NATIONAL TOXICOLOGY PROGRAM PROPOSAL TO LIST NICKEL NANOPARTICLES IN FUTURE EDITIONS OF THE ROC

Comments of the Nickel Producers
Environmental Research Association

October 17, 2013

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NiPERA INC.

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COMMENTS ON THE NTP PROPOSAL TO LIST NICKEL NANOPARTICLES IN FUTURE EDITIONS OF THE RoC

1.   EXECUTIVE SUMMARY

The Nickel Producers Environmental Research Association (NiPERA) is pleased to submit these Comments to the NTP regarding the nomination for possible review and listing of nickel nanoparticles in a future edition of the Report on Carcinogens (RoC). Please note that NiPERA is responding on behalf of the global nickel mining and refining companies as well as bulk nickel producers. Although NiPERA does not represent industries involved in the downstream production of nickel nanoparticles, we appreciate the opportunity to submit comments.

The nomination to review nickel nanoparticles as a potential cancer hazard for people in the United States does not appear to be justified based on the following:

- Nickel compounds are already listed in the RoC as "known human carcinogens" (dating from the 9th RoC in 2000), and that listing applies to all forms of nickel compounds (nanoparticles included).

- Nickel metal is already listed in the RoC as "reasonably anticipated to be a human carcinogen" (dating from the 10th RoC in 2002), and that listing applies to all forms of nickel metal (nanoparticles included).

- Based on the weight of evidence of current toxicological data and using proper dose metrics, the lung toxicity and carcinogenicity hazards of nanoparticles (including nickel nanoparticles) do not appear to be different from those of micron-sized particles of the same materials. With the publication of a negative carcinogenicity study of nickel metal powder in 2008 and the lack of positive human epidemiology data, the evidence for nickel metal being a carcinogen is weaker now than it was in 2002, with stronger evidence that it is not a carcinogen. There is at present no basis for distinguishing between nickel micron-sized particles and nickel nanoparticles in this regard.

- Information obtained from several sources indicate that nickel nanomaterials do not have a high production volume in the United States (<100 tons/year) and the number of people with the potential for significant inhalation, oral or dermal exposure to nickel nanomaterials in the U.S. is very low (~300 people).

- Altogether, there does not appear to be any demonstrable justification (based on exposure or hazard information) to consider a separate listing for nickel nanoparticles in NTP's RoC.

> *Recommendation: Nickel nanoparticles appear to present the same hazard as micron-sized nickel particles. Since nanomaterials of nickel compounds and nickel metal are already covered in the 9th and 10th RoC, respectively, there is no need for a separate listing.*

NiPERA's comments include information in support of our recommendation as per NTP's formal Request for Information.

2. DATA ON CURRENT PRODUCTION, USE PATTERNS, AND HUMAN EXPOSURE

2.1 Production volume of nickel nanoparticles in the United States

Although precise volumes of nickel nanoparticles are not known, estimates can be made from available data of multiple sources. Nanoparticles, in general, are manufactured worldwide for use in several consumer product categories, such as health and fitness, home and garden, automobile, food and beverage, cross cutting (coatings), electronics and computers, and appliances. According to the Project on Emerging Nanotechnologies online inventory (2013) of nanotechnology-based consumer products on the market, approximately 45% (741 out of 1628 products) of those nanotechnology-containing products are made in the U.S., with Europe (27%) and East Asia (17%) producing most of the remaining products. The most common nanocomponents referenced in this online inventory are silver, carbon, titanium, silicon/silica, zinc, and gold nanomaterials. Nickel was not specifically mentioned as one of the primary nanomaterials used in these products. A separate database, Nanowerk Nanomaterial Database (2013), reports 32 suppliers of nickel nanomaterials worldwide, with only nine of these suppliers (28%) located in the U.S. However, some additional companies that produce nickel nanoparticles did not provide information for the database and thus were not represented.

Approximately 10 to 20 companies produce nickel nanoparticles in the U.S.[1] However, a few additional companies were reported to produce nickel nanoparticle in surveys from 2008 and 2009 that are no longer producing nickel nanoparticles. The annual nickel nanoparticle production volume of some of these companies is only in the gram to kilogram range each, while a few other companies may produce ~1 ton per year, as confirmed by several experts in the field. For example, one U.S. company that produces nickel metal nanoparticles, QuantumSphere, indicates in its website[2] that it has 8 reactors that can generate up to a combined total of ~6 tons of nanoparticles of iron, silver, copper, nickel and manganese per year. Although the specific volume of nickel metal nanoparticles is not listed, it could be predicted to be no greater than ~1 ton a year for QuantumSphere. Based on this information, a high estimate of the total U.S. production of nickel nanoparticles is ~20 tons per year, assuming each company produced the high limit of 1 ton annually, although this is unlikely since several companies only produce nickel nanoparticles in kilogram quantities.

An inference of U.S. nanonickel production can be made from recent REACH registrations in the European Union. The REACH regulatory initiative requires registration of all chemicals manufactured in or imported to the European Union. As of May 2013, all chemicals placed on

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[1] NiPERA does not represent downstream producers of nickel nanoparticles. However, we are in the process of developing a list of the U.S. producers as a result of the NTP nomination for possible review and listing of nickel nanoparticles in the RoC. This information can be made available upon request.

[2] QuantumSphere website: http://www.qsinano.com/products_nanomaterials.html

the European Union market had to be registered if manufactured or imported at more than 100 tons/year by a manufacturer(s) or importer(s). As of October 2013, there were 23 registrations that covered nanomaterials or nanoforms of substances. Of those 23 registrations, no nickel nanomaterials were listed, indicating that any nickel nanomaterials produced in Europe would be less than 100 tons/year.[3] The registration information from REACH is not only evidence of low production volumes of nickel nanoparticles in Europe (<100 tons/year), it also suggests low volumes of imported nickel nanoparticles coming from the U.S. (<100 tons/year).

Data is scarce with regard to the production volume of nickel nanoparticles and specific producers in the U.S. However, as mentioned above, nickel nanoparticles are not as frequently produced as other types of nanoparticles. The information regarding producers of nickel nanoparticles in the U.S. and information gathered from REACH registrations indicate a rough estimate of 1 to 100 tons/year of total nickel nanoparticles produced in the U.S.[4]

2.2   Use patterns

Nickel nanoparticles have many commercial and industrial uses. Several worldwide nickel nanoparticle producers[5] report the use of these small particles in:

- Additives in ceramics, lubricants, and sintering
- Alloys
- Batteries
- Capacitor materials
- Catalysis reactions
- Ceramic and diamond tool production
- Electrical conductors / Conductive paste
- Fuel cell applications
- Fuel combustion
- Magnetic materials
- Metallic conductive coatings
- Pigmentations
- Uranium purification

The specific uses for nickel nanoparticles suggest that occupational exposure (via inhalation and dermal routes) is of primary concern. The various tasks involved with the production and handling of these nanoparticles needs to be considered with regard to the exposure of workers

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[3] Multi walled carbon nanotubes (MWCNTs), some of which may contain small amounts of nickel impurities, were registered with a production volume of 100-1000 tons/year (much less in terms of nickel).
[4] Carbon nanotubes (CNTs) are estimated to have a U.S production of ~75 tons/year (with ~278 tons/year worldwide) but there was no information on the proportion of CNTs that are catalyzed by nickel versus those catalyzed by other chemicals (Eklund *et al.*, 2007).
[5] The uses identified in the text were obtained from information provided from the websites of several producers of nickel nanoparticles: SkySpring Nanomaterials, Sun Innovations, NanoMaterial, Applied Nanotech Inc., QuantumSphere, MTI Corporation, US Research Nanomaterials Inc., CVMR Powders, NANONI, and EPRUI Nanoparticles and Microspheres Co.

in manufacturing and in research and development laboratories. The general public is typically not exposed to nickel nanoparticles through the use of consumer products. Nanoparticles are generally tightly-bound or well-integrated into most end-products for consumer use (Biskos and Schmidt-Ott, 2012; Chaudhry *et al.*, 2009), thus greatly reducing the potential for inhalation or dermal exposure to nickel nanoparticles on the part of the general public.

