Human Health Risk Assessment

Appendix 5

Bioaccessibility and Bioavailability of Metals Following Ingestion of Rodney Street Community Soils / Dusts and Other Environmental Media (Food, Drinking Water)

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A5-1 Bioavailability and Bioaccessibility

In risk assessment, oral exposures are typically stated in terms of the external dose or intake, instead of in terms of absorbed dose or uptake. Most regulatory exposure limits are expressed as intakes. The distinction between intake and uptake is an important one. The process of a chemical entering the body can be described in two steps - contact with an outer boundary (exposure or intake), followed by actual entry (absorption across the boundary into the bloodstream leading to some amount of the chemical reaching target tissues in the body, this is also called uptake). Intake is typically defined as the process by which an agent crosses the outer exposure surface of a human or animal *without passing an absorption barrier* (e.g., through ingestion or inhalation or soil contact with the skin), while uptake is the process by which an agent crosses an absorption barrier (the lining of the digestive system, the outer layer of the skin, or the lining of the nose, throat and lungs) into human or animal (IPCS, 2000).

In broad terms, the bioavailability ("absolute bioavailability") of a compound can be defined as the fraction of an administered dose that reaches the central (blood) compartment, whether through the gastrointestinal tract, skin, or lungs (NEPI, 2000). Bioavailability values are measured *in vivo* using animals or human volunteers. However, when evaluating potential differences in the bioavailability of a compound that is encountered *via* different routes of exposure, it is also useful to understand the "relative bioavailability" of the compound. Relative bioavailability refers to comparative bioavailabilities of different forms of a compound (e.g., metal species) or for different exposure media containing the chemical (e.g., bioavailability of a chemical bound to soil *versus* its bioavailability in water) (NEPI, 2000). Relative bioavailability is typically expressed as a relative absorption factor (RAF), which provides the absorbed fraction of the compound from a particular exposure medium relative to the fraction absorbed from the dosing vehicle used in the toxicity study for that particular compound (NEPI, 2000). Consideration of relative bioavailability allows variations in bioavailability of a chemical in a different exposure media to be adjusted to make intakes comparable.

Bioaccessibility is the fraction of a chemical in an environmental medium that is available for absorption based on *in vitro* extraction *but not necessarily absorbed*. This depends on the relation between *in vitro* chemical extraction systems and what is measured *in vivo* (in animals or humans). *In vitro* extraction methods were developed as an inexpensive alternative to more expensive *in vivo* experiments (Ruby et al., 1999). The term "bioaccessible" will be used to indicate the *in vitro* fraction of the chemical intake that is directly available for absorption. As the current assessment is primarily driven by the consideration of nickel ingestion, the focus of this discussion will be on the use of bioaccessibility in estimating oral exposures to nickel in different exposure media.

When considering exposure to a metal or other chemical contained in a complex environmental matrix such as soil, there are several aspects of how exposure to the chemical may be estimated.

First, there is the total chemical content of the soil. This is measured by harsh *in vitro* chemical extraction. Second, there is the fraction of the chemical which is extractable using gentler, *in vitro* chemical extraction procedures which simulate chemical conditions in the GI tract (the bioaccessible fraction). Third, there is the fraction of the chemical in the soil that can be absorbed *in vivo* (the bioavailable fraction).

Exposure assessment of the oral route of exposure considers ingestion of food, water and soil. The default relative bioavailability factor for intake estimates from ingestion is 1.0 in different exposure media. Use of this default value assumes that the bioavailability of the chemical being evaluated in any exposure medium will always be the same as its bioavailibilty using a different exposure medium than the one being assessed. More recently, as our understanding of how chemicals behave as they pass through the digestive tract is applied to exposure assessment methodologies, variations in relative bioavailability and the bioaccessible fraction of ingested chemicals is being taken into account. The US EPA has indicated that when the oral exposure limit is based on studies where the bioavailibilty of the chemical administered to the test animals differs from the exposure medium being considered, some adjustment of the oral exposure estimate is permitted (US EPA, 1989, 2001). What this means, in the case of soil ingestion, is that not all the metal that can be extracted from the soil using chemical methods that simulate the stomach and intestines is actually absorbed into the body, and that we can adjust the intake estimate using this information to account for differences in the relative bioavailability between that used in the toxicity study used to derive the R*f*D compared with the chemical in soil.

In the case of many metals, pH is an important variable controlling the bioaccessible fraction of ingested metals. As ingested materials containing these metals pass through the digestive tract, the low (acid) pH of the stomach can increase the bioaccessible fraction considerably while passing through this part of the digestive tract. After the stomach, the digestive tract reverts to higher pH (neutral to alkaline) and the bioaccessible fraction of metals may change. Investigators have tried to simulate conditions in the digestive tract experimentally using *in vitro* tests. These involve various degrees of complexity from simple acid solutions to controlled pH regimes and the addition of digestive enzymes, bile, food and other components of digestion (Ellickson et al., 2001, Rodriguez et al., 1999, Ruby et al., 1999). It should be kept in mind that the pH of the stomach can vary from pH 1 to pH 1.5 under fasting conditions and up to pH 4 during active digestion of food. The transit time of ingested materials is related to gastric emptying and can vary from less than an hour for liquids to over three hours for solid food. Whether the food is carbohydrate, protein or fat can also control transit time. Consequently, *in vitro* stomach acid tests using pH 1 to pH 1.5 represent fasting stomach conditions and are highly conservative. Bioaccessibility measured *in vitro* using simulated GI tract fluids may be a nonequilibrium process given the limited time for passage through the GI tract which may not allow the metal to completely dissolve. The validity of some *in vitro* models has been assessed by comparison with *in vivo* bioavailability assays in test animals.

Bioaccessibility of a metal is also affected by a large range of soil properties including particle size, whether the metal is part of the crystalline structure of a mineral in the soil or is adsorbed onto the surface of soil components, and soil pH. As noted in section A5-6, the preparation of the soil sample for bioaccessibility testing is important and should reflect as closely as possible the state of the soil that is likely to be ingested. Since hand-to-mouth activity accounts for most soil ingestion, particles of a size range that are most likely to adhere to skin on the hands, i.e., that pass through a 250 µm mesh screen, should be tested.

For nickel, our knowledge of oral bioavailability is based on soluble nickel salts administered to test animals and human subjects in the diet or in water (see discussion in sections A5-2 and A5-3). There is little information on oral exposure to insoluble nickel compounds. These studies indicate that soluble nickel salts administered under fasting conditions (stomach pH about 1), results in up to 30% absolute bioavailability (uptake will be 30% of intake). Under non-fasting conditions and in the presence of food (stomach pH about 4), bioavailability (uptake) of soluble nickel salts is much lower (2% to 5%). Limited information indicates that the bioavailability of insoluble nickel compounds is < 1%. Based on the data of Griffin et al., (1990) and Ishimatsu et al., (1995), it is assumed that soil bound insoluble nickel would be less bioavailable than soluble forms.

This appendix describes the available literature on the *in vivo* bioavailability (absolute and relative) of metals, particularly nickel. It should be noted that the focus of the exposure assessment part of the risk assessment is in developing intake estimates, not uptake estimates. The use of *in vitro* bioaccessiblity factors based on soil sieved to 250 µm(which is more likely to adhere to the skin and be transferred to the mouth by children) to adjust soil ingestion intakes will tend to overestimate relative bioavailability factors, and is a conservative and realistic method of estimating metal intakes from soil ingestion.

A5-2 Bioavailability of Nickel Ingested in Water or Food

As discussed in section A2-9, an important issue relating to nickel toxicity is its speciation (metallic, salt, oxide, etc.) and the solubility of different nickel compounds which strongly influences their absorption. There is little information on oral exposure to insoluble nickel compounds and it is assumed that insoluble forms of nickel would be less accessible than soluble forms. Several studies have indicate a lower bioavailability of insoluble forms of nickel are, Ishimatsu et al., (1995) and Griffin et al., (1990). These studies are cited and described in sections A5-3 and A5-4. None of these studies address *in vitro* bioaccessibility of nickel, though Griffin et al., (1990) provide indirect information on the relative bioavailability of soluble nickel in drinking water versus soil mixtures.

