Oral Bioaccessibility of Nickel in Various Particle Sizes of House Dust from Communities Close to Nickel Mining and Smelting Operations

by

Nancy Dai

A Thesis
presented to
The University of Guelph

In partial fulfillment of requirements
for the degree of
Master of Science
in
Environmental Sciences and Toxicology

Guelph, Ontario, Canada

© Nancy Dai, December, 2016
ABSTRACT

ORAL BIOACCESSIBILITY OF NICKEL IN VARIOUS PARTICLE SIZES OF HOUSE DUST FROM COMMUNITIES CLOSE TO NICKEL MINING AND SMELTING OPERATIONS

Nancy Dai
University of Guelph, 2016

Adviser:
Professor B. A. Hale

Particle size is known to influence leaching of Ni from soil and dust particles for total [Ni] and bioaccessibility. Little research has been done on Canadian dusts that contain Ni from mining and smelting sources, which may differ in speciation from that in the current literature. This work extends knowledge to a different set of samples. The relationship between particle size and oral Ni bioaccessibility was examined in pooled house dust previously studied in the Sudbury Soils Study. Dusts were separated into ranges of <10 µm, 10-41 µm, 41-70 µm, 70-105 µm, and 105-250 µm diameter fractions. Total [Ni] was determined following a modified reverse aqua regia acid digestion assay. Bioaccessibility was determined following the SBRC method. Both total and bioaccessible [Ni] increased inversely with particle size, though the relationship varied based on the dust group. Particle size and total [Ni] together explained much more of the variance in bioaccessible [Ni]. Bioaccessibility percentages were adjusted for their relative mass in each particle size range. Particles <10 µm and between 41 and 70 µm had high Ni bioaccessibility, and particles <10 µm and between 70 and 105 µm were highest after weight adjustment. Study results support that a more appropriate particle size for risk assessment use may be smaller than 250 µm. An upper limit of 105 µm is recommended.
**Acknowledgements**

Immense gratitude goes to my advisor, Beverley Hale, for her invaluable guidance, support, and patience throughout the past few years. My utmost gratitude goes to Luba Vasiluk for all her contributions to this project. I would like to thank Peter Smith for his lessons on using the FAAS and GFAAS and his technical support in data analysis.

I’ve had the pleasure to work alongside a delightful cohort of lab mates: Kim Zupfer, Elizabeth Jones, Jessica Sowa, Amanda Pellegrino, Pooja Aurora, Yamini Gopalapillai, Bianca Pereira, Amanda Laird, and Ryan Thorn. Thank you to all the Hale lab members for sharing this journey with me.

Thank you to my committee members Mike Dutton and Ron Brecher for their input to this thesis and helping to get me there.

Thank you to Mom and Dad for reminding me of the bigger picture and for all your support during my research. ‘Thanks’ goes to my friends for keeping me sane and making sure I had some non-educational fun once in a while.

I would like to acknowledge financial support from NSERC, Vale, and Glencore in funding this project.
Contents

Acknowledgements ........................................................................................................... iii
List of Tables ........................................................................................................................ vi
List of Figures ...................................................................................................................... vii
List of Abbreviations .......................................................................................................... viii
List of Symbols .................................................................................................................... ix

1 LITERATURE REVIEW .................................................................................................. 1
  1.1 Why indoor dust? ........................................................................................................ 1
  1.2 Why Ni? ..................................................................................................................... 2
    1.2.1 Carcinogenicity of Ni ........................................................................................... 2
    1.2.2 Reproductive toxicity of Ni ................................................................................. 3
  1.3 Characteristics of indoor dust .................................................................................. 5
    1.3.1 Elemental composition and variability ............................................................... 5
    1.3.2 Modifying factors ............................................................................................... 6
  1.4 Sources of dust and metals ...................................................................................... 7
    1.4.1 Outdoor sources ................................................................................................ 7
    1.4.2 Indoor sources .................................................................................................... 8
  1.5 Ni bioaccessibility .................................................................................................... 8

2 INTRODUCTION ........................................................................................................... 10
  2.1 Particle size and exposure ....................................................................................... 10
  2.2 Particle size and Ni concentration ........................................................................ 10
  2.3 Particle size and bioaccessibility .......................................................................... 11
  2.4 Research objectives ............................................................................................... 12

3 MATERIALS AND METHODS .................................................................................. 13
  3.1 Dust samples .......................................................................................................... 13
  3.2 Sieves ...................................................................................................................... 13
    3.2.1 Sieve validation ................................................................................................. 14
  3.3 Particle size separation ......................................................................................... 16
  3.4 Total Ni micro digestion .................................................................................... 17
3.5 Bioaccessibility assay ........................................................................................................ 17
3.6 Statistical analysis ............................................................................................................. 18
4 RESULTS AND DISCUSSION .............................................................................................. 19
  4.1 Dust masses of particle size fractions .............................................................................. 19
  4.2 Total and bioaccessible Ni concentrations ..................................................................... 20
  4.3 Ni bioaccessibility and weighted bioaccessibility ......................................................... 31
6 REFERENCES .......................................................................................................................... 37
Appendix I .................................................................................................................................. 41
Mass (g) of house dust ............................................................................................................ 41
Appendix II ............................................................................................................................... 42
Appendix III ............................................................................................................................. 43
Appendix IV ............................................................................................................................. 44
Appendix V ............................................................................................................................... 45
Appendix VI ............................................................................................................................. 46
List of Tables

Table 1. Pearson’s linear correlations. ................................................. 23
Table 2. ANOVA for total [Ni]. ......................................................... 24
Table 3. ANOVA for bioaccessible [Ni]. ............................................ 26
Table 4. Multiple linear regression statistical output. .......................... 29
List of Figures

Figure 1. Sieve mesh testing with glass bead SRMs... .......................................................... 15
Figure 2. Dust mass distribution by particle size ................................................................. 19
Figure 3. Total [Ni] by particle size ..................................................................................... 21
Figure 4. Bioaccessible [Ni] by particle size. ..................................................................... 22
Figure 5. Total [Ni] means by dust group .......................................................................... 24
Figure 6. Bioaccessible [Ni] means by dust group. ............................................................ 26
Figure 7. Multiple linear regression .................................................................................... 30
Figure 8. Ni bioaccessibility percentages........................................................................... 32
Figure 9. Weight adjusted Ni bioaccessibility.. ................................................................. 34
List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>IARC</td>
<td>International Agency for Research on Cancer</td>
</tr>
<tr>
<td>Ni</td>
<td>Nickel</td>
</tr>
<tr>
<td>SARA Group</td>
<td>Sudbury Area Risk Assessment Group</td>
</tr>
<tr>
<td>SAS</td>
<td>Statistical Analysis System</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for the Social Science</td>
</tr>
<tr>
<td>SRMs</td>
<td>Standard Reference Materials</td>
</tr>
<tr>
<td>U.S. EPA</td>
<td>United States Environmental Protection Agency</td>
</tr>
</tbody>
</table>
List of Symbols

[ ] the concentration of a substance
µm micrometer
1 LITERATURE REVIEW

1.1 Why indoor dust?

Dust ingestion contributes to oral exposure of humans to indoor pollutants, such as polychlorinated biphenyls (PCBs), brominated flame retardants (PBDEs), metals, pesticides, and industrial chemicals, in addition to mites, molds, and allergens (Roberts et al., 2009). Exposure to these can result in adverse health effects including asthma, irritated skin and mucous membranes, and cancers (Roberts et al., 2009). Fine suspended dust particles may be inhaled and can travel deep into the lungs and cross into the circulatory system or are transported into the gastrointestinal tract via the mucociliary escalator process. Particles less than 250 µm adhere to hands and thus may be ingested through hand to mouth behaviours (e.g., nail biting, finger sucking or licking) (Vasiluk, Dutton, & Hale, 2011). Since North Americans spend up to 90% of their time indoors (Garcia-Jares, Barro, Regueiro, Sanchez-Prado, & Llompart, 2010), the majority of their exposure to pollutants could be from indoor sources.

Children have the highest exposure due to increased proximity to dust when crawling on the floor or playing on the ground, as well as actively mouthing objects and fingers, and voluntarily ingesting soil and dust (Garcia-Jares et al., 2010). It is estimated that infants and children ingest 30-60 mg of dust per day, the highest among all age groups (Garcia-Jares et al., 2010; U.S. EPA, 2009). Infants and children consume twice as much dust as adults, but have a smaller body mass (approximately one sixth of an adult), making them up to ten times more vulnerable to dust exposure, according to U.S. EPA estimates (Roberts et al., 2009).

