably smaller litter sizes would be biased towards the inflation of treatment effects. The possibility of such bias calls into question the validity of using pup deaths from the second breeding of controls as a basis of comparison for treated groups, and suggests that historical control data should be used.…"

"The control group that was used for comparisons had significantly smaller litter sizes than several of the other treatment groups … At the very least, smaller litters present fewer opportunities for pup deaths."

"...[A] relatively large number of stillborn young may have been cannibalized by dams in the second breeding of the control group as indicated by the unusually low average number of pups per litter (10.6±0.98). This sample mean was smaller than that of any treatment groups in the study, and t-tests comparing it to other groups gave strong evidence to suggest that the differences were not due to chance alone: control group in the first breeding (12.9±0.40, $p=0.015$), 50 ppm group in the second breeding (13.3±0.69, $p=0.012$), and 250 ppm group in the first breeding (13.2±0.62, $p=0.011$)."

Response 8: Yes, it is possible that cannibalism in the study of Smith et al. (1993) may have resulted in lower numbers of pups per litter associated with a lower number of dead pups in the control group in the second breeding. The same may be true of the lower number of pups per litter and a lower number of dead pups in the 50 ppm group of the first breeding. In the first case this would have appeared to strengthen a possible effect, in the second it would weaken it. Hypotheses about the factors responsible for the putative effects can only be answered with more data. No historical data were available to us for this analysis. However, the relatively weak effect in this case is substantiated by results of several other studies.

We agree that the data could be examined from other perspectives. The means and standard deviations of number of pups per litter for the second breeding are 10.6±4.7, 12.5±4.0, 13.3±3.4, and 11.3±4.3 for the control, 10, 50, and 250 ppm, respectively (Smith et al., 1993). Student t-test results (one-tail test) indicate that in the second breeding, litter size of the control group is significantly lower than that of the 50 ppm group but not significantly different from the 10 ppm and 250 ppm groups ($p<0.05$). Consequently, although the control group size was significantly lower than the middle dose group, there was still a positive trend of dead pups across all dose groups.

Comment 9: "Using the data from the first breeding of controls [in evaluating the second breeding data], the comparison of the proportion of litters with dead pups between groups gave no evidence of treatment effects in any of the dose groups ($p = 0.334$, $p = 0.532$, $p = 0.146$, in ascending dose order). This is basically consistent with the result that was obtained for the first breeding (apart from the negative effect in the 50 ppm group)."

Response 9: Reproductive results are well known to differ between successive breedings, so the suggested comparison is not valid.

Comment 10: "An additional confounder observed in the rats from the Smith et al. (1993) study raises the question of the relevance of the observation in the Smith study for human risk assessment. Specifically, Smith et al. (1993) demonstrated decreases in plasma prolactin levels in the dams and pups at one week after weaning of the second litter." "There are, however, key differences in the functions of prolactin in rats and humans… In the rat, in early pregnancy, prolactin is luteotrophic and serves to maintain the corpus luteum (CL) and stimulates the CL to produce progesterone…. In this period, any event that results in a decrease in prolactin levels could potentially result in fetal death and subsequent decreases in litter size. In contrast, in humans, the primary role of prolactin is to stimulate the differentiation of the mammary gland in preparation for milk production…. Prolactin is not luteotrophic in humans…and normal CL function has been observed in prolactin-deficient women…. Therefore, any chemical that decreased prolactin levels would <u>not</u> be expected to result in fetal death in humans."

Response 10: As reported by Smith et al. (1993) and shown in the table below, prolactin levels measured in the dams of the control, low-dosed, and mid-dosed groups at one week after weaning of the second litter were not significantly different from each other. Nonetheless, Smith et al. (1993) observed significantly higher numbers of dead pups in the low- and mid-dosed groups compared with the controls in the second breeding. The data indicate that changes in prolactin levels may not be related to the adverse reproductive outcomes observed.

Plasma prolactin levels (in ng/ml) measured in dams and pups (from Smith et al., 1993)

	Concentration (ppm Ni)			
	0	**10**	**50**	**250**
Dams	6.6±2.1	5.8±1.8	6.3±1.8	5.2±1.1**
G1 male pups	17.9±12.3			17.5±10.6
G1 female pups	13.1±9.3			17.4±11.7
G2 male pups	6.0±2.9			5.0±2.3
G2 female pups	6.4±6.2			4.5±3.1

** $0.01 < p \leq 0.03$

Prolactin levels were only measured in the dams at one week after weaning of the second litter. Since estrogens are known to stimulate prolactin secretion by the pituitary (Neumann, 1991), it is not clear if the prolactin levels measured are representative of the prolactin levels during the first and second gestation periods.