There are several studies of nickel administered to human volunteers where urinary excretion of nickel was measured and absorption of the administered dose was calculated. A recent review

summarized most of these studies (Diamond et al., 1998). The following table (Table A5-1) updates that review. It should be noted that all estimates of % bioavailability of nickel which are primarily based on cumulative urinary excretion of nickel were calculated using simplified pharmacokinetic models or the cumulative excretion of nickel as a percentage of the ingested dose over three, four or five days.

The studies not described by Diamond et al., (1998) address orally administered isotopes of nickel (Templeton et al., 1994b; Patriarca et al., 1997; Nielsen et al., 1999). Templeton et al., (1994b) administered ^{61}Ni orally in water to a human volunteer after an overnight fast. Urinary excretion of ^{61}Ni demonstrated absorption of 30% of the administered dose. Pairs of human subjects were administered ^{62}Ni in water (Patriarca et al., 1997); urinary and fecal excretion of ^{62}Ni followed for five days. Gastrointestinal absorption of ^{62}Ni was estimated as the difference between ingested nickel and nickel excreted in urine and the feces. The absorbed dose was 33.1± 4.9%. Nielsen et al., (1999) performed two studies to examine the influence of fasting and food intake on the absorption and retention of nickel added to drinking water. In the first study, volunteers drank nickel dissolved in water either four hours or 1.5 hours before or during a scrambled egg breakfast, or 0.5 to one hour after the breakfast. The results show that bioavailability is lowest when nickel is co-administered with food, intermediate when food is taken within about an hour before or after the food and highest (approaching the high bioavailability shown by the fasting stomach) at four hours after eating.

All of these bioavailability studies involve ingestion of soluble nickel either in drinking water or mixed into food. Inspection of Table A5-1 shows fairly clearly that gastrointestinal absorption of soluble nickel in drinking water is maximal at 23% to 33% under fasting conditions (Cronin et al., 1980; Nielsen et al., 1999; Patriarca et al., 1997; Sunderman et al., 1989; Templeton et al., 1994b). Absorption of nickel is much lower in the presence of food (Christensen and Lagesson, 1981; Gawkrodger et al., 1986; Horak and Sunderman, 1973; McNeely et al., 1972; Menne et al., 1978; Nielsen et al., 1999; Spruit and Bongaarts, 1977; Sunderman et al., 1989). It is not clear whether the lowered absorption of nickel in the presence of food is due to nickel binding to food components or pH changes in the stomach associated with the presence of food. Patterns of peristalsis and gastric emptying into the intestines during ingestion may influence nickel absorption (Nielsen et al., 1999). No differences in nickel absorption related to gender or nickel sensitivity were found (Menne et al., 1978; Nielsen et al., 1999; Patriarca et al., 1997).

A5-3 Oral Bioavailability of Nickel in Animals

Studies in mice, rats and dogs indicate that over10% of nickel, given as soluble nickel, nickel sulphate, or nickel chloride in the diet or by gavage, is rapidly absorbed by the gastrointestinal tract (Ambrose et al., 1976; Ho and Furst 1973; Ishimatsu et al.,1995; Nielsen et al., 1993; Phatak and Patwardhan, 1952; Severa et al., 1995; Tedeschi and Sunderman, 1957). Many of these studies with high oral bioavailability results only tested single doses up to 48 hours,

whereas long term studies suggested that overall oral bioavailability is much lower (Ambrose et al., 1976; Severa et al., 1995).

Absorption by the gastrointestinal tract depends on the solubility of the nickel compound. In a study in which rats were treated with a single gavage dose of a nickel compound (10 mg nickel) in a 5% starch saline solution, the absorption was found to be directly correlated with the solubility of the compound (Ishimatsu et al., 1995). The percentages of the dose absorbed were 0.01% for green nickel oxide, 0.09% for metallic nickel, 0.04% for black nickel oxide, 0.47% for nickel subsulphide, 11.12% for nickel sulphate, 9.8% for nickel chloride, and 33.8% for nickel nitrate. Absorption was higher for the more-soluble nickel compounds. Unabsorbed nickel is excreted in the feces.

$^{57}NiCl_2$ was administered either orally by gastric intubation or by intraperitoneal injection to *ad libitum* fed mice in doses equivalent to the average human daily dietary nickel intake (Nielsen et al., 1993). Whole body retention was measured by whole body counting of the radiation emitted by ^{57}Ni. The intestinal absorption was estimated to be 1.7% to 10% after 20 to 50 hours.

Wistar strain rats were given 100 mg Ni/L (as nickel sulphate) in drinking water for six months (Severa et al., 1995). The urinary excretion of the orally administered nickel was estimated to be 2% assuming a 1% absorption of the administered dose, however, recalculation of the reported nickel concentrations in blood and urine suggest that the actual uptake may have been much lower than 1%.

A5-4 Bioavailability of Nickel Ingested in Soil

The oral bioavailability of nickel in soil is not well characterized. Currently, there are no published reports on the human bioavailability of nickel in soil. In the only study available, Griffin et al., (1990) compared the oral bioavailability of nickel chloride, a soluble salt, administered to rats either as an aqueous slurry with sandy loam, or clay loam soil, or in water. Nickel bioavailability was estimated from levels of nickel in the blood. The soil bound nickel had a reduced bioavailability relative to nickel chloride in water. The relative bioavailabilities of nickel in the sandy and clay loam slurries were 63% and 34%, respectively. The mean absolute bioavailabilities were 3% and 1.5% for the sandy and clay loam slurries, respectively. These absolute bioavailabilities are similar to those reported for nickel administered in animal diets.

A5-5 Bioavailability and Bioaccessibility of Other Metals in Soil

Recent papers discussing the oral bioavailability of metals in soil as assessed by *in vitro* and *in vivo* techniques focus on arsenic and lead because of their large historic database (Ellickson et al., 2001; Hamel et al., 1998; Ruby et al., 1999). For both lead and arsenic, the bioavailability in soil has been shown to be reduced when compared to the bioavailability of soluble forms, i.e., the relative bioavailability is less than the default value of 1.0. *In vivo* and *in vitro* studies have yielded similar results (Ruby et al., 1999).

Table A5-1: Summary of Studies to Determine Oral Bioavailability of Soluble Nickel Salts

Study	n	Exposure medium	Duration	Fasting Status	% Bioavailability
Sunderman et al., 1989	88	water breakfast	acute acute	fasted fasted	29.3 1.8
Cronin et al., 1980	5	gelatin capsule + 100 mL of water	acute	fasted	12 - 32
Christensen and Lagesson, 1981	8	capsule	acute	w/meal	5.7
Gawkrodger et al., 1986	3	capsule	acute	w/meal	2.7, 2.8
Menne et al., 1978	6	capsule	acute	not fasted	2.2 (women)
Menne et al., 1978	7	capsule	acute	not fasted	1.7 (men)
Spruit and Bongaarts, 1977	1	solution	acute	not fasted	0.8
Horak and Sunderman, 1973	10 - 50	food	chronic	not fasted	1
McNeely et al., 1972	1920	food + water food	chronic chronic	not fasted not fasted	1.6 1.2
Templeton et al., 1994a	1	1 L water	acute	fasted	30
Patriarca et al., 1997	4	capsules + water	acute	fasted	33.1 (men and women)
Nielsen et al., 1999	1	eggs 4.5 hours prior to Ni in 250 mL water	acute	fasted	23.2 (men)
Nielsen et al., 1999	1	eggs 1.5 hours prior to Ni in 250 mL water	acute	fasted	7.1 (men)
Nielsen et al., 1999	1	Ni in 250 mL water and eggs 0.5 or 1hour later	acute	fasted	12.8 (men) 16.7 (men)
Nielsen et al., 1999	1	Ni in 250 mL water and eggs together	acute	fasted	3.4 (men) 2.3 (men)
Nielsen et al., 1999	20 20	Ni in 250 mL water and eggs 4 hours later	acute acute	fasted fasted	10.8 (1.8 - 29.5) (women) 11.3 (4 - 25.1) (women)