2.3   Human exposure

For all nanomaterials, the routes of exposure of concern for carcinogenicity are inhalation, oral, and dermal.[6] Inhalation is the primary route of concern for carcinogenicity in workers exposed to nickel-containing materials, as only respiratory tract tumors have been consistently associated with exposure to nickel-containing compounds in animal (in studies via inhalation and oral routes) and epidemiological studies.

The dermal route of exposure is important when evaluating dermal sensitization for occupational exposure and consumer uses of nickel metal nanoparticles, nickel compound nanoparticles, and nickel-containing carbon nanotubes. Dermal exposure to sufficient amounts of nickel ions from nickel-releasing materials can trigger *de novo* dermal sensitization or elicit dermatitis in individuals who already are nickel-sensitized. For workers, there is also a possibility of oral exposure via the perioral region, but the absorbed oral dose of nickel will be low at these levels of exposure, and no toxicity is expected to occur.

Currently, data on the number of people exposed to nickel metal nanoparticles, nickel compound nanoparticles, and nickel-containing carbon nanotubes (CNTs) in the U.S. are not available. The commercial and industrial uses identified in Section 2.2 above indicate that workers in these industries would have the highest potential risk of inhalation and dermal exposure during the handling and processing of the raw materials. A rough estimate of U.S. workers exposed to nickel nanoparticles is ~300, with ~150 comprised of industrial workers and ~150 comprised of university workers.

The general public is expected to have limited exposure to nickel metal nanoparticles, nickel compound nanoparticles, and nickel-containing CNTs, especially when the nickel nanoparticles are contained in end-use products that are used by only a small subset of the general population. At most, their contact with these particles could be related to possible dermal exposures associated with handling batteries, possible physical contact with various types of surface coatings applied to consumer goods, and improper disposal methods. Even in those situations, the risk of general population exposure is expected to be minimal, as nickel nanocomponents will likely be tightly bound, no longer nano-sized, or otherwise wholly

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[6] Currently, no information is available to indicate that nickel nanoparticles are used in orthopedic or vascular devices. However, there has been some interest in the development of nickel nanoparticles for drug delivery via injection (Xu *et al.*, 2006; Klostergaard and Seeney, 2012). Biomedical use of nickel nanoparticle-containing drug delivery systems is experimental only and not approved by the FDA. However, if this application were to become FDA approved, it would only involve a small number of exposed people. Yet, because of the possibility of immunological reactions, this application should be very carefully monitored and it may be determined to be an inappropriate application for these materials.

integrated into most consumer end-use products, thereby reducing direct physical contact with the nickel nanoparticles themselves (Biskos and Schmidt-Ott, 2012; Chaudhry *et al.*, 2009; Meyer *et al.*, 2009).

In air, nanoparticles can exist as unbound, agglomerate, or aggregate particles. It is quite difficult for nanoparticles to remain in the unbound state, which is the most toxic form. Under normal environmental conditions, nanoparticles tend to agglomerate or aggregate, unless the nanoparticles are dispersed in specific media or have specific surface modifications designed to prevent such interactions (Card *et al*, 2008). Agglomerate and aggregate nanoparticles that have a total particle size over 100 nanometers will have different deposition fractions in the respiratory tract and most likely no longer exhibit nano-specific properties.

Nickel nanoparticles, primarily nickel metal and nickel oxide, can be used as catalysts in the formation of CNTs as well as coatings for CNTs (Chaudhry *et al.*, 2009). Although these specific uses in CNTs are listed above (*e.g.*, catalysis reactions and coatings) in Section 2.2, the issue of nickel nanocomponents in CNTs should be addressed briefly in light of recent publications that link nickel impurities to toxicity effects associated with CNTs in laboratory studies (*e.g.*, Morimoto *et al.*, 2013).

CNTs that could contain nickel nanoparticle impurities, as a result of their role as catalysts for the production of CNTs, are used often in textiles, plastics and electronics (*e.g.*, clothing, epoxy resins, batteries, electronic components, sporting equipment, airplane components, etc.). Overall, the general population's exposure to nickel nanoparticle impurities contained in end-use products is expected to be negligible because CNTs are typically tightly bound or embedded within these consumer products (Chaudhry *et al.*, 2009; Mueller and Nowack 2008). However, improper use and disposal methods (*e.g.*, incomplete incineration) as well as CNT-coated textiles are scenarios with potential dermal (and under some extreme circumstances inhalation) exposures. Research on CNTs is becoming increasingly popular in industrial and university settings, with workers in these research and development laboratories having a potential for inhalation and dermal exposure, especially during CNT synthesis. These workers represent a small subpopulation of the general public (*e.g.*, U.S. researchers in university and industrial research laboratories testing the safety or the applications of nickel-containing CNTs) and the exposure to nickel from these CNTs is likely to be very low (especially if workers utilize proper safety precautions), thus indicating that widespread exposure to CNTs containing nickel nanoparticles is limited (Chaudhry *et al.*, 2009).

In summary, manufacturing workers as well as industrial and university researchers have the highest potential risk of exposure to nickel nanoparticles. However, the number of exposed workers in the U.S. is unlikely to be higher than 300. Negligible or very low exposure (*e.g.*, CNT-coated textiles) is expected for the general population from consumer products.

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3. PUBLISHED STUDIES RELATED TO EVALUATING CARCINOGENICITY

3.1 Hazard comparison of nano- and micron-sized particles of the same materials

In recent years, significant efforts have been made to clarify the hazard profiles of nano- and micron-sized particulates, especially for pulmonary effects. At the same mass concentration, nano-sized particles have a greater surface area (SA) and particle number than micron-sized particles. The anatomy of the respiratory tract of animals and humans and their respective respiratory parameters together with the aerodynamic particle size distributions of the aerosols will determine in which part of the respiratory tract, and to what extent, the inhaled particles will be deposited (Geiser and Kreyling, 2010). The deposition of particles in the various regions of the respiratory tract is primarily controlled by physical mechanisms such as sedimentation, inertial impaction and diffusion. While the first two mechanisms are relevant for particles larger than 0.5 µm, the latter governs the lung deposition of nanoparticles (< 0.1 µm). Equal exposure levels (mass/volume) of nano- and micron-sized particles are expected to result in different deposited masses (as well as SA and number of particles) in various regions of the respiratory tract. However, it should be noted that nanoparticles may readily agglomerate, and thus then have a deposition pattern like micron-sized particles (Card et al., 2008). Furthermore, the clearance of nano- versus micron-sized particles will play a role in determining the ultimate retained dose. Differential uptake and bioavailability of the nano- and micron-sized particles in the target cells will influence the ultimate effective dose.

3.1.1 Biodurable substances

Oberdörster et al. (1994) performed a sub-chronic inhalation study with nano (20 nm) and fine (250 nm) anatase titanium dioxide (TiO_2) at similar mass concentrations (23.5 and 22.3 mg/m³ respectively). Lung inflammatory responses and changes in lung morphology were significantly more severe after exposure to nano TiO_2. Thus, the authors suggested that surface area (SA) and not mass or volumetric load was the more appropriate dose metric for nanoparticles in correlation with the examined endpoints. Along similar lines, Tran et al. (2000) analyzed the results from TiO_2, barium sulphate ($BaSO_4$), and carbon black inhalation studies (Cullen et al., 2000; Driscoll et al., 1996; and Oberdörster et al., 1994). The number of inflammatory cells in the lung in relation to the corresponding lung particle burdens expressed in terms of mass, SA, and particle numbers were compared. The surface-area burden was the most likely of the three measures to explain the difference in the numbers of inflammatory cells among the three different dusts. Stoeger et al. (2006) also found that pulmonary inflammation caused by six different carbonaceous nanoparticle types also correlated with SA over organic content and primary particle size.