If changes in prolactin levels were interfering with corpus luteum function (when the corpus luteum is producing progesterone) as suggested by the comment, the pregnancy would not be established in the first place, or there could be reduced litter size or number of litters. After placentation, the corpus luteum degenerates. As pup death was the endpoint in the Smith et al. (1993) study, this means that pups must have died late, maybe even after they were born. The observed results therefore should not be inferred to be secondary to changes in prolactin and effect on corpus luteum.

Comment 11: "Since the general population is exposed to nickel in drinking water overwhelmingly in non-fasting conditions, the NOAEL calculated from Nielsen et al. should be adjusted upward. Using the midpoint of the 10 to 30 times adjustment factor noted by OEHHA, the reference value based on Nielsen et al. would be 24 μg Ni/kg/day (20×1.2 μg Ni/kg/day)."

Response 11: OEHHA agrees that the general population is most likely exposed to Ni in the nonfasting state. A sentence is added to the PHG document to indicate that the reference values derived from the Nielsen et al. (1999) and Cronin et al. (1980) studies should be considered upper-bound estimates.

Comment 12: "The only animal studies where soluble nickel compounds have been shown to cause cancer are intraperitoneal studies… Because they employed the intraperitoneal route of exposure, they are irrelevant for regulatory hazard identification and risk assessment."

Response 12: OEHHA does not agree that all studies using the intraperitoneal route of exposure are irrelevant for regulatory hazard identification and risk assessment. However, these study results are more difficult to interpret and may not be easily extrapolated quantitatively to human exposures.

Comment 13: "In sum, while the issue of potential <u>oral</u> carcinogenicity of the nickel ion has not been definitively resolved, the data available to date indicate that this route of exposure is unlikely to present a carcinogenic hazard to humans. OEHHA's reference to IARC (1990) and NTP (1998) are not inconsistent with this view, since those evaluations both focus on potential carcinogenicity via inhalation, as opposed to oral exposure."

Response 13: As described in the PHG document, OEHHA acknowledges that there are uncertainties in the identification of soluble nickel as an oral carcinogen. OEHHA finds it is prudent to apply an uncertainty factor of ten (10) for the potential carcinogenicity of soluble nickel through the oral route because:

1. Soluble nickel compounds have been shown to be positive in mutagenic and clastogenic tests (both *in vitro* and *in vivo*) (see the section on genetic toxicity in the PHG document.
2. It has been shown in many epidemiological studies that inhalation exposure to soluble and insoluble nickel compounds was associated with increased incidence of nasal and lung cancers (see the section on carcinogenicity in humans in the PHG document.
3. There is limited evidence suggesting occupational exposure to nickel compounds was associated with increased chromosomal aberrations. This shows that soluble nickel compounds may be clastogenic in humans (Deng et al., 1988; Waksvik and Boysen, 1982 and 1984, as cited in IARC, 1990).

4. In two inhalation bioassays (NTP, 1996a, b), male and female rats exposed to nickel subsulfide or nickel oxide showed significantly higher incidence of pheochromocytomas of the adrenal medulla than the controls. These data indicate that some forms of nickel (e.g., free nickel ion or chelated nickel ion) were able to reach and induce cancer in a distal target organ.
5. Soluble nickel has been shown to be a complete transplacental carcinogen in rats. Increased pituitary tumors were observed in rats given nickel acetate prenatally (Diwan et al., 1992).

OEHHA also reviewed the "Draft RoC Background Document for Nickel Compounds" (NTP, 1998) and determined that the document not only addresses inhalation exposure to nickel compounds but also carcinogenicity of nickel compounds in general. For instance, the first two sentences of the document are: "Nickel compounds are known to be *human carcinogens* based on findings of increased risk of cancers in exposed workers and evidence of malignant tumor formation by multiple routes of exposure at various sites in multiple species of experimental animals. The combined results of epidemiological studies, carcinogenesis studies in rodents, and mechanistic data support the concept that nickel compounds act by the generation of nickel ions at critical sites in target cells of carcinogenesis and allow consideration and evaluation of these compounds as a single group."

In their overall evaluation, IARC (1990) determined that nickel compounds are Group 1 carcinogens. IARC noted that there is sufficient evidence in humans for the carcinogenicity of nickel sulfate, and of the combinations of nickel sulfides and oxides encountered in the nickel refining industry.