Pollutants in indoor dust are not subject to the same transformation and degradation forces that occur outside (Garcia-Jares et al., 2010), such as UV radiation from the sun or wind
dispersion and dilution. Pesticides and chemicals that were banned years ago are still prevalent in house dust, which suggests limited degradation and ongoing accumulation (Garcia-Jares et al., 2010).

1.2 Why Ni?

Although there are many dust pollutants of interest, Ni is important to consider due to its ubiquity (presence in stainless steel and other metal alloys). The majority of oral Ni exposure is from drinking water and diet (Cempel & Nikel, 2006; Das, Das, & Dhundasi, 2008), followed by ingestion of dust and soil.

1.2.1 Carcinogenicity of Ni

The International Agency for Research on Cancer (IARC) designates Ni compounds to be carcinogenic to humans (Group 1), citing sufficient evidence for carcinogenicity of nickel monoxides, nickel hydroxides, nickel (sub)sulfides, nickel acetate, nickel metal, and exposure to a mixture of nickel metal and nickel compounds (IARC, 2011). In contrast, there is inadequate evidence to declare the carcinogenicity of nickel titanate, nickel trioxide, and amorphous nickel sulfide (IARC, 2011).

The degree of Ni toxicity depends on the solubility of the Ni species, the route of exposure, and the mechanism of cellular uptake (Coogan, Latta, Snow, Costa, & Lawrence, 1989; Goodman, Prueitt, Dodge, & Thakali, 2009; Heim, Bates, Rush, & Oller, 2007). Nickel metal, sulfides, oxides, other water insoluble nickel compounds, and nickel salts including nickel sulfate hexahydrate are relatively low in toxicity and do not induce tumours when Ni is ingested through oral exposure (Heim, Bates, Rush, & Oller, 2007; National Academy of Sciences, 1975).
Epidemiological evidence suggests that laryngeal and pulmonary cancers and cancer of nasal cavities are linked with occupational exposure to nickel sulfide ore smelting and refining activities, seen in Ni mine and refinery workers around the world (IARC, 2011; National Academy of Sciences, 1975).

Short and long term oral exposure to soluble Ni leads to accumulation primarily in the kidney, followed by the lung and liver (Ambrose, Larson, Borzelleca, & Hennigar, 1976; Maria Cempel & Janicka, 2002). Nickel’s carcinogenic mode of action is to contribute to the creation of reactive oxygen species that may damage cell membranes, mitochondria, proteins, and DNA (Goodman et al., 2009; Rana, 2008).

1.2.2 Reproductive toxicity of Ni

Ten mg/kg body weight NiSO₄ was found to disrupt ovarian cycles in SPRD rats and 40 mg/kg body weight NiSO₄ inhibited their ovulation (Forgács, Paksy, Varga, Lazar, & Tátrai, 1997). Body mass, weights of ovaries, weights and histology of adrenal and pituitary glands, and kidneys, blood pressure, and ovarian blood flow were not affected by Ni treatment (Forgács et al., 1997).

An oral dose of 5 or 10 mg/kg body weight NiSO₄ administered via gavage to adult male mice for 35 days resulted in a dose dependent decrease in absolute and organ-to-body weight ratio of testes, epididymides, seminal vesicles, and prostate gland (Pandey, Kumar, Singh, Saxena, & Srivastava, 1999). Sperm count and motility were also decreased, but body weight gain was not affected by the treatment (Pandey et al., 1999). The exact same effects of reduction in male reproductive organ weights and sperm quality were also found in a separate but very similar study at 20 mg/kg body weight for oral dose of NiSO₄ and NiCl₂ via distilled
drinking water (Pandey & Srivastava, 2000). An increase in sperm abnormalities in the head, neck, and tail regions was observed (Pandey & Srivastava, 2000). Higher doses of 10 and 20 mg/kg affected body weight gain, while the lowest dose of 5 mg/kg did not (Pandey & Srivastava, 2000). In another study, Ni\textsuperscript{2+} treatment was found to adversely affect testicular structure, spermatozoa development, and steriodogenesis in mice (Massányi et al., 2007). Decreased sperm quality in bull, ram, boar, and bovine have also been found for Ni\textsuperscript{2+}, NiSO\textsubscript{4}, and NiCl\textsubscript{2} (Lindemann, Walker, & Kanous, 1995; Lukac et al., 2011; Massányi et al., 2004; Zemanova et al., 2007). It should be noted that other factors such as reduced food intake by the animals can also adversely affect reproduction endpoints and may confound toxicity results for the substance tested. For example, feed restriction has been shown to significantly decrease the number of sperm and spermatids in epididymides and testes of Swiss CD-1 mice (Chapin et al., 1993).

Ni\textsuperscript{2+} crosses the placenta and can directly affect the developing embryo/fetus as well as indirectly by altering hormonal balance in the rodent mother (Forgács, Massányi, Lukac, & Somosy, 2012). Twenty mg/kg body weight NiCl\textsubscript{2} administered via intraperitoneal injection to female mice resulted in a significantly lower implantation frequency (Storeng & Jonsen, 1981). Size and weight of litters were also reduced, along with higher instances of early and late resorptions, and stillborn or abnormal fetuses (Storeng & Jonsen, 1981). Ten and 300 µM NiCl\textsubscript{2} adversely affected development of Day 2 and Day 3 mouse embryos \textit{in vitro}, respectively (Storeng & Jonsen, 1980). In a separate experiment, Day 3 embryos ceased development after 48h of NiCl\textsubscript{2} exposure in culture, but the effect was reversible after transfer to Ni free medium (Storeng & Jonsen, 1984).
It is well known that some phenolic and carbon ring structures of organic compounds mimic estrogen and can bind to its receptors (endocrine disrupters). Certain metal ions, including Cd, Cu, Pb, Hg, Se, and Ni are also able to bind to estrogen receptors and exert agonistic effects (metalloestrogens) (Forgács et al., 2012). In one study, Ni activated estrogen receptor-α in human breast cancer cell line, MCF-7, in much the same way as estradiol (Martin et al., 2003). Treating cells with Co, Cu, Cr, Pb, Hg, and Ni stimulated cell proliferation, leading to a two to five times increase in cell number by day 6 (Martin et al., 2003). This showed that Ni has similar effects to estradiol and has the estrogenic potency equal to that of estradiol in cell culture (Martin et al., 2003). More research is needed to see whether there are impacts in whole organisms.

1.3 Characteristics of indoor dust

1.3.1 Elemental composition and variability

Indoor dust has been shown to contain three times the concentration of trace elements such as arsenic, copper, lead, chromium, antimony, cobalt, gold, and zinc compared to outdoor soil, of which dust is partly comprised (Fergusson, Forbes, Schroeder, & Ryan, 1986). The presence and/or elevated concentration of these metals in indoor dust cannot solely be attributed to soil, and are thus considered ‘pollution elements’ (Fergusson et al., 1986).

The contribution of indoor objects and processes to pollution elements in indoor dusts make the elemental composition of house dust highly variable, as compared to soil and street dust that are co-located (Fergusson et al., 1986). This variation among residences is seen across different geographic locations and even between different houses in the same area (Rasmussen, Subramanian, & Jessiman, 2001). On the other hand, soil-based elements contained in dust,
such as aluminum, iron, manganese, sodium, and potassium, are relatively uniform in variability and occur at the same concentrations as in the soil outside (Fergusson et al., 1986). The elevated indoor concentrations likely result from contribution of indoor metal sources, coupled with the limited degradation and thus ongoing accumulation.

Nickel speciation data on nine house dust samples from Sudbury analyzed using electron microprobe showed the following compounds to be present (approximately from highest to lowest relative mass): Pentlandite, NiS, NiO, Ni metal, NiSO4, NiMO, NiP, NiFeO, and Cr-Ni metal (SARA Group, 2007).

### 1.3.2 Modifying factors

Several factors have been investigated for their effect on elemental composition of house dust. The ones that did not account for differences in elemental composition included house age and construction material used (except for zinc and lead, as lead can come from paint) (Fergusson et al., 1986). In an Australian study, household income level, whether the residence was a unit or a house, and the number of occupants were not significant factors for variability in Ni concentration (Chattopadhyay, Lin, & Feitz, 2003).

The opposite was found to be true for dust samples from Istanbul, Turkey, where the number of occupants was the most significant factor in determining metal concentrations, including Ni (Kurt-Karakus, 2012). A likely explanation for this discrepancy is that the Sydney, Australia study sampled house dust from a suburban area whereas the Istanbul study included dust samples from both residential and office buildings located in urban, suburban, and rural areas. The difference in results is therefore not surprising, considering the variability of dust sampled across different geographic locations and varying proximity to traffic and urban...
pollution. This is supported by the finding that there were significant differences in Ni concentration between urban and suburban dust samples (Kurt-Karakus, 2012). In addition, the type of building and geographic location were found to be significant modifying factors in the Istanbul study (Kurt-Karakus, 2012). Median Ni concentrations in office dust (from administrative and retail offices) were higher than in residential house dust (Kurt-Karakus, 2012).