Further comparisons were performed by Sager et al. (2008) and Sager and Castranova (2009) who examined the lung response of nano-sized versus micron-sized TiO_2 and carbon black after intratracheal instillation to rats. This administration technique bypasses deposition differences between nano- and micron-sized particles and thus delivers an equivalent dose of each to the deep lung. They compared the dose-response relationship of nano- and micron-sized material either on a mass-based or a surface-based dose metric. They observed that on a mass dose

basis, nano-sized particles gave a 30–100-fold higher pulmonary response than the micron-sized particles of the same composition. However, when the dose was normalized to SA, the difference of the same sets of parameters was about an order of magnitude lower (e.g., 3–10-fold, Sager *et al.,* 2008).

Analysis of rat inhalation carcinogenicity studies with nano- and micron-sized particles of the same biodurable materials (e.g., talc, toner, titanium dioxide, diesel emissions) concluded that differences in carcinogenic potency between the two forms of these materials was low (*e.g.,* 2–2.5-fold, using mass concentration as the dose-metric) (Gebel, 2012).

A number of studies over the past 15 years on biodurable particles lacking specific toxicity suggest that the smaller the particle (*e.g.* the greater the SA dose), the greater the induced pulmonary inflammatory response. Additionally, the studies indicate that inflammatory processes are responsible for the pathogenic lung responses to these particles, and that mechanistically no difference between nano- or micron-sized particles exists, as both follow the same mode of action. There is no evidence to indicate that particles below 100 nm show any kind of step-change in their hazard status and for the onset of any novel nano-specific hazard (Donaldson and Poland, 2012).

3.1.2   Nickel substances

When nano nickel oxide particles were compared to micron-sized nickel oxide particles, the authors concluded that in terms of SA, the toxicity of the materials was similar. Importantly, when doses are expressed in terms of SA, nano- and micron-sized particles fit the same dose-response curve and did not display different hazard profiles (Mizuguchi *et al*., 2013).

3.1.3   Summary of all substances

A higher biological activity of smaller particles is not necessarily to be expected and, notwithstanding their smaller size, nanoparticles are no more hazardous than conventional particles. Normal toxicological principles can therefore be applied equally, and conventional particle toxicology data are useful and relevant to the determination of nanoparticle hazard evaluation. The OECD determined, after a six-year review, that existing international and national chemical regulatory frameworks can adequately manage the risk of nanomaterials, and "that the approaches for the testing and assessment of traditional chemicals are in general appropriate for assessing the safety of nanomaterials, but may have to be adapted to the specificities of nanomaterials" regarding dosimetry (OECD, 2013).

3.2   Summary of toxicological data for nickel-containing nanoparticles

3.2.1   Possible toxicological effects of nickel-containing nanoparticles compared to micron-sized nickel particles

As discussed in Section 3.1, evidence is mounting that to compare the effects elicited by nanoforms of metal and metal compounds to those elicited by micron-sized particles, it is critical

to consider the most appropriate metrics. Comparison of inhalation effects based on mass may not be appropriate, as equal masses of nano- and micron-sized particles will differ greatly in the number of particles present, the SA of the particles, and their deposition in the respiratory tract. In turn, the SA of particles will influence their interactions with macrophages and other lung cells as well as their release of metal ions in the different biological fluids and the clearance of the particles.

Studies done with poorly soluble particles in nano- and micron-sized ranges demonstrate that when equal masses of nano- and micron-sized particles are compared, nano forms show higher toxicity after instillation or inhalation *in vivo*. However, when doses are expressed in terms of equal number of particles or equal SA, the results are comparable or differ only by a few-fold (see Section 3.1). These results strongly indicate that the hazard of the two forms does not change, only the potency may differ as articulated by Donaldson and Poland (2012). Likewise, an examination of rat inhalation carcinogenicity studies with nano- and micron-sized particles of the same biodurable materials (*e.g.*, talc, toner, titanium dioxide, diesel emissions) concluded that differences in carcinogenic potency between the two forms of these materials was low (*e.g.*, 2–2.5-fold, using mass concentration as the dose-metric) (Gebel, 2012).

The following discussion examines *in vivo* studies comparing lung toxicity effects (and carcinogenicity when available) of nickel-containing nanoparticles and micron-sized particles of the same substance administered by routes relevant to human exposure (instillation and inhalation). Also considered are a few studies with nickel metal nanomaterials conducted by non-relevant routes of exposure. Emphasis is given to evidence related to the carcinogenicity of nickel metal.

Nickel-containing nanomaterials tested

The most common form of nickel nanoparticles examined in inhalation-instillation studies are nickel oxides. Fewer studies looked at the toxicity of nanoforms of nickel metal, nickel hydroxide, and nickel sulfate. Another group of studies looked at the toxicity of carbon nanoparticles (*e.g.*, single or multiwall nanotubes, fullerenes) some of which, depending on the manufacturing process, may contain nickel metal or nickel oxide impurities (Appendix, Table 1).

Types of in vivo studies with nickel-containing nanomaterials

The majority of the *in vivo* studies examined the lung effects of exposure to nickel-containing nanomaterials via inhalation or instillation. Often the responses elicited by the two routes of exposure were compared and/or the responses between nano- and micron-sized nickel particles of the same substances were contrasted. Sometimes the *in vivo* responses were compared to *in vitro* results.[7] In other studies, lung deposition and clearance of nickel nanoparticles were studied. Many of these studies included other metal nanomaterials in an effort to identify signature responses or differences in toxic potency for various metals. Nickel oxide nanoparticles were by far the most studied of the nickel-containing nanomaterials. A

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[7] Note: Exclusive *in vitro* studies with nickel nanoparticles were not systematically reviewed.

summary of the inhalation studies with nickel-containing nanoparticles is provided in Appendix, Table 2. No inhalation studies with nickel metal nanoparticles were identified.

The inhalation studies with nickel oxide nanoparticles demonstrated higher toxicity compared to nanoparticles of TiO_2 or carbon fullerenes (C_{60}) at equal particle number (Morimoto *et al.*, 2011b, Fujita *et al.*, 2009; Ogami *et al.*, 2011). This is expected based on similar differences in toxicity observed in two year inhalation studies with micron-sized particles of nickel oxide (Dunnick *et al.*, 1995) compared to micron-sized particles of TiO_2 or carbon black (Lee *et al.*, 1985; Mauderly *et al.*, 1994).

In a series of inhalation toxicity studies with nickel oxide nanoparticles, a range of lung inflammation responses was observed after 4 weeks of repeated exposure to the same concentration of micron-sized nickel oxide particles of what appears to be the same or very similar material. (Oyabu *et al.*, 2007; Fujita *et al.*, 2009; Ogami *et al.*, 2011; Morimoto *et al.*, 2011b; Mizuguchi *et al.*, 2013). In general, the toxicity of nano nickel oxide in these studies was described as not severe and did not progress after exposure. When nano nickel oxide particles were compared to micron-sized nickel oxide particles (Mizuguchi *et al.*, 2013), the authors concluded that in terms of SA, the toxicity of the materials was similar. This is consistent with previous reports for low solubility and low toxicity particles indicating nano- and micron-sized particles have similar hazard profiles when comparing exposures in terms of particle number or SA (Section 3.1).