A5-6 *In vitro* Bioaccessibility Testing

In vitro methods for assessing oral bioavailability of heavy metals found in impacted soils and subsequently in exposure assessments of the human digestive system have been under active development for the past decade. As noted in section A5-1, *in vitro* methods may overestimate *in vivo* measurements. Establishment of validated models for extraction of metals in digestive fluids to provide a tool for determining the metal exposure from any soil has been the focus of a number of groups (US Naval Facilities Engineering Command, 2000, Solubility/Bioavailability Research Consortium (Ruby et al., 1999), NEPI, 2000). Imitation biological fluids of the digestive tract can be prepared chemically, and their extraction capabilities examined to provide a measure of the maximum amount of the metal that can be made accessible in the gastrointestinal tract (bioaccessible). However, the amount that may cross the digestive tract membranes and be absorbed into the tissues requires an *in vivo* method. The artificial fluids evaluated in various studies of sequential extraction are 1) saliva, 2) gastric juice, and 3) duodenal fluid, which represent the major portions of the gastrointestinal tract involved in metal uptake by the body.

Ingested metals are transported by saliva to the stomach where the low pH enhances the dissolution of many metals. The first region of the small intestine, the duodenum, is the primary site of absorption of most metals, however, the conditions in the mouth and stomach may be important preconditions to uptake in this portion of the intestine. Enzymes in the prepared bio-fluids, the soil type chosen, and the soil matrix appear to also play a role in extraction capacity. Accurate simulation of the human digestive system includes consideration of fluid volumes, pH, additional digestive components (enzymes, salts, etc.), peristaltic behavior (fasting vs. presence and type of food) and residence time.

Saliva is 99.5% water and contains sodium, potassium and chloride as the dominant ions. The main digestive enzymes are ptyalin (salivary amylase) secreted by the salivary glands. Lingual glands secrete a lipase. Saliva also contains mucin, a glycoprotein which lubricates the food. The optimal pH for ptyalin is pH 6.7. The normal flow rate of saliva ranges from 0.25 mL/min in the un-stimulated state up to 1 mL/min following stimulation by chewing. Consequently, over a one hour period the maximal production of saliva would be about 60 mL. The role of saliva in absorbing metals in the mouth does not appear to have been studied widely but since the pH of saliva is close to neutral, it is not expected to facilitate metal dissolution from soil.

Gastric secretion is stimulated by the presence of food in the stomach. Gastric acid contains 0.2% to 0.5% HCl (approx. 0.17 N HCl). The lowest pH is 0.87, but the resting human stomach is pH 1 to pH 1.5. Gastric fluid is mainly water with mucin, inorganic salts, pepsin (activated by HCl), rennin and gastric lipases. The stomach can hold two to three L, and produces up to 2.5 L of gastric fluids daily (ICRP, 1984, Ganong, 1989).

The small intestine is the largest part of the gastrointestinal tract and is composed of the duodenum which is about one foot long, jejunum (five - eight feet long), and ileum (16 - 20 feet long). The duodenum is the major portion of the small intestine where enzyme secretion takes

place. The small intestine secretes sucrase, maltase, and lactase. It also secretes peptidase, and lipase. The duodenum receives bile from the liver and gallbladder. Lipase, amylase, trypsin, chymotrypsin and sodium bicarbonate to neutralize the acid chyme from the stomach are received from the pancreas. Epithelial cells in the small intestine secrete several litres/day of a neutral fluid. Brunner's glands in the duodenum secrete mucus. Almost 90% of the daily fluid intake is absorbed in the small intestine.

Altogether food can remain in the small intestine between three - ten hours normally. Depending on what is in the stomach (water, solid food or both), gastric emptying into the duodenum can occur within 75 minutes to 3.5 hours (up to five hours) (ICRP, 1984, Ganong, 1989).

MOE used two methods to assess the *in vitro* bioaccessibility of metals of concern in Rodney Street community soils. Initially MOE used fasting stomach simulation tests based on 24 hour extractions, which are described in section A5-6.1. In addition, to validate the MOE fasting stomach simulation tests, and to account for metal bioaccessibility in other parts of the gastrointestinal tract, Rodney Street community soil samples were also sent out for testing by Exponent using the Standard Operating Protocol developed by the Solubility / Bioavailability Research Consortium (Ruby et al., 1999). This protocol has been validated by comparison to animal studies for arsenic and lead, but not for nickel. The results of the bioaccessiblity testing by the external laboratory are reported in section A5-6.2.

A5-6.1 Simulated Stomach Acid Leachate Tests

MOE adapted the traditional Leachate Extraction Procedure (formerly described in Reg. 347), which is based on the Toxicity Characteristic Leaching Procedure, Method 1311 that appears in the United States Environmental Protection Agency Publication SW-846 entitled "Test Methods for Evaluating Solid Waste, Physical/Chemical Methods", as amended from time to time and available online at http://www.epa.gov/epaoswer/hazwaste/test/main.htm to simulate leaching of metals from soil under the acidic conditions found in the stomach. This method was originally designed to investigate the leaching behavior of various hazardous chemicals in municipal, industrial and hazardous wastes.

Soil samples were analyzed for bioaccessibility utilizing this adapted leaching method to simulate stomach digestion conditions. In this method bioaccessibility is estimated by comparing the amount of total metal extracted by routine ICP analysis with the amount of metal extracted from the soil samples using the simulated stomach acid digestion. The MOE Leachate Extraction Procedure was modified as follows:

- The 0.5 N acetic acid was replaced with 0.17 N HCL;
- 20 g dry weight soil sample used instead of 50 g;
- 400 ml acid used instead of 800 ml acid; and
- Initial pH adjusted to pH 1 instead of pH 5.

This approach has been used to assess the amount of metal that would be released into the

stomach from soil samples in other MOE community exposure studies.

Ten soil samples from the Rodney Street community containing very high levels of nickel were selected for simulated stomach acid leach testing. For each soil sample, 20g of dried, sieved material (355 µm mesh size) was added to 400 ml of 0.17 N HCl (pH 1.0). The samples were agitated for 24 hours on a rotary extractor. The mixture was then filtered through a 4.5 micron filter and the filtrate was analyzed for metals and hydrides. For each sample, the percentage leached was calculated by dividing the mass of the metal in the leachate by the mass in the original soil sample and then multiplying the ratio by 100. The results of the analyses are shown in Tables A5-2 through A5-7.

It is recognized that the use of a 24 hour digestion period, which is longer than the typical residency times for food in the stomach, will overestimate the amount of metal that will be released and available. However, these simulated stomach acid leach tests were run at room temperature not at body temperature. Subsequent extractions using this method at higher temperatures and for shorter time periods (not reported) did not result in substantially reduced results. It was believed that as a precautionary measure, that the potential overestimation of exposures was justified.

The Simulated Stomach Acid Leachate Test results displayed below (Tables A5-2 to A5-7) are the same laboratory data used in the March 2001 report. However, the data have been re-calculated to reflect the mass of metal extracted as a percentage of the mass of metal in the soil sample. Beryllium and cadmium levels in leachate were below detection limits.

