COMMENTS ON THE PROPOSED PRIORITIZATION OF NICKEL FOR THE POSSIBLE DEVELOPMENT OF HAZARD IDENTIFICATION MATERIALS RELATING TO LISTING AS A REPRODUCTIVE OR DEVELOPMENTAL TOXICANT UNDER PROPOSITION 65

Comments of the Nickel Producers
Environmental Research Association

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1. EXECUTIVE SUMMARY

In response to OEHHA's proposal to prioritize nickel for the possible preparation of hazard identification materials as a developmental or reproductive toxicant under Proposition 65, the Nickel Producers Environmental Research Association (NiPERA) wants to draw the DARTIC attention to the following issues:

- The forms of nickel to which people in California are mostly exposed do not correspond to the CAS number listed by OEHHA. Specifically, excluding occupational settings, exposure to metallic nickel (CAS# 7440-02-0) via inhalation or the oral route is negligible or non-existent. Similarly, despite dermal exposure to metallic nickel in some consumer items, dermal absorption is very low and the contribution of this exposure route to systemic blood nickel levels is essentially undetectable.
- Confirmatory evidence of reproductive effects in rats exposed to high levels of water soluble nickel salts has existed since 1970's with a definitive study sponsored by NiPERA in 2000.
- The current proposal for prioritization of nickel was based on new epidemiological studies available since 2007[1]. Of these studies, the case-control study manuscripts of workers at the Monchegorsk nickel refinery stand out due to the high external and internal nickel exposures of the workers (many fold higher than any ambient air study) and the studies' ability to detect potential causal exposures related to reproductive outcomes. Yet, these studies do not support a causal association between exposure to nickel and adverse reproductive outcomes.
- In summary, the totality of the current epidemiological data does not justify a high priority for developing reproductive hazard identification materials for nickel compounds and even less so for nickel metal.

2. INTRODUCTION

The Office of Environmental Health Hazard Assessment (OEHHA) is proposing that nickel (CAS# 7440-02-0, nickel in metallic form) be reviewed by the Developmental and Reproductive Toxicant Identification Committee (DARTIC) to determine if a preliminary evaluation of the evidence warrants the development of hazard identification materials for the possible listing of nickel as a reproductive or developmental toxicant under Proposition 65. NiPERA welcomes the opportunity to provide comments on this proposal for consideration by the DARTIC and discussion at the November 9, 2015 meeting.

The potential for exposure to nickel metal in California was reported as being confirmed. However, this is misleading since human exposure to nickel metal in California and other locations is almost exclusively dermal in nature which does not result in significant systemic nickel ion absorption and hence does not lead to nickel ion reproductive toxicity as identified in animals. According to OEHHA, the assertion that human exposure to nickel metal in California occurs was based primarily on data concerning production and use of nickel compounds, or monitoring data for nickel compounds.

[1] OEHHA used an epidemiology literature review to assess the evidence of reproductive hazard posed by metallic nickel to humans. However, many of the studies reviewed by OEHHA involved other forms of nickel. These comments, nonetheless, address the scientific value of all these studies.

Further, the prioritization proposal for metallic nickel was based on the outcome of a prioritization process adopted by OEHHA in 2004. OEHHA used a focused epidemiology literature review to assess the evidence of possible reproductive hazard of nickel compounds (not nickel metal which was specified by CAS number). The review identified three human studies (Ebisu *et al.*, 2012; Bell *et al.*, 2010; and Guo *et al.*, 2010) as providing sufficient human data to pass the screen. None of these studies involved exposure to metallic nickel.

2.1 Background information

2.1.1 Exposure Profile

The chemical forms of nickel predominantly present in ambient air (PM_{10}) are water soluble sulfates and oxidic nickel including nickel monoxides and complex Ni-Mg and Ni-Fe oxides (Galbreath *et al.*, 2003). Nickel (II) is essential to microorganisms, plants and certain mammals (Eskew *et al.*, 1983; ATSDR, 2005). Nickel (II) is present in plants as complex organic molecules and in drinking water as the hydrated Ni(II) ion (water soluble form of nickel). Thus, nickel is present in food, yielding an average daily dietary intake of 100-300 µg Ni via ingestion. Owing to nickel ingestion through the diet (and to a much lesser extent from air), nickel levels can be detected in blood and urine of the general population, even without any exposure to nickel in the workplace. Another example of natural nickel exposure from plants is cigarette smoking which can contribute to the daily nickel exposure (nickel oxides). However, it should be noted that results are mixed as to whether smoking can significantly increase blood nickel levels.

Nickel metal (CAS# 7440-02-0) is not present in ambient air or soils to any significant extent. Therefore, for the general population, in California or anywhere else, exposure to nickel metal from ambient air (or from dust or soil) is negligible. Occupational situations where exposure to nickel metal may be significant include: nickel refining and the manufacturing and use of nickel metal powders. Minor exposures to nickel metal may occur during other operations such as plating and alloy manufacturing. Welding of nickel-containing alloys such as stainless steel mostly generates exposure to spinels and complex nickel oxides, rather than to nickel in elemental form.