The studies conducted with nickel hydroxide nanoparticles demonstrated toxicity similar to that of nickel sulfate, and these results are consistent with the high solubility of this particular sample of nickel hydroxide. However, it is unclear if the high solubility of this sample is due to the process of synthesis of the hydroxide or to the particle size. No micron-sized particles of nickel hydroxide were tested in parallel. Nickel hydroxide samples can vary in composition [*e.g.*, Beta-NiO(OH) and $Ni_3O_2(OH)_4$, content of Ni(III) and Ni (VI), *etc.*] (Gmelin, 1966). Therefore, the present results do not allow us to conclude whether the effects of nickel hydroxide nanoparticles are any different from those of micron-sized particles of the same material.

A summary of the instillation or oropharyngeal aspiration (OPA) studies with nickel-containing nanomaterials is provided in Appendix, Table 3. As observed for inhalation, instillation studies with nickel oxide nanoparticles demonstrate higher toxicity compared to nanoparticles of the other metal oxides tested (see Appendix, Table 3) at equal SA doses. Considering both particle size/SA dose as well as surface characteristics, Duffin *et al.* (2007) tested the pulmonary toxicity of different particles indicated by polymorphonuclear leukocyte (PMN) content in bronchioalveolar lavage fluid (BALF) of rats after instillation. Low-toxicity biodurable materials, metal nanoparticles (metallic nickel and cobalt), and quartz as an example of a particle with a highly reactive surface were compared. Low-toxicity biodurable particles produced a greater inflammatory response as particle SA dose increased. Compared to these materials, quartz, at the same SA dose, induced a far greater level of inflammation. The inflammatory response of the metals fell midway between the effect of the low-toxicity biodurable particles and the quartz particles. Thus the specific characteristics of nanoparticles are determined by SA and functionalization (Duffin *et al.* 2007).

The results obtained by Hamilton *et al.* (2012) with multiwall carbon nanotubes (MWCNT) containing 3–5% of nickel as an impurity are unexpected in light of other results. The authors attributed the observed toxicity of the materials administered to mice by OPA (*i.e.*, mild to extensive inflammation) to nickel metal, and they reported a dose-response for toxicity with increasing nickel content of the MWCNT. However, instillation exposure to nickel metal nanoparticles at 10-fold higher mass than present in Hamilton's MWCNT, using the same OPA method (Poland *et al.*, 2012), did not seem to elicit as great a toxic response in mice as observed with MWCNT. The Hamilton *et al.* (2012) results with MWCNT (0.15 mg/mouse) are different from those obtained by Lam *et al.* (2004) with single wall carbon nanotubes (SWCNT). These researchers found that at equally instilled mass doses, SWCNT without nickel resulted in a higher incidence of inflammation and fibrosis than SWCNT with 26% nickel content, when observed 90 days after instillation. The SWCNT with 26% nickel content showed granulomas and high mortality at the high dose (0.5 mg/mouse) but had no adverse effects at the low dose (0.1 mg/mouse), and no incidence of lung inflammation at either exposure level.

Several investigators looked at the importance of route of exposure for toxic effects (Ogami *et al.*, 2011; Fujita *et al.*, 2009; Mizuguchi *et al.*, 2013). While instillation of nickel nanoparticles can recapitulate the same toxicity effects of inhalation, it took repeated inhalation exposure for 4 weeks at relatively high levels of nickel oxide nanoparticles (0.2 mg/m^3) to elicit the same toxic effects as a single bolus dose of 0.2 mg/rat via instillation. In terms of SA/lung, the same PMN effects could be seen at lower doses of nano nickel oxide particles after repeated exposure (5h/d x 5d/w x 4 w) via inhalation (1/10) than via a single instillation. These researchers also compared the effect on BALF PMNs of instillation of nickel oxide particles of different particle size (nano- and micron-sized). Importantly, when doses are expressed in terms of SA, nano- and micron-sized particles fit the same dose-response curve and did not display different hazard profiles (Mizuguchi *et al.*, 2013).

Cho *et al.*, (2012a,b) compared the *in vivo* effects of nano nickel oxide to other metal oxide nanoparticles (*e.g.*, zinc oxide, copper oxide, cobalt oxide, and chromium oxide) at similar SA doses. Different profiles of BALF cells were found. Researchers concluded that the *in vivo* results cannot be predicted from the *in vitro* tests. Nickel oxide nano-sized particles at 150 cm^2/rat caused an immune response, inflammation and proteinosis. Nickel substances in micron-sized particles have also been shown to induce proteinosis after repeated exposure via inhalation (Benson *et al.*, 1995; Oller *et al.*, 2008). Cho *et al.* also compared the effects of nano nickel oxide particles to their aqueous extract. The extracts had no toxicity. These results are not surprising as researchers showed that dissolution of this nano nickel oxide sample at neutral pH (used to generate extracts) was low (1–2%). By contrast, release of nickel ion after 4 weeks at pH 5.5 (as could be found in lysosomes *in vivo*) was 34%.

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3.2.2 Possible carcinogenicity effects of nickel-containing nanoparticles compared to micron-sized nickel particles

The study of Hansen *et al.* (2006) (also reported in Gatti *et al.*, 2008) is the only study which specifically looked at the ability of nickel metal nanoparticles (compared to massive forms of nickel metal) to induce tumors (Appendix, Table 4). Both samples produced rhabdomyosarcomas in rats but did not induce tumors when injected into sheep knee joints. In rats, tumors were generally surrounded by fibrous capsules. The bulk material (discs) implantation sites showed a central cystic component and no particles. The authors noted that the presence of calcium (Ca) and phosphorus (P) precipitates in spherical shape seems to be part of the evolution of the pathology. There are some issues with this study, but most importantly, the authors themselves stressed that rat implantation tumors are not a good model for humans (Hansen *et al.*, 2006). No difference in cancer hazard between nano and massive forms of nickel metal could be made based on the results from this study.

Existing data on the carcinogenicity of nickel metal

Intratracheal instillation of bolus doses of nickel metal micron-sized particles have been shown in early studies to cause lung tumors (*e.g.,* Ivankovic *et al.*, 1988; Pott *et al.*, 1987). Injection studies of metallic nickel at non-physiological doses by non-relevant routes of exposure have also shown induction of tumors (see IARC, 1990 and Sivulka *et al.*, 2005, for complete list). These studies provided the basis for the 2002 listing of nickel metal as "reasonably anticipated to be a human carcinogen" in NTP's 10th RoC. The 10th RoC entry regarding nickel metal states:

> *"A variety of carcinogenicity studies in rodents indicate that metallic nickel powder can produce tumors when given by intratracheal instillation or subcutaneous, intramuscular, or intraperitoneal injection. Intratracheal instillation of metallic nickel powder induces primarily adenocarcinoma, whereas injection most frequently induces sarcoma, demonstrating that metallic nickel can induce both epithelial and connective-tissue tumors. Tumors have been produced by metallic nickel exposure in both rats and hamsters (IARC 1990). The available data from human studies of metallic nickel exposures are less informative. The available epidemiological studies of workers are limited by inadequate exposure information, low exposures, short follow-up periods, and small numbers of cases."*

A review of the numerous studies investigating excess respiratory cancer risk in the nickel refining and nickel-using industries generally suggests that exposures to nickel metal do not increase workers' respiratory cancer risk. Epidemiological studies have found an increased respiratory cancer risk among workers involved in refining and processing sulfidic nickel ores where there were mixed inhalation exposures to both water soluble nickel compounds (*e.g.*, nickel sulfate or nickel chloride) and water insoluble nickel compounds (*e.g.*, nickel subsulfides, oxides or mixed nickel-copper oxides).[8] However, no association between metallic nickel exposure and respiratory cancer risk was found in several studies (ICNCM, 1990; Egedahl *et al.*, 2001; Egedahl and Collins, 2009; Goldberg, 1994; Sorahan and Williams, 2005). Only two studies analyzing the data from the Clydach and Kristiansand nickel refineries show hints of

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[8] Epidemiological studies have consistently identified the inhalation route and respiratory tract tumors as the only relevant route and sites associated with exposure to nickel compounds. This is why in the EU, nickel compounds are classified as Category 1A carcinogens by the inhalation route only (*Carc. 1A; H350i via inhalation,* EC, 2009).

possible statistical correlations between excess cancer risk and nickel metal exposure (Easton *et al.*, 1992; Grimsrud *et al.*, 2002). However, these associations were either not reproduced or lost statistical significance after accounting for confounding exposures. Moreover, no association between inhalation exposure to any nickel species and increased respiratory cancer risk has been found outside of nickel refineries (*e.g.*, in nickel alloy industries or barrier manufacturing), although, clearly, metallic nickel would have been present (Cragle *et al.*, 1984; Arena *et al.*, 1998; Moulin *et al.*, 2000; Sivulka, 2005; Sorahan, 2004; Sivulka and Seilkop, 2009).

