A Health Risk Assessment on the Consumption of Trace Metals Found in Crops Grown on Biosolids-Amended Soil

By: Jamal Harb

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree of

Master of Science

Faculty of Medicine
Department of Community Health Sciences
University of Manitoba
Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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ABSTRACT

Statement of the Problem: The City of Winnipeg produces an average of approximately 655000 cubic meters of biosolids annually. Biosolids are solid residues that are produced by primary (physical/chemical) and secondary (biological) treatments of raw sewage. Biosolids production poses a major problem, as disposal in the landfill is costly and requires careful and responsible management to avoid potential environmental health problems that may include contamination of underground water aquifers. An alternative to disposal in the landfills is to recycle the biosolids through the application to agricultural land. Biosolids contain nutrients such as nitrogen and phosphorous that are required for plant growth. However, biosolids also contain trace metals such as cadmium (Cd), zinc (Zn), copper (Cu), chromium (Cr), lead (Pb) and nickel (Ni). The uptake of such trace metals by crops grown on biosolid-amended soil could pose a health hazard to humans consuming the crops or their by-products. This study assessed the potential increase in health risk to humans from the consumption of trace metals in crops grown on biosolids-amended soil.

Methods: Using the principles of toxicology, biostatistics and epidemiology, the study design consisted of a health risk assessment (HRA) process that identified the kinds of adverse outcomes that may be associated with oral exposure to potentially harmful substances (trace metals). The health risk assessment also predicted the likelihood that a specific human population will experience such effects at given exposure levels. The model used in the health risk assessment included the following steps: hazard identification, dose-response assessment, exposure assessment and health risk estimation.

Results: The HRA process demonstrated that out of the six heavy metals (Cd, Pb, Cr (III), Zn, Ni and Cu) present in the biosolids and taken up by the plants when added to the soil, Cd and Zn have the potential of posing a health hazard if consumed in sufficient quantities. For Cd in crops grown on soil amended with 100000 kg/ha of biosolids, an average individual weighing 70 kg would have to consume 0.98 kg of wheat (or 7.0 loaves of 100% whole wheat bread [280g/loaf]) or 4.62 kg of oats (or 32.34 loaves of bread [280 gram loaf containing 51% (w/w) oats]) per day for a lifetime to reach the benchmark level that is considered to be without deleterious risk to health. For the metal Zn found in crops grown on soil amended with 100000 kg/ha of biosolids, an average individual

weighing 70 kg would have to consume 0.45 kg of wheat (or 3.1 loaves of 100% whole wheat bread [280g/loaf]) or 0.70 kg of oats (or 4.90 loaves of bread [280 gram loaf containing 51% (w/w) oats]) per day for a lifetime to reach the benchmark level that is considered to be without deleterious risk for oral exposure to Zn. Lead has also been identified as a health hazard. However, laboratory analysis of the crops revealed Pb concentrations too low (<0.1 mg Pb per Kg of grain) to support a quantitative risk assessment. Lead does not appear to be a problem in crops grown on biosolids-amended soil since the data from the literature and the City of Winnipeg study suggest that lead is not taken up by the crops. Ni, Cu and Cr (III) were not identified as health hazards and thus do not pose a health risk to humans consuming wheat and oats grown on biosolids-amended soil.

Conclusion: The health risk to humans from the consumption of heavy metals in wheat and oats grown on biosolids-amended soil is negligible. The HRA process revealed that oral exposure to cadmium, lead or zinc could pose a potential health hazard to humans. However, the quantities of these metals found in the crops grown on biosolids-amended soil are minimal thus making the risk to human health negligible. Therefore, application of biosolids to agricultural land provides environmental and economic benefits with negligible increase in risk to human health.

Acknowledgements

For all the guidance, assistance and support that I have received in this project, I would like to thank my committee members: Dr. M. Fast, Dr. Allen Kraut, Dr. A. Farenhorst and especially Dr. Annalee Yassi who guided me all through the process. Many thanks also go to Bob Ross, Bob Tate, Sylvia Cosway and especially Vivian Lee for her ongoing support.

The data for this thesis would not have been possible without the assistance from the City of Winnipeg, Water and Waste Department and I thank them for access to their valuable data.

Thesis Outline

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Chapter 1: Introduction

1.1 Background

The City of Winnipeg, Water and Waste Department conducted a ten-year research project (1986-1996) using biosolids as a substitute for chemical fertilizers on agricultural land. The primary objective of the project was to establish an economic analysis of various biosolids application rates to agricultural plots as a cost-effective method of disposal. This included establishing soil lifetime loading limits and fate of plant nutrient and metals after biosolids application. Benefits to the crops in terms of yield and protein content were also studied. An important issue not studied in this project was the potential health risks posed to humans consuming the crops.

The advantages of applying biosolids to agricultural land have been recognized since the beginning of humankind (Gras, 1925). Until recently, the economical advantages have masked the potential disadvantages (possible environmental and health effects) associated with biosolids use on agricultural land. In Manitoba, studies have been carried out to determine the fate of biosolid constituents when applied to agricultural land (Hastie, 1993). These studies and others have shown that various crops will, in addition to using the nutrients found in biosolids, also absorb other biosolids constituents such as heavy metals (Bitton et al., 1980; Chaney, 1973). The introduction of heavy metals into the food chain could significantly contribute to the total dietary intake of these metals (Bitton et al., 1980). Heavy metals have been shown to cause various adverse health effects in animals and humans if consumed in sufficient quantities (Kawai et al., 1976). How much of these metals will reach the human body via crops grown on biosolids-amended soil and what are the health implications are important questions that need to be answered.

In this study, a health risk assessment process will be applied to identify the adverse health effects that may be associated with exposure (from consumption) to the potentially harmful metals that are present in biosolids and are absorbed by the crops. The last step of the health risk assessment will predict the likelihood that the human population

consuming the crops grown on biosolid-amended soil will experience the adverse health effects at given consumption levels.

1.2 Objective

The overall objective of this study is to assess the potential health risk to humans from the consumption of trace metals in crops grown on biosolid-amended soil. To achieve the overall objective, the study will:

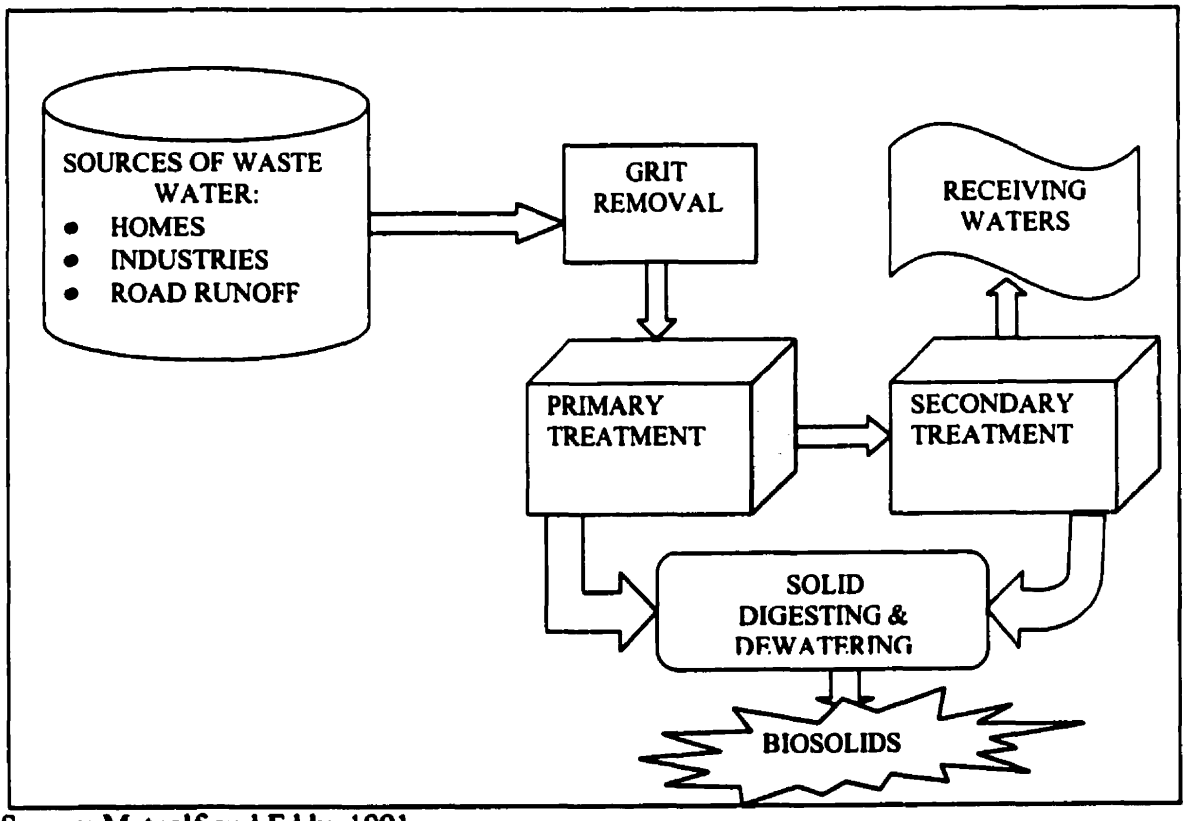
1. Review the hazardous effects of the trace metals present in crops grown on soil amended with biosolids.
2. Review the dose-response/dose-effect relationships between the hazard and the associated health effects.
3. Report the concentration of the hazards (toxic metals) in the crops grown on untreated and treated (chemical fertilizer) soil amended with the different application rates (0, 10000, 25000, 50000 and 100000 kg of biosolids per hectare) of biosolids.
4. Estimate the level of hazard (toxic metals) exposure due to crop consumption over an average life span that is required to produce the health effect (i.e. determine the maximum X amount of plant Y grown on biosolids-amended soil which can be consumed by an average person over his/her life span without any known adverse health effects).
5. Determine if a significant difference exists in the metal content of the crops grown on soil amended with different biosolids application rates (i.e. does the health risk increase according to the consumption of crops grown on soil amended with increasing amounts of biosolids).

Chapter 2 Biosolids: An Overview

2.1 Generation of Biosolids

Biosolids are a viscous residues produced by the treatment of wastewater. The typical wastewater treatment process and the sources of the wastewater are outlined in Figure 1. The sources of the wastewater include those from industries, residential homes, urban run-off and leaching from plumbing fixtures (Metcalf and Eddy, 1991). When the wastewater reaches the treatment plant, the influent undergoes primary and secondary treatment. The primary treatment process removes the solids that settle out of the wastewater by gravity. This process generates about 2500 to 3000 liters of sludge per million liters of wastewater treated. Primary sludge contains 3-7 percent solids, 60 to 80 percent of which is organic matter.

Figure 1: Typical wastewater treatment process.



Source: Metcalf and Eddy, 1991

Secondary treatment produces sludge generated by biological treatment processes. Biological treatment processes utilize microbes to break down and convert the organic substances in the wastewater to sludge and methane gas. This process removes up to ninety percent of the organic matter in the wastewater to produce sludge (Metcalf and Eddy, 1991).

2.2 Biosolids Constituents

The composition of biosolids is influenced by many factors including the composition of the wastewater influent and the type of treatment process in the plant. In many cases, the wastewater influent is generated by residential homes, industries and urban run-off. These sources and others such as leaching from plumbing fixtures can significantly affect the composition of the influent (Bolton and Klein, 1971).

In general, biosolids are composed of heavy metals, organic compounds, microorganisms and nutrients such as phosphorus and nitrogen. The main reasons for applying biosolids to agricultural land are that biosolids contain nitrogen, phosphorous and organic matter and exhibit soil conditioning properties (Weber, 1984). Other favorable effects of applying biosolids include increasing soil temperature, greater activity of microbes, increase aeration porosity, increase organic carbon, increase cationic exchange capacity and increase water retention characteristics that allows nutrients in biosolids to be retained in the plant rooting zone (Kladivko, E.J., and Nelson, D.W., 1979). Phosphorous and nitrogen are nutrients required for healthy plant growth (Hinsley et al., 1982). A study of biosolids use on agricultural crops in one area of Oregon found that the return per acre of sludge application when compared to traditional chemical fertilizer ranged from a gain of $6 to $15 per acre. Overall, the producers gained net savings in the cost of chemical fertilizer through replacement with biosolids (Chaney, 1973). In another study carried out in the mid-west U.S.A., nine crop species including wheat were seeded on soil having a pH ranging from 6.0 to 7.5. Crop yields and qualities were greater on biosolids-amended soil than control soils without biosolids (Hinsley et al., 1982).

Heavy metals are defined in the periodic table as elements having densities greater than that of iron. The typical heavy metal concentration range in sludge is illustrated in Table 1.

Table 1: Typical concentration of heavy metals in biosolids.