Table A5-2: Simulated Stomach Acid Leachate Test: Antimony

Level in Soil (ppm)[1]	Mass in 20 g (µg)	Amount Leached (ppm)[1]	Mass in Leachate (µg)	% Leached by Mass
2.5	49.1	0.004	1.6	3.3
1.8	36.4	0.0035	1.4	3.9
2.1	41.9	0.0033	1.3	3.2
2.3	46.7	0.0033	1.3	2.8
2.8	56.5	0.0036	1.4	2.6
2.5	50.5	0.003	1.2	2.4
2.2	44.2	0.0033	1.3	3.0
2.0	40.2	0.0026	1.0	2.6
2.4	48.3	0.0025	1.0	2.1
2.1	41.0	0.0023	0.9	2.2
Averaged Values				
2.3	45.5	0.00314	1.3	2.8
Minimum % Leached				2.1
Maximum % Leached				3.9

[1] ppm is equivalent to µg/g in soil and µg/ml in leachate.
Note: numbers may not equate due to rounding.

Table A5-3: Simulated Stomach Acid Leachate Test: Arsenic

Level in Soil (ppm)[1]	Mass in 20 g (µg)	Amount Leached (ppm)[1]	Mass in Leachate (µg)	% Leached by Mass
52.0	1040	0.7	281.6	27.1
39.1	782	0.6	222.4	28.4
45.0	900	0.6	230.4	25.6
50.2	1004	0.7	275.6	27.5
63.1	1262	0.4	158.4	12.6
44.8	896	0.4	154.4	17.2
43.1	862	0.4	172.0	20.0
42.2	844	0.5	210.4	24.9
62.3	1246	0.5	217.6	17.5
37.6	752	0.5	205.6	27.3
Averaged Values				
47.9	958.8	0.5	212.8	22.8
Minimum % Leached				12.6
Maximum % Leached				28.4

[1] ppm is equivalent to µg/g in soil and µg/ml in leachate.

Note: numbers may not equate due to rounding.

Table A5-4: Simulated Stomach Acid Leachate Test: Cobalt

Level in Soil (ppm)[1]	Mass in 20g (µg)	Amount Leached (ppm)[1]	Mass in Leachate (µg)	% Leached by Mass
200	4000	2.0	784	19.6
180	3600	1.7	664	18.4
130	2600	1.2	468	18.0
140	2800	1.2	492	17.6
210	4200	2.4	940	22.4
160	3200	1.7	684	21.4
220	4400	1.7	676	15.4
150	3000	1.9	740	24.7
230	4600	1.4	576	12.5
120	2400	1.3	516	21.5
Averaged Values				
174	3480	1.6	654	19.2
Minimum % Leached				12.5
Maximum % Leached				24.7

[1] ppm is equivalent to µg/g in soil and µg/ml in leachate.
Note: numbers may not equate due to rounding.

Table A5-5: Simulated Stomach Acid Leachate Test: Copper

Level in Soil (ppm)[1]	Mass in 20g (µg)	Amount Leached (ppm)[1]	Mass in Leachate (µg)	% Leached by Mass
990	19800	17.2	6880	34.8
770	15400	17.1	6840	44.4
1000	20000	19.1	7640	38.2
780	15600	14.2	5680	36.4
1000	20000	15.9	6360	31.8
840	16800	14.7	5880	35.0
1000	20000	20.5	8200	41.0
980	19600	20.7	8280	42.2
970	19400	16.1	6440	33.2
640	12800	14.0	5600	43.8
Averaged Values				
897	17940	17.0	6780	38.1
Minimum % Leached				31.8
Maximum % Leached				44.4

[1] ppm is equivalent to µg/g in soil and µg/ml in leachate.

Note: numbers may not equate due to rounding.

Table A5-6: Simulated Stomach Acid Leachate Test: Lead

Level in Soil (ppm)[1]	Mass in 20g (µg)	Amount Leached (ppm)[1]	Mass in Leachate (µg)	% Leached by Mass
400	8000	15.6	6240	78.0
480	9600	21.1	8440	87.9
350	7000	12.8	5120	73.1
310	6200	11.1	4440	71.6
400	8000	13.3	5320	66.5
370	7400	14.4	5760	77.8
300	6000	9.17	3668	61.1
350	7000	11.4	4560	65.1
360	7200	15.4	6160	85.6
290	5800	13.1	5240	90.3
Averaged Values				
361	7220	13.7	5495	75.7
Minimum % Leached				61.1
Maximum % Leached				90.3

[1] ppm is equivalent to µg/g in soil and µg/ml in leachate.

Note: numbers may not equate due to rounding.

Table A5-7: Simulated Stomach Acid Leachate Test: Nickel

Level in Soil (ppm)[1]	Mass in 20g (μg)	Amount Leached (ppm)[1]	Mass in Leachate (μg)	% Leached by Mass
8800	176000	86.2	34480	19.6
9200	184000	107	42800	23.3
11000	220000	93	37200	16.9
11000	220000	87.9	35160	16.0
12000	240000	88.5	35400	14.8
13000	260000	96.9	38760	14.9
14000	280000	127	50800	18.1
14000	280000	115	46000	16.4
16000	320000	104	41600	13.0
17000	340000	99.9	39960	11.8
Average				
12600	252000	100.5	40216	16.5
Minimum % Leached				11.8
Maximum % Leached				23.3

[1] ppm is equivalent to µg/g in soil and µg/ml in leachate.

Note: numbers may not equate due to rounding.

A5-6.2 External Bioaccessibility Studies
A5-6.2.1 Methods

The following sections describe the manner in which the soil samples were prepared for analysis, the methods by which the bioaccessibility analyses were performed, and the results of these analyses.

Sample preparation included some preparation by the MOE Phytotoxicology Laboratory (described below). Further sample preparation and bioaccessibility extractions were performed in Exponent's laboratory in Boulder, Colorado (Exponent, 2001). Analyses for total metals concentrations for the eight metals of interest in the sample substrates and extraction fluids were conducted by Columbia Analytical Services, Inc. (CAS) in Kelso, Washington.

A5-6.2.2 Sample Preparation and Analysis

Twenty samples were received at Exponent's Boulder laboratory in two separate shipments in May, 2001. The first shipment consisted of ten samples. Prior to receipt by Exponent, these samples had been dried, ground, and sieved to <350 µm, and each sample had been labeled with a three or four digit sample number. Exponent assigned six digit sample numbers; "SLO" was prefixed to the three digit sample numbers, and "SL" was prefixed to the four digit sample numbers. The dry samples were then sieved to <250 µm. These samples are referred to as "ground soil" in the tables.

The second shipment also consisted of ten samples. The soil samples in this shipment were "bulk" samples which had not been ground and sieved to <350 µm. Each sample had been similarly labeled with a three or four digit sample number. Again, Exponent assigned six digit sample numbers; "CSO" was prefixed to the three digit sample numbers, and "CS" was prefixed to the four digit sample numbers. The samples were then dried in the oven at 100˚C for 24 hours, after which they were sieved to <250 µm. These samples are termed "sieved fine soil" in the tables.

The <250 µm soil size fraction was used for bioaccessibility testing, because it is believed to represent the fraction of soil that is most likely to adhere to human hands and become ingested during hand-to-mouth activity (Maddaloni et al., 1998). A one gram aliquot of each substrate was collected and subjected to the *in vitro* extraction procedure (described below). The rest of the sample was used for analysis of total metal concentrations for the eight metals of interest. As a quality control measure, an additional aliquot of one soil sample (sample number CS3740) was submitted as a triplicate for total metal analysis.

A5-6.2.3 Bioaccessibility Testing

The sieved soil samples (<250 µm size fraction) were subjected to bioaccessibility testing according to the Standard Operating Procedure (SOP) developed by the Solubility/ Bioavailability Research Consortium (SBRC) (Ruby et al., 1999, Exponent, 2001). This protocol

is provided below *(in italics)*. The testing included extraction and analysis of one sample in triplicate (sample number SLO 415).