Overall, there is little, if any, evidence to suggest that exposures to metallic nickel increase respiratory cancer risks in workers employed in either the nickel-producing or nickel–using industry sectors, encompassing > 80,000 workers. However, as recognized in the 10th RoC, the epidemiological studies have limitations related to the power of each study to detect an effect. Thus, in studies where nickel metal exposures were high, the number of exposed workers generally has been low; while a high proportion of the workers in most studies have been exposed to relatively low concentrations of nickel metal. Therefore, in order to arrive at a more definitive conclusion regarding the carcinogenicity of nickel metal, it is necessary to consider the epidemiological data together with information from animal studies by a relevant route of exposure and knowledge of mode of action for the carcinogenicity of nickel substances.

A post-2002 carcinogenicity study by a relevant route of exposure (inhalation) with nickel metal powder did not show induction of respiratory tumors (Oller *et al.*, 2008). This 30-month inhalation study (OECD guideline and GLP compliant study) with Wistar rats investigated the potential carcinogenic responses to inhaled nickel metal powder (*i.e.,* MMAD =1.8 µm; purity = 99.9% purity) over a period of exposure of up to two years, with a follow-up senescence period of 6 months. The study demonstrated that exposure levels up to 0.4 mg/m^3 of nickel metal (maximum tolerated dose, MTD) did not induce respiratory tumors in male and female Wistar rats. However, these exposures induced significant lung inflammation and resulted in a retained nickel burden of 60 µg/lung at the MTD.[9] Despite the presence of chronic lung inflammation in these animals, no respiratory tract tumors were observed.

A dose-related increase of adrenal gland pheochromocytomas in male rats and a dose-related increase for combined adenomas/carcinomas of the adrenal cortex in female rats were the only neoplastic findings in this study. In both cases, statistical significance was achieved only in the 0.4 mg/m^3 exposure group. These findings are considered to be treatment-related (secondary to lung toxicity) in the case of pheocromocytomas (Ozaki *et al.*, 2002), or within historical

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[9] The highest lung burden that can be achieved in Wistar rats at the MTD in a chronic inhalation study with nickel metal powder (60 µg/lung) is much lower than the burden achieved by Pott *et al.* (1987) in the intratracheal instillation studies at which local tumors were detected (single instillation of 300 or 900 µg nickel metal, 10 instillations totaling 3,000 and 9,000 µg of cumulative doses) in the same strain of rats. Similarly, Ivankovic *et al.* (1988) observed a statistically significant increase in tumors in hamster instilled with 40,000 but not 10,000 µg of nickel metal powder. Intratracheal studies will result not only in higher dose rates but also in higher local doses that could never be achieved by inhalation. Instillation produces hotspots and more centralized particle deposition than inhalation. In studies where the lung burden achieved by intratracheal instillation is massive, there is a potential for affecting lung defense mechanisms and affecting the animal's ability to eliminate the material. These conditions can lead to false positive results when extrapolated to realistic inhalation exposure levels. Muhle *et al.* (1992) did not observe significant tumor induction in hamsters instilled with a cumulative dose of 10,000 µg nickel metal.

background levels (in the case of cortical tumors) (Bomhard, 1992), but not nickel-related.[10] Increased incidence of either type of adrenal tumors was not observed in a rat oral carcinogenicity study with nickel sulfate (Heim *et al.*, 2007), in which blood nickel levels were much higher than in the nickel metal study. If the adrenal tumors were related to systemic exposure to nickel ion, the oral study would have replicated the results from the inhalation study (MTD in oral study = 50 mg nickel sulfate/kg).

Therefore, the information from epidemiological and animal carcinogenicity studies is consistent in not identifying a carcinogenic potential associated with exposure to nickel metal by relevant routes. Differences in carcinogenic potential between nickel metal and nickel compounds can be attributed to their distinct physico-chemical and surface properties.

The bioavailability model for lung cancer induction by nickel compounds has been proposed by many researchers (*e.g.*, Haber *et al.*, 2000; Costa *et al.*, 2003; Hack *et al.*, 2007) and has been recently articulated in Goodman *et al.*, (2011). This model proposes that lung tumors will occur when sufficient amounts of bioavailable nickel ion can reach the nucleus of target respiratory cells. This bioavailability will depend on the characteristics of the nickel exposure such as: retained particle dose, uptake of particles into the cells and intracellular dissolution. In the case of micron-sized nickel metal powder, the maximum lung levels that can be tolerated are limited by the high respiratory toxicity. The resulting relatively low retained dose, combined with very poor intracellular uptake (also observed *in vitro* with several micron-sized metals in elemental state, Costa *et al.*, 1981) and low intracellular dissolution (*i.e.*, the particles need to be oxidized as reflected in their relatively low *in vitro* cell transformation potency) results in a low predicted nuclear bioavailability *in vivo*. This is consistent with the lack of respiratory tumors observed in the Oller *et al.* (2008) rat study.

In a 2011 review of existing animal, human and other types of mechanistic data by an expert panel convened by *TERA (Toxicology Excellence for Risk Assessment)*, it was stated that the weight of the evidence of the animal and epidemiology data provides "fairly strong evidence that metallic Ni is not carcinogenic."[11] In summary, the evidence for the carcinogenicity of micron-sized nickel metal is even more limited now than it was 10 years ago and is consistent with no classification for carcinogenicity for nickel metal or nickel-containing alloys.

While micron-sized particles of nickel metal did not manifest a carcinogenic hazard at the MTD of 0.4 mg/m^3 in a rat inhalation study, one could speculate that because nano-sized particles of nickel metal have a higher particle number and SA at equal mass, an inhalation carcinogenicity study with nickel metal nanoparticles (at the same exposure levels) might have had a different outcome. However, because the toxicity of the nickel nanoparticles is also related to the particle number and the SA per equal mass, it is logical to predict that such a study would have capped

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[10] The nickel metal inhalation carcinogenicity study was initiated in 2000 at the request of the European Union (EU) and Germany's BK Tox (Beraterkreis Toxikologie). In 2004, the tasks of the BK Tox Group were taken over by the German Subcommittee III of the AGS. Members of the BK-Tox and AGS III committees were part of the Expert Group overseeing the study. The Expert Group accepted this study as a negative study for the inhalation carcinogenicity of nickel metal.