The amount of organic matter (such as moulds and fungi) in house dust as a proportion of total mass influenced its metal concentrations, likely because organic matter acts as a sink for metals (Rasmussen et al., 2001). Estimates show that indoor dust contains approximately 40-50% organic matter, as compared with 3-20% for street dust, and 9% for garden soil, although percentages will vary based on source location and soil type (Fergusson & Schroeder, 1985; Fergusson & Kim, 1991). This is one reason metal concentrations in house dusts are elevated compared to soil. It also illustrates the distinctiveness of indoor dust compared to street dust and soil.

1.4 Sources of dust and metals

1.4.1 Outdoor sources

House dust is partially generated from outdoor soil and street dust brought indoors through trek-in from bottoms of shoes, especially from attached garages, transferred on clothing, and transported via air drafts and wind (Kurt-Karakus, 2012). According to a New Zealand urban house dust study, 2-3% can be attributed to tire wear, concrete, and car emissions, and 1% from road salt (Fergusson et al., 1986). High levels of lead have been found in socks of lead industry workers, in conjunction with 3 to 26 times higher lead concentrations
in the houses of occupationally exposed individuals (Fergusson & Kim, 1991). Nickel concentration has been found to be positively correlated with concentrations of cadmium, chromium, copper, zinc, and iron, and their co-occurrence suggests heavy traffic to be the source (Hassan, 2012). Outdoor soil contributions to indoor dust composition are estimated to be 20-40% when comparing ratios of elemental concentrations in soil versus in dust (Fergusson & Kim, 1991; Rutz, Valentine, Eckart, & Yu, 1997), but can range up to 70% for sites where soil is a major contributor to dust (U.S. EPA, 1994).

1.4.2 Indoor sources

House dust is also generated from within the home from renovation activities, through wear and tear of furniture, household products, wallpaper, wall paint, dust fall from aerosol, and combustion of fossil fuels and tobacco products, central heating and cooling, and humidifiers (Hassan, 2012; Rasmussen et al., 2013). Chattopadhyay et al. (2003) found electrically heated houses to have higher lead and mercury levels, compared to heating by oil, gas, and coal. However, heating with coal and other fossil fuels was found to correlate with higher overall metal concentrations (Rasmussen et al., 2001). Indoor Ni sources include nickel-plated products, and nickel plating and alloys on cars (Hassan, 2012).

1.5 Ni bioaccessibility

To assess potential human health risk of Ni toxicity from dust ingestion, the Ni exposure must be determined, which can be estimated in three different ways. The first estimate is total Ni concentration in the dust. The second estimate is bioaccessible Ni in the dust, which is the amount that becomes potentially available for absorption in the stomach and intestines after
digestion processes. Bioaccessibility is typically expressed as a percent of total concentration ([bioaccessible/total] x 100%). The third estimate is bioavailable Ni, the amount that enters the systemic circulation and is either accumulated in tissues or excreted in urine. Ninety percent of ingested Ni is not absorbed but passed out through feces (National Academy of Sciences, 1975).

The use of bioaccessibility to estimate exposure has many advantages. First, bioaccessibility measures Ni which is solubilized in some simulation of gastric conditions (i.e., in vitro testing), and thus is likely more related to toxicity than total Ni concentration (Niu, Rasmussen, Hassan, & Vincent, 2010). Bioaccessibility testing is also relatively easy to carry out. On the other hand, bioavailability testing uses laboratory animals (i.e., in vivo studies), which is costly, time consuming, and has considerations for animal welfare. The bioaccessible concentration is assumed to be greater than the bioavailable concentration, in which case, risk would be overestimated if exposure was based on the bioaccessible concentration. For Ni, it is not known whether this assumption is true. Bioaccessibility of an ingested metal could be used in Health Canada’s typical exposure equations for “contaminated site” exposure assessment, as follows, where:

\[
\text{Dose} \ (mg/kg/day) = \frac{C_s \times IR_s \times RAF_{GIT} \times ET}{BW}
\]

RAF_{GIT} = relative absorption factor from gastrointestinal tract
\(C_s\) = concentration of contaminant in soil (mg/kg); in this case, the total Ni concentration in dust
\(IR_s\) = soil ingestion rate (kg/day); dust ingestion in this case
ET = exposure term
BW = body weight (kg)
If properly validated against in vivo studies of bioavailable Ni, bioaccessible Ni could be used to estimate the relative absorption factor (RAF\textsubscript{GIT}).

2 INTRODUCTION

2.1 Particle size and exposure

Particle size is a key factor to consider in dust exposure. For instance, particles \(<250\ \mu m\) adhere to hands upon contact and are ingested through hand to mouth behaviours. Inhalation is the other route of oral exposure, where particles \(>6.7, 2.7-6.7, 1.3-2.7, 0.80-1.3,\) and \(<0.80\ \mu m\) (including PM10 and PM2.5 of air quality guidelines) can progressively reach deeper into the pulmonary system, from the nasal cavity and throat, to the trachea, bronchi, and the alveoli (Samara & Voutsa, 2005). The respiratory tract rids a portion of foreign, inhaled particles via the mucociliary escalator followed by coughing or swallowing (Bright, Richardson, & Dodd, 2006; Das et al., 2008). Thus, entry into the circulatory system can occur through both routes of ingestion and inhalation. Absorption of toxicants after inhalation may result in similar health effects as those resulting from ingesting contaminated particles (De Miguel, Iribarren, Chacón, Ordoñez, & Charlesworth, 2007). It is estimated that 58% of house dust particles are between 44-149 \(\mu m\) in size while 6-35% are 30-63 \(\mu m\), which are all small enough to be inhaled and/or ingested (Kurt-Karakus, 2012; Lidia, 2004).

2.2 Particle size and Ni concentration

Particle size is an important source of variation in total metal concentration in dust. Samples not sorted by particle size (known as bulk) may yield different metal concentrations
than some or all the constituent size fractions.¹ For instance, Sudbury soil particles <70 µm in
diameter contained almost twice the total [Ni] as the <250 µm bulk fraction (Vasiluk, Dutton, &
Hale, 2011). Metals tend to concentrate on a mass/mass basis in smaller dust particles, due to
their increased surface area to mass ratio (Rasmussen et al., 2008). Total Ni concentrations
tended to increase progressively as particle size decreased (Fedotov, Ermolin, Karandashev, &
Ladonin, 2014; Hassan, 2012; Niu et al., 2010). There is evidence that metal accumulation by
finer dust particles occurs to a greater extent when it is from anthropogenic sources (Fedotov
et al., 2014).

2.3 Particle size and bioaccessibility

Two important factors that influence Ni bioaccessibility are the solubility of Ni
compounds and particle size. Dust contains metals in various organic and inorganic phases with
their own physical and chemical properties that determine their dissolution and adsorption to
dust, and to some extent, these particles are the dust itself. Distribution of these phases
among various particle sizes determines whether bioaccessible metals such as Ni increase or
decrease with particle size. In an Ottawa indoor dust study, Ni bioaccessibility increased with
decreasing particle size, with 58% bioaccessible Ni in the smallest fraction (<36 µm) versus 43%
in the largest fraction (80-150 µm) (Rasmussen et al., 2008). Little research has been done on
Canadian dusts that contain Ni from mining and smelting sources, which may differ in
speciation and hence in bioaccessibility compared to Ni from non-industrial sources.

¹ Bulk samples do undergo some sieving to rid of extraneous objects such as rocks and twigs in soil, and hair and
nail clippings in dust. Bulk samples contain a large range of particles and are not further sieved to obtain a specific
size range (i.e., fine or coarse fractions; bulk is a mix of both).
2.4 Research objectives

The focus of this research was on dust ingestion as one route of human exposure to Ni. The objective was to examine the relationship between particle size and oral bioaccessibility of Ni in Sudbury house dusts collected near Ni mining and smelting industry. It was hypothesized that prior studies of Ni in urban house dust and exterior soils would not be replicated in these house dusts, due to influence of nearby industrial facilities on Ni speciation. The null hypotheses were that total and bioaccessible Ni concentration, and relative bioaccessibility of Ni would be constant with particle size. Thus, the present study tested the alternate hypothesis that smaller particle sizes would contain higher Ni concentrations and bioaccessibility compared to larger particles.
MATERIALS AND METHODS

3.1 Dust samples

Dust samples were collected from 91 Sudbury houses as part of the Indoor Dust Survey of 2004 that was commissioned with the Sudbury Soils Study (SARA Group, 2008). A high volume surface vacuum sampler was used to vacuum three 1 m² carpeted areas in each home (SARA Group, 2008). High traffic areas and areas where children would spend long periods of time were targeted (e.g., in front of the television, in the child’s bedroom, playroom or recreational room) (SARA Group, 2008). The vacuum was disassembled and cleaned with alcohol wipes, rinsed with methanol, and air dried before each sampling (SARA Group, 2010).