2.1.2 Toxicokinetics of Nickel and Nickel Compounds

The gastrointestinal absorption of nickel (II) from food is low since nickel is present in plants as complex organic molecules and is not easily bioaccessible in gastric or intestinal fluids (Olivares Arias *et al.*, 2015). Ni (II) in water is ~100% bioaccessible, but its absorption is modulated by the presence of food in the stomach. Oral absorption of nickel from water ranges from 1-5% (ingested with food) to 12-27% (ingested under fasting) in studies of human volunteers (*e.g.*, Nielsen *et al.*, 1999).

The oral absorption of non-water soluble compounds is much lower than that of water soluble nickel compounds as described in rat studies and predicted by their much lower bioaccessibility in gastric fluids (Ishimatsu *et al.*, 1997; Henderson *et al.*, 2012).

Absorption of nickel ion via the dermal route is very low. Dermal absorption is estimated at ~2% for water soluble nickel compounds and 0.2% for insoluble compounds and the metallic form. Absorption of nickel via inhalation will depend on the particle size and respiratory tract deposition of the aerosol as well as on the bioaccessibility of nickel in lung fluids. Larger particles will be deposited in the upper respiratory tract, swallowed and absorbed from the gastrointestinal tract. Smaller particles of water soluble nickel compounds that are deposited in the lower respiratory tract are assumed to be completely absorbed, while water insoluble particles will be absorbed to a much lower extent.

There is a good correlation between oral exposures to 100% bioaccessible nickel in water and blood and urine nickel levels in rats (Heim *et al.*, 2007). There is also a good correlation between inhalation exposure to water soluble aerosols and blood-urine nickel levels in humans (Thomassen *et al.* 1999). It should be noted, however, that the internal exposures achievable in the rat gavage studies are not achievable in humans, even in the most highly exposed workers. See further discussion on this point in section 4.3.

3. DISCUSSION OF HUMAN AND ANIMAL REPRODUCTIVE STUDIES

3.1 Review of epidemiological reproductive studies

A discussion on the most relevant human studies is provided in Table 1 below. A relevancy score of Low, Medium, or High was assigned to each study based on the reliability of the study to establish a causal association between exposure to nickel and adverse reproductive effects. Assignment of scores took the following factors into consideration: strength of study design and reporting, size of study, and availability of relevant exposure data.

The studies are grouped in two major categories: studies of the non-occupationally exposed general population and studies of workers exposed to nickel substances (mainly by inhalation with some oral exposure). Studies #1, #2 and #5 (blue font in Table 1) are the ones identified by OEHHA as being good analytical studies that fulfill the criterion for prioritization of nickel metal (CAS#7440-02-0) even though nickel metal exposures did not occur in those studies. Other epidemiological studies considered by OEHHA to report statistically significant increases in adverse developmental or reproductive outcomes are studies #4, #11, #13, and #20. Study #16 corresponds to a study also considered by OEHHA as reporting an increased risk of adverse developmental or reproductive outcome, with findings that were not statistically significant.

TABLE 1. EPIDEMIOLOGICAL STUDIES

No	Studies	Findings	Critique	Relevancy
General Population Studies				
1	Prenatal exposure to fine particulate matter and birth weight: variations by particulate constituents and sources. Bell M. L., Belanger K., Ebisu K., Gent J. F., Lee H. J., Koutrakis P. and Leaderer B. P. Epidemiology. 2010;21(6):884-91	Study conducted in counties in Connecticut and Massachusetts (USA). ~77,000 births were included. Birth weight information gathered from birth certificates. A change in Ni levels of 2 ng/m^3 in PM2.5 was associated with 11% increase in risk of low birth weight	Personal exposures not measured, blood or urinary nickel levels not measured. County-wide air monitoring measurements were used which do not reflect personal measurements or personal activity pattern.	**Low.** If the link between Ni exposures and low birth weight reported in this study was causal, the study of 3,000 female refinery workers exposed to much higher Ni levels (respirable fraction aerosol levels 400 to 30,000-fold higher than ambient air) should also have demonstrated a statistically significant association, but did not (see study #15).
2	Airborne PM2.5 chemical components and low birth weight in the northeastern and mid-Atlantic regions of the United States. Ebisu K. and Bell M. L. Environ Health Perspect. 2012;120(12):1746-52.	Study conducted in northeastern and mid-Atlantic USA. Compared to the previous study (#1), this study covered a much larger area and population and expanded the components considered. Study includes 1.2 million infants. Birth weight information gathered from birth certificates. A change in Ni levels of 6 ng/m^3 in PM2.5 was associated with 5.7% increase in risk of low birth weight	Personal exposures not measured, blood or urinary nickel levels not measured In this larger study, Ni air levels 3-fold higher than in previous study by same authors are associated with 2-fold lower risks.	**Low**. Same comment as above.
3	Does the metal content in soil around a pregnant woman's home increase the risk of low birth weight for her infant? McDermott S., Bao W., Aelion C. M., Cai B. and Lawson A. B. Environ Geochem Health. 2014;36(6):1191-7.	Retrospective cohort study in South Carolina (USA). Study included 10,000 infants. Birth weight information gathered from birth certificates. No association between Ni in soil and low birth weight was found.	Personal exposures not measured, blood or urinary nickel levels not measured. Ni levels in soil varied by 43-fold between quartile 1 and 3. Soil samples were obtained from the neighborhood, not at the maternal residence, and were collected post-delivery. Authors did not account for season in their models; only 8 trace metals were examined.	**Low**. Personal biological exposures not measured