[11] http://www.tera.org/Peer/NiBioavailability/

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**COMMENTS ON THE NTP PROPOSAL
TO LIST NICKEL NANOPARTICLES IN
FUTURE EDITIONS OF THE RoC**

the MTD at a much lower concentration (in terms of mg/m^3). This will be compounded by the fact that clearance of nanoparticles of nickel metal may be faster compared to clearance of micron-sized particles.[12] In a 2011 *in vitro* study, Pietruska and coworkers report that in contrast to metallic nickel micron-sized particles, nickel metal nanoparticles caused a rapid and prolonged activation of the HIF-1a pathway, which is the same response elicited *in vitro* by the "carcinogenic" nickel compounds. The results of this study are interesting because even if nickel metal nanoparticles are able to elicit the same qualitative response (which nickel metal micron-sized particles would probably have also elicited if tested at higher concentration or for longer periods), significant differences in potency between nano nickel metal and nano nickel oxide were reported in this study. The nano-sized particles of nickel metal were noted to have higher uptake into cells and mobilized nickel ion at higher levels than micron-sized particles (*i.e.*, after 48 hours ~0.6% of nickel content was released from nano-sized nickel metal particles, while <0.1% was released from micron-sized particles), the nickel metal nanoparticles displayed >80-fold lower mobilized nickel ion and several-fold lower uptake into cells than nano-sized nickel oxide particles (*e.g.*, ~50% mobilization of nickel from nano NiO after 48 hours). Furthermore, to elicit the same HIF response as nano nickel oxide, the nickel metal nanoparticles needed to be exposed for longer time periods (Pietruska *et al.*, 2011). When likely *in vivo* differences in toxicity and clearance are added to these *in vitro* differences in potency, it is very unlikely that inhalation exposure to nano nickel metal would have resulted in a different tumor outcome compared to micron-sized nickel metal particles. Together these data suggest that the ultimate effective dose to target cells that can be achieved *in vivo* may not necessarily be higher for nanoparticles than for nickel metal micron-sized particles. Other *in vitro* studies with nickel-containing nanoparticles are not reviewed here to any significant extent. These studies often show the same types of responses for nano- and micron-sized particles of nickel compounds. Whether the types of responses observed *in vitro* (*e.g.*, induction of HIF) under the conditions of exposure employed in these studies, play a significant role in the induction of tumors *in vivo* is impossible to evaluate with the present studies.

Therefore, based on the weight of evidence, there is no indication to suggest that the carcinogenic potential of nano-sized nickel metal would be different from that of micron-sized particles. This is consistent with results reported for biodurable particles lacking specific toxicity (Donaldon and Poland, 2012; Gebel, 2012).

4. SCIENTIFIC ISSUES IMPORTANT FOR ASSESSING THE CARCINOGENICITY OF NICKEL NANOPARTICLES

Several issues must be considered when analyzing data from studies with nanomaterials. These factors are identified below with regard to assessing the carcinogenicity of nickel nanoparticles.

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[12] In rats, repeated exposure to nano-size nickel oxide particles resulted in a retention T1/2 = 62 days (Oyabu *et al.*, 2007). By contrast, micron-size nickel oxide particles had a retention T1/2 = 116-346 after repeated exposure (Benson *et al.*, 1995).

- **Quality of studies with nickel nanoparticles. Physical and chemical properties of nickel nanoparticles**: It is imperative that nanoparticles (as well as micron-sized nickel particles) be well-characterized when they are use in toxicological studies. Acceptable studies for consideration with regard to carcinogenic potential should include information on properties of the nanomaterial such as mass, SA, particle number, surface chemistry, and agglomeration state (Card and Magnuson, 2010). Studies that lack such pertinent information should be considered less reliable. These properties can affect the toxicokinetics and can help the interpretation and comparison of results across studies.

- **Relevance of route of exposure to humans**: At present, the main routes of exposure for carcinogenicity studies with nanomaterials are inhalation, oral, and dermal. The primary route of concern for nickel nanoparticles is inhalation – for two reasons: (1) Inhalation is the primary potential route of human exposure to nickel nanoparticles; and (2) Only respiratory tract tumors have been consistently associated with exposure to nickel compounds in animal and epidemiological studies, while inhalation studies involving nickel metal exposures have been negative in both animals and humans.

- **Dosimetric modeling of exposure to airborne nanoparticles**: Once nickel nanoparticles are released into the air, the individual particles tend to become agglomerated. These larger agglomerated particles are inhaled and deposited in the upper respiratory tract, where they are expected to have similar effects as micron-sized nickel particles. The use of dosimetric models to compare deposited and retained doses of nanomaterials in various regions of the respiratory tract should be considered.

- **Dose metrics**: Current data indicates that micron-sized and nano-sized particles (including nickel-containing particles) present the same hazard. When doses were expressed in terms of SA, both types of particles had similar dose-response curves and hazard profiles for non-carcinogenic toxic effects. This suggests that evaluations and comparisons of nano- and micron-sized nickel particles should be made in terms of equal number of particles or equal SA, rather than, or in addition to, mass.[13]

- **Carcinogenicity classification for nano-sized particles of nickel compounds**: The potential carcinogenicity of nickel compounds in nano-sized forms is not presently open to question at NTP, because nickel compounds (whether in nano-sized or micron-sized forms) were classified as "known to be human carcinogens" more than a decade ago in the 9th RoC.

- **Carcinogenicity classification for nano-sized particles of nickel metal:** Since 2002, the RoC has classified nickel metal as "reasonably anticipated to be a human carcinogen," and a weight-of-evidence approach, considering all the additional data

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[13]While exposure limits typically are expressed as mass/volume of air, it should be possible to do the risk assessments for air limits taking SA and particle number into account and then converting that to a mass/volume based limit value.

generated since 2002, would suggest that the "reasonably anticipated" classification is, if anything, overly conservative. In particular, a 2008 inhalation carcinogenicity study in rats with nickel metal powder and a 2007 oral carcinogenicity study in rats with nickel sulfate have found that nickel metal does not increase the incidence of respiratory tumors after inhalation and that exposure to the Ni (II) ion systemically (as it could be present after oral exposure to nickel metal or nickel compounds) does not induce tumors either. Rhabdomyosarcomas were observed at the implantation site in mice (but not goats) implanted with nickel metal nanoparticles or discs of pure nickel metal. However, implantation is not a relevant route of exposure for assessing human carcinogenicity via inhalation, oral or dermal routes. Accordingly, when a weight-of-evidence approach is applied to the totality of the data, a "reasonably anticipated" classification is the most that can be justified.

5.   CONCLUSIONS

The available data on nickel metal nano-sized particles does not indicate that these particles will present a toxicity hazard, including carcinogenicity that is different from that of micron-sized particles when results are compared using proper metrics. Furthermore, nanoparticles tend to agglomerate under normal environmental conditions, unless chemically or physically inhibited. These agglomerated (hence larger) particles tend to act similarly to micron-sized nickel particles. In addition, the evidence for the lack of carcinogenicity of nickel metal to humans exposed through inhalation or oral routes is much greater now than it was 10 years ago. Finally, the production volume of all nickel nanoparticles (oxide, metal, etc.) in the U.S. is estimated below 100 tons/year, and the number of U.S. residents exposed to nickel-containing nanoparticles appears to be quite small.

Nickel nanoparticles should not be considered for separate listing in future editions of the RoC, as they are already covered under the 9th and 10th RoC listings of nickel compounds (*e.g.*, nickel sulphate, nickel oxide, nickel chloride, etc.) as "known to be human carcinogens" and nickel metal as "reasonably anticipated to be human carcinogen." Overall, the production and exposure information as well as the hazard evidence for nickel nanoparticles does not justify consideration of a separate listing.

6.   SCIENTIST(S) WITH EXPERTISE OR KNOWLEDGE OF NICKEL

Günter Oberdörster, DVM, Ph.D. (University of Rochester, New York) is an expert in the field of inhalation toxicology (including the characterization and evaluation of nanoparticles) and has extensive knowledge of inhalation toxicity with regard to nickel.