Comment 14: "OEHHA's own calculations demonstrate that if soluble nickel presents any carcinogenic risk at all to humans via oral ingestion, its carcinogenic potency would be extremely low, and any risk of cancer would be negligible. Accordingly, there is no scientific justification for applying an additional 10-fold safety factor to account for potential carcinogenicity of soluble nickel through oral exposure."

Response 14: In the PHG document, OEHHA did not provide an estimate of the carcinogenic potency of soluble nickel through the oral route. Given the limited data on oral carcinogenicity of nickel, no specific cancer potency can be computed. The evidence of a potential effect is strong enough that OEHHA considers it to be appropriate to apply an uncertainty factor of ten to the non-cancer PHG to account for the potential carcinogenicity of soluble nickel through the oral route.

Comment 15: "In calculating the PHG for nickel, OEHHA applied a Relative Source Contribution (RSC) adjustment factor of 20 percent to account for the fact that 'food is an important source of nickel'. Food is indeed an important source of nickel, but that does not justify making an arbitrary 20 percent RSC adjustment in calculating the PHG. Instead, the logical and rational approach would be to subtract from the total daily acceptable intake of nickel the amount that is reasonably expected to be ingested in food."

Response 15: It is a challenge to implement the suggested approach because, as stated in the PHG document, bioavailability of soluble nickel through ingestion is affected by the medium and the fasting state of the subject. Sunderman et al. (1989), Nielsen et al. (1999) and several other researchers demonstrated that when soluble nickel was ingested with water and the subject was fasting, the percentage of administered soluble nickel absorbed could be as high as 30 percent.

However, when the subject was not fasted and the soluble nickel was ingested with food, the percentage of administered soluble nickel absorbed could be as low as 1 percent. For this reason, the oral bioavailability of soluble nickel is highly variable and is dependent on the water consumption pattern (also see the response to Comment #16). Our review of amounts of nickel available from food and from California drinking water indicates that relative source contribution from water justifies a value of 30 percent for the final PHG calculation.

Comment 16: "The PHG proposed by OEHHA of 1 μg Ni/L is not supported by the scientific evidence and cannot pass the rigors of a comparison to the sum of existing data on oral exposure to nickel. It is based on the premise that daily intake of nickel in excess of 10 μg Ni/day is unsafe. But, as OEHHA recognizes, average dietary intake of nickel is more than an order of magnitude higher than this."

Response 16: The PHG has been changed to 12 μg Ni/L (or 12 ppb).

Using a PHG of 12 μg/L and assuming a water consumption rate of 2 L/day, the intake from water is estimated to be 24 μg/day. Assuming a dietary intake of approximately 200 μg/day (Myron et al., 1978; Nielsen and Flyvholm, 1984; Smart and Sherlock,, 1987) the contribution of water to total intake of nickel would be about 11 percent. Actual mean level of nickel in California drinking water is about 20 μg/L, which would correspond to 40 μg/day, or 17 percent of total exposure. Considering that the bioavailability of soluble nickel in water is higher than the bioavailability of soluble nickel in food, a RSC of about 30 percent seemed to be a reasonable estimate and was used in the calculation of the PHG.

Comments from the Metal Finishing Association of Southern California (MFASC)

Comment 1: In the Smith et al. (1993) study, the most plausible mechanism for the reproductive effects observed is an effect on prolactin secretion. There are, however, key differences in the functions of prolactin in rats and humans. If the decrease in prolactin levels were causally related to the adverse reproductive outcomes observed in rats, similar decrease is not expected to result in fetal death in humans.

"Because of the possible role of changes in prolactin secretion in the observed effects in rats in Smith et al. (1993), and the questionable significance of these changes to human health, the uncertainty factor of 10 currently used for animal to human extrapolation is not supported. Given the greater susceptibility of the rats to these effects, an uncertainty factor of 3 would be adequate."

Response 1: The revised PHG is based on three reproduction toxicity studies in rat (Smith et al., 1993; Springborn Laboratory, 2000a, 2000b). OEHHA identified the oral dose of 1.12 mg Ni/kg-d as the appropriate NOAEL value, from the lower dose-range Springborn Laboratory (2000b) study. This NOAEL is lower than the doses at which early pup mortality was observed (a LOAEL of 2.23 mg/kg-d was identified in the preliminary study reported by Springborn Laboratory (2000a) and a LOAEL of 1.3 mg/kg-d was identified in the study reported by Smith et al. (1993)).