No	Studies	Findings	Critique	Relevancy
4	Effect of exposure to trace elements in the soil on the prevalence of neural tube defects in a high-risk area of China. Huang J., Wu J., Li T., Song X., Zhang B., Zhang P. and Zheng X. Biomed Environ Sci. 2011;24(2):94-101.	This is not an epidemiological study but a model-building study. Study conducted in the Lvliang region (China). Cases of neural tube defects (identified in hospital records) were matched with soil samples from each village. >6,000 births were included. Results show that both lower and higher than average levels of Ni in soil were associated with increased risk of neural tube defects (a form of muscle-skeletal malformation).	Personal exposures not measured, blood or urinary nickel levels not measured. No information is provided on the number of cases assessed, population characteristics, *etc*. Soil mean levels: 41.38 +/ 6.39 µg Ni/g soil. Higher prevalence of adverse effects at Ni levels < 30 µg Ni/g soil.	**Low**. No biological measurements of exposure were conducted.
5	Monitoring of lead, cadmium, chromium and nickel in placenta from an e-waste recycling town in China. Guo Y., Huo X., Li Y., Wrocu K., Liu J., Huang J., Zheng G., Xiao Q., Yang H., Wang Y., Chen A. and Xu X. Sci Total Environ. 2010;408(16):3113-7.	Study conducted in the Guiyu region (China). 101 mother-infant pairs (e-waste recycling) and 119 pairs from control. Higher levels of Ni in placenta were associated with lower gestational age. No significant Ni effects on birth weights were detected. Higher placenta Ni levels detected among controls than among exposed women.	Nickel in placenta was measured. Guiyu's placentas (n=101) had lower levels of Ni (median: 7.64 ng/g) than placentas from controls (median: 14.3 ng/g), even though Ni in Guiyu soil was reported to be extremely high. The finding of an association between higher levels of Ni in placenta and lower gestational age includes all women (exposed and unexposed), not just from the e-recycling area. This finding may be driven by the controls with higher placenta Ni levels.	**Medium**. Levels of Ni in placenta were measured.
6	Levels of heavy metals and trace elements in umbilical cord blood and the risk of adverse pregnancy outcomes: a population-based study. Zheng G., Zhong H., Guo Z., Wu Z., Zhang H., Wang C., Zhou Y. and Zuo Z. Biol Trace Elem Res. 2014;160(3):437-444	Study conducted in Xiamen (China). The correlation between metals in umbilical cord blood and adverse pregnancy outcomes (identified in hospital records) in China was studied. There were no significant findings related to Ni and adverse pregnancy outcomes. .	The adverse pregnancy group (n=73) had slightly lower median Ni levels in cord blood than the control group (n=106). Median values: 11.6 µg Ni/l (n=58) & 12.7 µg Ni/l (n=68), respectively.	**Medium**. Levels of Ni in cord blood were measured.

No	Studies	Findings	Critique	Relevancy
7	Associations between blood metals and fecundity among women residing in New York State. Bloom M. S., Louis G. M., Sundaram R., Kostyniak P. J. and Jain J. Reprod Toxicol. 2011;31(2):158-63	80 women studied prospectively in New York (USA). Fecundity (assessed through the use of diaries and pregnancy tests) was not associated with blood Ni levels	Nickel levels in blood were measured (6.8-6.9 µg Ni/l). A time to pregnancy study is highly relevant to reproductive outcomes.	**Medium**. Levels of Ni in blood were measured.
8	Heavy metals (Pb, Cd, Ni) concentration in the hair of mothers of preterm and small for gestational age (SGA) infants. Peitrzyk J. J., Nowak A., Mitkowska Z., Petko M., Zachwiejowa Z., Chelopicka J., Kroasniak M., Glianska A., Strzelecki T., Dobosz P. and *et al.* Pediatr Res. 1994;36(1 Pt 2).	Case-control study in Poland (104 preterm cases and 74 SGA cases). No significant correlation was found for Ni in hair of mothers and cases of small for gestational age infants.	Reported as Abstract (few details). Personal exposure of mothers measured in hair. Values not reported	**Low**. Lack of detailed information.
9	Urinary nickel concentrations and selected pregnancy outcomes in delivering women and their newborns among arctic populations of Norway and Russia. Odland J. O., Nieboer E., Romanova N., Thomassen Y., Norseth T. and Lund E. J Environ Monit. 1999;1(2):153-61.	A prospective cross-sectional study of delivering women and their outcomes in Murmansk and Arkhangelsk Counties (Russia) and Finnmark and Bergen (Norway). Study included 50 mother-infant pairs. Birth weight information identified in hospital records. Maternal urinary Ni had no impact on birth weight.	Urinary Ni levels were higher in Russian communities than in Norway, independent of nickel refinery employment. Questionnaire used was translated; there is also potential recall bias during interviews.	**Medium**. Levels of Ni in urine were measured. Small study size.
10	Elements in placenta and pregnancy outcome in arctic and subarctic areas. Odland J. O., Nieboer E., Romanova N. and Thomassen Y. Int J Circumpolar Health. 2004;63(2):169-87	Additional analyses for the study described above. No relationships between placenta, maternal blood, and cord blood levels of Ni and birth weights of newborns was found.	Relationships between placenta, maternal blood, and cord blood levels of various metals were found but not for Ni. Maternal body mass index, maternal age, placental lead, or maternal blood lead, and smoking were found to be predictors of birth weight	**Medium**. Biological levels of Ni were measured. Small study size.