After the Sudbury Soils Study was completed, the residual dust samples were grouped into a smaller number of composite samples due to the small sample size, based on prior analyses of Ni concentration ranges. One house sample was excluded as it was almost all sand. The remaining 90 samples were combined into seven groups based on similar nominal total [Ni], and sieved to retain only particles <250 µm in diameter. The sieved dusts were stored in transparent plastic vials with snap caps.

3.2 Sieves

For particle size separation, plastic sieves (Scienceware mini sieve set CAT no. F37845-1000) were custom made with plastic meshes of nominal sizes 10 µm, 41 µm, 70 µm, 105 µm, and 150 µm in diameter (Spectrum Labs Spectra/Mesh Nylon filters) using a glue gun. A thin layer of glue was applied around the ridge located midway inside the sieve. Circular meshes were cut to fit snugly on the ridge and positioned tautly over the glue. Some slack in the mesh was necessary to withstand vigorous operation of the sieve shaker for dust separation. Another
A ring of glue was applied on top, to sandwich the edges of the mesh and secure it to the sieve. The glue was allowed to dry and harden overnight.

### 3.2.1 Sieve validation

The following standard reference materials (SRMs), sourced from the National Institute of Standards and Technology (NIST), were used to validate the custom sieves: Glass Beads 1021, 1003c, and 1004b, with certified bead sizes of 2-12 µm, 20-50 µm, and 40-150 µm, respectively. Sieves with a mesh size that fell within an SRM’s range were tested using that SRM (e.g., the 10 µm mesh was tested using Glass Beads 1021 2-12 µm).

Ten grams of glass beads (1.00 g for SRM 1021 2-12 µm) were transferred using plastic disposable spatulas into 10 mL beakers and weighed on a precision balance (Mettler AT250 K12373). Beaker contents were poured onto the mesh, brushing off beads stuck to the sides with an antistatic brush. Sieves were capped tightly and shaken on a sieve shaker (Endecotts M100) for 20 min at highest intensity. Beads which passed through the sieve and those retained on the mesh were each weighed. Mass values were converted into percentages and compared to the expected values described in the SRM certificate (Figure 1), to corroborate the nominal mesh sizes. Each sieve was tested with four repetitions.
Figure 1. Sieve mesh testing with glass bead SRMs. Pink data points show certified values for percent glass beads that should pass through mesh sizes of 10, 41, 70, 105, and 150 µm. Black data points are the average percent of glass beads that passed through during testing of custom sieves. Error bars are standard deviations.
3.3 Particle size separation

Sieves were soaked in a 10% (v/v) HNO₃ (certified ACS grade, Fisher Scientific) acid bath for 30 minutes, rinsed with 3 volumes of distilled, deionized water and 4 volumes of Type I water (Nanopure Diamond, model D-11901, Barnstead), and allowed to air dry for 24-72 hours. Two sieves were stacked together as a pair, with the largest mesh on top and the next largest on the bottom. Dusts were poured onto the topmost sieve to a maximum fill of midway, to avoid tearing the mesh due to excessive weight placed on it. Sieves were then capped tightly at both ends, reinforced with masking tape, and shaken for 20 min at highest intensity on the sieve shaker.

Dusts retained on top of the two meshes were weighed and recorded. Dusts which passed through both sieves were transferred onto the sieve stack with next largest mesh openings (by removing bottom cap and using it as the top cap for the next sieve). The bottom sieve contents were transferred onto a pre-weighed plastic, disposable weigh boat, brushing off dusts stuck to the sides with an antistatic brush. The weigh boat mass was subtracted from the total mass obtained. The top sieve contents were weighed by inverting the sieve to shift dusts onto the attached, pre-weighed sieve cap, which was subtracted from the total mass afterwards.

After a dust group was sieved in its entirety, the used sieves were soaked overnight in a plastic tub with water and laboratory detergent (Fisher Scientific “Sparkleen”), then placed in the acid bath and washed according to the description earlier, before using the sieves for the next dust group. This procedure was repeated until all dust groups were sieved through all
mesh sizes, and weighed. This produced dust samples (n=41) in the following particle size ranges: <10 µm, 10-41 µm, 41-70 µm, 70-105 µm, 105-150 µm, and 150-250 µm.

### 3.4 Total Ni micro digestion

A version of the pseudo-total metal acid digestion developed by Topper and Kotuby-Amacher (1990) was used to prepare samples for total [Ni] determination. A dust mass of 5 mg was used for this open vessel, reverse *aqua regia* (3:1 HNO₃-HCl) digestion. The mass was scaled down from the original procedure to accommodate the small mass of some particle size fractions.

Dust samples were oven dried at 60°C overnight and placed in a desiccator for 30 minutes before weighing. Five milligrams of dust and 500 µL of acid solution (3 HNO₃:1 HCl) were added to 2 mL volumetric flasks. Flasks were inserted into a silica sand bath heated to 150 ± 0.8°C on a hot plate in the fume hood. Flasks were swirled once or twice during the 5 hour digestion. Afterwards, they were topped up with nanopure water to a final volume of 4 mL. Laboratory glassware was cleaned after each use with detergent and acid washed in the manner described above. Digestions were syringe filtered with 3 mL syringes and 0.1 µm pore size filters (GE Whatman, PTFE filter media) into polypropylene tubes, and stored at room temperature.

### 3.5 Bioaccessibility assay

A version of the Solubility/Bioavailability Research Consortium (SBRC) method (Ontario Ministry of Environment, 2002; Ruby et al., 1999) was used to prepare samples for bioaccessible [Ni] determination. The original procedure was scaled down to 50 mg of dust and
5 mL of extraction fluid, but otherwise followed the same steps. Dust samples were placed in scintillation vials with the extraction fluid (0.4 M glycine in nanopure water, pH-adjusted to 1.50 ± 0.05 with hydrochloric acid). The extraction fluid was prepared at 37°C (body temperature) and had the same pH as stomach acid. Vial caps were wrapped in laboratory film to prevent leakage, placed in a water bath at 37 ± 2°C, and shaken from side to side in a Precision Dubnoff Metabolic Shaking Incubator for an hour. Samples were then syringe filtered the same way as for total Ni digestion, and stored under refrigeration. The post-extraction pH of each sample was measured to ensure it was within ± 0.5 units of the starting pH. Nickel concentration was analyzed using graphite furnace atomic absorption spectrometer (GFAAS) with Zeeman background correction for both total Ni and bioaccessibility assays.

3.6 Statistical analysis

Mean and median values were determined using Excel, and Pearson’s linear correlations were calculated using SPSS. Analysis of variance for total and bioaccessible [Ni] was calculated using SAS PROC GLM and Tukey’s test of mean separation was calculated using the ‘lsmeans’ function. Multiple linear regression was calculated using SAS PROC REG.
RESULTS AND DISCUSSION

4.1 Dust masses of particle size fractions

Dust masses obtained after sieving ranged from 0.0007 to 7.6 g with a median mass of 2.2 g (Appendix I). Dust G had the smallest masses for almost all particle size fractions (Figure 2). The largest and smallest masses occurred in the <10 µm fraction (of Dust A and F, respectively). There was insufficient mass in the <10 µm fraction of Dust F for any Ni analysis. The <10 µm fraction of Dust B contained enough mass for total Ni determination only.

Figure 2. Dust mass distribution by particle size
4.2 Total and bioaccessible Ni concentrations

Total [Ni] when all size fractions of Sudbury house dust were compared ranged from 43.9 to 832 mg/kg with a median of 209 mg/kg (Appendix II). In comparison, background total [Ni] in Ottawa house dust\(^2\) ranged from 16.0 to 243 mg/kg, with a median of 51.5 mg/kg (Rasmussen et al., 2001). Sudbury dusts in the current study contained approximately 3 times higher total [Ni] than Ottawa dusts. Bioaccessible [Ni] in Sudbury house dust ranged from 12.6 to 184 mg/kg with a median of 56.0 mg/kg (Appendix III). By comparison, background solubilized [Ni] in Ottawa house dust ranged from 7 to 47 mg/kg with a median of 16 mg/kg (Rasmussen et al., 2008). Sudbury dusts in the current study contained approximately 2 to 4 times higher bioaccessible [Ni] than the Ottawa dusts. The elevated total and bioaccessible [Ni] in this study were to be expected, given the elevated [Ni] in the soil of the Sudbury area (SARA Group, 2004). Total [Ni] of house dusts in the Sudbury area reported in the Sudbury Soils Study (SSS) were in the same ranges as those found in the pooled samples of this study (which were derived from the SSS samples).