Deviations from the SBRC method with regard to sample preparation and analysis included the following:

- Analyses were conducted for antimony, beryllium, cadmium, cobalt, copper, and nickel, in addition to the analyses for arsenic and lead as specified in the SOP.

- The bioaccessibility test was modified to include a simulation of the small intestinal environment (i.e., a second phase, at neutral pH, was added to the extraction procedure). This was done to evaluate whether an extraction procedure that simulates the environment of the small intestine would influence the bioaccessibility of metals from the sample substrates (e.g., by affecting either the metal solubility or the integrity of the soil matrix that contains the metals). This was accomplished by adding the following steps at the end of the standard SBRC extraction procedure:

1. At the end of the one hour extraction, a 5mL sample of the extraction fluid was collected and preserved with nitric acid for analysis (as in the methods described in the SOP).

2. The extraction fluid in each bottle was then titrated to pH 7.0 ± 0.2 with NaOH (50% w/w) (this required approximately 20 drops of NaOH solution).

3. Once the extraction fluid had been neutralized, 175 mg of bile salts and 50 mg of pancreatin were added to each extraction bottle, and the bottles were returned to the extractor for an additional four hours of extraction time.

4. At the end of the small-intestinal-phase extraction, a 10mL sample of the extraction fluid was collected from each bottle and filtered for analysis.

5. Matrix interference required that the arsenic analyses be conducted by graphite furnace atomic absorption (GFAA) rather than the inductively coupled plasma (ICP) method as specified in the SOP.

All of the extracts produced from the bioaccessibility testing were shipped to CAS for analysis of total concentrations of antimony, arsenic, beryllium, cadmium, cobalt, copper, lead and nickel.

It should be noted that the Exponent extraction method includes both acid and neutral pH extractions with realistic residency times in the stomach (one hour) and intestines (four hours).

Solubility/Bioavailability Research Consortium Standard Operating Procedure

In vitro Method for Determination of Lead and Arsenic Bioaccessibility

Procedure

Sample Preparation *- All soil/material samples should be prepared for testing by oven drying (<40˚C) to reduce volatilization of any volatile metals and sieving to <250 µm. The <250-µm size fraction is used because this particle size is representative of that which adheres to children's hands. Sub-samples for testing in this procedure should be obtained using a sample splitter.*

Apparatus and Materials

Equipment *- The main piece of equipment required for this procedure consists of a Toxicity Characteristic Leaching Procedure (TCLP) extractor motor that has been modified to drive a flywheel. This flywheel in turn drives a Plexiglass block situated inside a temperature controlled water bath. The Plexiglass block contains ten 5cm holes with stainless steel screw clamps, each of which is designed to hold a 125mL wide mouth high density polyethylene (HDPE) bottle. The water bath must be filled such that the extraction bottles are immersed. Temperature in the water bath is maintained at 37±2˚C using an immersion circulator heater (for example, Fisher Scientific Model 730). Additional equipment for this method includes typical laboratory supplies and reagents, as described in the following sections.*

The 125mL HDPE bottles must have an air tight screw cap seal (for example, Fisher Scientific 125mL wide mouth HDPE Cat. No. 02-893-5C), and care must be taken to ensure that the bottles do not leak during the extraction procedure.

Standards and Reagents *- The leaching procedure for this method uses a buffered extraction fluid at a pH of 1.5. The extraction fluid is prepared as described below.*

The extraction fluid should be prepared using ASTM Type II deionized (DI) water. To 1.9 L of DI water, add 60.06 g glycine (free base, Sigma Ultra or equivalent). Place the mixture in a water bath at 37˚C until the extraction fluid reaches 37˚C. Standardize the pH meter using temperature compensation at 37˚C or buffers maintained at 37˚C in the water bath. Add concentrated hydrochloric acid (12.1 N, Trace Metal grade) until the solution pH reaches a value of 1.50 ±0.05 (approximately 120 mL). Bring the solution to a final volume of 2 L (0.4 M glycine).

Cleanliness of all reagents and equipment used to prepare and/or store the extraction fluid is essential. All glassware and equipment used to prepare standards and reagents must be properly cleaned, acid washed, and finally, rinsed with DI water prior to use. All reagents must be free of lead and arsenic, and the final fluid should be tested to confirm that lead and arsenic concentrations are less than 25 and 5 µg/L, respectively.

Leaching Procedure *- Measure 100 ±0.5 mL of the extraction fluid, using a graduated cylinder, and transfer to a 125mL wide mouth HDPE bottle. Add 1.00 ±0.05 g of test substrate (<250 µm) to the bottle, ensuring that static electricity does not cause soil particles to adhere to the lip or outside threads of the bottle. If necessary, use an antistatic brush to eliminate static electricity*

prior to adding the soil. Record the volume of solution and mass of soil added to the bottle on the extraction test checklist. Hand tighten each bottle top, and shake/invert to ensure that no leakage occurs, and that no soil is caked on the bottom of the bottle.

Place the bottle into the modified TCLP extractor, making sure each bottle is secure and the lid(s) are tightly fastened. Fill the extractor with 125mL bottles containing test materials or Quality Control samples.

The temperature of the water bath must be 37±2˚C. Record the temperature of the water bath at the beginning and end of each extraction batch on the appropriate extraction test checklist sheet.

Rotate the extractor end over end at 30±2 rpm for 1 hour. Record start time of rotation.

When extraction (rotation) is complete, immediately remove bottles, wipe them dry, and place them upright on the bench top.

Draw extract directly from reaction vessel into a disposable 20ml syringe with a Luer-Lok attachment. Attach a 0.45-μm cellulose acetate disk filter (25 mm diameter) to the syringe, and filter the extract into a clean 15mL polypropylene centrifuge tube or other appropriate sample vial for analysis. Store filtered sample(s) in a refrigerator at 4˚C until they are analyzed.

Record the time that the extract is filtered (i.e., extraction is stopped). If the total elapsed time is greater than1 hour 30 minutes, the test must be repeated.

Measure and record the pH of fluid remaining in the extraction bottle. If the fluid pH is not within ±0.5 pH units of the starting pH, the test must be discarded and the sample re-analyzed as follows.

If the pH has dropped by 0.5 or more pH units, the test will be re-run in an identical fashion. If the second test also results in a decrease in pH of greater than 0.5 s.u., the pH will be recorded, and the extract filtered for analysis. If the pH has increased by 0.5 or more units, the test must be repeated, but the extractor must be stopped at specific intervals and the pH manually adjusted down to pH 1.5 with dropwise addition of HCl (adjustments at 5, 10, 15 and 30 minutes into the extraction, and upon final removal from the water bath [60 minutes]). Samples with rising pH values must be run in a separate extraction, and must not be combined with samples being extracted by the standard method (continuous extraction).

Extracts are to be analyzed for lead and arsenic concentration using analytical procedures taken from the US EPA publication (US EPA, 2000), Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, SW-846 (current revisions). Inductively coupled plasma (ICP) analysis, method 6010B (December 1996 revision) will be the method of choice. This method should be adequate for determination of lead concentrations in sample extracts, at a project required detection limit (PRDL) of 100 µg/L. The PRDL of 20 µg/L for arsenic may be too low for ICP analysis for some samples. For extracts that have arsenic concentrations less than five

times the PRDL (e.g., <100 µg/L arsenic), analysis by ICP-hydride generation (method 7061A, July 1992 revision) or ICP-MS (method 6020, September 1994 revision) will be required.