No	Studies	Findings	Critique	Relevancy
Workers' Studies				
11	Semen quality of Indian welders occupationally exposed to nickel and chromium. Danadevi K., Rozati R., Reddy P. P. and Grover P. Reprod Toxicol. (Elmsford, NY). 2003;17(4):451-6.	Study conducted in a welding plant in south India. Welding material was not described. No relationship between Ni blood levels and sperm count was found. An association between blood Ni levels in 28 exposed welders and sperm tail defects, motility changes, and vitality was reported. A nickel association with sperm tail defects was also detected among 27 controls. The same effects were also associated with increased Cr levels (both Ni and Cr levels were correlated).	Blood Ni levels in exposed welders (123.3 µg/l) and among controls (16.7 µg/l). *Something seems amiss since control blood levels of Cr and Ni are usually much lower (e.g., 1.1 µg Ni/l, 92 µg Cr/l in Iarmarcovai et al., 2005).* Interestingly tail defects was the only sperm parameter that was not different between controls and welder groups, even when Ni levels differ by > 7-fold.	**Medium**. Exposure levels are unusual. Other possible confounders exposures (*e.g.*, Pb, heat) were not reported, and the levels of Ni and Cr were correlated.
12	Stainless steel welding and semen quality. Jelnes J. E. and Knudsen L. E. Reprod Toxicol. 1988;2(3-4):213-5.	Study conducted in a welding plant in Denmark. 145 study participants. The sperm quality (concentration, viability, motility, abnormalities) of stainless steel welders (n=77) or manual metal arc welders (n=20) was not different from those of controls within and outside the plant.	Blood and urinary Ni levels were measured but the values were not reported in this manuscript. In a follow up study (Knudsen et al 1992), air, serum & urine levels of Ni are reported for welders. Ni serum levels were not elevated among welders even though their air exposures to Ni were 10-fold higher than for unexposed controls	**Low.** Lack of information on biological exposure levels.
13	Congenital defects, abortion and other health effects in nickel refinery workers. Chashschin V. P., Artunina G. P. and Norseth T. Sci Total Environ. 1994;148(2-vak):287-91.	The study of ~800 males and ~800 females at a Russian nickel refinery reported adverse reproductive outcomes like spontaneous abortions and malformations. This was the first human study to report a possible association between Ni and reproductive outcomes.	The results are not clear from the tables in the manuscript. The journal editor included a disclaimer about this study: "*Although the results are incompletely documented and thus must be considered inconclusive they identify a concern that requires a more comprehensive and quantitative epidemiologic investigation*"	**Low**. The "*comprehensive and quantitative epidemiologic investigation"* mentioned by the journal editor was carried out in the 1998-2005 time period with the results from these studies reported in several manuscripts (#14 to #20 shaded) below.

No	Studies	Findings	Critique	Relevancy
14	Genital malformations in newborns of female nickel-refinery workers. Vaktskjold A., Talykova L. V., Chashchin V. P., Nieboer E., Thomassen Y. and Odland J. O. Scan J Work, Environ Health. 2006;32(1):41-50	Register-based, nested case-control study (n= 103 cases; 23,038 controls). Each record was assigned a categorical nickel exposure rating according to the occupation of the delivering woman (at the time of becoming pregnant), using, as guidelines, the water-soluble inhalable exposures or the urinary nickel concentrations No negative effect of maternal exposure to water-soluble nickel on the risk of delivering a newborn with malformations of the genital organs was found.	Ni exposure during pregnancy estimated based on personal air samples or urinary levels. Urinary levels: Background 5.9 µg/l; Low up to 70 µg/l; high up to 179 µg/l	**High**. Large group of Ni-exposed workers with 10- to 30-fold higher internal exposure levels than background.
15	Small-for-gestational-age newborns of female refinery workers exposed to nickel. Vaktskjold A., Talykova L. V., Chashchin V. P., Odland J. O. and Nieboer E. Int Occ Med Environ Health. 2007;20(4):327-38.	Register-based, nested case-control study (n= 2,096 cases; 20,740 controls). Each record was assigned a categorical nickel exposure rating according to the occupation of the delivering woman (at the time of becoming pregnant), using, as guidelines, the water-soluble inhalable exposures or the urinary nickel concentrations. Study found no adverse effect of maternal occupational exposure to water-soluble Ni in the first part of pregnancy on the risk of delivering a small-for-gestational-age newborn	Ni exposure during pregnancy estimated based on personal air samples or urinary levels. Urinary levels: Background 5.9 µg/l; Low up to 70 µg/l urine (air 160 µg Ni/m^3); high up to 179 µg/l urine Low Ni exposure: 146 SGA cases/1672 births High Ni exposure: 75 SGA cases/1242 births	**High**. Large group of Ni-exposed workers with 10- to 30-fold higher urinary levels than non-occupationally exposed and >1000 higher air levels than ambient air.
16	Spontaneous abortions among nickel-exposed female refinery workers. Vaktskjold A., Talykova L. V., Chashchin V. P., Odland J. O. and Nieboer E. Int J Environ Health Res. 2008;18(2):99-115.	A case-control study (n=184 cases, 1,691 controls). There was no statistical association between maternal occupational exposure to water-soluble Ni in early pregnancy and the risk of self-reported spontaneous abortions.	Ni exposure during pregnancy estimated based on personal air samples or urinary levels. Urinary levels: Background 5.9 µg/l; Low up to 70 µg/l; high up to 179 µg/l	**High**. Large group of Ni-exposed workers with 10- to 30-fold higher internal exposure levels than background.