Both total and bioaccessible [Ni] in the current study increased as particle size decreased for all dust groups (Figure 3, Figure 4). Pearson’s linear correlations showed that particle size was negatively correlated at moderate to strong strength with all three Ni measures, especially bioaccessible [Ni] (Table 1). Bioaccessible [Ni] was also more strongly correlated with total [Ni] than with particle size.

\(^2\) Nickel concentrations in Ottawa house dust are similar to natural background concentrations in south-eastern Ontario (i.e., differs only by a factor of 1.2) (Rasmussen et al., 2001)
Figure 3. Total [Ni] by particle size. Note the difference in scale between the two graphs. Error bars are standard deviations.
Figure 4. Bioaccessible [Ni] by particle size. Note the difference in scale between the two graphs. Error bars are standard deviations.
Analysis of variance was calculated to test for non-linear relationships, including statistical interaction among independent variables in their effect on the dependent variable. A main effect of both dust group and particle size, and a significant interaction between the two factors were identified for total Ni concentration (Table 2). Thus the dependence of total [Ni] on particle size had to be separately considered for each dust group (Appendix II).

Comparisons of all possible pairs of means in each dust group would have been challenging to interpret. Instead, the arithmetically highest and lowest means in each dust group were compared for significant differences with Tukey’s least-squares mean separation to see how variable the dusts were (Figure 5, Figure 6). The larger particle size fraction was chosen when there were multiple non-significant means tied for highest or lowest value. For total [Ni] in general, the highest means ranged across different particle sizes depending on the dust group (Figure 5). For instance, the highest mean in Dust D for total [Ni] (637 mg/kg) occurred in the 105-150 µm fraction, while the highest mean in Dust C and E (467 and 342 mg/kg, respectively) were in the 10-41 µm fraction (p<0.05 with Tukey’s test). The lowest mean for total [Ni] in all dust groups occurred in the 150-250 µm fraction.

<table>
<thead>
<tr>
<th></th>
<th>Bioaccessible [Ni]</th>
<th>Particle size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total [Ni]</td>
<td>0.86**</td>
<td>-0.40**</td>
</tr>
<tr>
<td>Bioaccessible [Ni]</td>
<td>-</td>
<td>-0.68**</td>
</tr>
<tr>
<td>Bioaccessibility %</td>
<td>-</td>
<td>-0.36**</td>
</tr>
</tbody>
</table>

** p < 0.01

Table 1. Pearson’s linear correlations among the factors of total and bioaccessible Ni concentration, bioaccessibility, and particle size.
Table 2. **ANOVA for total [Ni]**. Analysis of variance for the contributions of dust group and particle size to variance in total Ni concentration.

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of freedom</th>
<th>Type III sum of squares</th>
<th>Mean square</th>
<th>F value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dust group</td>
<td>6</td>
<td>4567088</td>
<td>761181</td>
<td>148.11</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Particle size</td>
<td>5</td>
<td>1022592</td>
<td>204518</td>
<td>39.80</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Dust group*Particle size</td>
<td>29</td>
<td>437856</td>
<td>15098</td>
<td>2.94</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

![Figure 5](image_url)

**Figure 5. Total [Ni] means by dust group**. Highest and lowest significantly different means for total Ni concentration for each dust group are shown, with standard error bars. There are no data for Group F.
Analysis of variance showed a main effect of both dust group and particle size, and a significant interaction between the two factors in their effect on bioaccessible Ni concentration (Table 3). Thus the dependence of bioaccessible [Ni] on particle size had to be separately considered for each dust group (Appendix III). For bioaccessible [Ni], the size fraction containing the highest mean depended on the dust group (Figure 6). For example, the highest mean in Dust A occurred in the <10 µm fraction (70.9 mg/kg), while in Dusts B, D, F, and G, it occurred in the 41-70 µm fraction (76.3, 165, 59.2, and 93.4 mg/kg, respectively). The lowest mean for bioaccessible [Ni] in all dust groups occurred in the 150-250 µm fraction, as was the case for total [Ni]. While Ni concentrations decreased in the smallest (<10 µm) fraction compared to the next largest (10-41 µm) fraction for some of the dust groups (Figure 3, Figure 4), these differences were not statistically significant (Tukey’s test of mean separation).

The inverse relationship between particle size and Ni concentration found in this study is consistent with the literature on dust and soil of various types and origin (e.g., urban house dust, urban street dust, landfill soil, and brownfield soil from Egypt, Moscow, England, and Canada) (Fedotov et al., 2014; Hassan, 2012; Niu et al., 2010; Qin, Nworie, & Lin, 2016; Rasmussen et al., 2008; Siciliano, James, Zhang, Schafer, & Peak, 2009). The most likely explanation for the increase in [Ni] with decreasing particle size has to do with specific surface area (surface area per unit of mass). The increased specific surface area of smaller particles means a greater area to which Ni can adhere, plus greater access to the Ni by the dissolution solution in a time-limited digestion assay.
<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of freedom</th>
<th>Type III sum of squares</th>
<th>Mean square</th>
<th>F value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dust group</td>
<td>6</td>
<td>47857</td>
<td>7976</td>
<td>54.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Particle size</td>
<td>1</td>
<td>59142</td>
<td>59142</td>
<td>405</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Dust group*Particle size</td>
<td>6</td>
<td>7588</td>
<td>1265</td>
<td>8.66</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Table 3. ANOVA for bioaccessible [Ni]. Analysis of variance for the contributions of dust group and particle size to variance in bioaccessible Ni concentration.

![Figure 6](image)

Figure 6. Bioaccessible [Ni] means by dust group. Highest and lowest means for bioaccessible Ni concentration for each dust group are shown, with standard error bars.
Four subsamples of each particle size fraction in all dust groups were taken for total [Ni] digestions. The number of subsamples was reduced for the bioaccessible [Ni] digestions in light of the small variability found for total [Ni] (Appendix II). Five subsamples were taken once for every particle size fraction, chosen based on relative high dust mass in each group. The 70-105 µm fraction of the six fractions was selected more than once to cover the seventh dust group. Two subsamples were taken for the remaining five fractions in each dust group. Small standard deviations for both the two and five subsamples of bioaccessible [Ni] (Appendix III) showed that the following regression results were not sensitive to lack of five subsamples in every sample.

Multiple linear regression was conducted on the continuous variables in this study. The regression described results for current data and provided predictive data for future bioaccessibility work using same methods on a different sample of house dust. Multiple linear regression was used to model the continuous relationship among particle size, total [Ni] (the independent variables), and bioaccessible [Ni] (the dependent variable), for all particle size fractions of all dust groups. Averages of the four subsamples for total [Ni] and all subsamples of bioaccessible [Ni] were used as data points in SAS, resulting in 101 observations used in the regression model and a missing value of 1. Including all subsamples instead of the average for total Ni] would have yielded 94 used observations and 74 missing values that were omitted from the model, due to mismatch between the numbers of subsamples. Reducing the missing values in this way yielded a larger adjusted R² value.

The multiple factors of particle size and total [Ni] together in the regression model explained much more of the variance in bioaccessible [Ni] than either Pearson linear correlation. Total [Ni] was more than twice as important as particle size in explaining variance in
bioaccessible [Ni] (based on the standardized regression coefficients) and both were significant contributors to variance in bioaccessible [Ni] (Table 4). Adding an interaction between total [Ni] and particle size yielded a significant, though slight improvement to the fit of the regression model (Figure 7). Total [Ni] was by far the most important contributing factor, followed by particle size and the interaction term which had equal weight (Appendix IV).