Element	Concentration (mg/kg dry weight)
Cadmium	<1-3410
Chromium	8-40600
Copper	50-8000
Nickel	6-5300
Lead	29-3600
Zinc	91-4900

Source: Environment Canada, 1984

Heavy metals in sludge arise mainly from domestic and industrial input to the sewage system. Their presence in biosolids poses a problem when considering application to agricultural land in that they as elements are persistent in the environment and are readily taken up by plants (Page et al., 1989).

Heavy metal uptake by plants has been demonstrated in numerous studies (Hinsley et al., 1984; Houda, 1987; Page et al., 1989). In these studies, metals have been shown to accumulate in the soil, plant roots, stems, leaves and grain (Page et al., 1989). In an Illinois study, the uptake of Cd and Zn by corn that received repeated sludge application resulted in additive increases of both Cd and Zn in the corn leaves and grains (Hinsley et al., 1984). Houda (1987) examined the accumulation of trace metals in wheat, carrots and spinach grown on soil amended with different amounts of biosolids. This study showed that as the amount of metals in the soil is increased, the amount of heavy metal uptake by the crops also increases. The accumulation of Cd, Ni and Zn in the plants showed the greatest increases with increasing application rates of biosolids. The Cu and Pb accumulation in the plants showed only small increases. The study concluded that in order to control the accumulation of metals in food plants, their concentration in the soil must be limited and monitored when applying biosolids (Houda, 1987).

In addition to the organic and inorganic compounds, biosolids also contains microorganisms. Although microorganisms are not the focus of this thesis, only a brief

description will follow to provide a broad picture of the overall constituents found in biosolids.

Microorganisms in biosolids include bacteria, viruses, protozoa and helminth ova. These organisms can cause diseases, usually enteric diseases through direct human contact with the organism or through the ingestion of meats from infected animals or contaminated crops (Bitton et al., 1980). Pathogens in biosolids that pose hazards to human and animal health are derived from a variety of sources. These sources include humans infected with enteric diseases, effluents from abattoirs and animal feces carried into the sewage system via surface water drainage. Sewage treatment practices reduce the number of pathogenic organisms but studies have demonstrated that detectable amounts of most types of pathogenic organisms can occur in biosolids (Environment Canada, 1984).

2.3 Fate of Biosolids: Use, Disposal and Risks of Improper Disposal

2.3.1 Uses of Biosolids

The organic and nutrient content of biosolids makes it a valuable resource to use both in improving marginal lands and as a supplement or replacement to fertilizers and soil conditioners. The beneficial uses of sludge are not limited to the production of agricultural commodities. Biosolids are used in silviculture to increase forest productivity and to revegetate and stabilize harvested forestland (Page et al., 1989). The use of biosolids can be grouped into two categories:

1) Land application to agricultural land

Biosolids application to land is used to improve the growing conditions and nutrient content of soil. The method or rate of application depends on the physical characteristic of the sludge, soil and the crops grown. Liquid sludge may be applied using irrigation systems or customized application vehicles. Dewatered sludge is typically applied by equipment (hoppers with spray arm attachments) similar to that used for applying chemical fertilizers. Generally, dewatered sludge is applied to the land surface and then incorporated by tillage.

2) Land application to non-agricultural land

Biosolids application to non-agricultural land includes forests, parks, cemeteries and golf courses. When used to stabilize or re-vegetate land, amounts in excess of that used on agricultural land are applied at one time to ensure sufficient nutrients are available to support vegetation.

2.3.2 Disposal Methods

The most common methods of biosolids disposal include surface disposal, land filling and incineration (Environment Canada, 1984).

Biosolids surface disposal is a method of disposal where large quantities of sludge are left on land surface and include land application to dedicated non-agricultural land. Generally, surface disposal sites do not have a vegetative or soil cover (i.e. uncovered). In many cases, surface disposal sites are areas of land where biosolids has been placed for many years without consideration for subsequent removal of the disposal.

Land filling is another disposal method where sludge is deposited in a dedicated area and buried beneath a soil cover. Similar to the surface disposal method, land filling does not attempt to use the nutrient content of the sludge for beneficial use.

Finally, incineration is a method of disposal that destroys the organic pollutants and reduces the volume of biosolids.

2.3.3. Risks Associated With Improper Disposal

Disposal of biosolids in landfills or on land surfaces may pose human health problems if the pollutants leach from the sludge into the ground water. These pollutants include nitrogen, heavy metals, chlorinated hydrocarbons and pathogenic organisms. The

potential for ground water contamination is greater when the water table is closer to the soil surface.

Incineration of biosolids will add to the community's air pollution problem by releasing particulates, heavy metals, and toxic organic compounds to the environment. Incineration may also contribute to global warming (green house effect) by releasing carbon dioxide and methane.

2.4 Metal-Soil Interaction and Plant Availability

When heavy metals are added to the soil environment, the ions can undergo a number of reactions. These reactions affect how the metal in the soil is distributed into the mobile (dynamic) or immobile (stationary) phase. The reactions or processes that determine how a metal is distributed into the mobile or immobile phases in the soil environment are:

Precipitation: is the process by which a soluble metal ion reacts with other soluble ions to form a solid product. Metals precipitate with compounds such as hydroxides and carbonates and are dependent on pH. Some of the precipitates are very stable and unlikely to dissolve once formed. Depending on the physical size of the precipitate formed, the specific metal may be immobilized by precipitation.

Sorption (adsorption and absorption): Adsorption is the process by which a compound in solution becomes attached to the surface of a solid particle. The nature of the particle surface determines the particle affinity for the compound. Some particles such as clay minerals have an overall negative surface charge. Compounds with a positive charge, such as metal ions, are thus more susceptible to adsorption to these particles than ions with a negative charge.

Absorption is the process by which a compound in solution moves into the interior of the solid particle by diffusion into the inner lattice structure of the particle.

Sorption of heavy metals is pH dependent. At low pH values, metal ions compete with protons for the available sorption sites. The sorption increases as pH increases. Also, metals compete with each other and with other cations for the available sorption sites.

Complexation: Complexation is a process by which a metal ion combines with an inorganic or organic compound to form a soluble complex. Typical inorganic ligands in the soil include hydroxides, nitrates and carbonates. Complexation may enhance the solubility of the specific heavy metals and reduce the fraction that precipitates or adsorbs.

2.5 Soil Properties Influencing Metal Uptake By Plants

The uptake of trace elements by plants is influenced by the physical and chemical properties of the soil. When biosolids are added to the soil, the chemical and physical constituents of the biosolids may affect the soil properties by:

- Increasing the total content of nitrogen and carbon.
- Increasing the cationic exchange capacity
- Decreasing pH (depending on the pH of the sludge)
- Increasing the total concentration of heavy metals
- Increasing organic matter
- Increasing soil temperature

The soil properties affecting the availability of the trace elements to the plants include:

1) Soil pH: the sorption of heavy metals by plants is heavily dependent on soil pH. In general, sorption of metals by the plants increases with increasing pH. The lower the pH value, the more metal can be found in solution and therefore, the more mobile the metal is.

2) Physical properties: soil structure and texture determine the porosity, permeability and drainage rates of the soil. These properties in turn influence soil moisture content and aeration, which impact the rates of microbial activity, chemical reactions and plant root development.

When biosolids are mixed into the soil, the organic matter from the biosolids may increase soil aggregate formation and stability therefore improving aeration and drainage properties of the soil. The added organic content also increases soil water retention that may improve water uptake by plants.

3) Cation exchange capacity (CEC): CEC of a soil is a measure of the negative charge density of a soil as a function of the soil's ability to adsorb positively charged ions. Therefore a high CEC reflects a soil with a high sorption capacity. High CEC is desirable because it lessens nutrient loss by leaching.

   CEC is normally improved by sludge addition to soil because of the high CEC of organic matter.

The fraction of the total metal content in a soil, which is available for plant uptake, is considered to be the sum of the water-soluble and exchangeable metal. The factors affecting the uptake of heavy metals by plants include:

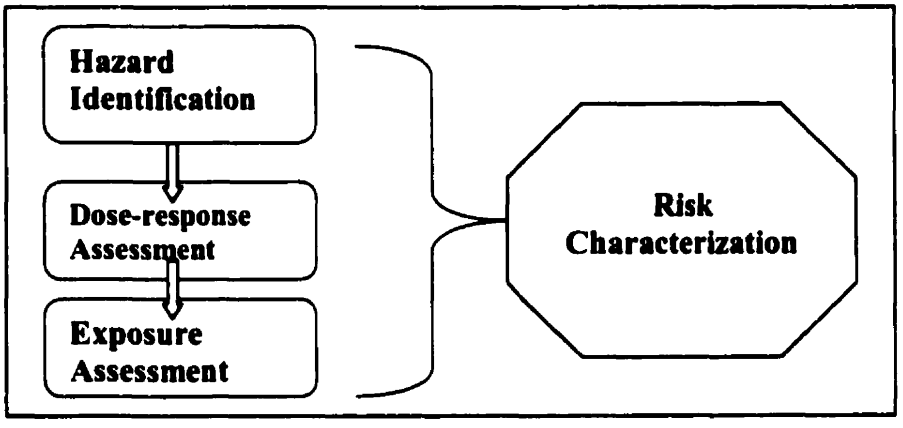
- Soil temperature: when the temperature rises, the metal activity in the soil solution may increase and the plant roots may be more active and have faster absorption rates. In addition, the absorption rate of the roots may be increased as a result of higher evapotranspiration from the plant (Houda, 1987).
- Metal concentration: the metal concentration level in plants increases with increasing metal concentration in the soil (Houda, 1987).
- Soil pH: sorption of metals by plants increases as the soil acidity increases (lower pH values).
- Type of crop: In general, leafy crops (ex. Spinach) uptake metals more than non-leafy crops.
- Cationic Exchange Capacity (CEC): high CEC reflects a soil with high sorption capacity.

Chapter 3. Health Risk Assessment: An Overview

3.1 Health Risk Assessment Process

Health risk assessment is a process that seeks to identify the kinds of adverse outcomes that may be associated with exposure to potentially harmful substances and to predict the likelihood that a specific human population will experience such effects (U.S.EPA, 1987). The steps involved in the risk assessment process are shown in Figure 2.

Figure 2: Schematic diagram of the risk assessment process



Source: U.S.EPA, 1987

Hazard Identification. This initial risk assessment activity is directed at determining the nature of the effects that may be experienced by an exposed human from an identified pollutant. Hazard identification is used to identify through the available literature whether the pollutant poses a hazard and whether sufficient information exists to perform a quantitative risk assessment. Hazard identification consists of reviewing all relevant data (i.e. epidemiological and toxicological studies) to demonstrate whether a pollutant poses a specific hazard, then qualitatively evaluating those data on the basis of the type of health effect produced, the conditions of exposure, and the metabolic processes that govern pollutant behavour within the body (U.S.EPA, 1987).

Dose-response Assessment. This step seeks to identify the quantitative relationship between a dose level and the resulting incidence of injury or disease. With noncarcinogens, the normal working assumption is that biological effects occur only after a threshold level of exposure has been exceeded (U.S.EPA, 1987). Thresholds include:

lowest-observable-effect-level (LOEL), the smallest dose that causes any detectable health effect; no-observed-effect-level (NOEL), the dose at or below which no biological effects (good or bad health effects) of any type are detected; and no-observed-adverse-effect-level (NOAEL), the dose at or below which no harmful effects are detected (U.S.EPA, 1987).

Exposure Assessment. This step attempts to identify the exposure level along with the duration and extent of exposure in a given circumstance to the risk agent or hazard. The exposure assessment could be based on current exposures, past exposures or those anticipated in the future. The steps involved in the exposure assessment vary widely because the circumstances differ with respect to how much is known about existing exposures. Also, numerous pathways may exist through which exposures can occur (U.S.EPA, 1987).

Risk Characterization. This final step in the risk assessment process combines the findings of the previous three steps into an integrated picture of the nature and expected frequency of the adverse health effects in exposed populations in a given situation (U.S.EPA, 1987).

3.2 Data Sources for Health Risk Assessment

The data used in health risk assessment processes are derived from two main types of studies: principal studies and supportive studies.

1. Principal studies are those that contribute most significantly to the qualitative assessment of whether or not a chemical or biological agent is potentially hazardous to humans. In addition, principal studies may be used in the quantitative dose-response assessment phase of the risk assessment process. The two types of principal studies are:

- Toxicological studies. For most chemical or biological agents, information on their adverse health effects on humans is lacking. In such cases, information is drawn from

experiments conducted on animals. Toxicological studies examine how the potentially toxic substances behave (distribution in the body and the resultant health effects) at different exposure levels in the animal.

Toxicological studies are designed to provide information on the appropriate dosage range for the hazards and the probable adverse health effect on the target organ or system. Such studies yield data that can be used to assess the NOAEL of the toxicant. In turn the NOAELs are used to derive various safety benchmark levels used in the assessment of health hazards. The NOAEL and the safety benchmarks will be discussed further in chapter 4.