As reported by Smith et al. (1993) and shown in the table below, prolactin levels measured in the dams of the control, low-dosed, and mid-dosed groups at one week after weaning of the second litter were not significantly different from each other. Nonetheless, Smith et al. (1993) observed significantly higher numbers of dead pups in the low- and mid-dosed groups compared with the

controls in the second breeding. The data indicate that changes in prolactin levels may not be related to the adverse reproductive outcomes observed.

Plasma prolactin levels (in ng/ml) measured in dams and pups (from Smith et al., 1993)

	Concentration (ppm Ni)			
	0	**10**	**50**	**250**
Dams	6.6±2.1	5.8±1.8	6.3±1.8	5.2±1.1**
G1 male pups	17.9±12.3			17.5±10.6
G1 female pups	13.1±9.3			17.4±11.7
G2 male pups	6.0±2.9			5.0±2.3
G2 female pups	6.4±6.2			4.5±3.1

** $0.01 < p \leq 0.03$

Prolactin levels were only measured in the dams at one week after weaning of the second litter. Since estrogens are known to stimulate prolactin secretion by the pituitary (Neumann, 1991), it is not clear if the prolactin levels measured are representative of the prolactin levels during the first and second gestation periods.

If changes in prolactin levels were interfering with corpus luteum function (when the corpus luteum is producing progesterone) as suggested by the comment, the pregnancy would not be established in the first place, or there could be reduced litter size or number of litters. After placentation, the corpus luteum degenerates. As pup death was the endpoint in the Smith et al. (1993) study, this means that pups must have died late, maybe even after they were born. The observed results therefore should not be inferred to be secondary to changes in prolactin and effect on corpus luteum.

Comment 2: "Information on the potential carcinogenicity of soluble nickel from animal and epidemiological data indicate that a low concentrations soluble nickel would not be carcinogenic. Therefore, the factor of 10 for evidence of carcinogenicity would be eliminated."

Response 2: As described in the PHG, OEHHA acknowledges that there are uncertainties in the identification of soluble nickel as an oral carcinogen. OEHHA finds it is prudent to apply an extra uncertainty factor of ten for the potential carcinogenicity of soluble nickel through the oral route because:

1. Soluble nickel compounds have been shown to be positive in mutagenic and clastogenic tests (both *in vitro* and *in vivo*) (see the section on genetic toxicity in the PHG document.
2. It has been shown in many epidemiological studies that inhalation exposure to soluble and insoluble nickel compounds was associated with increased incidence of nasal and lung cancers (see the section on carcinogenicity in humans in the PHG document.
3. There is limited evidence suggesting occupational exposure to nickel compounds was associated with increased chromosomal aberrations. This shows that soluble nickel compounds may be clastogenic in humans (Deng et al., 1988; Waksvik and Boysen, 1982 and 1984 as cited in IARC, 1990).

4. In two inhalation bioassays (NTP, 1996a, b), male and female rats exposed to nickel subsulfide or nickel oxide showed significantly higher incidence of pheochromocytomas of the adrenal medulla than the controls. These data indicate that some forms of nickel (e.g., free nickel ion or chelated nickel ion) were able to reach and induce cancer in a distal target organ.
5. Soluble nickel has been shown to be a complete transplacental carcinogen in rats. Increased pituitary tumors were observed in rats given nickel acetate prenatally (Diwan et al., 1992).

Comment 3: "Due to the limitations in the Smith et al. (1993) study, and the fact that the toxicity observed in this study was not reproduced in similar studies (RTI, 1987; Ambrose et al., 1976; Schroeder and Mitchener, 1971) until much higher concentrations (>250 ppm) of soluble nickel compounds were administered, it could be argued that a different study should be evaluated as possible alternative to the public health goal derived based on the Smith et al. (1993) study."

Response 3: The PHG has been revised. It is now based on three reproduction toxicity studies in rat (Smith et al., 1993; Springborn Laboratory, 2000a, 2000b). OEHHA identified the oral dose of 1.12 mg Ni/kg-d as the appropriate NOAEL value, from the lower dose-range Springborn Laboratory (2000b) study. This NOAEL is lower than the doses at which early pup mortality was observed (a LOAEL of 2.23 mg/kg-d was identified in the preliminary study reported by Springborn Laboratory (2000a) and a LOAEL of 1.3 mg/kg-d was identified in the study reported by Smith et al. (1993)).