The adjusted R² showed that total [Ni] and particle size explained 88% of the variance in bioaccessible [Ni], leaving only 12% unexplained variance. This remainder can be attributed to other factors known to affect Ni bioaccessibility, such as proportion of organic matter, clay content, and speciation. House dust samples in this study were likely similar in terms of these three factors because of their shared geographic origin, in which case these additional factors would not have made a significant difference had they been characterized in this study. However, this is not known for sure. These regression results can be predicative of similar bioaccessibility studies using the SBRC method and a different set of house dust samples containing elevated Ni; total [Ni] and particle size together could be expected to account for a similar amount of variance in Ni bioaccessibility.
<table>
<thead>
<tr>
<th></th>
<th>Adjusted $R^2$</th>
<th>Root MSE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.884</td>
<td>14.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Sum of squares</th>
<th>DF</th>
<th>Mean square</th>
<th>$F$ value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>148558</td>
<td>2</td>
<td>74279</td>
<td>381</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>19082</td>
<td>98</td>
<td>195</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>167640</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unstandardized coefficients</th>
<th>Standardized coefficients</th>
<th>t value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$B$</td>
<td>Standard error</td>
<td>$\beta$</td>
<td>$t$</td>
</tr>
<tr>
<td>Intercept</td>
<td>43.6</td>
<td>4.00</td>
<td>0</td>
<td>10.9</td>
</tr>
<tr>
<td>Total [Ni]</td>
<td>0.154</td>
<td>0.00802</td>
<td>0.735</td>
<td>19.2</td>
</tr>
<tr>
<td>Particle size</td>
<td>-0.180</td>
<td>0.0203</td>
<td>-0.340</td>
<td>-8.87</td>
</tr>
</tbody>
</table>

**Table 4. Multiple linear regression statistical output.** Note that the standardized $\beta$ is positive for total Ni concentration and negative for particle size. See Appendix IV for the multiple linear regression with interaction statistics.
Figure 7. **Multiple linear regression.** Particle size and total [Ni] together explain much more of the variance in bioaccessible [Ni]. Standardized and unstandardized coefficients are presented in Table 4. The interaction between total [Ni] and particle size accounts for the tilt of the predicted plane. Although the interaction is significant, it only improves the fit of the model slightly (Appendix IV).

\[ Z = 43.6 - 0.180x + 0.154y - 0.000379x*y \]
4.3 Ni bioaccessibility and weighted bioaccessibility

Bioaccessibility is expressed as a percentage of the total concentration (bioaccessible [Ni]/total [Ni]*100%) to enable comparison across studies. Nickel bioaccessibility in this study ranged from 10.9% to 46.0% with a median of 24.7% (Appendix V). In comparison, background Ni bioaccessibility from Ottawa house dust ranged from 7% to 76% with a median of 41% (Rasmussen et al., 2008). Dust from the current study, averaged across the minimum, maximum, and the median, contained 1.3 times lower percentages than Ottawa dust. This difference is likely due to variation in Ni compounds, such as more soluble and more bioaccessible forms of Ni present in Ottawa dust.

The highest and lowest percentage means in each dust group were dispersed across particle sizes. The highest means occurred in particles <105 µm and the lowest means were in particles >105 µm (Appendix V). For instance, the highest mean in Dust B (46.0%) was found in the 41-70 µm fraction while the lowest mean for Dust B, C, E, and G (28.6%, 21.2%, 16.6%, and 20.6%, respectively) were in the 150-250 µm fraction. On average, bioaccessibility percentages peaked for particles <10 µm and between 41 and 70 µm (Figure 8).
Figure 8. **Ni bioaccessibility percentages.** Percentages averaged across the 7 dust groups are shown with the line plot. Error bars are standard deviations.

Bioaccessibility reported in the Sudbury Soils Study (SSS) ranged from 0-3.3% as opposed to the range of 10.9-46.0% found in this study. This large difference can be attributed to numerous method differences in the SSS’s bioaccessibility testing and calculation. These included adding 1 g of pepsin to the extraction fluid (no justification was given for this deviation from the original SBRC method), addition of an intestinal phase with a pH of 7 to 8, Ni contamination in their blank bottles (mean of 166 μg/L Ni), and subtraction of Ni in the blanks from Ni in the extraction fluid (their equivalent of bioaccessible Ni). In comparison, a mean of
7.58 µg/L Ni was found in the blanks of the present study. Credit for this low contamination goes to various Quality Assurance/Quality Control measures employed, such as using nanopure water, non-metal (plastic) sieves, disposable laboratory spatulas and syringes, cleaning equipment in detergent and acid, and utmost care and attention to preventative measures. Other notable differences in the SSS include a low sample number with no replication, a 2h extraction time, and a single particle size fraction of <60 µm. For these reasons, the SSS bioaccessibility results would be expected to differ from results of this study.

Adjusting the bioaccessibility of a trace element for the relative mass contribution of their particle size fraction (and hence, relative surface area) to the total mass of each dust group provides a better estimate of risk. Particle size fractions with high Ni bioaccessibility but a small mass relative to the total make a smaller contribution to exposure than would be indicated by bioaccessibility alone. Weight-adjusted bioaccessibility was calculated according to the following equation:

\[
\text{Weight adjusted bioaccessibility} = \% \text{mass in each size fraction out of total mass in dust group} \times \text{Ni bioaccessibility} \%
\]

Raw data are provided in Appendix I and sample calculations are in Appendix VI. The highest weight adjusted percentages for Dust A, C, E were found in particles <10 µm, and between 70 and 105 µm for Dust B, D, and G (Figure 9).
Figure 9. Weight adjusted Ni bioaccessibility. Highest and lowest significantly different means for each dust group are shown, with standard error bars. There are no data for Group F.

Standard practice for human health risk assessments is to sieve to <250 µm to assess human exposure to pollutants in particulates. Sieving to <45 µm has been recommended as a better estimate of exposure, due to the tendency of that size fraction to adhere to hands and its elevated metal concentrations (Bright et al., 2006; Siciliano et al., 2009). Sieving to ≤70 µm has also been suggested to be more inclusive of soil composition (soil particles ≤ 70 µm included sand in addition to clay and silt found in particles ≤45 µm) (Vasiluk et al., 2011).
In this study, particles <10 µm and between 41 and 70 µm had the highest Ni bioaccessibility. Particles <10 µm and between 70 and 105 µm made the greatest contribution to exposure, after weight adjustment. These results corroborated suggestions that a more appropriate particle size cut-off for risk assessment may be smaller than <250 µm, based on what adheres to hands. To maximize estimated bioaccessible exposure, 105 µm is suggested as the upper limit, given the high Ni bioaccessibility and the high weight-adjusted values associated with particles <105 µm.

Concern about using glycine in bioaccessibility testing has been raised due to its ability to form aqueous complexes with Ni, which enhances extraction of Ni at pH of 7, and may overestimate bioaccessibility by four times (Fischer, Rainer, Bieniek, & Kettrup, 1992; Ontario Ministry of Environment, 2002). However, glycine did not form Ni complexes at typical gastric pH values of 1.5 or less (Ontario Ministry of Environment, 2002). Since the SRBC method used in this study has a pH of 1.5, overestimation in Ni bioaccessibility due to use of glycine was not expected. It is assumed that there was minimal Ni complexation in the bioaccessibility acid solution, and if present, consisted of highly insoluble forms such as oxides. In addition, large complexes may not have passed through the syringe filter.
The relationship between particle size and oral bioaccessibility of Ni in Sudbury house dust was determined in this study. It was found that both total and bioaccessible [Ni] increased as particle size decreased. As hypothesized, smaller particles contained higher [Ni] than larger particles did. The negative correlations between particle size and all three measures of Ni (total [Ni], bioaccessible [Ni], and bioaccessibility) showed that particle size was a factor in influencing house dust [Ni], especially bioaccessible [Ni]. The relationship between total [Ni] and particle size was dependent on the dust group. Total [Ni] and particle size explained 88% of the variance in bioaccessible [Ni]. Total [Ni] was more than twice as important as particle size in explaining variance in bioaccessible [Ni], and was by far the most important contributing factor, followed by particle size and the interaction term which had equal weight. These regression results can be predictive of similar bioaccessibility studies using the SBRC method and a different set of house dust samples containing elevated Ni; total [Ni] and particle size together could be expected to account for a similar amount of variance in Ni bioaccessibility. The current study corroborated the suggestion from prior research that a more appropriate particle size for risk assessment of oral ingestion should be much smaller than the <250 µm upper limit that is commonly used at the present. An upper limit of 105 µm is recommended due to the high Ni bioaccessibility and high relative mass contained in that size fraction.
REFERENCES


Niu, J., Rasmussen, P. E., Hassan, N. M., & Vincent, R. (2010). Concentration distribution and bioaccessibility of trace elements in nano and fine urban airborne particulate matter:


Appendix I

Mass (g) of house dust

<table>
<thead>
<tr>
<th>Particle size (µm)</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>150-250</td>
<td>3.34</td>
<td>3.64</td>
<td>2.15</td>
<td>3.36</td>
<td>1.59</td>
<td>2.69</td>
<td>0.702</td>
</tr>
<tr>
<td>105-150</td>
<td>2.32</td>
<td>2.24</td>
<td>1.29</td>
<td>2.34</td>
<td>1.47</td>
<td>3.73</td>
<td>0.664</td>
</tr>
<tr>
<td>70-105</td>
<td>3.98</td>
<td>6.60</td>
<td>2.23</td>
<td>4.60</td>
<td>2.98</td>
<td>2.17</td>
<td>1.63</td>
</tr>
<tr>
<td>41-70</td>
<td>0.253</td>
<td>2.64</td>
<td>0.535</td>
<td>1.86</td>
<td>0.736</td>
<td>1.33</td>
<td>0.693</td>
</tr>
<tr>
<td>10-41</td>
<td>0.243</td>
<td>3.59</td>
<td>1.46</td>
<td>3.53</td>
<td>0.632</td>
<td>2.80</td>
<td>0.194</td>
</tr>
<tr>
<td>&lt;10</td>
<td>7.58</td>
<td>0.0185</td>
<td>3.11</td>
<td>1.74</td>
<td>5.83</td>
<td>0.0007</td>
<td>0.761</td>
</tr>
<tr>
<td>Total mass</td>
<td>17.7</td>
<td>18.7</td>
<td>10.8</td>
<td>17.4</td>
<td>13.2</td>
<td>12.7</td>
<td>4.64</td>
</tr>
</tbody>
</table>

Relative mass (% of total mass) of house dust

<table>
<thead>
<tr>
<th>Particle size (µm)</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>150-250</td>
<td>0.189</td>
<td>0.194</td>
<td>0.199</td>
<td>0.193</td>
<td>0.120</td>
<td>0.211</td>
<td>0.151</td>
</tr>
<tr>
<td>105-150</td>
<td>0.131</td>
<td>0.120</td>
<td>0.120</td>
<td>0.135</td>
<td>0.111</td>
<td>0.293</td>
<td>0.143</td>
</tr>
<tr>
<td>70-105</td>
<td>0.225</td>
<td>0.352</td>
<td>0.207</td>
<td>0.264</td>
<td>0.225</td>
<td>0.171</td>
<td>0.351</td>
</tr>
<tr>
<td>41-70</td>
<td>0.0143</td>
<td>0.141</td>
<td>0.0497</td>
<td>0.107</td>
<td>0.0557</td>
<td>0.105</td>
<td>0.149</td>
</tr>
<tr>
<td>10-41</td>
<td>0.0137</td>
<td>0.192</td>
<td>0.136</td>
<td>0.202</td>
<td>0.0477</td>
<td>0.220</td>
<td>0.0419</td>
</tr>
<tr>
<td>&lt;10</td>
<td>0.428</td>
<td>0.000988</td>
<td>0.288</td>
<td>0.0996</td>
<td>0.441</td>
<td>0.000551</td>
<td>0.164</td>
</tr>
<tr>
<td>Total</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>
### Appendix II

Total Ni concentrations (mg/kg)

<table>
<thead>
<tr>
<th>Particle size (µm)</th>
<th>B</th>
<th>A</th>
<th>F</th>
<th>G</th>
<th>E</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>150-250</td>
<td>43.9*</td>
<td>57.8*</td>
<td>82.7</td>
<td>111*</td>
<td>151*</td>
<td>164*</td>
<td>301*</td>
</tr>
<tr>
<td></td>
<td>(9.21)</td>
<td>(19.2)</td>
<td>(16.4)</td>
<td>(38.1)</td>
<td>(27.9)</td>
<td>(145)</td>
<td>(105)</td>
</tr>
<tr>
<td>105-150</td>
<td>100</td>
<td>110</td>
<td>172</td>
<td>154</td>
<td>191</td>
<td>175</td>
<td>637**</td>
</tr>
<tr>
<td></td>
<td>(50.5)</td>
<td>(66.3)</td>
<td>(78.0)</td>
<td>(58.7)</td>
<td>(117)</td>
<td>(84.5)</td>
<td>(130)</td>
</tr>
<tr>
<td>70-105</td>
<td>147**</td>
<td>112</td>
<td>163</td>
<td>225</td>
<td>239</td>
<td>229</td>
<td>632</td>
</tr>
<tr>
<td></td>
<td>(56.5)</td>
<td>(51.8)</td>
<td>(38.1)</td>
<td>(78.7)</td>
<td>(74.8)</td>
<td>(66.6)</td>
<td>(76.1)</td>
</tr>
<tr>
<td>41-70</td>
<td>165</td>
<td>210**</td>
<td>190</td>
<td>279**</td>
<td>273</td>
<td>319</td>
<td>767</td>
</tr>
<tr>
<td></td>
<td>(24.0)</td>
<td>(28.3)</td>
<td>(37.5)</td>
<td>(125)</td>
<td>(29.5)</td>
<td>(54.7)</td>
<td>(36.9)</td>
</tr>
<tr>
<td>10-41</td>
<td>207</td>
<td>232</td>
<td>228</td>
<td>298</td>
<td>343**</td>
<td>467**</td>
<td>824</td>
</tr>
<tr>
<td></td>
<td>(29.4)</td>
<td>(41.2)</td>
<td>(133)</td>
<td>(56.0)</td>
<td>(14.4)</td>
<td>(55.4)</td>
<td>(117)</td>
</tr>
<tr>
<td>&lt;10</td>
<td>189†</td>
<td>200</td>
<td>No</td>
<td>209</td>
<td>256</td>
<td>383</td>
<td>832</td>
</tr>
<tr>
<td></td>
<td>(83.3)</td>
<td>sample</td>
<td>(87.8)</td>
<td>(39.9)</td>
<td>(70.1)</td>
<td>(44.1)</td>
<td></td>
</tr>
</tbody>
</table>

Dust groups are arranged from least to greatest total [Ni]. Means (in bold) and standard deviations (in parentheses) are shown. Single (lowest mean) and double asterisk values (highest mean) are significantly different.

† single measurement
## Appendix III

Bioaccessible Ni concentrations (mg/kg)

<table>
<thead>
<tr>
<th>Particle size (µm)</th>
<th>B</th>
<th>A</th>
<th>F</th>
<th>G</th>
<th>E</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>150-250</td>
<td><strong>12.6</strong></td>
<td><strong>13.1</strong></td>
<td><strong>21.9</strong></td>
<td><strong>22.9</strong></td>
<td><strong>25.0</strong></td>
<td><strong>34.7</strong></td>
<td><strong>47.9</strong></td>
</tr>
<tr>
<td></td>
<td>(0.974)</td>
<td>(3.34)</td>
<td>(3.89)</td>
<td>(5.40)</td>
<td>(0.462)</td>
<td>(0.537)</td>
<td>(6.81)</td>
</tr>
<tr>
<td>105-150</td>
<td><strong>27.0</strong></td>
<td><strong>23.4</strong></td>
<td><strong>31.7</strong></td>
<td><strong>44.7</strong></td>
<td><strong>36.1</strong></td>
<td><strong>43.5</strong></td>
<td><strong>69.4</strong></td>
</tr>
<tr>
<td></td>
<td>(2.44)</td>
<td>(3.04)</td>
<td>(6.37)</td>
<td>(7.41)</td>
<td>(0.934)</td>
<td>(2.25)</td>
<td>(7.58)</td>
</tr>
<tr>
<td>70-105</td>
<td><strong>42.0</strong></td>
<td><strong>34.2</strong></td>
<td><strong>43.5</strong></td>
<td><strong>55.0</strong></td>
<td><strong>47.1</strong></td>
<td><strong>57.0</strong></td>
<td><strong>122</strong></td>
</tr>
<tr>
<td></td>
<td>(2.20)</td>
<td>(1.13)</td>
<td>(3.79)</td>
<td>(5.70)</td>
<td>(1.84)</td>
<td>(0.429)</td>
<td>(6.83)</td>
</tr>
<tr>
<td>41-70</td>
<td><strong>75.7</strong></td>
<td><strong>45.6</strong></td>
<td><strong>57.6</strong></td>
<td><strong>93.4</strong></td>
<td><strong>57.8</strong></td>
<td><strong>85.2</strong></td>
<td><strong>166</strong></td>
</tr>
<tr>
<td></td>
<td>(2.56)</td>
<td>(2.66)</td>
<td>(0.640)</td>
<td>(4.30)</td>
<td>(1.45)</td>
<td>(9.74)</td>
<td>(14.7)</td>
</tr>
<tr>
<td>10-41</td>
<td><strong>80.9</strong></td>
<td><strong>47.9</strong></td>
<td><strong>65.3</strong></td>
<td><strong>104</strong></td>
<td><strong>78.8</strong></td>
<td><strong>110</strong></td>
<td><strong>184</strong></td>
</tr>
<tr>
<td></td>
<td>(6.26)</td>
<td>(1.02)</td>
<td>(3.15)</td>
<td>(3.72)</td>
<td>(7.62)</td>
<td>(7.23)</td>
<td>(4.63)</td>
</tr>
<tr>
<td>&lt;10</td>
<td>No data</td>
<td><strong>70.9</strong></td>
<td>No</td>
<td><strong>91.1</strong></td>
<td><strong>67.3</strong></td>
<td><strong>115</strong></td>
<td><strong>157</strong></td>
</tr>
<tr>
<td></td>
<td>(1.08)</td>
<td>sample</td>
<td>(1.70)</td>
<td>(2.93)</td>
<td>(6.81)</td>
<td>(0.566)</td>
<td></td>
</tr>
</tbody>
</table>