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8. APPENDIX

Appendix, Table 1. Nickel-containing nanoparticles tested in *in vivo* toxicological studies

Chemical form of nickel-containing nanoparticles	Supplier	Sample Characteristics	Examples of Studies Using this Material
NiO*	Nanostructured and Amorphous Material, TX USA	99.8% purity. Nominal particle diameter =20 nm, SA ~ 30-170 m^2/g, Dh-a ~ 60-800 nm Black in color. Partially soluble in water-based solutions. Dispersed NiO particles medium (pH ~7) had 13-35% of nickel content released as nickel ion	Oyabu *et al.*, 2007; Fujita *et al.*, 2009; Ogami *et al.*, 2009; 2011; Mizuguchi *et al.*, 2013; Morimoto *et al.*, 2010; 2011a,b; Horie *et al.*, 2011; 2012; Lu *et al.*, 2009; Nishi *et al.*, 2009
NiO*	Nanostructured and Amorphous Material,TX USA	Primary particle size =5 nm (TEM) Dh-a ~ 92 nm; -21 Zeta potential in saline-serum Solubility (24h): saline 1.6%; pH 7.4, 1.2%; pH 5.5, 9% Solubility (4 w), pH 7.4, 2.5%; pH 5.5, 34%	Cho *et al.*, 2010; 2012a,b
Ni(OH)$_2$	Generated by electric arc discharge between 2 rods of metallic nickel in the presence of argon	Count median diameter ~ 40 nm Calculated SA= 40 m^2/g Solubility (24h): pH 7.4, 83%; pH 4.5, 95%	Kang *et al.*, 2011a,b; Gillespie *et al.*, 2010; Cuevas *et al*, 2010; Liberda *et al.*, 2010; 2011
NiSO$_4$ 6H$_2$O	Generated by nebulizing a nickel sulfate solution	Count median diameter= 38 nm	Kang *et al.*, 2011b
Ni^o	Nanostructured and Amorphous Material, TX USA	Mean dry particle diameter =15 nm (Dh-a =57 nm)	Poland *et al.*, 2012
Ni^o	Generated by closed circuit pulverization in controlled (Argon) atmosphere of Ni^o power of 99.9% of 3–7 µm-sized.	Non-agglomerated crystallites of nominal average diameter 50-140 nm. Upon injection it formed aggregates of 10 µm. SA/volume =600 mm^{-1}.	Gatti *et al.*, 2008; Hansen *et al.*, 2006
Ni^o	Inabta and Co., Vacuum Metallurgical Co., Ltd. Japan	Nominal particle size = 20 nm. >99% of particles under 40 nm. SA = 43.8 m^2/g.	Zhang *et al.*, 2008a,b; Dick *et al.*, 2003; Duffin *et al.*, 2007
Impure Ni^o (NiO)	Vacuum Metallurgical Co. Japan	85-90% Ni^o and 10-15% NiO Nominal particle size = 20 nm; SA=44 m^2/g	Wan *et al.*, 2011
Ni^o nanowires (NW), short and long	Produced electrochemical template synthesis using alumina membranes	Short nickel NW had average length of 4.3 µm, long NW had length of 24 µm. Diameter was 0.2 µm.	Poland *et al.*, 2012; Murphy *et al.*, 2011
MWCNT with nickel impurities	Several commercial sources**	Purity 94-97%. nickel content determined by XRF = 3-5%. Length 1-15 µm. Diameter 24-45 nm	Hamilton *et al.*, 2012
SWCNT with nickel impurities	CarboLex, Inc KY USA	Nanotubes contained 26% Ni, 0.5% Fe, 5% Y, 0.15% Al, 0.15% Zn, 0.02% Co by weight No data on particle size	Lam *et al.*, 2004

*Information about calcining temperature not provided; ** MK Impex (Canada), Nanostructured and Amorphous Material (USA), Nano Lab (USA), Sigma Aldrich (USA), Sun Innovations (USA), Cheap Tubes (USA, Helix Material (USA); NiO: nickel oxide; Ni^o: nickel metal; Ni(OH)$_2$: nickel hydroxide; NiSO$_4$ 6H$_2$0; nickel sulfate hexahydrate; MWCNT: multiwall carbon nanotubes; SWCNT: single wall carbon nanotubes; XRF: X-ray fluorescence; Dh-a: hydrodynamic or aerodynamic diameter; SA: Surface Area; d: days; w: weeks.

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Appendix, Table 2. Inhalation studies

Chemical form of nickel-containing nanoparticles	Species	Administration & examination	Concentration/ dose	Comparison	References
NiO	rat	4 w Animals examined 4 d, 1 m and 3 m after exposure	1×10^5 particles/cm³ (0.2 mg/m³)	Histopathology not severe right after exposure and did not progress. Lung burden at 4 d post exposure=30 µg Ni/lung. T1/2 =62 days	Oyabu et al., 2007
NiO [C₆₀]	rat	6h/d x 5d/w x 4 w Animals examined 3 d to 1 m after exposure	0.9×10^5 particles/cm³ (0.2 mg/m³)	Increased gene expression for inflammatory, immune and chemotactic responses in lung.	Fujita et al., 2009
NiO [C₆₀]	rat	6h/d x 5d/w x 4 w Animals examined 3 d, 1 m, 3 m after exposure	0.9×10^5 particles/cm³ (0.2 mg/m³)	Macrophage accumulation in the alveoli with infiltration of inflammatory cells was observed 3 d after termination of exposure. Hyperplasia of the terminal bronchiole and alveolar epithelial cells was also seen. These findings decreased 3 m after inhalation. No granulomas were observed.	Ogami et al., 2011
NiO [NiO median diameter 1.3]	rat	6h/d x 5d/w x 4 w Animals examined 3 d to 1 m, 3 m after exposure.	0.9×10^5 particles/cm³ (0.2 mg/m³)	Increases in BALF PMNs were noted. Lung burden with nano NiO was 17 µg/lung.	Mizuguchi et al., 2013
NiO [TiO₂]	rat	6h/d x 5d/w x 4 w Animals examined 4 d, 1 m, 3 m after exposure	1×10^5 particles/cm³ (0.2 mg/m³)	No changes in gene expression and minimal lung inflammation.	Morimoto et al., 2011b
Ni(OH)₂ NiSO₄ [Carbon graphite, TiO₂]	mice	Single 4 h Animals examined 0.5 and 24 h after exposure	NiOH up to 2.8×10^7 particles/cm³ (1.2 mg/m³, ~0.8 mg Ni/m³) NiSO₄ 4.5×10^6 particles/cm³ (3.7mg/m³, ~0.8 mg Ni/cm³) [1.8×10^7 particles/cm³, 0.56 mg/m³ C graphite & 2.5×10^7 part/cm³, 0.56 mg/m³ TiO₂]	Lots of inflammation was observed. Lung burden =1 µg/lung (41-46% clearance in first 24 hs confirming this is a very soluble compound). Nano nickel hydroxide gave similar response as nano nickel sulfate based on deposited doses but was more toxic based on equivalent nickel mass conc. Both nickel nanos were more toxic than nano carbon or TiO₂.	Kang et al., 2011b
Ni(OH)₂	mice	Single 4 h Animals examined 0.5 and 24 h after exposure 5h/d x 5d/w x (1 w, 3 m or 5 m) Animals examined 24 h after last exposure	2.8×10^7 particles/cm³ (1.2 mg/m³) ~0.125 mg/m³	Lung inflammation observed in 4 h study. Lung inflammation and histopathology examined in the long term study. After 5 m exposure, mild focal inflammatory infiltrates were observed.	Gillespie et al., 2010

NiO: nickel oxide; Niᵒ: nickel metal; Ni(OH)₂: nickel hydroxide; TiO₂: titanium dioxide; C₆₀: carbon fullerenes; h: hour; d: day; w: week; PMN: polymorphonuclear leukocytes; BALF: bronchioalaveolar lavage fluid.