Toxicological studies are generally divided into three categories: 1) acute toxicity studies 2) short-term toxicity studies and 3) long-term toxicity studies.

Acute toxicity studies involve either a single administration of the chemical or agent under test or several administrations within a 24-hour period. These studies are designed to either determine the median lethal dose (LD50) of the toxicant or provide an estimate of LD50. The LD50 is defined as the statistically derived expression of a single dose of material that can be expected to kill 50% of the animals. In addition, acute toxicity studies may also be used to indicate the probable target organ of the chemical and its specific toxic effect.

After the toxicant has been administered to the animals, examinations are made for the number and times of death in order to estimate the LD50. When the percent response (proportion of population killed) is plotted against the dose on a logarithmic scale, an S-shaped curve appears. This plot is then used to estimate the various lethal doses.

Short-term toxicity (also known as sub-acute or sub-chronic) studies involve repeated administration, usually on a daily or a five times per week basis over a period of about 10% of the life span of the animal. Long term toxicity studies, on the other

hand, involve repeated administration of the toxicant to the animal over the entire life span or at least a major fraction of it.

Short term and long term toxicity studies are conducted because humans are more often exposed to agents at low levels over longer periods of time. The procedures involved for these two types of studies are very similar except for their duration.

Short term and long term toxicity studies provide information on the toxicity of the agent with respect to the target organs. From these studies, the dose-effect and dose-response relationships for the agents can be determined. The NOAELs from these studies can again be used for determining the acceptable daily intake of that specific agent for humans.

In order to make use of the toxicological data from animal studies, the data are extrapolated accordingly to make them applicable to humans. Extrapolation from animals to humans has many sources of uncertainties. These uncertainties will be discussed in chapter 4.

- Epidemiological studies. Epidemiology is the study of the distribution and determinants of disease in populations. The data derived are based on the collective experience of the subjects in question. When information exists on the exposure levels in human populations that are associated with a certain health effect and the exposed population can be well defined, epidemiology provides the most direct way of determining the effects of a risk agent on human subjects. The availability of such data alleviates the necessity to extrapolate from animal studies to humans.

  Epidemiological studies are useful in hazard identification but as level of exposure is not provided or quantified, they are less useful in establishing dose-response relationships which requires quantification of the exposure levels. Available human studies on ingestion of hazards are usually of this nature and only provide support for the choice of critical toxic effect (effect observed at lowest level of toxicant).

The limitation of epidemiological studies is that essential data such as the level of exposure to the risk agent, the resultant adverse effect or the ability to define an exposed population are often not met or provided. In addition, the presence of confounding factors such as simultaneous exposure to other substances can make identifying the health effect of an agent difficult. Nonetheless, epidemiological studies play a crucial role in hazard identification which is a crucial step in the risk assessment process (Lu, 1996).

2. Supporting studies. Supportive studies provide supportive, rather than definitive, information about the adverse effects of potential hazards. Thus, supporting studies are useful for the hazard identification and dose-response assessment steps of the risk assessment process. The types of supportive studies include:

- Structure activity studies. These studies seek to evaluate toxicity based on the substance chemical structure. Structure activity studies can provide insights into the chemicals' potential for biologic activity. The structure-activity relationship between a chemical and other structurally related compounds can be studied to provide clues to the chemical's possible toxicity.

  Metabolic and pharmacokinetic studies also provide insights into the mechanism of action of a particular compound. For example, the metabolism of the chemical exhibiting the toxic effects in animals is compared with the metabolism found in humans to assess the potential for toxicity in humans.

- Exposure data and exposure modeling. The identification of the various pathways in which the hazard enters the body is a crucial input to the risk assessment process. Exposure data is needed to estimate the amount of the substance that reaches the cells, tissues or organs of exposed individuals. This type of information is useful for assessing the dose-response relationship as well as assessing the exposure to the substance.

Therefore, health risk assessments draw on several or all of these types of studies. Additional studies can also provide valuable information. Such studies include pharmacokinetics, metabolic research and the mechanisms of toxicity. These studies are used to evaluate the relevance of the above approaches in predicting adverse health effects in humans (Lu, 1996).

Chapter 4: Threshold Levels and Other Benchmarks: Origin, Uses and Limitations

4.1 Introduction

Humans are constantly exposed to environmental hazards. The duration, dose and route of exposure to these hazards will determine whether adverse health effects will be experienced or not. To protect the health of the human population, official government agencies (e.g. Health Canada, U.S. Environmental Protection Agency [EPA]) have established guidelines and standards for known hazards. In deriving these guidelines and standards, the agencies have used various threshold parameters to develop safe levels. In this chapter, threshold parameters (i.e NOAEL, LOAEL and NOEL) and other benchmarks (i.e. Allowable Daily Intake [ADI], Reference Doses [RfD] and Minimal Risk Levels [MRL]) used in the health risk assessment of chapter 6 will be discussed.

4.2 NOAEL, LOAEL and NOEL

The NOAEL is the maximum dose level that has not induced any sign of toxicity (adverse health effect) in the most susceptible species of animal tested. The NOAEL is not necessarily an absolute no-effect level, but rather a no adverse effect level or no documented effect. Using more sensitive indicators or a more susceptible animal may reveal a lower NOAEL. In addition, an effect might be demonstrated if sufficiently large numbers of animals were used in the tests (Health Canada, 1993).

In cases where NOAEL has not been demonstrated experimentally, the LOAEL is used. The LOAEL is the lowest level at which some symptoms are found. A NOEL, on the other hand, is the level at which no effect (positive or adverse) is detected (Health Canada, 1993).

4.3 Allowable Daily Intake (ADI)

The term ADI was coined by the joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives in 1961 (WHO, 1962).

This term has then been used at all subsequent meetings of FAO/WHO in their toxicological evaluation and reevaluation of food additives and pesticides that leave residues in food (WHO, 1962).

ADI is defined as the amount of daily intake of a chemical over an entire lifetime (average life-span of 70 years) that can be consumed without appreciable risk on the basis of all known facts at the time. It is expressed in milligrams of the chemical per kilogram of body weight (mg/kg) (WHO, 1962).

Estimates of ADIs are commonly derived using the following steps:

1. compile data from relevant principal and supporting studies
2. select NOAEL
3. divide by a safety factor (SF) to obtain ADI

The SF is used in the derivation of ADIs to reflect the uncertainty when extrapolating from animal data to human populations. The effect of the hazard on the animals may not apply to humans due to physiological differences between the species. The SF is therefore intended to allow for differences in sensitivity of the animal species and humans, to allow for wide variation in susceptibility among the human population and to allow for the fact that the number of animals tested is small compared to the size of the human population that may be exposed (NAS, 1970).

The size of the SF to be used in calculating ADIs is based on the nature of the toxicity and the adequacy of dose-response data for that particular hazard. The National Academy of Science (NAS) and U.S.EPA have developed safety/uncertainty guidelines. These guidelines are outlined in Table 2.

Table 2: Safety/Uncertainty Factors

Factor	Application
10X	Used when extrapolating from valid experimental results on prolonged human intake. This factor is intended to account for the variation in sensitivity among the members of the human population.
100X	Used when experimental results from studies of human intake are not available or are inadequate. This factor accounts for the uncertainty involved in extrapolating from animal data to humans.
1000X	Applied when there are no long-term human data and only scanty results on experimental animals are available. This factor accounts for the uncertainty involved in extrapolating from animal data to humans and from short-term to long-term effects as well as protecting sensitive members of the population.

Source: Dourson and Stara, 1983

4.4 Reference Dose (RfD)

A reference dose is an estimate of a daily exposure to the human population (including sensitive sub-groups) that is likely to be without appreciable risk of deleterious effects during a lifetime. The RfD is commonly expressed in units of mg/kg of body weight per day. Similar to the ADI, the RfD is operationally derived from the NOAEL with the use of SF. The RfD differs from the ADI in that a modifying factor (MF) is sometimes used and is based on a professional judgement of the data on the hazard. The magnitude of the MF ranges from 0 to 10 and depends upon the professional assessment of scientific uncertainties of the study and the chemical database (Dourson and Stara, 1983).

The RfD is computed using the following formula:

- RfD= NOAEL/(UF X MF) where UF is the Uncertainty Factor.

It is important to note that the RfD is a minor variation of the ADI. The RfD is derived by the U.S. EPA using essentially the same procedures as the ADI, except the safety factor is called an Uncertainty Factor. The ADI is used widely on an international level as well as by regulatory agencies in many nations, including the U.S. EPA. whereas the RfD is used mainly by the U.S.EPA.

4.5 Minimal Risk Levels (MRL)

The Agency for Toxic Substances and Disease Registry (ATSDR) along with the U.S. EPA were required by the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) to prepare toxicological profiles for substances included on the priority list of hazardous substances in the environment. As a response to the mandate, ATSDR developed MRLs. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse health effects over a specified duration of exposure (ATSDR, 1990).

ATSDR adopted a practice similar to that of the EPA RfD for deriving MRL. The NOAEL divided by the UF approach is used to derive MRLs for hazardous substances. MRLs are set below levels that, based on current information, might cause adverse health effects in the people most sensitive to such substance-induced effects. Oral MRLs are expressed as daily human doses in units of mg per kg per day (mg/kg/day).

Most MRLs contain some degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive to the effects of hazardous substances. ATSDR uses a conservative approach to address these uncertainties. MRLs are often based on animal studies because relevant human studies are lacking. In the absence of human studies, ATSDR assumes that humans are more sensitive than animals to the effects of hazardous substances. Thus, the resulting MRL may be as much as a hundred-fold below levels shown to be nontoxic in laboratory animals (ATSDR, 1990).

4.6 Limitations/Difficulties in Using Benchmark Levels

As noted above, the ADI, RfD and MRL are derived from an experimentally determined NOAEL. In addition to the NOAEL, a SF or an UF is used to compensate for inadequate data. Both the NOAEL and the SF (or the UF) have their limitations. As such, the benchmark doses, ADI, RfD and MRL will have inherent limitations leading the user of the benchmarks to be careful in the interpretations of the obtained results. Limitations or uncertainties associated with the benchmark levels include:

- The experimental dose NOAEL is based on scientific judgement. As such, experimentally determined doses for a hazard producing a statistically significant adverse effect may differ amongst researchers resulting in different NOAELs for the same hazard.
- NOAEL is limited to the doses tested experimentally.
- Guidelines have not been developed to take into account that some studies use larger number of animals and are thus more reliable than studies that use a lower number of test animals.
- Measuring techniques in laboratories have their limitations (human and instrumental limitations), which may skew the determination of the NOAELs.
- NOAELs for a specific substances may differ between animal species
- As scientific knowledge increases, questions about the selection of the appropriate health effect arise.
- Data relating to the upper and lower ends of the dose-response may be difficult to obtain because large exposures are relatively rare and low level exposures may be too small to detect.
- Safety factors used for calculating ADI, MRL and RfD may be somewhat arbitrary.
- Since the use of SF is a judgement call, different values for ADI, MRL and RfD may exist for the hazard.
- The term SF suggests the notion of absolute safety. In the majority of cases, a firm experimental basis for this notion does not exist.

Chapter 5: Background and Toxicological Profile of Trace Metals

5.1 Zinc

Background. Zn is one of the most common elements in the earth's crust. It is an abundant element and constitutes approximately 0.004% of the earth's substance (Health Canada, 1989). Thus it is ubiquitous in the environment, present in most food products, water, and air. Pure Zn is a bluish white shiny metal. When Zn combines with other elements, it forms various compounds that include Zn oxides, Zn sulfates and Zn chlorides. The most common Zn mineral is sphalerite (ZnS), which is often associated with the sulphides of other metallic elements e.g., Pb, Cu, Cd, and iron. Zn is also found as calamine ($ZnCO_3$) in carbonate sediments; other forms of Zn are usually products of the oxidation of sphalerite (Health Canada, 1989). Zn is also ubiquitous in the soil environment. The average soil concentration of Zn in Canada is 90 mg/kg (Health Canada, 1989).

Zinc is an essential nutrient in humans and animals that is necessary for the function of a large number of metalloenzymes (Cousins, 1985). Thus, an insufficient amount of Zn in the human diet can be harmful. Zn is required for normal nucleic acid, protein and cell growth and division. Therefore, certain levels of Zn intake are recommended. The Recommended Dietary Intake (RDI) for Canadians is 2 mg/day for young infants, 3 to 7 mg/day for children to age 12, and from the age of 13, 9 mg/day for males and 8 mg/day for females (Department of National Health and Welfare, 1983). The different rates among the adult males and females reflect the different metabolic rates and hormonal capacity between the two genders.