Comments from the Office of Water, U.S. Environmental Protection Agency

Comment: "Smith et al. (1993) reported that the proportion of dead pups per litter was significantly increased at the highest dose (250 ppm) in the first breeding and at 10 and 250 ppm [low- and high-dosed groups, respectively] in the second breeding. In the second breeding, the proportion of dead pups per litter at 50 [mid-dosed group] was marginally significant (P = 0.076). The authors noted that if only one additional female, drinking 50 ppm with no dead pups, had lost one pup at birth, the probability level for the analysis at this dose would change from 0.076 to 0.04. U.S. EPA considered that this was a personal interpretation of the authors and is not supported by experimental data."

Response: Table 11 of the PHG is duplicated below. Adverse reproductive outcomes observed in the control, low-dosed (10 ppm), mid-dosed (50 ppm), and high-dosed (250 ppm) groups were 1 percent, 4.3 percent, 4.6 percent, and 8.8 percent, respectively. There is a positive trend in the dose-response relationship (p=0.02).

Reproductive outcome of second breeding of female rats drinking nickel chloride solutions (from Smith et al., 1993).

Conc. of nickel in water (ppm Ni)	Sperm positive females	No. of viable litters	Average no. of pups per litter (live and dead)	No. litters with dead pups at birth	Total dead pups on postnatal day 1 (% dead pups per litter)
0 (29[a])	28	23	10.6	2[b]	2 (1.0)
10 (29)	28	22	12.5	7†	11** (4.3)**
50 (30)	29	24	13.3	6	16* (4.6) †
250 (31)	31	25	11.3	10**	22*** (8.8)***

[a]Number of females bred for second time.
[b]Number of litters with at least one dead pup.
Significant levels, pairwise comparison to the control.
† $0.05<P\leq0.10$, * $0.03<P\leq0.05$, ** $0.01<P\leq0.03$, *** $0.001<P\leq0.01$.

Based on these data, Smith et al. (1993) stated: "We conclude that 10 ppm Ni represents the lowest observed adverse effect level (LOAEL) in this study." The difference in the number of pup deaths per litter between the low-dosed and the mid-dosed groups was small. Smith et al. (1993) offered a plausible explanation: "The data suggest that the dose-response curve for these measures at Ni levels below 250 ppm is shallow, possibly reflecting a homeostatic mechanism regulating nickel absorption." The Springborn Laboratory studies (2000a,b) substantiate the identification of a LOAEL and NOAEL at the low end of this concentration range.

Comments University of California, Berkeley

Comment 1: "For trace metals such as nickel, it may be more reasonable to set the acceptable dose as a fraction or percentage of the amount that is estimated to be in the "normal" average American diet. For example, a average of 5% or 10% of the "average" intake of 150 µg/day might be a more realistic number to consider."

Response 1: The proposed PHG has been changed to12 µg Ni/L. Assuming a water consumption rate of 2 L/day, an intake of 24 µg/day can be estimated. There are data to indicate the average dietary intake of nickel is approximately 200 µg/day. Using these estimates, the contribution of water to the total intake of soluble nickel would be 11 percent, which should be corrected for relative bioavailability. Due to a higher bioavailability of soluble nickel in water than that in food, a relative source contribution (RSC) of 30 percent is assumed in the PHG determination. The approach used in the development of the PHG is similar to the one suggested.

Comment 2: "Under the section on absorption: Is there skin absorption from nickel-plated materials and from the 5 cent piece?"

Response 2: No relevant data are located regarding skin absorption from nickel-plated materials. However, a small section has been added to the document discussing the dermal absorption of soluble nickel from aqueous solutions.

Comment 3: "Nickel compounds administered sub-chronically through gavage seem to be more toxic than those administered through the drinking water. American Biogenics Corporation (1988 as cited in ATSDR, 1997) administered 1.2 and 8.6 mg Ni/kg_{bw}/day nickel chloride hexahydrate to Sprague-Dawley rats by gavage for 91 days and found 2/60 and 6/52 died at the end of the study, respectively. What was the mortality rate for controls?"

Response 3: The information is not provided by ATSDR (1997). No changes were made to the PHG document as a result of this comment.

Comments from University of California, Davis

Comment 1: "It is surprising that no attempt was made to compare doses used in the Smith study with human exposures in the study by Chashschin, mentioned on page 41, third paragraph. Here we have human data (increase in spontaneous abortions) and presumably some exposure data (Ni concentrations in air) that could be used to calculate some possible human exposures. It would be interesting to see how presumed LOAEL's between rats and human compare."