Calculation of the Bioaccessibility Value *- A split of each solid material (<250 µm) that has been subjected to this extraction procedure should be analyzed for total lead and/or arsenic concentration using analytical procedures taken from the US EPA publication (US EPA, 2000), Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, SW-846 (current revisions). The solid material should be acid digested according to method 3050A (July 1992 revision) or method 3051 (microwave-assisted digestion, September 1994 revision), and the digestate analyzed for lead and/or arsenic concentration by ICP analysis (method 6010B). For samples that have arsenic concentrations below ICP detection limits, analysis by ICP-hydride generation (method 7061A, July 1992 revision) or ICP-MS (method 6020, September 1994 revision) will be required.*

The bioaccessibility of lead or arsenic is calculated in the following manner:

$$\text{Bioaccessibility value} = \frac{(\text{Concentration in vitro extract, mg/L})(0.1\text{L})}{(\text{Concentration in solid, mg/kg})(0.001\text{ kg})} \times 100$$

Chain-of-Custody/Good Laboratory Practices *- All laboratories that use this SOP should receive test materials with chain-of-custody documentation. When materials are received, each laboratory will maintain and record custody of samples at all times. All laboratories that perform this procedure should follow good laboratory practices as defined in 40 CFR Part 792 (US EPA, 1999) to the extent practical and possible.*

Data Handling and Verification *- All sample and fluid preparation calculations and operations should be recorded in bound and numbered laboratory notebooks, and on extraction test checklist sheets. Each page must be dated and initialed by the person who performs any operations. Extraction and filtration times must be recorded, along with pH measurements, adjustments, and buffer preparation. Copies of the extraction test checklist sheets should accompany the data package.*

Quality Control Procedures

Elements of Quality Assurance and Quality Control (QA/QC) *- A standard method for the in vitro extraction of soils/solid materials, and the calculation of an associated bioaccessibility value, are specified above. Associated QC procedures to ensure production of high quality data are as follows:*

- *Reagent blank—Extraction fluid analyzed once per batch.*
- *Bottle blank—Extraction fluid only run through the complete extraction procedure at a frequency of no less than 1 per 20 samples or one per extraction batch, whichever is more frequent.*
- *Blank spikes—Extraction fluid spiked at 10 mg/L lead and/or 1 mg/L arsenic and run*

through the extraction procedure at a frequency of no less than every 20 samples or one per extraction batch, whichever is more frequent. Blank spikes should be prepared using traceable 1,000 mg/L lead and arsenic standards in 2 percent nitric acid.

- *Duplicate—duplicate extractions are required at a frequency of 1 for every 10 samples. At least one duplicate must be performed on each day that extractions are conducted.*
- *Standard Reference Material (SRM)—National Institute of Standards and Technology (NIST) material 2711 (Montana Soil) should be used as a laboratory control sample (LCS).*

Control limits for these QC samples are delineated in Table A5-8, and in the following discussion.

Table A5-8: Summary of QC Samples, Frequency of Analysis, and Control Limits

QC Sample	***Minimum Frequency of Analysis***	***Control Limits***
Reagent Blank	*Once per batch (min. 5%)*	*<25 µg/L lead* *<5 µg/L arsenic*
Bottle Blank	*Once per batch (min. 5%)*	*<50 µg/L lead* *<10 µg/L arsenic*
Blank Spike	*Once per batch (min. 5%)*	*85–115% recovery*
Duplicate	*10%*	*±20% RPD*
SRM (NIST 2711)	*2%*	*9.22 ±1.50 mg/L Pb* *0.59 ±0.09 mg/L As*

QA/QC Procedures *- Specific laboratory procedures and QC steps are described in the analytical methods and should be followed when using this SOP.*

Laboratory Control Sample (LCS) *- The NIST SRM 2711 should be used as a laboratory control sample for the in vitro extraction procedure. Analysis of 18 blind splits of NIST SRM 2711 (105 mg/kg arsenic and 1,162 mg/kg lead) in four independent laboratories resulted in arithmetic standard deviations of 1.50 mg/L lead and 0.09 mg/L arsenic. This SRM is available from the National Institute of Standards and Technology, Standard Reference Materials Program, Room 204, Building 202, Gaithersburg, Maryland 20899 (301/975-6776).*

Reagent Blanks/Bottle Blanks/Blank Spikes *- Reagent blanks must not contain more than 5 µg/L arsenic or 25 µg/L lead. Bottle blanks must not contain arsenic and/or lead concentrations greater than 10 and 50 µg/L, respectively. If either the reagent blank or a bottle blank exceeds these values, contamination of reagents, water, or equipment should be suspected. In this case, the laboratory must investigate possible sources of contamination and mitigate the problem before continuing with sample analysis. Blank spikes should be within 15% of their true value. If recovery of any blank spike is outside this range, possible errors in preparation, contamination, or instrument problems should be suspected. In the case of a blank spike outside specified limits,*

the problems must be investigated and corrected before continuing sample analysis.

Analytical Methods

Extraction fluids (from both the stomach-phase and intestinal-phase extractions) were analyzed for metals (antimony, arsenic, beryllium, cadmium, cobalt, copper, lead and nickel). Inductively coupled plasma/mass spectrometry (ICP/MS) (EPA Method 200.8), ICP/atomic emission spectrometry (ICP/AES), and graphite furnace atomic absorption (GFAA) (EPA Method 7060A) were used for the analyses. The laboratory reported arsenic results by GFAA for selected samples because of matrix interference encountered for arsenic during the ICP analyses. Most of the data were reported from the ICP/MS analysis, however, the laboratory used the ICP/AES method for samples in which high levels of target analytes were present. All solid and aqueous/fluid samples were shipped to CAS under chain of custody.

A5-6.2.3.1 Results

The results of the external bioaccessibility testing for metals of concern in the Rodney Street community soils are shown in Tables A5-9 to A5-16. All averages are reported as two significant figures.

Table A5-9: *In vitro* Bioaccessibility Testing of Antimony

Ground Soil				
Soil Sb content	acid extract		neutral extract	
(µg/g)	actual (µg)	%	actual (µg)	%
1.3	0.2	17	0.3	21
1.3	0.3	21	0.5	36
0.5	0.2	32	0.2	36
1.1	0.1	11	0.2	15
1.4	0.3	19	0.4	24
1.2	0.3	21	0.3	23
0.9	0.2	25	0.2	26
3.0	0.5	18	0.9	29
2.2	0.2	7	0.5	21
4.1	1.8	44	2.6	64
min		7		15
max		44		64
avg		22		30

Sieved Fine Soil				
Soil Sb content	acid extract		neutral extract	
(µg/g)	actual (µg)	%	actual (µg)	%
1.3	0.2	13	0.2	18
0.8	0.2	32	0.3	39
3.4	0.2	5.2	0.2	6.1
1.0	0.1	14	0.4	42
1.1	0.3	28	0.4	40
1.4	0.5	35	0.6	44
0.8	0.6	84	0.2	31
2.3	0.5	23	0.8	33
1.5	0.1	6.6	0.3	20
2.4	0.8	34	1.1	45
min		5.2		6.1
max		84		45
avg		28		32

Table A5-10: *In vitro* Bioaccessibility Testing of Arsenic

Ground Soil				
Soil As Content (µg/g)	Acid Extract Actual (µg)	%	Neutral Extract Actual (µg)	%
31.8	8.6	27	8.3	26
50.1	21.5	43	23.1	46
30.5	8.9	29	9.2	30
20.4	4.5	22	7.1	35
49.0	13.2	27	10.8	22
29.2	5.6	19	5.0	17
35.3	9.9	28	6.7	19
30.3	9.4	31	5.2	17
37.2	19.0	51	13.4	36
34.4	11.4	33	10.0	29
min		19		17
max		51		46
avg		31		28

Sieved Fine Soil				
Soil As content (µg/g)	acid extract actual (µg)	%	neutral extract actual (µg)	%
46.0	12.4	27	9.2	20
28.9	13.9	48	13.3	46
38.9	10.9	28	3.3	8.5
19.3	5.0	26	10.8	56
46.7	17.3	37	16.8	36
42.5	9.8	23	5.1	12
20.9	7.3	35	1.9	9.2
31.8	11.8	37	6.0	19
29.1	13.7	47	8.4	29
33.4	13.0	39	7.4	22
min		23		8.5
max		48		56
avg		35		26