Human exposure to Zn results from a variety of sources. These include:

- Drinking water contaminated with Zn from nearby industries or waste sites
- Ingesting small amounts that are present in food and water
- Drinking contaminated water that has been stored in metal containers or flow through pipes that have coated with Zn to prevent rust
- Consuming vitamin supplements containing Zn, and
- Breathing Zn particles in the air at manufacturing sites.

(ATSDR, 1994).

Toxicokinetics. About 20 to 30 percent of ingested Zn is absorbed. In humans, the small intestine absorbs Zn by a carrier-mediated mechanism. Because Zn is also secreted into the gut, the fraction of Zn absorbed is difficult to determine, however, 33% of the total ingested Zn is the average Zn absorption in humans (Health Canada, 1989). Zn may be more bioavailable from drinking water than from food. With increasing dietary Zn, Zn absorption increases, up to a maximum rate, indicating a saturated carrier-mediated mechanism (Health Canada, 1989). An individuals Zn status may influence Zn absorption. Humans on a high Zn diet show a reduced efficiency of absorption whereas Zn-deprived humans absorb Zn with increased efficiency. Zn absorption can be influenced by many regulatory and dietary factors, with phytate (myoinositol hexaphosphate) being one of the most important (Health Canada, 1989). The phytate content of cereals and legumes greatly reduces the bioavailablility of Zn from these foods. Some components of dietary fibre may also reduce Zn absorption. When phytate is ingested with calcium, phytate reduces Zn absorption by forming insoluble precipitates (Health Canada, 1989).

Zn and iron appear to antagonise each other's absorption at high doses. The doses of iron required to inhibit Zn absorption are well above those found in food and the competitive interaction between iron and Zn is unlikely to be significant under ordinary dietary conditions (Valberg et al., 1984). Valberg et al. (1984) found that Cu in a dose of 5 mg had no significant effect on Zn absorption in humans, which implies that Cu at levels ordinarily found in the diet is unlikely to have any important effect on the absorption of Zn.

Zn toxicity from excessive ingestion is uncommon, however, gastrointestinal distress and diarrhea have been reported following ingestion of beverages standing in galvanised cans or from use of galvanised utensils (Moore, 1978). Evidence of hematologic, hepatic, or renal toxicity has not been observed in individuals ingesting as much as 12 g of elemental Zn over a two-day period (Murphy, 1970). Metal fume fever resulting from inhalation of freshly formed fumes of Zn presents the most significant effect. The disorder has been

most commonly associated with inhalation of Zn oxide fumes. There have been reports of teratogenic effects in sheep, and disrupted cholesterol metabolism in humans, both thought to be due to the adverse effects of high Zn concentrations on Cu metabolism (Campbell and Mills, 1979).

5.2 Cadmium

Background. Cd is a silvery- white metal that is soft and ductile. Chemically, Cd closely resembles Zn and is a natural element in the earth's crust. Most commonly it is found as the sulphide, also known as Cd blende, which is often associated with the Zn ore, ZnS. Canadian Zn ores contain from .001 to .067 percent Cd (Lymburner, 1974).

Cd is a relatively rare element that is uniformly distributed in the earth's crust where it is estimated to be present at an average concentration of between 0.15 to 0.2 mg/kg soil (Hiatt and Huff, 1975). Cd occurs in nature in the form of various inorganic compounds and as complexes with naturally occurring chelating agents (Nordberg, 1974).

Uses of Cd are primarily for electroplating other metals to protect them against corrosion. It is employed extensively in the production of low melting-point alloys and solders. In Canada, Cd is used in the manufacture of stabilizers for plastics and pigments (Environment Canada, 1976). Other applications consuming Cd include fungicides, control rods for nuclear reactors, motor oils and Ni-Cd batteries (Riihimaki, 1972).

Human exposure to Cd results from a variety of sources. These sources include:

- Breathing contaminated workplace air (e.g. battery manufacturing)
- Eating foods containing Cd (liver and kidney meats)
- Breathing Cd in cigarette smoke
- Drinking contaminated water

- Breathing contaminated air near the burning of fossil fuels

(ATSDR, 1993)

The main source of Cd intake for humans is food. Estimates of the mean daily intake from food have been made in a number of countries and range from .02 to .06 mg (Sherlock, 1984). A survey of Canadian diets found that the mean daily intake of Cd was in the range of .007 to .034 mg (Dabbeka et al., 1987).

Cd is not known to have any beneficial effects, but can cause a number of adverse health effects. Long term exposure to lower levels of Cd in air, food and water Pbs to a build up of Cd in the kidneys and possible kidney disease (Kitamura et al., 1970). A detailed evaluation of the health effects of Cd will be covered in more detail in the health identification section of Chapter 7 Section 7.1.

Toxicokinetics:

- **Absorption.** Studies on human subjects have indicated that 4 to 7 percent of a single dose of ingested Cd is absorbed from the intestines (Fulkerson and Goeller, 1973; Nordberg, 1974). The absorption of Cd nitrate in animal studies ranged from 0.5 to 3 percent (Friberg et al., 1974). Results of animal experiments have indicated that intestinal absorption is dependent on age and diet (Gauvin, 1986). The amount absorbed depends on the components of the diet such as iron, calcium and protein (Hallenbeck, 1984). In animal studies (rats), females have been found to absorb more dietary Cd than males (Buhler, 1985).

- **Distribution.** Absorbed Cd accumulates in the renal cortex and liver. The pancreas, gall bladder and testes can also contain relatively high concentrations of Cd (World Health Organization, 1974). The total body burden of a person of 50 years of age ranges from 5 to 40 mg (Laureys, 1978). About half the body burden is found in the kidneys and liver. The Cd concentration of the cortex of the kidney ranges form .005 to .1 mg/kg. Concentrations of Cd in the renal cortex are normally 5 to 20 times those in the liver (Fleisher, 1974).

- **Excretion.** Only a small portion of absorbed Cd (less than 10 percent) is excreted mainly in the urine and the feces. Negligible amounts are eliminated through hair, nails and sweat (Friberg, 1974).

- **Metabolism.** Cd is not known to undergo any direct metabolic conversion. The Cd ion does bind to anionic groups in proteins and other molecules (Nordberg, 1985).

5.3 Chromium (III)

Background. Cr is a naturally occurring element in the earth's crust and exists in oxidation states ranging from Cr^{2+} to Cr^{6+}. Cr is present in the environment mainly in the trivalent or hexavalent forms, which appear to be of significance in biological systems. The trivalent form (Cr[III]) is the more common naturally occurring state of Cr and is essential in humans and animals for efficient lipid, glucose, and protein metabolism. However, the hexavalent forms of chromate compounds are of greater industrial importance (Health Canada, 1989). Trivalent chromium (Cr[III]) is not considered to be toxic, however, if present in raw water, it may be oxidised to hexavalent Cr (Cr[VI]) during chlorination (Health Canada, 1989).

Chromium (VI) does not occur naturally in the environment but is produced from anthropogenic sources such as in the chemical industries. Chromium (VI) is toxigenic to humans. Breathing high levels (>2ug/m^3) of Cr (VI) can cause irritation to the nose, including sneezing, itching, nose bleeds and ulcers. Cr (VI) is also believed to be primarily responsible for the increased lung cancer rates observed in workers who were exposed to high levels of Cr in the workplace (ATSDR, 1993a).

Cr is present in Canadian soils at concentrations ranging from 20 to 125 mg/kg and is found in trace quantities in most plant and animals tissues. Most of the Cr in soils is present in the form of highly insoluble chromites (Health Canada, 1989).

Cr is widely used in industry. For the production of all Cr chemicals, sodium chromate and dichromate are the principal substances. Sodium dichromate is produced industrially by the reaction of sulfuric acid on sodium chromate. The major uses of sodium dichromate are for the production of chrome pigments, for the production of trivalent chrome salts used for tanning leather, textile dyeing, wood preservatives, and as an anticorrosive in cooking systems. Trivalent chrome salts are also used in the ceramic and glass industry, and in photography. The hexavalent Cr compounds are used in the metallurgical industry. Metallurgic-grade chromite is usually converted into one of several types of ferrochromium or other Cr alloys containing cobalt or Ni (ATSDR, 1993a).

Foods vary considerably in Cr content. The main dietary sources of this element are:

- milk and dairy products (mean, 0.06mg/kg),
- meat (0.07 mg/kg),
- cereal (0.17 mg/kg),
- potatoes (0.05 mg/kg),
- fruits (0.06 mg/kg),
- sugars (0.34 mg/kg)
- seafood commercially available in Canada (0.13 to 0.85 mg/kg)
- carbonated beverages and fruit juices generally contain less than 0.01 mg/L
- imported and domestic wines available in Canada (between 0.02 and 0.06 mg/L)

(Health Canada, 1989)

The Canadian mean dietary intake of Cr was found to be approximately 0.055 mg/day from an analysis of Canadian diets (including drinking water), with a range of approximately 0.01 to 0.16 mg/day (Health Canada, 1989). The estimated daily intake by humans is under 100 ug (0.1 mg), mostly from food, with trivial quantities (less than 10%) from most water supplies and ambient air (ATSDR, 1993a).

Based on the food, air, and water considerations, the total daily intake of Cr would be about 0.06 mg (Health Canada, 1989). Intake for smokers may be higher because of the presence of Cr in cigarettes.

Toxicokinetics:

- **Absorption.** Approximately 0.5 to 2% of the ingested Cr is absorbed via the GI tract of humans. This absorption rate or efficiency is highly dependent on the dietary intake. At low levels of dietary intake (10 ug), around 2% of the Cr is absorbed. At dietary intakes of greater than 10 ug of Cr, the absorption efficiency drops to around 0.5% (Anderson, 1986).

- **Distribution**. Studies have indicated that Cr concentrations in the body are highest in the kidney, liver, lung, aorta, heart, pancreas and spleen (Schroeder et al., 1962). The distribution of Cr in human body tissue after acute oral exposure was determined through an autopsy of a 14-year-old boy who ingested 7.5 mg Cr/kg as potassium dichromate. The Cr concentrations were as follows: liver 2.94 mg/100cc (normal, .016 mg/100cc) and brain, .06 mg/100cc (normal .002 mg/100cc) (Kaufman et al., 1970).
- **Excretion**. In a study, radioactive Cr was administered to human subjects to determine the level of Cr excretion. After six days of fecal collection, around 99.6% of the ingested Cr were recovered. The amount of the Cr in the urine collected was around 0.5% of the ingested dose (Donaldson and Barreras, 1966).

5.4 Lead

Background. Pb is one of the most ubiquitous and persistent heavy metals in the environment. It has been detected in the air, soil, sediment, surface and groundwater and biological systems. Pb occurs naturally in the environment and from human activities. In surface water, Pb may form insoluble compounds with other substances in the water. In

soil and sediment, Pb complexes with soil particles, which reduces its bioavailability to organisms living in those environments (Health Canada, 1989).

Routes of human exposure to Pb are oral ingestion, inhalation, dermal contact or transfer via the placenta. The oral ingestion route will be focused on in this thesis. The primary routes of exposure vary for children and adults. For adults, inhalation of Pb-containing dusts and fumes in occupational settings, particularly during mining, smelting, and refining operations is the primary route of exposure (Juberg et al., 1997). A child's primary route of Pb exposure is oral ingestion of Pb-based paint, Pb-containing dust and Pb-contaminated soil. Exposure to Pb may also occur through eating or smoking in Pb-contaminated environments (Juberg et al., 1997).

The most common source of Pb exposure for young children remains to be Pb based paints (Juberg et al., 1997). Children can be exposed through ingestion of flaking, chipped paint from playground equipment, toys, furniture, interior and exterior residential surfaces. In the 1940's white Pb paint containing up to 50% Pb was more common.

The other potential source of Pb exposure to children is the elevated concentration of Pb in the soil, particularly on homes located in close proximity to Pb smelters or industries involved in the manufacture of Pb products. For Pb in soil and dust, the gastrointestinal absorption rate in children has been estimated at 30% (Ziegler et al., 1978). Ingestion of drinking water with elevated Pb levels from Pb-containing pipes and fixtures may also contribute to human exposure of Pb.

An additional route of Pb exposure is the ingestion of food. Pb occurs in and on food naturally from atmospheric deposition, or introduced through harvesting, transportation, processing, packaging or preparation. Pb can contaminate food through dust, metals used in grinding, crushing or sieving, solder used in packaging and water used on cooking. Young children absorb from 40% to 53% of Pb ingested from food (Mushak and Crocetti, 1989).

The daily dietary intake of Pb has decreased significantly since 1940's from as high as 400-500 Φg / day to under 20 Φg /day for the United States (US) population (Juberg et al., 1997). This decrease has been attributed to the recognition of the adverse health effects of Pb and the implementation of regulations that prevent exposures to Pb. The global average daily intake of Pb, estimated by the United Nations Environment Program is 80 ug/day from food and 40 ug/day from drinking water. The continuing decline in dietary intake of Pb world wide over the years has contributed to the decline in blood-Pb levels (Juberg et al., 1997).