Response 1: The calculation of inhalation dose received by workers in the Chashschin et al. (1994) study has been added to the document. Based on the occupational exposure data provided by Chashschin et al. (1994), a human LOAEL of 20 µg/kg-day can be estimated. The estimate is made by assuming an average air concentration of 0.2 mg Ni/m^3, an inhalation rate of 10 m^3/day, an exposure regime of 5 days a week, and an adult body weight of 70 kg. This value is considerably lower than the animal LOAELs of 1,300 µg/kg-day estimated from the Smith et al. (1993) study and 2,230 µg/kg-day estimated from the Springborn (2000a) study. However, it is important to note that the study lacks adequate statistical and sampling details and Chashschin et al. (1994) consider the data preliminary in nature. Chashschin et al. (1994) also noted two possible confounders, exposure to chlorine gas and lifting heavy objects.

Comment 2: "The Cronin study used 3 doses and so an uncertainty factor of 10 to derive a NOAEL from LOAEL seems OK; however, the Nielsen study used only one dose and, in view of missing information on the possible shape of a dose response curve, it is not possible to simply divide this dose, which can only be assumed to be a LOAEL, by 10 to derive a NOAEL."

Response 2: The fact that only one dose was used in the Nielsen et al. (1999) study increases the uncertainty in identifying the LOAEL and NOAEL. It should be noted that the human LOAEL (12 µg/kg) identified in the Nielsen et al. (1999) study is similar to the human LOAEL (8.6 µg/kg) identified in the Cronin et al. (1980) study. These two studies were listed in Table 24 of the final PHG document for comparison purpose, neither of the studies was used in the PHG calculation. No changes were made to the PHG document as a result of this comment

Comment 3: "Table 19 also compares apples and oranges - the rat data are developmental toxicity, but the human data deal with an allergic disorder (Ni eczema). On page 43, second para, a study is mentioned (Sjovall) where apparently increased Ni doses decreased the toxic effect. So, the apparent good correlations between animal and man, as presented in table 19, may be coincidence or fortuitous."

Response 3: OEHHA recognizes that the study results listed in the table are not easily compared. The studies differ in species tested, dose regime, state of fasting, and toxicological end-points observed. The main purpose of the table is to list the candidate studies that can be used for PHG development. No changes were made to the PHG document as a result of this comment.

Comment 4: "Table 19 brings up an additional point. On page 45 it is (correctly) pointed out that the human data were derived from the sensitive subpopulation, i.e. people who are allergic to Ni and therefore represent the most sensitive population. Yet the reference values derived are remarkably close to the ones calculated from animal studies. In other words, the most sensitive humans appear to be as sensitive as the average rat. Yet, in several places (e.g., p.51) an uncertainly factor of ten (10) is introduced for interspecies extrapolation. If the sensitive human population is about as sensitive as rats, why should the average human population be 10 times more sensitive?"

Response 4: As pointed out correctly by the reviewer, the reference values derived from the human and rat studies are based on different toxic end-points. Consequently, the reference values derived from the human studies (nickel eczema) can not be directly compared nor used to support reference values derived from the animal studies (adverse reproductive effects).

Comment 5: "The methodology used is the one that is commonly used. However, it is disappointing that (as outlined above) commonly held assumptions are used even in presence of data to the contrary. Nickel toxicology should provide enough human data as to allow a critical reappraisal of the usual uncertainty factors, such as 10 for interspecies extrapolation (which does not stand scrutiny, as per above) or interspecies (what is the difference between allergic and non-allergic people? Can it be guesstimated - is it 10? More? Less? Would the human data allow to come at some conclusions here?)"

Response 5: There are no data to indicate the relative susceptibilities of humans and rodents to the reproductive hazards posed by soluble nickel through the oral route. There are also no data to indicate the range of susceptibility in the human population to this effect. In the development of the PHG, OEHHA used an uncertainty factor of 10 for the interspecies extrapolation and another factor of 10 for intraspecies variability. No changes were made to the PHG document as a result of this comment.

Comment 6: "Page 14, line 11 from bottom: increased body weights without some pathology are useless information."

Response 6: This appears to relate to the subchronic study of RTI (1987) which states: "At approximately 50 mg Ni/kg-day, they found increased lung and kidney weights in females." Even without detailed pathological information, OEHHA recognizes the information reported as useful. No changes were made to the PHG document as a result of this comment.

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