Table A5-11: *In vitro* Bioaccessibility Testing of Beryllium

Ground Soil

Soil Be content (µg/g)	acid extract actual (µg)	%	neutral extract actual (µg)	%
0.7	0.3	42	0.08	11
0.5	0.3	53	0.08	16
0.6	0.3	57	0.08	14
0.5	0.3	48	0.08	15
0.8	0.4	43	0.08	10
0.9	0.4	39	0.08	8.6
0.7	0.3	45	0.07	9.6
1.1	0.7	61	0.08	7.0
4.3	3.4	79	0.08	1.9
0.7	0.4	49	0.07	10
min		39		1.9
max		79		16
avg		52		10

Sieved Fine Soil

Soil Be content (µg/g)	acid extract actual (µg)	%	neutral extract actual (µg)	%
0.8	0.4	48	0.08	11
0.5	0.3	58	0.08	16
0.7	0.4	59	0.07	11
0.5	0.3	55	0.08	16
0.8	0.5	59	0.08	10
1.0	0.5	51	0.08	7.8
0.9	0.5	58	0.08	8.8
1.2	0.8	70	0.08	6.8
4.2	3.4	81	0.08	1.9
0.9	0.4	52	0.08	9.4
min		48		1.9
max		81		16
avg		59		10

Table A5-12: *In vitro* Bioaccessibility Testing of Cadmium

Ground Soil

Soil Cd content (µg/g)	acid extract actual (µg)	%	neutral extract actual (µg)	%
1.5	1.0	63	0.9	59
1.4	1.2	83	0.7	51
1.6	0.9	58	1.0	62
2.3	1.6	70	1.1	48
2.2	1.6	69	1.1	49
1.2	0.9	73	0.7	56
1.8	1.2	68	1.3	75
4.7	3.4	72	2.8	59
3.4	2.4	69	0.3	9
2.5	1.9	77	1.5	62
min		58		9
max		83		75
avg		70		53

Sieved Fine Soil

Soil Cd content (µg/g)	acid extract actual (µg)	%	neutral extract actual (µg)	%
2.2	1.5	68	1.2	54
1.2	1.0	86	0.7	57
1.5	1.0	65	1.2	79
2.2	1.7	78	1.5	69
2.5	2.0	79	1.5	59
1.9	1.4	73	1.2	62
1.5	1.1	74	1.2	75
5.3	4.3	80	3.2	60
2.4	1.7	71	0.1	3.8
1.9	1.6	83	1.2	63
min		65		3.8
max		86		79
avg		76		58

Table A5-13: *In vitro* Bioaccessibility Testing of Cobalt

Ground Soil

Soil Co content	acid extract		neutral extract	
(µg/g)	actual (µg)	%	actual (µg)	%
78.6	14.9	19	8.7	11
46.2	11.6	25	7.9	17
63.6	16.5	26	14.6	23
171	39.3	23	30.8	18
113	17.0	15	10.7	9.5
71.7	10.8	15	8.6	12
100	18.0	18	16.0	16
111	37.7	34	27.8	25
56.6	10.8	19	8.5	15
82.3	20.6	25	14.8	18
min		15		9.5
max		34		25
avg		22		16

Sieved Fine Soil

Soil Co content	acid extract		neutral extract	
(µg/g)	actual (µg)	%	actual (µg)	%
109	21.8	20	13.1	12
28.7	10.1	35	6.0	21
72.5	23.2	32	18.9	26
163	53.8	33	12.2	7.5
66.6	21.3	32	12.7	19
90	18.9	21	14.4	16
60.1	19.2	32	14.4	24
102	32.6	32	24.5	24
37.1	11.5	31	8.5	23
65.8	15.8	24	11.2	17
min		20		7.5
max		35		26
avg		29		19

Table A5-14: *In vitro* Bioaccessibility Testing of Copper

Ground Soil

Soil Cu content (µg/g)	Acid Extract actual (µg)	%	Neutral Extract actual (µg)	%
438	114	26	149	34
359	111	31	176	49
436	166	38	209	48
631	177	28	227	36
880	273	31	370	42
453	149	33	181	40
798	239	30	303	38
601	162	27	240	40
820	3	0.4	90	11
520	239	46	255	49
min		0.4		11
max		46		49
avg		29		39

Sieved Fine Soil

Soil Cu content (µg/g)	Acid Extract actual (µg)	%	Neutral Extract actual (µg)	%
637	178	28	236	37
247	104	42	124	50
592	207	35	255	43
628	232	37	377	60
819	360	44	401	49
656	249	38	282	43
737	280	38	332	45
647	265	41	304	47
494	3.4	0.7	43	8.8
460	207	45	216	47
min		0.7		8.8
max		45		60
avg		35		43

Table A5-15: *In vitro* Bioaccessibility Testing of Lead

Ground Soil

Soil Pb content (µg/g)	Acid Extract actual (µg)	%	Neutral Extract actual (µg)	%
187	114	61	15.7	8.4
239	165	69	33.5	14
120	94	78	3.7	3.1
231	176	76	13.9	6.0
383	260	68	23.0	6.0
209	134	64	4.2	2.0
343	240	70	17.2	5.0
1030	680	66	36.9	3.6
838	285	34	14.6	1.7
911	738	81	79.3	8.7
min		34		1.7
max		81		14
avg		67		6

Sieved Fine Soil

Soil Pb content (µg/g)	acid extract actual (µg)	%	neutral extract actual (µg)	%
232	167	72	8.8	3.8
211	167	79	12.0	5.7
190	150	79	7.0	3.7
222	178	80	21.1	9.5
430	314	73	20.6	4.8
389	335	86	14.4	3.7
320	253	79	12.8	4
1210	1016	84	48.4	4
973	487	50	12.7	1.3
511	393	77	19.9	3.9
min		50		1.3
max		86		9.5
avg		76		4

Table A5-16: *In vitro* Bioaccessibility Testing of Nickel

Ground Soil

Soil Ni content	Acid Extract		Neutral Extract	
(µg/g)	actual (µg)	%	actual (µg)	%
5170	517	10	517	10
3130	657	21	657	21
5010	852	17	1002	20
7870	944	12	1023	13
10500	945	9	1050	10
4670	514	11	514	11
9550	726	7.6	879	9.2
4610	922	20	1014	22
5450	763	14	763	14
4750	760	16	713	15
min		7.6		9.2
max		21		22
avg		14		15

Sieved Fine Soil

Soil Ni content	Acid Extract		Neutral Extract	
(µg/g)	actual (µg)	%	actual (µg)	%
7310	804	11	804	11
1840	515	28	442	24
5370	1074	20	1074	20
6410	1154	18	1090	17
5620	1293	23	1124	20
5730	917	16	917	16
6200	682	11	744	12
5290	1058	20	1005	19
3040	608	20	638	21
4270	769	18	726	17
min		11		11
max		28		24
avg		19		18

A5-7 Discussion of Bioaccessibility Studies for Metals of Concern in Rodney Street Community Soils

The mean percent bioaccessibilities and the range from minimum to maximum values for the MOE and external laboratory tests are shown in Table A5-17. In the case of the external laboratory test results, the highest combined bioaccessibility value for either the acid or neutral pH extractions for both the ground and sieved and the sieved only soil samples is shown. In this context, perusal of Tables A5-9 to A5-16 shows that for arsenic, beryllium, cobalt, lead, cadmium and nickel, the extraction of the metal was greatest under acid conditions, and that under subsequent neutral pH extraction conditions, the overall bioaccessibility of these metals did not change appreciably (arsenic, cobalt, copper, nickel) or was much less (beryllium, lead). This lowered bioaccessibility at neutral pH following an acid extraction suggests that these metals become less available in the small intestine. The other metals (antimony and copper) showed further extraction of the metal under neutral pH subsequent to the acid extraction indicating that the combined acid and neutral pH extraction gives a more realistic value for overall bioaccessibility. The MOE and the external laboratory's bioaccessibility results for cobalt, copper, lead and nickel are comparable.