Toxicokinetics

Absorption and Distribution:

Pb begins to accumulate in human bodies either during prenatal development (from placental transfer due to maternal exposure) or following birth as a result of trace level exposure from a variety of sources. Adults absorb 5 to 15% of ingested Pb and generally retain less than 5% of what is absorbed (Goyer et al., 1996). Young children absorb approximately 30-40% more ingested Pb than adults due to physiological and metabolic differences (Goyer et al., 1996). Once Pb is in the blood, it is distributed primarily among the soft tissues (blood, kidney, spleen, bone marrow, liver, and brain), and mineral tissues (bone and teeth). The distribution of Pb in bone increases with age from about 70% of body Pb in childhood to as much as 95% with advancing age. The distribution of Pb in bone is fractional compared with other body stores. Pb that is not absorbed by the body is excreted primarily through the feces. Studies have confirmed that Pb absorption is highly dependent on the form of Pb ingested (Pb sulphide, Pb-contaminated soil, or Pb acetate) and the medium (soil, dust, or as Pb itself) in which it is consumed (Goyer et al., 1996).

The potential health effects of Pb in soil were studied by U.S.EPA to determine the effect of soil abatement on children and to quantify the relationship between soil or dust Pb content and blood-Pb levels (U.S. EPA, 1996). Results indicated that soil Pb is not a major determinant of blood Pb. Researchers noted that soil Pb abatement, by itself, has

minimal impact on blood-Pb status and concluded that Pb in soil is not very bioavailable (Juberg et al., 1997). The absorption and toxicity potential of Pb to humans is also influenced by the overall nutritional status and eating behaviour. Pb intake from consumption of water and other beverages tends to be absorbed to a greater degree than Pb in food (Pb complexes with food particles), while Pb ingested during fasting (no food consumption) conditions is absorbed to a greater extent than Pb ingested during food consumption (Juberg et al., 1997; Mahaffey, 1990). The absorption of Pb is greatly increased when the intakes of calcium and phosphorus are low. Pb also interacts and competes physiologically with three essential elements: namely calcium, iron, and Zn. (Juberg et al., 1997; Goyer, 1996). Pb may be released from bone in humans. This is an important consideration in regards to blood-Pb levels, particularly since various physiological and pathological conditions (e.g., osteoporosis, chronic disease, pregnancy, and lactation) may cause mobilisation of Pb stored in bone into the bloodstream (Juberg et al., 1997 and Goyer, 1996).

5.5 Nickel

Background. Ni is an abundant element that is found in soil and constitutes about .008 % of the earth's crust (NAS, 1975). It is found primarily bound to oxygen or sulfur. Pure Ni is a hard, silvery white metal that normally occurs in the 0 and 2+ valence state. Ni and its compounds have no characteristic odor or taste (NAS, 1975).

The chemical properties of Ni make it desirable for combining with other metals to form alloys. These alloys are used to make metal coins, jewelry, stainless steel, color ceramics and batteries (NAS, 1975).

Ni is considered an essential element to maintaining good health in animals. However, to date, it has not been recognized as an essential element to humans (ATSDR, 1997). The health effects from exposure to Ni in humans are covered at the end of the section.

The major source of exposure to Ni for humans not working in Ni related industries are through the ingestion of food. Other sources of exposure include:

- Breathing air or smoking tobacco containing Ni
- Drinking water containing traces amounts of Ni
- Skin contact with coins and other metals containing Ni

Toxicokinetics

- **Absorption.** In humans, Ni is sparsely absorbed from the GI tract. Studies by Sunderman et al. (1989) have shown that when Ni is consumed in food, only 0.7% (+/- 0.4%) is absorbed from the GI tract. When Ni was given in drinking water, 27%(+/-17%) was absorbed through the GI tract.

- **Distribution.** Once absorbed form the GI tract, Ni is transported in the plasma to serum albumin and multiple small organic ligands (surface of organ cells). Autopsy studies of individuals not occupationally exposed to Ni has shown the highest concentration of Ni to be in the lungs, followed by thyroid, adrenals, kidney, heart, liver, brain, spleen and pancreas (Rezuke et al., 1987).

- **Excretion.** Regardless of the route of exposure, absorbed Ni is mostly excreted in the urine. Excretion in the urine is nearly complete in 4 or 5 days. The remainder of the ingested Ni is excreted in the feces (Sunderman et al., 1989).

5.6 Copper

Background. Cu is a reddish metal that occurs naturally in soil, water, plants, and rocks. It has an average concentration of 50 parts per million (ppm) in the earth's crust. Cu is an essential element for all known living organisms including humans and animals (NAS, 1977).

Alloys of Cu are extensively used for the manufacture of wire, sheet metal, pipes and other metal products. Cu is also used for water treatment and as a preservative for wood, leather and fabrics (NAS, 1977).

Cu deficiency can cause a variety of health effects. Since Cu is necessary for the absorption and use of iron, deficiency is likely to Pb to ruptured blood vessels, osteoporosis and bone and joint problems. Other problems with Cu deficiency include brain impairment and hindered immune function (ATSDR, 1990).

Like all other heavy metals, Cu is potentially toxic. Exposure to Cu may occur by breathing air, drinking water, eating food and by skin contact with the metal or Cu containing products. Workplace exposure is also likely during Cu mining and ore processing. Other workplace exposures may occur in industries such as agriculture and electroplating (ATSDR, 1990).

Toxicokinetics:

- **Absorption**. Cu is absorbed in the stomach and the small intestines. The site of maximal absorption is not known for humans. A study by Strickland et al. (1972) revealed that around 60% of an oral dose of Cu as Cu acetate was absorbed from the GI tract.

- **Distribution**. Absorbed Cu loosely binds to plasma albumin and amino acids in the portal blood and is then taken to the liver (Marceaw, 1970). In the liver, Cu is incorporated into ceruloplasmin and released into the plasma (Owen, 1965).

- **Excretion**. Bile is the major pathway for the excretion of Cu. After oral administration of radioactive Cu in healthy humans, 72 % was excreted in the feces (Bush, 1955). Another study has shown that 0.5-3.0% of daily Cu intake is excreted in the urine (Cartwright, 1964).

Chapter 6: Methodology

6.1 Study Design

The basic design of this study entails the application of a health risk assessment process to quantify the human health risks associated with the consumption of trace metals in crops grown on biosolids-amended soil. The health risk assessment model employed in this study was adopted from the U.S. EPA. The steps involved in the health risk assessment are described in detail in Chapter 3, Section 3.1.

For hazard identification, the initial risk assessment activity, the published literature on the health effects of the metals was evaluated to determine whether or not each specific metal was known to pose a hazard to human health. The literature included a review of both animal and human studies and consisted mostly of epidemiological and toxicological studies. The data in these studies were evaluated on the basis of the type of health effect produced by the metals, the conditions of exposure (oral route) and the metabolic processes that governed the metals' behavior within the body. Thus, employing this initial risk assessment activity assisted in qualitatively determining whether the metals have the potential to pose a human health hazard and describing the nature of the effects that may be experienced by humans consuming the metals.

In the second stage of the health risk assessment process, dose-response assessment, the quantitative relationship between the dose level of the metal and the resulting health effect was identified. Similar to the hazard identification step, the dose-response assessment relied on the available research data to characterize the relationship between oral exposure to the metals and their related health effects. Therefore, this stage of the process identified the level or oral dose of the metals to which humans may be exposed without risk of deleterious health effects. The safe levels or doses identified are subject to a number of uncertainties that must be considered when interpreting these safe levels. These uncertainties related to the benchmark levels were discussed in section 4.6.

In the third stage of the health risk assessment process, exposure assessment, characterization of the exposure to each metal was conducted. This involved gathering and analyzing data collected by the City of Winnipeg, Water and Waste Department on the trace metals found in the crops grown on sewage-sludge amended soil. The concentrations of the metals in the crops were compared to the benchmark levels derived in the dose-response assessment stage. This comparison allowed for calculating maximum safe levels of the crops that can be consumed by humans. The calculated levels of crop consumption were based on the oral route of exposure only and are an estimate of daily exposure to the metal that is likely to be without appreciable risk of deleterious health effects during a lifetime.

In the final step of the risk assessment process, risk characterization, the findings of step 2 (dose-response assessment) and step 3 (exposure assessment) were combined into an integrated picture that described and estimated the adverse health effects in humans exposed (orally) to each of the metals. Metals not identified as potential health hazards as a result of oral exposure were described accordingly.

6.2 Sources of Data

The database used for this study is compiled from a project conducted by the City of Winnipeg, Water and Waste Department on the economic analysis of various sewage sludge application rates to agricultural land. As part of the project, the department's Laboratory Services Division established two 5.6-hectare test plots in the Rural Municipality of Rockwood, Manitoba. The two 5.6-hectare of land was used as follows:

- Each test plot was randomly subdivided into five 1.1-hectare strips.
- Biosolids were applied to five strips in a random block design at rates of 0,10000, 25000, 50000 and 100000 kg per hectare.
- Each of the five strips in the 5.6-hectare was further subdivided into two sub-plots for a total of 10 sub-plots. Each sub-plot was referred to as either fertilized (chemically) or non-fertilized (free of chemical fertilizer). The fertilizer was added at a rate to

ensure a minimum of 100kg/ha of plant-available nitrogen. Thus, the non-fertilized half of the test plot relied entirely on the biosolids nutrients for crop growth

- Each hectare of land was seeded with either wheat or oats (therefore, 10 sub-plots on one hectare plot was seeded with wheat and the other 10 sub-plots on the other hectare were seeded with oats).
- At harvest, five randomly selected, one square meter crop samples were collected from each of the sub-plots
- The grains from the crop samples were analyzed for heavy metal content using atomic absorption spectroscopy.

The design of the test plot is illustrated as Appendix A.

The data employed for the health risk assessment on the consumption of trace metals in crops grown on biosolid amended soil were therefore secondary data. As part of the project, the following information was collected by the City of Winnipeg and used in this thesis:

- Concentration of trace metals in the soil of the test plots prior to biosolids application
- Concentration of trace metals in biosolids prior to land application
- Level or concentration of the trace metals taken up by the plants (grain and straw) grown on soil amended with different biosolid application rates (0, 10000, 25000, 50000, and 100000 kg of sewage sludge per hectare) and with and without chemical fertilizer.

The analysis of the trace metal content in the crops was conducted by the City of Winnipeg, Laboratory Services Department. The methodology for the analysis was adopted from the American Public Health Association (1998). A description of the method is attached as Appendix A-1.

6.3 Statistical Analysis

Statistical analyses of the data from the study conducted by the City of Winnipeg were performed using Number Cruncher Statistical Systems 1997 (NCSS97). The data

required to achieve the objectives of this study were exported from Excel and imported into NCSS 97.

In order to detect whether a significant difference exists in the amount of metal content in the crops grown on biosolids-amended soil at different biosolids application rates, multivariate analysis (one-way analysis of variance) was used. This method was chosen because only one possible cause of variation (differences in metal content due to the different application rates of biosolids) was being tested. In addition, the designs of the plots were randomized which further validates the use of multivariate analysis. The proposition that the metal content found in crops grown on soil amended with different amounts of biosolids are equivalent is known as the null hypothesis (Ho). If significant differences existed in the metal content of the crops due to the different application rates (alternative hypothesis or H_1), Tukey's multiple comparison tests was used to identify treatment differences. In all cases, significance was assessed to be at the 0.05 level.

Some of the average concentrations of cadmium in the grains grown on biosolids-amended soil (without chemical fertilizer) have been reported as having values of <0.005 mg/kg. These values have been replaced with absolute values of 0.005. This is done so as not to under-estimate the value of the means. For example, the cadmium content in the grains grown on soil free of biosolids were recorded as 0.005, <0.005, <0.005, 0.08 and 0.082. If the values had been left as <0.005, the NCSS97 program would treat them as missing values because only absolute values are sensible for calculating the means. In this case the mean would be 0.0334 ([0.08+0.082+0.005+0+0]/5). Hence, under-estimation of the mean would occur. If the <0.005 values were replaced with 0.005 (conservative approach i.e. maximum possible concentration), then the mean would be calculated as 0.0354. The "less than" values in the data set indicate that the detection limit was set to the maximum level and thus concentrations below the detection limit were recorded as less than the value of the detection limit in the data set.

Chapter 7: Results

The average background concentration of heavy metals in the soil and biosolids used in this study are reported in Table 3.