Dust groups are arranged from least to greatest bioaccessible [Ni]. Means (in bold) and standard deviations (in parentheses) are shown. Single (lowest mean) and double asterisk values (highest mean) are significantly different.
## Appendix IV
SAS output for multiple linear regression model with interaction

<table>
<thead>
<tr>
<th></th>
<th>Adjusted $R^2$</th>
<th>Root MSE</th>
<th>Sum of squares</th>
<th>DF</th>
<th>Mean square</th>
<th>$F$ value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model</strong></td>
<td>0.895</td>
<td>13.3</td>
<td>150482</td>
<td>3</td>
<td>50161</td>
<td>284</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Error</strong></td>
<td></td>
<td></td>
<td>17158</td>
<td>97</td>
<td>177</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Corrected Total</strong></td>
<td></td>
<td></td>
<td>167640</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unstandardized coefficients</th>
<th>Standardized coefficients</th>
<th>$t$ value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>36.4</td>
<td>0</td>
<td>8.27</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total [Ni]</td>
<td>0.184</td>
<td>0.881</td>
<td>15.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Particle size</td>
<td>-0.111</td>
<td>-0.210</td>
<td>-3.89</td>
<td>0.0002</td>
</tr>
<tr>
<td>Total [Ni] x Particle size</td>
<td>-0.000379</td>
<td>-0.180</td>
<td>-3.30</td>
<td>0.0014</td>
</tr>
</tbody>
</table>
**Appendix V**

Ni bioaccessibility

(bioaccessible [Ni]/total [Ni]*100%)

<table>
<thead>
<tr>
<th>Particle size (µm)</th>
<th>B</th>
<th>A</th>
<th>F</th>
<th>G</th>
<th>E</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>150-250</td>
<td>28.6%*</td>
<td>22.7%</td>
<td>26.5%</td>
<td>20.6%*</td>
<td>16.6%*</td>
<td>21.2%*</td>
<td>15.9%</td>
</tr>
<tr>
<td></td>
<td>(2.23)</td>
<td>(5.77)</td>
<td>(4.69)</td>
<td>(4.86)</td>
<td>(0.309)</td>
<td>(0.329)</td>
<td>(2.26)</td>
</tr>
<tr>
<td>105-150</td>
<td>26.9%</td>
<td>21.3%*</td>
<td>18.4%</td>
<td>29.1%</td>
<td>18.9%</td>
<td>24.9%</td>
<td>10.9%*</td>
</tr>
<tr>
<td></td>
<td>(2.43)</td>
<td>(2.76)</td>
<td>(3.71)</td>
<td>(4.82)</td>
<td>(0.488)</td>
<td>(1.29)</td>
<td>(1.19)</td>
</tr>
<tr>
<td>70-105</td>
<td>28.6%</td>
<td>30.5%**</td>
<td>26.7%</td>
<td>24.5%</td>
<td>19.7%</td>
<td>24.9%</td>
<td>19.3%**</td>
</tr>
<tr>
<td></td>
<td>(1.50)</td>
<td>(1.01)</td>
<td>(2.32)</td>
<td>(2.53)</td>
<td>(0.770)</td>
<td>(0.189)</td>
<td>(1.08)</td>
</tr>
<tr>
<td>41-70</td>
<td>46.0%**</td>
<td>21.7%</td>
<td>30.3%</td>
<td>33.5%</td>
<td>21.2%</td>
<td>26.7%</td>
<td>21.7%</td>
</tr>
<tr>
<td></td>
<td>(1.56)</td>
<td>(1.26)</td>
<td>(0.339)</td>
<td>(1.54)</td>
<td>(0.532)</td>
<td>(3.05)</td>
<td>(1.92)</td>
</tr>
<tr>
<td>10-41</td>
<td>39.1%</td>
<td>20.6%</td>
<td>28.6%</td>
<td>34.8%**</td>
<td>23.0%**</td>
<td>23.5%</td>
<td>22.3%</td>
</tr>
<tr>
<td></td>
<td>(3.03)</td>
<td>(0.438)</td>
<td>(1.38)</td>
<td>(1.25)</td>
<td>(2.22)</td>
<td>(1.55)</td>
<td>(0.561)</td>
</tr>
<tr>
<td>&lt;10</td>
<td>No data</td>
<td>35.5% sample</td>
<td>43.5%</td>
<td>26.3%</td>
<td>30.1%**</td>
<td>18.9%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.541)</td>
<td>(0.814)</td>
<td>(1.15)</td>
<td>(1.78)</td>
<td>(0.0680)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Standard deviations are in parentheses. Single (lowest mean) and double asterisk values (highest mean) are significantly different.
Appendix VI
Weight-adjusted Ni bioaccessibility

<table>
<thead>
<tr>
<th>Particle size (µm)</th>
<th>B</th>
<th>A</th>
<th>F</th>
<th>G</th>
<th>E</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>150-250</td>
<td>5.56%</td>
<td>4.29%</td>
<td>5.61%</td>
<td>3.12%</td>
<td>1.99%*</td>
<td>4.23%</td>
<td>3.07%</td>
</tr>
<tr>
<td></td>
<td>(0.432)</td>
<td>(1.09)</td>
<td>(0.992)</td>
<td>(0.735)</td>
<td>(0.0370)</td>
<td>(0.0654)</td>
<td>(0.437)</td>
</tr>
<tr>
<td>105-150</td>
<td>3.23%*</td>
<td>2.79%</td>
<td>5.40%</td>
<td>4.16%</td>
<td>2.10%</td>
<td>2.99%</td>
<td>1.47%*</td>
</tr>
<tr>
<td></td>
<td>(0.291)</td>
<td>(0.362)</td>
<td>(1.09)</td>
<td>(0.689)</td>
<td>(0.0542)</td>
<td>(0.155)</td>
<td>(0.160)</td>
</tr>
<tr>
<td>70-105</td>
<td>10.1%**</td>
<td>6.85%</td>
<td>4.55%</td>
<td>8.58%**</td>
<td>4.43%</td>
<td>5.16%</td>
<td>5.09%**</td>
</tr>
<tr>
<td></td>
<td>(0.527)</td>
<td>(0.227)</td>
<td>(0.396)</td>
<td>(0.889)</td>
<td>(0.173)</td>
<td>(0.0391)</td>
<td>(0.285)</td>
</tr>
<tr>
<td>41-70</td>
<td>6.48%</td>
<td>0.310%*</td>
<td>3.17%</td>
<td>4.99%</td>
<td>1.18%</td>
<td>1.33%*</td>
<td>2.32%</td>
</tr>
<tr>
<td></td>
<td>(0.220)</td>
<td>(0.0180)</td>
<td>(0.0354)</td>
<td>(0.230)</td>
<td>(0.0296)</td>
<td>(0.151)</td>
<td>(0.205)</td>
</tr>
<tr>
<td>10-41</td>
<td>7.50%</td>
<td>0.282%</td>
<td>6.31%</td>
<td>1.46%*</td>
<td>1.10%</td>
<td>3.19%</td>
<td>4.51%</td>
</tr>
<tr>
<td></td>
<td>(0.581)</td>
<td>(0.00600)</td>
<td>(0.304)</td>
<td>(0.0524)</td>
<td>(0.106)</td>
<td>(0.210)</td>
<td>(0.114)</td>
</tr>
<tr>
<td>&lt;10</td>
<td>No data</td>
<td>15.2%**</td>
<td>No</td>
<td>7.14%</td>
<td>11.6%**</td>
<td>8.67%**</td>
<td>1.88%</td>
</tr>
<tr>
<td></td>
<td>(0.232)</td>
<td>sample</td>
<td>(0.133)</td>
<td>(0.506)</td>
<td>(0.513)</td>
<td>(0.00678)</td>
<td></td>
</tr>
</tbody>
</table>

Standard deviations are in parentheses. Single (lowest mean) and double asterisk values (highest mean) are significantly different.

Sample calculation using Group A <10 µm fraction:

Weight adjusted bioaccessibility %
= % mass in each size fraction out of total mass in dust group x Ni bioaccessibility %
= 0.428 x 35.5%
= 15.2%