Table A5-17: Comparison of Corrected MOE and Exponent Bioaccessibilities (%)

Metal	MOE Mean (range)	Exponent* Mean (range) (Ground & Sieved)	Exponent* Mean (range) (Sieved Only)	Bioaccessibility Value used for Exposure Estimates
Antimony	2.8 (2.1 - 3.9)	30 (7 - 64)**	32 (5-84)	32
Arsenic	22.8 (12.6 - 28.4)	31 (17 - 51)	35 (23-56)	-
Beryllium	N/A	52 (39 - 79)	59 (2-81)	59
Cadmium	N/A	70 (9 - 83)	76 (4-86)	76
Cobalt	19.2 (12.5 - 24.7)	22 (10 - 34)	29 (8-35)	29
Copper	38.1 (31.8 - 44.4)	39 (<1 - 49)	43 (1-60)	43
Lead	75.7 (61- 90.3)	67 (2 - 81)	76 (10-86)	-
Nickel	16.5 (11.8 - 23.3)	15 (9 - 22)	19 (11-28)	19

* = highest bioaccessibility value from either acid or neutral pH extractions

** = based on only 21% recovery from NIST soil standard
N/A = Not Available

The external laboratory (Exponent, 2001) notes that while neither the measured concentrations of metals in the soils nor the bioaccessibility differed significantly between the ground and unground samples, the data suggest that there is a slight trend toward higher bioaccessibility in the unground soil samples. On this basis, and given the small number of samples analyzed, the bioaccessibility values used to estimate the intake of metals from ingestion of Rodney Street community soils (Section A3-1.5) were selected from the unground (sieved only) soil sample data set (Table A5-17). Even though the external laboratory had difficulty extracting and analyzing antimony, the highest % bioaccessibility for antimony measured by them was selected. The mean bioaccessibility value was selected to account for the wide range of bioaccessibility values obtained which was assumed to reflect the considerable heterogeneity of the soil samples. In general, the percent metal bioaccessibility decreased with increasing soil nickel concentration. This latter trend is, in part, due to the non-equilibrium conditions involved in simulating processes in the gastrointestinal tract, which are themselves non-equilibrium processes, and, the solubility of the form or species of nickel present in the soils containing the higher soil nickel concentrations.

There is some information on the bioaccessibility of metals in other soils, however, the emphasis is on arsenic and lead (Ellickson et al., 2001; Ruby et al., 1999; Hamel et al., 1998). Hamel et al., (1998) do provide some information on the acid extraction of cadmium and nickel from a Standard Reference Material (SRM) 2710 from the US National Institute of Standards and Technology (NIST) and nickel in a chromium contaminated soil from Jersey City, NJ. NIST SRM 2710 is a Montana soil with a certified nickel content of 14.3 mg/kg. The percentage bioaccessibility of nickel in the SRM 2710 ranged from 11% to 14%, and was 23% to 40% in the Jersey City soil sample. The external laboratory that performed the bioaccessibility testing also performed the acidic or stomach phase part of the bioaccessibility protocol on another NIST SRM 2711 as part of the validation of their approach. NIST SRM 2711 is also a Montana soil, however, its certified nickel content is 20.6 mg/kg. The results of the acid bioaccessibility testing of SRM 2711 are shown in Table A5-18. Without further information on the speciation of nickel in these other soils, direct comparison with the bioaccessibility of nickel in Rodney Street community soils is difficult.

It should be noted that while concentrations of some metals are certified in NIST SRMs, not all metals have certified concentrations. For example, concentrations of antimony, beryllium and cobalt are not certified. Other publications (Roelandts and Gladney, 1998 and Church et al., 1999) report antimony concentrations of 12 mg/kg for NIST 2711, beryllium concentrations of 1.6 to 2 mg/kg, and cobalt concentrations of 9.5 and 12 mg/kg. The external laboratory's analytical procedure resulted in only a 21% recovery of antimony based on the certified value of 19.4 mg/kg. However, as noted another laboratory found 12 mg Sb/kg (Roelandts and Gladney, 1998). This would bring the recovery up to 34.6%, which is still low.

Two technical issues have been raised concerning the *in vitro* extraction methods used. It has been claimed that MOE and Exponent used too low a ratio of extraction fluid to soil sample and that the bioaccessibility value would be larger for greater extraction fluid:soil sample ratios, e.g., if a larger extraction fluid volume had been used, more metal would have been extracted and a

larger bioaccessibility value obtained. The paper by Hamel et al., (1998) contains detailed information on the effect of different soil: extraction fluid ratios on the bioaccessibility of nickel and other metals in contaminated soils. The authors examined soil: extraction fluid ratios from 100:1 up to 5000:1 and concluded that the bioaccessibility of metals in the soils extracted *in vitro* with synthetic extraction fluids will only be affected slightly by changes in extraction fluid to solid ratios for the range 100:1 to 5000:1. This study was undertaken by a university laboratory in New Jersey with no connection to Exponent or MOE and provides independent validation of this aspect of the Exponent and MOE extraction methodology. The data of Hamel et al., (1998) indicates that the extraction method of Exponent is unlikely to underestimate the bioaccessibility of nickel in Rodney Street community soils and that the use of a 100% bioaccessibility value is unnecessary.

Table A5-18: Bioaccessibility Testing of Standard Reference Material NIST 2711

		Soil		Stomach-phase Extraction	
Metal	Certified Value (mg/kg)	Measured Value (µg/g)	% Recovery of Lab. Analysis	Measured Value (µg/100 mL)	% Bioaccessiblity[1]
Antimony*	19.4	4.15	21	1.59	38.3
Arsenic	105	91.1	87	54.2	59.5
Beryllium*	1.6	0.92	57.5	0.383	41.6
Cadmium	41.7	37.7	90	35.2	93.4
Cobalt*	10	7.8	78	3.5	44.9
Copper	114	106	93	49.1	46.3
Lead	1162	1080	93	864	80
Nickel	20.6	15.9	77	4.08	25.7

1 = measured value (stomach extract) as a percentage of measured soil value

* = not certified values

The other issue concerned the role of glycine buffer in nickel bioaccessibility tests. Inco has provided information that the glycine buffer used in the Exponent bioaccessibility test method may form aqueous complexes with nickel which enhance the extraction of nickel at pH 7, and thus overestimate the bioaccessibility by a four-fold factor. The new extraction data performed by Inco and the information in Fischer et al., (1992) do indicate that the presence of glycine in extraction fluids results in greater extraction of nickel at pH 7. This effect also occurs for copper, cadmium and zinc but not for lead (Fischer et al., 1992). The effect does not seem to occur at pH 1.5 (Inco's data) and is not so pronounced at pH 4.5 (Fischer et al., 1992). This loss of glycine's ability to form complexes with nickel and other metals at more acidic pH is related to the dissociation of glycine below its isoelectric point of pH 6. This affects glycine's ability to form

complexes with nickel at low pH.

Inspection of Tables A5-7, A5-16 and Inco's data clearly indicate that the percentage of nickel extracted from Rodney Street community soils at low pH either exceeds or is equivalent to the amount of nickel extracted at neutral pH (pH 7). As indicated in section A5-6, MOE selected the highest average bioaccessibility value for either the acid or neutral pH extractions of the sieved not ground soil samples analyzed by Exponent. Extraction of nickel was greatest under acid conditions. The 19% bioaccessibility value was obtained under acid extraction and therefore the presence of glycine in the extraction fluid would have no influence on the percentage of nickel extracted. The MOE stomach acid leachate data which was obtained without glycine (Table A5-7) and the Inco data for acid extraction of nickel *without glycine* from Rodney Street community soils support MOE's selection of the 19% bioaccessibility value obtained under acid pH conditions.

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