Table 3: Background concentration of heavy metals in soil and biosolids:

Biosolids Application Rate	Background Concentration (mg/kg dry weight)					
	Cd	Cu	Zn	Ni	Pb	Cr(III)
Soil (no biosolids)	0.33	24.00	74.00	33.00	9.60	54.00
10000 kg/ha	12.40	726.00	2360.00	61.00	2430.00	2040.00
25000 kg/ha	11.50	716.00	2003.00	64.00	1367.00	2033.00
50000 kg/ha	13.20	815.00	1990.00	68.00	630.00	2370.00
100000 kg/ha	14.00	825.00	2180.00	66.00	1350.00	2130.00

The above table demonstrates that addition of biosolids does significantly contribute to the heavy metal load in the soil. To determine if the addition of biosolids to soil results in an increased health risk, a health risk assessment process was applied to cadmium, lead, chromium (III), zinc, copper and nickel. For the purpose of this study, the health risk assessments of the metals were based on the oral route of exposure since the concern deals with the ingestion of the metals in the crops.

7.1 Cadmium

Hazard Identification:

Epidemiological studies have demonstrated kidney effects from long term low level exposure to cadmium from both oral and inhalation routes (ATSDR, 1993). Most occupational studies have assessed kidney dysfunction by measuring the low molecular – weight protein in urine (typically ß2 microglobulin) as well as retinal binding protein (RBP) and N-acetyl- ß-D-glucosominidase (NAG). These proteins are readily filtered by the glomerulus and are normally reabsorbed in the proximal tubule of the kidney.

Therefore, elevated urinary excretion of these proteins is symptomatic of proximal tubular damage (Jung et al., 1993).

Human and animal studies for the incidence of kidney dysfunction based on cumulative cadmium exposure, kidney cadmium levels and urinary cadmium are available and will be discussed in this section. Studies on kidney effects reported in this section will focus on oral exposures since the risk assessment is based on the ingestion of the metal.

I) Human Studies:

- **Renal Effects**. Numerous studies have indicated that the kidney is the main target organ of cadmium toxicity following oral exposure to cadmium (Buchet et al., 1990; Hayano et al., 1996; Nogawa et al., 1989).

  Buchet et al. (1990) conducted a cross-sectional study of Belgians from cadmium polluted and non-polluted urban and rural areas. The cadmium intake by this population occurred primarily via ingestion of contaminated water and food. Buchet found abnormal rates of urinary excretion of ß2 microglobulan, retinal binding protein, amino acids and calcium in individuals with cadmium excretion rates greater than 2 ug/day.

  Nogawa et al. (1989) evaluated kidney function and cadmium exposure in Japanese subjects who lived in areas where the water was contaminated with cadmium leading to cadmium contamination of the rice grown in that area. Analysis of the prevalence of elevated ß2 microglobulan (ß2m) as a function of cadmium ingestion indicates that after a total intake of approximately .0021mg/kg-day of cadmium, renal damage will occur.

- **Bone disorders.** Bone disorders such as osteoporosis and spontaneous bone fracture have been observed in humans that have been chronically exposed to cadmium in foods (Kjellstrom, 1992). In a cadmium-contaminated river basin in Japan, Itai-Itai

disease (osteomalacia with skeletal pains and pseudo-fractures) has afflicted women of the region with several risk factors such as poor nutrition and multiparity (Nagawa and Kido, 1993). A study by Kido et al. (1990) found that Japanese populations with dietary cadmium exposure had elevated osteoporosis and osteomalacia in both men and women. Kido et al. (1990) also noted that the degree of loss in bone density is correlated with urinary excretion of ß2 microglobulan, an index of renal injury.

- **Gastrointestinal Effects**. Human and animal studies have shown that oral exposure to cadmium in high concentrations has caused severe irritation to the GI epithelium. Common symptoms in humans following ingestion of food or water containing high concentration of cadmium include nausea, vomiting, abdominal pain, cramps and diarrhea (Buckler, 1986). Although exact doses have not been measured, GI symptoms have been caused in children by 16 mg/L cadmium in soft drinks (Nordberg, 1974).

- **Hematological Effects**. Ingestion of cadmium reduces GI uptake of iron, which can result in anemia if dietary intake of iron is low. Anemia has been found among humans with chronic dietary exposure to cadmium (Kagamimori et al., 1986).

II) Animal Studies

Studies in animals have confirmed that the ingestion of cadmium can cause kidney tubular damage. These studies have also related the kidney damage to the cadmium concentration in the kidney (Kotsonis and Klassen, 1978; Prigge, 1978).

Prigge (1978) administered various doses (0, 25, 50, and 100 ppm) of cadmium orally to female Wistar rats for 90 days. Prigge noted that the body weight of the Wistar rats significantly decreased at greater than or equal to 50 ppm and significant increases in urinary protein were observed at or greater than or equal to 50 ppm. The kidney cadmium concentration ranged from 27-36 ppm in the Wistar rats who were administered 50 and 100 ppm oral cadmium (Prigge, 1978).

In another study by Kawai et al. (1976), 5 rats were orally given 0, 10, 50, 100, or 200 ppm cadmium for 37 weeks. Kidney lesions were observed at greater than or equal to 100 ppm. Spontaneous nephropathy was reported at 10 and 50 ppm in the animals.

Dose-Response Assessment:

There are two main studies that have shown the kidney as the primary target of oral exposure to cadmium and have quantified the dose-response relationship. The first by Nogawa et al. (1989) reported on a study in a Japanese population that consumed high levels of cadmium in rice and drinking water. The incidence of kidney dysfunction was determined as a function of cumulative cadmium intake. The study used a relatively insensitive measure of kidney dysfunction, ß2m levels in urine as opposed to a more sensitive measure such as NAG. Therefore, this study was not used as the primary basis for deriving the reference dose. However, this study did provide qualitative support for the study by Buchet et al. (1990) which was used as the principal study for deriving the reference dose.

Buchet et al. (1990) conducted an epidemiological study in a Belgian population that was exposed to cadmium via the oral route. The authors related urinary cadmium levels to ß2 microglobulan, NAG and retinal binding protein. For the most sensitive marker, NAG, the study reported that a 10% increase in the incidence of abnormal urinary excretion of NAG would occur at a urinary cadmium level of 2.7 ug/day. This study was conducted in a population that included sensitive sub-populations including diabetics and people up to 80 years old. Therefore, this study was considered the most appropriate basis for the reference dose.

Buchet et al. (1990) demonstrated the dose-response relationships between urinary cadmium levels and various urinary markers of renal effects in the general population. The authors estimated that the urinary cadmium level at which >10% of the population would have abnormally high excretion of the urinary markers was 2.7, 2.8, 3.05 and 4.29 ug cadmium/day for NAG, ß2 microglobulan, retinal binding protein and amino acids,

respectively. The increased levels of NAG are very likely associated with tubular breakdown. Bernard et al. (1990) demonstrated two enzymes of NAG and found the form associated with tubular breakdown to be predominant in the urine of cadmium workers nd non-exposed healthy subjects. The reference dose was therefore calculated based on excretion of cadmium associated with abnormal levels of NAG excretion at 2.7 ug-cadmium/day urine.

As noted above, urinary cadmium is reflective of the internal body burden of cadmium, which is related to the cumulative cadmium dose. To calculate the lifetime daily oral intake of cadmium that would result in a urinary excretion of 2.7 ug cadmium per day, absorption from the gastrointestinal tract was estimated at 5% with the other 95% of the ingested dose being eliminated in feces. Using this information, a urinary cadmium level of 2.7 ug cadmium per day was determined to correspond to a daily oral intake of 0.84 ug/kg-day assuming that all cadmium intakes are via the oral route (ATSDR, 1993).

This critical effect level (2.7 ug/day) is not a NOAEL but rather an estimate of a circumstance at which 10% of a population would be affected with abnormal urinary indicators. Until such time that information becomes available, the 10% probability of response for this endpoint in the human population is treated as a NOAEL (ATSDR, 1993).

No uncertainty factor (UF) was proposed for this estimate for several reasons. The study by Buchet et al. (1990) is based on a sensitive endpoint and a chronic lifetime exposure in a general population that included sensitive sub-populations. The absence of an UF is not meant to imply that the value would not change. General population studies with larger cohorts may yield different estimates.

The intake of attaining the 0.84 ug cadmium/kg-day level is non-specific for source and therefore should be inclusive of all routes of exposure and also inclusive of background levels. The principal background source of cadmium is dietary with the current mean life time exposure estimate to total cadmium from all food being 0.14 ug/kg-day (FDA,

1993). Based on this estimate, an individual, on the average, will consume 0.14 ug/kg-day in their normal diet from all food sources. Without any further exposure to dietary Cd, this background dietary exposure level corresponds to 16.7% (0.14/0.84 x 100) of the required or estimated critical affect level associated with the abnormal urinary indicators. Therefore, this background level of daily dietary intake should be adjusted for to arrive at the following RfD:

* RfD = 0.84 ug/kg-day - 0.14 ug/kg-day = 0.7 ug/kg-day. This equates to 49 and 7 ug/day for persons weighing 70 and 10 kg respectively. Therefore this total dose takes the estimated background dietary exposure to cadmium into consideration but does not include exposure to cigarette smoke (ATSDR, 1993). If the estimated Cd contributions from the diet were not taken into consideration, the value for the RfD would be over inflated resulting in a level over which is estimated to be without appreciable risk of deleterious effects.

The reference dose value of 0.7 ug/kg-day is an estimate of a daily exposure to a hazard above the background dietary level by the human population that is likely to be without appreciable risk of deleterious effects during a lifetime.

There is clear evidence in humans that smoking increases cadmium intake by as much as the daily dietary intake. Smokers have been shown to have 2-3 times higher cadmium concentration in their kidneys than similar aged non-smokers (Chung et al., 1986). This assessment acknowledges that smoking significantly adds to the burden of cadmium and thus smoking-related intake of cadmium is not considered in this assessment.

Exposure Assessment:

Sources of Cadmium Exposure:

1. Background sources:

   I) Diet. A study by Ellen et al. (1990) in a total diet study of 110 individuals reported that an average daily cadmium intake was around 10 ug/person/day. The major food sources were identified as green leafy vegetables, milk, potatoes, and liver. The total diet study by the FDA suggested the mean lifetime exposure to cadmium from all food to be 0.14 ug/kg/day or 10 ug/person/day (FDA, 1993). These estimates are comparable to the study by Dabbeka et al. (1987) of 24 individuals in 5 Canadian cities. The authors reported an average value of 13.8 ug/person/day or 0.19 ug/kg/day of dietary cadmium intake from food.

   Another major source of dietary cadmium is from cigarette smoke and shellfish (ATSDR, 1993). The FDA reported that smoking one pack of cigarettes per day may result in the exposure to approximately 10 ug of cadmium (FDA, 1993). Hence this amount is equivalent to the amount of cadmium from the diet.

II) Air, dust and water. Exposure to cadmium from potable water sources is very small. The FDA (1993) reported the average consumption of cadmium in water to be approximately 0.5 ug cadmium per liter of drinking water. This value was accounted for in the 10 ug/person/day value reported in the FDA total diet study (FDA, 1993).

2. Additional sources. For the purpose of this assessment, the main additional source of cadmium that will be considered is from the consumption of crops (wheat and oats) grown on biosolids-amended soil. The addition of biosolids which contains cadmium, to the soil has resulted in the absorption of the cadmium metal by the crops grown on such soil. The average concentrations of cadmium in such crops have been calculated and reported in Table 3-A. The complete data set from which the means are derived is attached as Appendix A-2.

Table 3-A: Average cadmium content in crops grown on biosolids-amended soil.

Sludge Application Rate (x1000 kg/ha)	Average (Avg.) Grain Cadmium Content (mg/kg)							
	Wheat				Oats			
	Fertilized (chemical and biosolids)		Not fertilized (biosolids only)		Fertilized (chenical and biosolids)		Not fertilized (biosolids only)	
	Avg	Std. deviation	Avg.	Std. deviation	Avg.	Std. deviation	Avg.	Std. deviation
0	0.014	0.003	0.042	0.055	0.008	0.001	0.020	0.024
10	0.023	0.003	0.018	0.006	0.007	0.001	Not available	
25	0.017	0.004	0.027	0.005	0.007	0.003	0.0060	0.001
50	0.024	0.011	0.028	0.010	0.007	0.002	0.0058	0.002
100	0.053	0.024	0.049	0.019	0.017	0.009	0.0106	0.004

The variability in results of metal content in the crops between fertilized and non-fertilized and within the range of sludge concentrations will be discussed in Chapter 8, Section 8.1.

Comparison analysis of cadmium content in crops grown on soil amended with different rates of biosolids:

One-way analysis of variance (ANOVA) statistical test was applied to the cadmium levels found in the crops grown on biosolids-amended soil. The purpose of applying ANOVA was to determine whether a significant difference exists between the levels of cadmium in the crops that are grown using different biosolids application rates and hence, determine if there is an increased health risk of consuming crops grown on soil with different levels of biosolids.