No	Studies	Findings	Critique	Relevancy
17	Maternal nickel exposure and congenital musculoskeletal defects. Vaktskjold A., Arild V., Talykova L. V., Ljudmila V. T., Chashchin V. P., Valerij P. C., Odland J. O., Jon O. O., Nieboer E. and Evert N. Am J Ind Med. 2008;51(11):825-33.	Register-based, nested case-control study (n=341 cases, 22,624 controls). Each record was assigned a categorical nickel exposure rating according to the occupation of the delivering woman (at the time of becoming pregnant), using, as guidelines, the water-soluble inhalable exposures or the urinary nickel concentrations. The incidence of defects in the musculoskeletal system at birth was high, especially for feet deformities, but no effect of maternal exposure to water-soluble Ni was found.	Ni exposure during pregnancy estimated based on personal air samples or urinary levels. Urinary levels: Background 5.9 µg/l; Low up to 70 µg/l; high up to 179 µg/l	**High**. Large group of Ni-exposed workers with 10- to 30-fold higher internal exposure levels than background
18	[Influence of nonindustrial risk factors on congenital abnormalities formation]. Talykova L. V. Med Tr Prom Ekol. 2009;5:35-8.	Reports on results from epidemiologic study of women in Russian refinery. No evidence that nickel compounds influence the reproductive health in females engaged in nickel purification in Murmansk refinery; nor did it reveal increased risk of abnormalities.	Article in Russian by one of the authors of above cited papers	**Low**. From English abstract, study appears to be a review of results from studies described in entries above.

Two additional studies that are not epidemiological studies but are relevant to the Monchegorsk epidemiological studies (# 13-17) are summarized below.

No	Studies	Findings	Critique
19	The Kola Birth Registry and perinatal mortality in Monchegorsk, Russia. Vaktskjold A.,, Talykova L., Chashchin, V., Nieboer E. and Odland J. O. Acta Obstet. Gynecol. Scand. 2004, 83: 58-69 *Not in DARTIC*	Describes the setting of a population-based birth registry (> 21,000 births covering 96% of all births) for the town of Monchegorsk. The quality of the registry is assessed and the perinatal mortality rates in the period 1973-97 are estimated. The perinatal mortality in Monchegorsk was lower than the overall rate in Russia.	This registry is needed to properly assess if reproductive risks in female workers are associated with Ni exposures

No	Studies	Findings	Critique
20	The prevalence of selected pregnancy outcome risk factors in the life-style and medical history of the delivering population in north-western Russia. Vaktskjold A., Paulsen E. E., Talykova L., Nieboer E. and Odland J. O. Int J Circumpolar Health. 2004;63(1):39-60.	Describes the health status of the delivering population in Monchegorsk based on the birth registry. Compared to women in Norway, the women in Monchegorsk were younger, had lower obesity and smoking but higher history of abortion and pelvic inflammatory disease	This registry is needed to properly assess if reproductive risks in female workers are associated with Ni exposures. Of the delivering women, 9,000 were employed at the nickel company

Of the three studies (#1, #2 and #5) identified by OEHHA as being good analytical studies that fulfill the criterion for prioritization of nickel, two of them (#1, #2) do not have measurements of internal exposures to nickel ion. About 98% of nickel ion in blood levels (and consequently nickel levels in reproductive organs) comes from nickel in the diet (food and water), with 1% coming from inhaled ambient air when exposures to PM_{10} nickel are ≤ 20 ng/m^3 (De Brouwere *et al.*,2012). Thus, the lack of assessment of internal exposure makes the attribution of a causal association between changes of 2-6 ng Ni/m^3 in $PM_{2.5}$ (which are not expected to affect blood nickel levels at all) and increases in low birth weight suspect, even if correlations are statistically significant.

Furthermore, if the association between increases of 2-6 ng Ni/m^3 in air and 6-11% increases in low birth weight was causal, statistical increases in low birth weights should have been observed in study #15 where women had >1000-fold higher air exposures to nickel compounds and where their urinary levels reflecting systemic exposures (all sources including workplace nickel in air) were 10- to 30-fold higher than background levels, but no such effect was seen. In short, we do not believe that studies #1 and #2 provide sufficient evidence to justify moving nickel metal or nickel compounds through the epidemiologic screen.

Study #5 included measurements of nickel ion in placenta and reported a link between higher nickel levels and lower gestational age; no statistically significant effects of nickel exposure on birth weight were noted in this study. Interestingly, low birth weights which were not a nickel exposure-related outcome in the most highly exposed worker population were also not observed in the definitive rat reproductive studies with nickel (SLI, 2000).