Appendix, Table 3. Instillation or oropharyngeal aspiration (OPA) studies

Chemical form of nickel-containing nanoparticles	Species	Administration	Concentration/dose	Comparison	References
SWCNT with 26% Ni, 0.5% Fe, 5% Y by weight [CB, quartz, raw & purified CNTs]	mice	single	0.1 and 0.5 mg/mouse Animals examined 7 d & 90 d after exposure	After 90d, mice exposed to low dose showed no lung inflammation of granulomas. Increased mortality and granulomas (but no inflammation) were observed in animals exposed to the high dose. Nanotubes without nickel induced granulomas and inflammation at low and high doses.	Lam *et al.,* 2004
MWCNT with 3-5% Ni⁰ impurities	mice	single (OPA)	0.15 mg/mouse (0.8 to 5 µg Ni) Animals examined 7 d & 56 d after exposure	Authors concluded that toxicity correlated with nickel content and was targeted towards macrophages. Mild to extensive inflammation and some granulomas were observed.	Hamilton *et al.,* 2012
Ni⁰ nanowires (NW), short and long [CNT]	mice	intrapleural	5 µg /mouse Animals examined 1 d -24 w after exposure	Long fibers cause inflammation & fibrosis. Short fibers did not. Inflammation for nickel NW was similar to that elicited by CNTs and seemed to be driven by length of fibers.	Murphy *et al.,* 2011
Ni⁰ Ni⁰ nanowires, short and long	mice	single (OPA)	50 µg/mouse Animals examined 24 h & 7 d after exposure	All materials (nano particles and wires) caused inflammation with increased PMN counts. Ni⁰ particles caused mild diffuse alveolitis, mild collagen staining, mild alveolar wall thickening. Longer nickel NW had a moderate inflammation and stronger granulomatous response.	Poland *et al.,* 2012
Ni⁰ (diameter = 20 nm) [nano Co, nano and micron TiO₂ and CB, polystyrene, quartz]	rats	instillation	125 µg/rat	Ni⁰ nano particles induced more BALF PMN than low-toxicity biodurable particles but less than surface-active quartz when compared by SA dose.	Duffin *et al.,* 2007
NiO (Dh-a =0.8 µm) [NiO median diameter =4.8 µm, Cryst silica GM =1.6 µm, TiO₂ GM =1.5 µm]	rats	instillation	2 mg/rat Animals examined 3 d, 1 w, 1 m, 3 m, & 6 m after exposure	Inflammation and alveolitis were seen with nano NiO but not with µm NiO particles when compared at equal mass. Nano NiO had similar toxicity and histological features as µm-sized crystalline silica.	Ogami *et al.,* 2009
NiO (median diameter = 26 nm) [C₆₀] [NiO median diameter 0.8, 1.3, 4.8 µm]	rats	instillation	0.1 & 0.2 mg/rat [1 & 2 mg/rat] Animals examined 3 d & 1 m after exposure	NiO exposure elicited higher toxicity than C₆₀. Nano NiO caused similar elevation in BALF PMN as µm-sized NiO but at lower mass doses. When doses are expressed in terms of SA, nano- and µm-sized particles fit the same dose-response curve. Similar PMN responses were observed for repeated exposure to NiO via inhalation (4 w x 0.2 mg/m³) and a single instillation (0.2 mg/rat) when compared in terms of SA/rat lung.	Ogami *et al.,* 2011; Fujita *et al.,* 2009; Mizuguchi *et al.,* 2013
NiO (20 nm) [TiO2]	rats	instillation	0.2 mg/rat Animals examined 1 h & 1 w after exposure	Similar response in this instillation study as observed *in vitro.* Similar response for nano NiO and NiCl₂, although nano NiO total nickel mass dose was higher. Oxidative stress related to nickel ion release. On mass basis, equal dose of µm-sized NiO did not have an effect via	Horie *et al.* 2011; 2012

				instillation.	
NiO [Other metal oxides: CeO_2, TiO_2, CB, SiO_2, ZnO, CuO, Co_3O_4, Cr_2O_3, amine-modified polystyrene beads]	rats	instillation	50-150 cm^2/rat; 0.055-0.163 mg/rat Animals examined 24 h & 4 w after exposure	Compared toxicity effects of nano NiO to other metal oxide particles at similar SA doses looking at BALF and histopathology. Many metal oxides (including NiO) induced toxicity but with different profiles. NiO nano at 150 cm^2/rat caused immune response, inflammation & proteinosis. Particle coating influenced the toxicity.	Cho *et al.*, 2010, 2012a
NiO [Other metal oxides: ZnO, CuO, Co_3O_4, Cr_2O_3]	rats	instillation	150 cm^2/rat; 0.163 mg/rat of particles or their aqueous extract Animals examined 24 h & 4 w after exposure	Studied effects on BALF parameters of nano NiO particles compared to their aqueous extract. Extracts had no toxicity.	Cho *et al.*, 2012a,b
NiO (10-20 nm) [Other metal oxides: CeO_2, TiO_2, MgO, Co_3O_4, ZnO, Al_2O_3, SiO_2, CB]	rats	instillation	250 cm^2/rat Animals examined 24 h after exposure	Only NiO and alumina 2 induced lung inflammation at this dose as evidenced by BALF parameters. When *in vitro* and instillation results were compared in terms of SA doses, the hemolytic *in vitro* test was found to predict the *in vivo* responses.	Lu *et al.*, 2009
NiO (Dh-a =26 nm) [TiO_2 GM=1.5 µm, cryst silica GM=1.6 µm]	rats	instillation	0.1-0.2 mg/rat Animals examined 3 d & up to 6 m after exposure	Characterized the inflammatory response and cytokine production by BALF and histopathology. Negative (TiO_2) and positive controls (cryst silica) (1-2 mg/rat) were not toxic. Persistent inflammation was observed. Definite granuloma, fibrosis or emphysematous changes were not observed.	Morimoto *et al.*, 2010; Nishi *et al.*, 2009
NiO (Dh-a =1.3 µm)	rats	instillation	1 mg/rat Animals examined 3 d & up to 6 m after exposure	Micron-sized NiO nanoparticle agglomerates were formed in solution. These agglomerates induced persistent inflammatory responses by BALF and histopathology.	Morimoto *et al.*, 2011a

NiO : nickel oxide; Ni^o : nickel metal; $NiCl_2$: nickel chloride; TiO_2 : titanium dioxide; C_{60} : carbon fullerenes; CNT : carbon nanotubes; MWCNT : multiwall carbon nanotubes; SWCNT : singlewall carbon nanotubes; CB : carbon black; Co : cobalt; Cr : chromium; Cu : copper; Zn : zinc; Ce : cerium; Si : silicon; Al : aluminum; Mg : magnesium; h : hour; d : day; w : week; PMN : polymorphonuclear leukocytes; BALF : bronchioalaveolar lavage fluid.

The bracketed information in the first column indicates other materials that were tested that are not nickel nanoparticles.

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Appendix, Table 4. Injection and implantation studies

Chemical form of nickel-containing nanoparticles	Species	Administration	Concentration/dose	Comparison	References
Ni^o [Ni^o disk of 1 cm diameter x 1 mm]	rats	i.m. implantation	Unclear what exact doses were given to each animal (10-125 mg covering 2 cm^2) [1 disk/rat]	Rhabdomyosarcomas (mesenchymal tumor) observed at site of implantation. Agglomerates of nano nickel (10 μm) were noted.	Hansen *et al,.* 2006; Gatti *et al.,* 2008
Ni^o	sheep	knee joint injection	Not provided	No tumors were induced.	Hansen *et al.,* 2006
Ni^o Ni^o nanowires, short and long	mice	i.p injection	0.5-50 μg/mouse	Examined 24 hs after injection. Inflammation was observed at 50 μg/mouse nano Ni^o particles, but no effect on macrophage function and no granulomas. Long nanowires displayed greatest response and affected macrophage function.	Poland *et al.,* 2012

Ni^o: nickel metal; i.m.: intramuscular; i.p.: intraperitoneal.