One-way analysis of variance was conducted on the following four different groups of plots:

1. Wheat grown on soil amended with biosolids and fertilizer
2. Wheat grown on soil amended with biosolids (no fertilizer)
3. Oats grown on soil amended with biosolids and fertilizer
4. Oats grown on soil amended with biosolids (no fertilizer)

The analysis of variance tests on the above plots revealed the following:

Group 1) Wheat grown on soil amended with biosolids and fertilizer:

- The cadmium content in the wheat grown on soil amended with an application rate of 100000 kg (biosolids)/ha is significantly different (p=0.00032) from the cadmium content in wheat grown on soil with 0, 10000, 25000 and 50000 kg (biosolids)/ha. Thus, the null hypothesis of the five means (from the different biosolids application rates) of cadmium content being equal is therefore rejected.
- The cadmium content in wheat grown on soil amended with 0, 10000, 25000 and 50000 kg (biosolids)/ha are not significantly different from each other. Thus, the null hypothesis of the four means (from the different biosolids application rates) of cadmium content being equal is therefore accepted.

Detailed calculations of the analysis of variance for the above plot are attached as Appendix- B.

Group 2) Wheat grown on soil amended with biosolids (no fertilizer):

- The cadmium contents in wheat grown on soil amended with 0, 10000, 25000, 50000 and 100000 kg (biosolids)/ha are not significantly different. Thus, the null hypothesis of the five means (from the different biosolids application rates) of cadmium content in wheat being equal is therefore accepted.

Detailed calculations of the analysis of variance for the above plots are attached as Appendix C.

Group 3) Oats grown on soil amended with biosolids and fertilizer:

- The cadmium content in the oats grown on soil with an application rate of 100000 kg (biosolids)/ha is significantly different (p=0.003502) from the cadmium content in oats grown on soil with 0, 10000, 25000 and 50000 kg (biosolids)/ha.
- The cadmium content in oats grown on soil amended with 0, 10000, 25000 and 50000 kg (biosolids)/ha are not significantly different from each other. Thus, the null hypothesis of all four means (from the different biosolids application rates) of cadmium content been equal is therefore accepted.

Detailed calculations of the analysis of variance for the above plot are attached as Appendix D.

Group 4) Oats grown on soil amended with biosolids (no fertilizer):

- The cadmium content in the oats grown on soil amended with 0, 10000, 25000, 50000 and 100000 kg (biosolids)/ha are not significantly different. Thus, the null hypothesis of the five means (from the different biosolids application rates) of cadmium content in oats been equal is therefore accepted.

Detailed calculations of the analysis of variance for the above plot is attached as Appendix E.

Risk characterization

This risk analysis characterizes the risks associated with the oral exposure of cadmium from crops grown on biosolids-amended soil. The risk characterization is based on average individuals weighing 70 kg (average adult) and 10 kg (child) with a RfD of 0.049 and 0.007 mg cadmium per day, respectively. The RfD takes background diet into consideration but does not take exposure to cigarette smoke into account.

The maximum amount of grain (harvested from crops grown on biosolids-amended soil) that can be consumed by humans per day for a lifetime without experiencing an increased risk of deleterious effects is computed using the following formula:

$$\textit{kg of grain that can be consumed per day during a lifetime} = \textit{RfD (mg / day)} \div \textit{cadmium content in grain (mg / kg)}$$

As an example, the kg of wheat grown on soil amended with 10000 kg biosolids per hectare (without chemical fertilizer) that can be consumed per day by a 70-kg person during a lifetime is calculated as follows:

$$\textit{kg of grain that can be consumed per day during a lifetime}^{*} = \textit{RfD (mg / day)}^{**} \div \textit{cadmium content in grain (mg / kg)}$$
$$= 0.049\text{mg/day} \div 0.018\ \text{mg/kg}$$
$$= 2.72\ \text{kg/day or } 0.039\ \text{kg/kg-day}$$

* daily consumption for a non-smoking 70 kg person

** reference dose for oral cadmium = 0.7 ug/kg-day. For a 70 kg person, the RfD converts to 49 ug/day or 0.049 mg/day.

The kilograms of grain that can be consumed per day during a lifetime for individuals weighing 70 and 10 kg without deleterious effects are reported in Table 4. Note that these levels will fluctuate as the individuals gain or lose weight. That is, the allowable consumption level increases as the weight of the individual increases and conversely.

Table 4: Maximum daily intake of grain grown on biosolids-amended soil for individuals weighing 70 and 10 kg without increased risk of deleterious health effects from cadmium.

Sludge Application Rate (x1000 kg/ha)	Maximum Daily Intake of Grain (kg) for individuals weighing 10 and 70 kilograms							
	Wheat				**Oats**			
	Fertilized (chemical and biosolids)		Not fertilized (biosolids only)		Fertilized (chemical and biosolids		Not fertilized (biosolids only)	
	10 kg	**70 kg**	**10 kg**	**70 kg**	**10 kg**	**70 kg**	**10 kg**	**70 kg**
0	0.50	3.50	0.17	1.19	0.88	6.16	0.34	2.38
10	0.30	2.10	0.39	2.73	1.00	7.00	Not Available	
25	0.41	2.87	0.26	1.82	1.00	7.00	1.20	8.40
50	0.29	2.03	0.25	1.75	1.00	7.00	1.20	8.40
100	0.13	0.91	0.14	0.98	0.41	2.87	0.66	4.62

To put these numbers in perspective, an example of the same individuals consuming 100% whole wheat bread will be used. One hundred percent whole wheat bread contains on the average 51% (w/w) wheat and is packaged as 280, 450 or 570 grams per loaf. For the purpose of this example, the 280 gram loaf of bread will be considered. Therefore, the same individuals can consume the following amounts of bread (280 gram loaf) per day (Table 5) made with wheat grown on biosolids-amended soil without increased risk of deleterious health effects:

Table 5: Maximum daily intake of 100% whole wheat bread made from wheat grown on biosolids-amended soil for individuals weighing 70 and 10 kg without increased risk of deleterious health effects from cadmium.

Sludge Application Rate (x1000 kg/ha)	Maximum Daily Intake of wheat (kg) and number of 100% whole wheat loaves of bread for individuals weighing 10 and 70 kg							
	Wheat				**loaves of 100% whole wheat bread**			
	Fertilized (biosolids and chemical)		Not fertilized (biosolids only)		Fertilized (biosolids and chemical)		Not fertilized (biosolids only)	
	10 kg	**70 kg**	**10 kg**	**70 g**	**10 kg**	**70 kg**	**10 kg**	**70 kg**
50	0.29	2.03	0.25	1.75	2.0	14.0	1.75	12.3
100	0.13	0.91	0.14	0.98	0.9	6.3	1.0***	7.0

***Sample calculation for wheat grown on soil amended with 100000 kg/ha of biosolids:

- Amount of wheat in a 280 g loaf of bread:

  (280 g of bread/loaf)x(51 g of wheat/100g of bread) = 143 g of wheat/loaf

- For a 10 Kg person, the maximum daily intake of wheat grown on soil amended with 100000 kg/ha of biosolids= 0.14 Kg or 140 grams
- Therefore, the number of loaves that can be consumed:

  (140 grams of wheat)/(143 grams of wheat/loaf) = 1.0 loaves

Using the same example but substituting oats for wheat, the same individual can consume the following amounts of bread (280 gram loaf containing 51%(w/w) oats) per day (Table 5-A) made with oats grown on biosolids-amended soil without increased risk of deleterious health effects:

Table 5-A: Maximum daily intake of bread made from oats grown on biosolids-amended soil for individuals weighing 70 and 10 kg without increased risk of deleterious health effects from cadmium.

Sludge Application Rate (x1000 kg/ha)	Maximum Daily Intake of oats (kg) and loaves of bread (280 gram loaf containing 51%(w/w) oats) for individuals weighing 10 and 70 kg							
	Oats				**loaves of bread**			
	Fertilized (biosolids and chemical)		Not fertilized (biosolids only)		Fertilized (biosolids and chemical)		Not fertilized (biosolids only)	
	10 kg	**70 kg**	**10 kg**	**70 g**	**10 kg**	**70 kg**	**10 kg**	**70 kg**
50	1.00	7.00	1.20	8.40	6.99	48.93	8.39	58.73
100	0.41	2.87	0.66	4.62	2.87	20.09	4.62**	32.34

**Sample calculation for oats grown on soil amended with 100000 kg/ha of biosolids:

- Amount of oats in a 280 g loaf of bread:

  (280 g of bread/loaf)x(51 g of oats/100g of bread) = 143 g of oats/loaf

- For a 10 Kg person, the maximum daily intake of oats grown on soil amended with 100000 kg/ha of biosolids= 0.66 Kg or 660 grams
- Therefore, the number of loaves that can be consumed:

  (660 grams of oats)/(143 grams of oats/loaf) = 4.62 loaves

The above data (Table 5 and Table 5-A) demonstrate that individuals weighing 10 kg (child) and 70 kg (adult) must consume a substantial amount of bread made with wheat and oats grown on soil amended with different application rates of biosolids to reach the derived oral cadmium reference dose of 0.7 ug per kg-day. These daily oral exposures to cadmium are likely to be without appreciable risk of deleterious health effects during the person's lifetime. Usually, oral doses less than the RfD are not likely to be associated with adverse health risks. As the frequency and/or magnitude of oral exposure to cadmium increases, the probability of adverse health effects increases. However, since the RfD is only an estimate, it should not be categorically concluded that oral doses

below the RfD are "acceptable" (risk free) and that all doses in excess of the oral RfD are "unacceptable" (will result in adverse health effects).

7.2 Lead

Hazard Identification:

Lead has been reported to have a wide spectrum of effects in humans. The health effects span the sub-cellular levels as well as the level of general function that encompasses all systems in the body (ATSDR, 1993b). Although it is not possible to report all of the studies linking adverse health effects to lead exposure, this section will focus on the major studies to establish that lead is a potential health hazard to humans if consumed.

Toxic effects of lead may involve several organ systems within the body and vary from subtle biochemical effects, which are not adverse but rather indicators (biomarkers) of exposure, to clinical or overt effects such as lead poisoning (plumbism). Frank anemia may occur at blood-lead levels of 80 ug/dL, while reduced hemoglobin production may occur at lower blood lead levels (above 50 ug/dL lead in blood in adults and 40 ug/dL in children) (Juberg et al., 1997).

Lead is a cumulative general poison. Fetuses, infants, children up to six years of age and pregnant women (because of their fetuses) are the most susceptible to adverse health effects. The central nervous system can be seriously affected by lead. Overt signs of acute intoxication include dullness, restlessness, irritability, poor attention span, headaches, muscle tremor, hallucinations and loss of memory, with encephalopathy occurring at blood lead levels of 100 to 120 ug/dL in adults and 80-100 ug/dL in children (U.S. EPA, 1986a).

Chronic signs and symptoms of lead toxicity include tiredness, sleeplessness, irritability, headaches, joint pain and gastrointestinal symptoms. These signs may appear in adults with blood lead levels of 50 to 80 ug/dL (Hanninen et al., 1979). In occupationally exposed populations at blood lead levels of 40-60 ug/dL, it has been observed that after

one or two years of exposure, symptoms include muscle weakness, gastrointestinal symptoms, lower scores on psychometric tests, disturbances in mood peripheral neuropathy. Furthermore, there were significant reductions in nerve conduction velocity noted at levels of 30 to 50 ug/dL (Seppalainen et al., 1983).

Lead has been shown to interfere with calcium metabolism, both directly and by perturbation of the heme-mediated generation of the vitamin D precursor 1,25-dihydroxycholeralciferol. The endocrine system plays a major role in the maintenance of extra and intracellular calcium homeostasis, bone remodelling, intestinal absorption of minerals, cell differentiation and immunoregulatory capacity. Dose-related significant decreases ($p<0.001$) in circulating 1,25-dihydroxy vitamin D levels were observed in children with blood lead concentrations ranging from 33 - 55 ug/dL compared with children with blood lead levels ranging from 10-26 ug/dL. A regression analysis indicated that significant decreases in 1,25-dihydroxy vitamin D levels were associated ($r = -0.88$) over the entire range of blood lead concentrations from 12 to 120 ug/dL with no evidence of a threshold (Mehaffey et al., 1982). In calcium-deficient persons, tissue lead content is increased. This is important when considering the increased propensity to lead exposure that could result from the calcium-deficient status of the pregnant women. It has also been shown that interactions between calcium and lead were responsible for a significant portion of the variance on the scores on general intelligence ratings, and that calcium had a significant effect on the deleterious effect of lead (Lester et al., 1986).