The epidemiological reproductive studies of female workers at a nickel refinery in Monchegorsk, Kola Peninsula (Russia), merit detailed discussion. As a direct consequence of very preliminary observations reported by Chashschin *et al.* (1994) (study #13 above), a retrospective series of analyses of reproductive outcomes among employees at a nickel refinery started in 1995 and culminated in the publication of several manuscripts describing the results from these studies (studies #14-#20). These studies were co-funded by the Monchegorsk Municipal Environmental Fund, the Norwegian Ministry of Health through the Barents Health Co-operation Program, the Nickel Producers Environmental Research Association (NiPERA), the Ontario Workplace Safety and Insurance Board (WSIB), and the Norwegian Research Council. A feasibility study conducted during 1995 and 1996 concluded that based on medical and company occupational records a full epidemiological study of reproductive outcomes among nickel refinery workers at the Monchegorsk nickel refinery complex was feasible (Odland *et al.*, 1999). This cohort

afforded the unique opportunity of studying a group of female workers exposed to relatively high levels of water soluble nickel salts and other nickel compounds; female workers in similar refining operations do not occur in North America and Europe (*i.e.*, many of the technologies used at the Monchegorsk facility were outdated). While workplace exposures included all chemical forms of nickel (and other metals such as Cu and Co), the soluble forms of nickel predominated in many of the departments. This allowed for high systemic absorption of nickel from the air and a higher systemic exposure of the female reproductive organs to nickel.

Occupational exposure assessments were conducted within the nickel refinery using personal samplers and biomonitoring data. The occupational hygiene component of the work was reported in five publications (Thomassen *et al.*, 1999; Höflich *et al.*, 2000; Weinbruch *et al.*, 2002; Koch *et al.*, 2002; Thomassen *et al.*, 2004). In January 1997, a birth registry was set up in Monchegorsk that included all deliveries (over 24,500) covering the period March 1973 to December 2001 (Vaktskjold *et al.*, 2004, study #19). A worker questionnaire was administered to current workers in the Monchegorsk nickel electrorefinery and to control women. The purpose of the worker questionnaire was to obtain personal data and information about occupational, reproductive and gynecological histories, as well as about lifestyle issues. The worker questionnaire was compatible with the birth registry and was linked to it. A careful assessment of data entry errors and coverage completeness was conducted for the period March 1973 to December 1997, followed by an examination of pregnancy risk factors in the life-style and medical history of the delivering Monchegorsk population (Vaktskjold *et al.*, 2004, study #20). In January 2001, the birth registry was expanded to include deliveries for the period 1998-2001, inclusive.

By 2005 the data elements necessary to conduct the actual assessments of reproductive impairment were in place. A. Vaktskjold, one of the original study researchers, conducted additional epidemiologic investigations using the pregnancy outcome and exposure databases that had been established. A birth-registry-based case-control study was carried out in order to determine whether pregnant women employed in the nickel refinery were at elevated risk of delivering a newborn with the sentinel genital malformation hypospadias (Vaktskjold *et al.*, 2006, study #14). No effect of maternal exposure to water-soluble nickel was observed for this outcome. In this paper, a categorical exposure matrix was employed based on the exposure assessments conducted.

In 2007 and 2008 Vaktskjold *et al.* published three additional manuscripts on the results of their further examination of reproductive impairment in nickel workers at Monchegorsk. These manuscripts examined spontaneous abortions, small-for-gestational-age newborns, and musculosketal effects in newborns of female refinery workers exposed to nickel (Vaktskjold *et al.*, 2007 study #16; Vaktskjold *et al.*, 2008a study #15; Vaktskjold *et al.*, 2008b study #17). The authors found no correlation between nickel exposure (Thomassen *et al.*, 1999) and the observed reproductive impairments such as spontaneous abortions (spontaneous abortions and stillbirths are the human reproductive outcomes most closely related to effects observed in animal reproductive studies), genital malformations (sensitive endpoints for malformations), small for gestational age, and musculoskeletal defects.

3.2 Review of definitive animal reproductive studies

OEHHA reports finding thirty-five animal studies of nickel reporting reproductive or developmental toxicity associated with nickel exposure, as well as five studies reporting no reproductive or developmental toxicity. Two additional reproductive developmental study in rats with nickel sulfate hexahydrate (gavage) funded by NiPERA and conducted at Springborn Laboratories are summarized below.

TABLE 2. ADDITIONAL RAT REPRODUCTIVE STUDIES NOT LISTED BY OEHHA

	NOAEL mg Ni/kg b.w. day	LOAEL mg Ni/kg b.w. day	Results	Comments
1-generation Oral (gavage) Rat (Sprague Dawley) 8M + 8F/group Ni sulphate hexahydrate 0, 10, 20, 30, 50 and 75 mg/kg b.w. per day = 0, 2.2, 4.4, 6.6, 11 and 17 mg Ni/kg b.w. per day	Parental and reproductive toxicity: 17 Offspring toxicity: 4.4	Parental and reproductive toxicity: - Offspring toxicity: 2.2	Parents: no effect on F0 survival, growth, mating behavior, copulation, fertility, precoital intervals, gestation lengths or gross necropsy findings. Increase mean post-implantation loss at ≥ 30 mg/kg b.w. per day Pups: increase incidence of dead pups on LD 0 and decrease mean live litter size at HD (and lower mean live litter size than historical controls at ≥ 30 mg/kg b.w. per day). No effect on growth of surviving F1 pups during lactation, no effect on survival or growth of F1 pups from post-natal day 22 for several weeks following weaning	Klimisch: 2 Equivalent to OECD TG415. GLP study. Meets generally accepted scientific standards with acceptable restrictions
2-generation Oral (gavage) Rat (Sprague Dawley) F0 & F1: 28 rats/sex/group Ni sulfate hexahydrate 0, 1.0, 2.5, 5.0 and 10.0 mg/kg b.w. per day = 0, 0.2, 0.6, 1.1 and 2.2 mg Ni/kg b.w. per day	Parental and reproductive toxicity: 1.1	Parental, reproductive toxicity: - Offspring toxicity: 2.2	Parents: No effect on F0 or F1 survival, growth, mating behavior, fertility, gestation, parturition or lactation. No treatment- related mortality or clinical signs of toxicity in F0 or F1 rats. No effect on estrous cycling, sperm parameters, copulation and fertility indices, precoital intervals, gestation lengths, gross necropsy findings or onset of sexual maturation in F1 rats. Pups: no effect on F1 or F2 pup viability and growth.	Klimisch: 2 Equivalent to OECD TG416. GLP study. Meets generally accepted scientific standards with acceptable restrictions. HDL did not result in toxicity of the parental animals

These studies are consistent with previous findings of perinatal mortality associated with high oral exposure to soluble nickel compounds and identified a NOAEL of 1.1 mg Ni/kg b.w. per day. The SLI (2000) studies have been selected by several government agencies (including OEHHA in the setting of an oral nickel REL in 2010) as the definitive studies for the setting of regulatory standards. These studies, as previous guideline studies, have not shown nickel to cause malformations. The effects of nickel on fertility (even if possible at higher exposure

levels), were not shown to occur at the levels tested in this study, including doses of water soluble compounds resulting in urine levels of ~2400 µg Ni/L (males + females) (Rush *et al.*, 2005).

OEHHA's synopses of these studies is as follows:

> "NiPERA (2000a) sponsored a one-generation reproduction study in Sprague-Dawley rats with nickel sulfate hexahydrate. Eight animals per sex/dose group were administered 0, 10, 20, 30, 50 or 75 mg/kg-d by daily aqueous gavage to the F0 parental animals and selected F1 offspring. Dosing of the F0 animals began two weeks prior to mating and dosing of F1 offspring began on PND 22. All doses were given at constant volume of 10 mL/kg. Both F0 and F1 animals were examined for indications of toxicity. Experimental endpoints for F0 animals included clinical observations, body weights, food and water consumption, mating, parturition, lactation and offspring growth and viability. Experimental endpoints for selected F1 animals included survival, clinical observations and body weight during the F1 dosing phase. All F0 and F1 animals were subjected to gross necropsy examination at time of death or terminal sacrifice. For the F0 animals post-implantation loss (implantation scar count minus the number of live pups on Day 0) was significantly increased at the 30, 50, and 75 mg/kg-d dose levels and increased at the 10 and 20 mg/kg-d levels (mean loss values: 0.4 control; 2.6; 1.5; 2.3($P<0.05$); 2.7 ($P<0.01$); 4.8($P<0.01$)). For F1, pup viability was significantly decreased at all dose levels except 50 mg/kg-d compared to the control (dead/live: 1/128 control; 12/100; 10/106; 10/92; 4/89; 23/80 all $P < 0.01$ except 50 mg/kg-d). For this study a LOAEL of 10 mg/kg-d equivalent to 2.1 mg Ni/kg-d was identified.
>
> NiPERA (2000b) sponsored a two-generation reproduction study in Sprague-Dawley rats with nickel sulfate hexahydrate. Twenty-eight animals per sex per group were administered 0, 0.22, 0.56, 1.12, or 2.23 mg Ni/kg-d by aqueous gavage. The animals were exposed from ten weeks prior to mating for F0, through gestation, and until PND 21 (13 weeks to delivery of F1 offspring). Exposure for F1 was from *in utero*, during lactation, through development from PND 22 to about PND 92 (a minimum of 70 days of treatment). In contrast to the one-generation study the F0 animals showed no statistically significant effects of nickel administration on implantation and post-implantation losses. Statistically significant differences in F0 organ weights consisted of decreased absolute and relative liver weights in the high dose males, decreased absolute brain weight in the mid dose females, and increased relative liver weight in 0.56, 1.12 and 2.23 mg/kg-d group females. The investigators did not consider these organ weight effects to be toxicologically meaningful. The percent of dead pups/total in the respective dose groups were: 2.2 (control); 3.7; 2.2; 2.1; 4.2 ($P = 0.105$ vs. control by one-sided Fisher exact test). For this study a NOAEL of 2.23 mg Ni/kg-d was identified by the authors."

3.3 Comparison of animal and human studies: exposure levels and reproductive outcomes

As indicated above, there are multiple rat studies with soluble nickel compounds (including the NiPERA-sponsored reproductive study, SLI, 2000a,b) showing increased perinatal mortality at daily oral exposures above 1.1 mg Ni/kg b.w. (administered by gavage). The mode of action is not currently known. Studies of female workers exposed to high levels of water soluble nickel compounds via inhalation failed to show an association between exposures to nickel and observed adverse reproductive effects (see studies #14-17 in Table 1).

To place the animal and human results in context, it is of interest to compare the urine nickel levels in the workers' cohort with the urine levels in rat reproductive studies. Background urinary nickel levels in female Monchegorsk population had a geometric mean of 5.9 µg/l, the low exposure refinery workers had urinary levels up to 70 µg/l (~12-fold increase in urinary levels) and the high exposure workers had urinary levels between 70 and 179 µg /l (30-fold increase in

urinary levels). Urinary levels of 70 µg/l in low exposure workers corresponded to approximately 160 µg soluble nickel inhalable exposure/m^3 (>1000-fold over ambient air levels). How comparable were the exposures in female workers to those of the rats in the reproductive study? Comparisons can be done by examining urinary nickel levels.

In a rat oral chronic study with nickel sulfate (100% bioaccessible nickel), rats were exposed to 2.2 to 10 mg Ni/kg and blood and urinary nickel levels were measured after a two-year exposure (Heim *et al.*, 2007; Rush, 2005). A linear dose-response between oral intake of nickel and urinary nickel levels was found. At the lowest exposure of 2.2 mg Ni/kg (the NOAEL for chronic oral toxicity), the mean urine value was 2300 µg Ni/L (males + females). Blood peak levels of ~70 µg/l were measured. The exposure level of 2.2 mg Ni/kg corresponds to the LOAEL for perinatal mortality effects in the definitive rat study (SLI, 2000). The rat urinary nickel level corresponding to the LOAEL for reproductive effects is 33-fold and 13-fold higher than those measured in Low and High exposure nickel refinery workers, respectively.

In conclusion, the reproductive effects observed in rats 1) may not be relevant to humans or 2) may not have been observed in the exposed human population because even the highest achievable human exposures are lower than those achieved in rats. In either case, the relevance of the positive results in rats (at unachievable human exposures) needs to be considered together with the negative results in human studies at Monchegorsk (for the highest exposed human population), realizing that human exposures to nickel in California (where no nickel refinery exists) are likely to be much lower than those in the workers studied at Monchegorsk.

4. SUMMARY AND CONCLUSIONS

People living in California are exposed to nickel compounds through air, soil, and diet. Dietary nickel is the main contributor to systemic exposure. Exposures to nickel occur in the form of soluble nickel compounds (water, air), complex oxides (air, soil), complex organic molecules (vegetables, food). Excluding occupational settings, exposure to metallic nickel (CAS# 7440-02-0) via inhalation or the oral route is negligible or non-existent. Similarly, even though there is dermal exposure to metallic nickel in consumer items in contact with the skin, dermal absorption is very low and the contribution of this exposure route to systemic blood nickel levels is undetectable.

The animal toxicity data from one- and two-generation rat reproductive studies indicate the possibility of increased perinatal mortality at exposures > 1.1 mg of 100% bioaccessible Ni/kg b.w. [*this corresponds to a human intake of ~70 mg Ni/day fully bioaccessible Ni, compared to a dietary amount of 0.1-0.3 mg/day of less bioaccessible Ni*]. The definitive two-generation reproductive study did <u>not</u> detect malformations or fertility effects at exposure levels ≤2.2 mg (100% bioaccessible) Ni/kg b.w.). Epidemiological studies (case-control) of the most highly exposed cohort of women (refinery workers in Monchegorsk, Russia) did not show significant associations between inhalation exposures to the most bioavailable of the nickel forms (evaluated by absorbed and excreted urinary nickel levels) and the sentinel endpoints of spontaneous abortions, genital or muscle-skeletal malformations, and small for gestational age. Even the most highly exposed worker population could not reach the high urinary levels (>10-fold higher) found in the rat studies.

In conclusion, the evidence indicates that the reproductive effects observed in rats either are not relevant to humans or, if they are relevant, they will not be seen in humans because even the highest achievable levels of systemic nickel ion exposure in humans are far lower than those achieved in rats. Furthermore, the results in rats (at internal exposure levels unachievable in humans) need to be considered in light of the negative results in human studies at Monchegorsk (involving the highest exposed human population and the most bioavailable form of nickel). The negative results at Monchegorsk are particularly telling for present purposes, since human exposures to nickel in California (where no nickel refinery exists) will be much lower than the nickel exposures of the workers studied at Monchegorsk.

The current proposal to prioritize nickel for possible consideration as a reproductive or developmental toxicant under Proposition 65 was based on the new epidemiological studies that have become available since 2007. Among these studies, the case-control investigations of workers at the Monchegorsk nickel refinery stand out due to the high external and internal nickel exposures of the workers (many fold higher than any ambient air study) and the studies' ability to detect potential causal associations between nickel exposures and reproductive outcomes. Yet, these studies do not support a causal association between exposure to nickel and adverse reproductive outcomes. In summary, the totality of the current epidemiological data does not justify prioritizing nickel for the development of reproductive hazard identification materials.

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