The central and peripheral nervous systems are principal targets for lead toxicity. These include subencephalopathic neurological and behavioural effects in adults and electrophysiological evidence of both central and peripheral effects on the nervous system in children with blood lead levels well below 30 ug/dL. Aberrant electroencephalograph readings were significantly correlated ($p<0.05$) with blood lead levels down to 15 ug/dL, with effects at non-significant levels noted down to 6 ug/dL (Otto et al., 1982). Significant reductions in maximal motor nerve conductivity velocity (MNCV) have been observed in five to nine year old children living near a smelter, with a threshold occurring at a blood lead level around 20 ug/dL. A 2 % decrease in the

MNCV was seen for every 10-ug/dL increase in the blood lead level (Schwartz et al., 1988).

The primary concern for adults with excess occupational exposure to lead is neurotoxicity and chronic kidney toxicity. Nerve conduction velocity is reversibly slowed in peripheral nerves at blood lead levels of 30 ug/dL, whereas overt effects on the nervous system such as wrist drop, require blood-lead levels of 60 ug/dL or greater (Juberg et al, 1997).

The adverse effects of lead on the kidney have been well documented. Acute lead poisoning in both humans and experimental animals produces similar functional and morphological changes in the proximal renal tubular living cells. It has been suggested that chronic and excessive lead exposure may result in end-stage renal disease (Juberg et al., 1997).

Chronic nephropathy requires relatively heavy exposure to lead. Blood lead levels in the range of 40-80 ug/dL are associated with the formation of nuclear inclusion bodies in the renal tubular epithelium, the first manifestation of lead accumulation in the kidney. Results of occupational studies indicate that maintaining blood-lead levels of below 60 ug/dL will prevent biologically relevant renal charges in the majority of lead-exposed workers.

A major organ for lead deposition is bone. Skeletal lead has been used as a measure of cumulative lead exposure (Juberg et al., 1997). It has also been suggested that lead affects bone formation by altering growth and stature and disrupting vitamin D metabolism. It is unclear whether these represent direct or secondary effects of lead exposure.

Questions have been raised about the possible effects of lead upon vitamin D metabolism, an effect that could be mediated through the kidney. Associations between blood lead and decreasing levels of vitamin D metabolite over blood lead ranging in concentrations from 12 to 120 ug/dL have been reported (Juberg et al., 1997). No threshold for this

effect could be demonstrated and it was speculated that lead at low exposure levels may result in an interference with vitamin D metabolism with possible adverse effects on bone growth in children.

The reproductive toxicity potential associated with lead has been recognised for some time. Severe lead intoxication is associated with sterility, abortion, stillbirths, and neonatal morbidity and mortality from exposure in utero (Juberg et al., 1997).

The potential effect of lead overexposure on the nervous system of children has received the most attention and discussion. Studies have associated lead overexposure with decreased intelligence, reduced short term memory, reading disabilities, and deficits in vocabulary, fine motor skills, reaction time, and hand-eye co-ordination (Juberg et al., 1997).

The WHO established a provisional tolerable weekly intake (PTWI) for lead for children of 25 ug/kg bodyweight, equivalent to an acceptable daily intake (ADI) of approximately 3.5 ug/kg bodyweight per day. The PTWI was established on the premise that lead is a cumulative poison and that there should be no increase in the body burden of lead from any source, thus avoiding the possibility of adverse biochemical and neurobehavoural effects in infants and young children. It was based on metabolic studies in infants showing that a mean daily lead intake of 3-4 ug/kg bw was a NOAEL and was not associated with an increase in blood lead levels or in the body burden of lead, whereas a daily intake of 5 ug/kg bw or more resulted in lead retention.

Over the years, the CDC has lowered the recommended blood-lead action level (concentration at which action is implemented) from 55 to 40 ug/dL in 1970, followed by a move to 30 ug/dL in 1975, then to 25 ug/dL in 1985, and finally to the current level of 10 ug/dL (1991) (Juberg et al., 1997). The 10 ug/dL level is the point at which some public health intervention or monitoring activity begins (Juberg et al., 1997). During the decades of the 1970's and 1980's, nearly 9 out of every 10 American children age 5 and under had serum blood lead levels exceeding 10 ug/dL and by today's definition would

have been considered lead poisoned. Today, fewer than 5 % of children in the 1-5 age group have blood-lead levels in excess of 10 ug/dL (Juberg et al., 1997).

Dose-Response Assessment:

EPA has compiled an extensive review of the literature that deals with the adverse health effects of lead exposure. Table 6 lists some of the health effects associated with exposure to lead and internal lead doses in humans.

Table 6: Health effects associated with exposure to lead and internal lead doses in humans.

Duration of exposure	Effect	Blood lead levels at which effect observed (ug/dl)
Not specified	Colic (abdominal pain, cramps, nausea, vomiting and weight loss)	40-200
Not specified	Colic in children (abdominal pain, cramps, nausea, vomiting and weight loss)	60-100
Not specified	decreased ALAD	3-56 (adults)
Not specified	Alteration in peripheral nerve function	20-30 (children)
2 weeks to 1 year	Increased blood pressure	30-120
Not specified	Decreased hemoglobin	>40 (children)
Not specified	Anemia	>20 (children)
Not specified	Chronic nephropathy	40-100
Not specified	Adverse effects on testes	40-50
Not specified	Encephalopathy (adults)	50-300

Source: ATSDR, 1993

To assess the health risk from exposure to lead, the relationship between a particular health outcome and the lead levels in the environment must be known. Most studies of lead exposure in the environment use measures of body-lead-burden such as blood-lead-concentration. As a result, there are no studies relating environmental exposure to lead and particular health effects.

Benchmark levels such as MRL, RfD and RfC (reference concentration for inhalation of pollutants) do not exist for lead or its inorganic compounds. Government agencies such as U.S.EPA are reluctant to set such levels because no thresholds have been demonstrated for the most sensitive effects in humans.

The only agency setting a benchmark level for the oral intake of lead is the World Health Organization (WHO). The WHO established a provisional tolerable weekly intake (PTWI) for lead for children at 25 ug/kg or an ADI of 3.5 ug/kg bw. The rationale for this value is based on the premise that lead is a cumulative poison and therefore there should be no increase in the body burden of lead from any source to avoid any adverse health effects in children. The 3.5 ug/kg bw value was chosen because a mean daily intake of 3-4 ug/kg bw was a NOAEL and was not associated with an increase in the body burden of lead, whereas a daily intake of 5 ug/kg bw or more resulted in lead retention (Health Canada, 1989).

Exposure Assessment

For the purpose of this assessment, the main additional source of lead that will be considered is from the consumption of crops (wheat and oats) grown on biosolids-amended soil. The data on the lead content in crops as an additional source of oral lead exposure were taken from the study conducted by the City of Winnipeg, Water and Waste Department, as explained in Chapter 6, Section 6.2.

The analysis of the crops for lead content by the City of Winnipeg, Laboratory Services reported values of <0.1 mg lead per kg grain for all crops at all biosolids application rates. This value may indicate either no lead uptake by the crops or that the sensitivity of the atomic absorption was not sufficient to detect lead levels below 0.1 mg/kg. As a result, the data reported for the lead content in the crops do not lend themselves to a quantitative assessment for determining the body-lead-burden attributed to oral exposure to lead in the crops.

Risk Characterization

There is no doubt in the scientific community as to the status of lead as a human health hazard. Lead is a systemic poison causing a wide variety of adverse health effects, including, in extreme cases, death.

In this risk analysis, quantification of the health hazard due to the oral exposure of lead from crops grown on biosolids-amended soil was not possible because all lead concentrations in the grains were reported to be at <0.1 mg lead per kg of grain (see Appendix A-2). The health risks from oral lead exposure in crops grown on biosolids-amended soil could, therefore, not be characterized.

7.3 Chromium (III)

Hazard Identification:

Human Data. Trivalent chromium is an essential element for lipid, protein and fat metabolism in animals and humans. Thus, chromium (III) deficiency causes changes in the metabolism of glucose and lipids and may be associated with cardiovascular diseases (Anderson, 1995). Although the essential role of chromium (III) in glucose and lipid metabolism has been widely studied, only one study in the literature addressed the oral toxicity of chromium (III) in humans. Due to increased mortality of stomach cancers in Canadian miners, Kusiak et al. (1993) investigated the possible explanation for the excess stomach cancer. Exposures to chromium and other metals were possible explanations. The authors found that the incidence of stomach cancer was best associated with the exposure to chromium in the miners. The authors concluded and suggested that chromium or a substance associated with chromium may be the causative agent for the stomach cancers (Kusiak et al., 1993).

Animal Data. Ivankovic and Preussman (1975) fed 60 male and female rats 0, 1, 2,or 5 % chromium (III) in baked bread 5 days/week for 120 weeks. The authors estimated that the rats consumed 360 g/kg body weight (bw), 720g/kg bw and 1800 g/kg bw of total chromium (III) over the duration of the study. The authors noted no adverse effects at any feeding levels.

Mackenzie et al. (1958) provided rats with 25 ppm chromium (III) in drinking water for 12 months and noted no change in body weight and macroscopic or macroscopic pathology. This study suggested a NOEL at 8.2 ppm chromium (III) or .82 mg chromium (III) per kg bw per day (assuming an average rat weighs 0.35 kg and consumes 0.035 liters of water).

Anderson et al. (1997) fed rats 0-100 mg/kg chromium (III) in the diet for 24 weeks. Histological examination of the rats in the high dose groups did not reveal any detectable differences. No statistical differences in body weight or blood variables were noted among the groups examined at 17 and 24 weeks.

Elbeticha and Al-Hamood (1997) examined fertility following chromium (III) exposure in mice. The male and female mice were exposed to 1000, 2000 or 5000 mg/L chromium (III) in drinking water for 12 weeks. At the end of the study period, the authors noted no mortality or clinical signs of toxicity in any group of male or female mice exposed at any concentration.

Dose-Response Assessment

There is insufficient data on the adverse effects of oral exposure to chromium (III) in humans. The animal studies on oral exposure to chromium (III) have reported no adverse health effects. Given the low absorption rate of chromium (III) in humans and the fact that it is an essential element with no proof of animal toxicity, oral dose-response data are not available for chromium (III). Since oral dose-response data are not available for chromium (III), benchmark or safe dietary levels for chromium (III) have not been

established. Therefore, based on the available literature, a dose-response assessment of chromium (III) can not be conducted at this time.

Exposure assessment

Oral exposure to chromium (III) was assessed based on the levels of chromium (III) found in crops grown on biosolids-amended soil. The chromium (III) concentrations in such crops were derived from the study conducted by the City of Winnipeg, Water and Waste Department as explained in Chapter 6, Section 6.2.

The analysis of the crops for chromium (III) content by the City of Winnipeg, Laboratory Services, reported values of <0.1 mg chromium (III) per kg grain for all crops at all biosolids application rates. This value may indicate either no chromium (III) uptake by the crops or that the sensitivity of the atomic absorption spectroscoper was not sufficient to detect chromium (III) levels below 0.1 mg/kg. Since all values were reported as <0.1, it is difficult to determine how close (i.e. 0.09, 0.08, etc.) or how far (i.e. 0.0001) the true values lie from 0.1 and thus cannot approximate 0.1 for the reported values of <0.1 as was done for the Cd assessment. Thus, an exposure assessment of the oral exposure to chromium (III) from crops grown on biosolids-amended soil could not be conducted.

Risk Characterization

In this risk analysis, quantification of the health hazard due to the oral exposure of chromium (III) from crops grown on biosolids-amended soil was not possible. Analysis of the grains for chromium (III) concentrations has revealed chromium (III) concentrations at <0.1 mg per kg of grain. In addition, an oral reference dose or other benchmark level for chromium (III) is not available. Therefore, based on these findings, the health risks from oral chromium (III) exposure in crops grown on biosolids-amended soil cannot be characterized quantitatively, but are extremely low.

7.4 Zinc

Hazard Identification:

Zinc is an essential nutrient in humans that is necessary for the function of metalloenzymes including alcohol dehydrogenase, carbonic anhydrase, deoxyribonucleic acid and ribonucleic acid polymerase. Zinc deficiency has been associated with anorexia, growth retardation, poor wound healing and impaired immune function. As such, certain levels of zinc intake are recommended for proper functioning of metalloenzymes. These enzymes include alcohol dehydrogenase, ribonucleic acid polymerase and superoxide dismutase (ATSDR, 1994). The recommended dietary allowance (RDA) for zinc is 15 mg/day for men, 12 mg/day for women and 10 mg/day for children. Higher RDAs are recommended for women during pregnancy (15 mg/day) to prevent such adverse health effects as growth retardation in the offsprings (NRC, 1989).

Zinc toxicity from excessive ingestion is uncommon, however, gastrointestinal distress and diarrhea have been reported following ingestion of beverages standing in galvanised cans or from use of galvanised utensils (ATSDR, 1994). There have been no reports of toxicity from dietary zinc. Evidence of hematologic, hepatic, or renal toxicity has not been observed in individuals ingesting as much as 12 g of elemental zinc over a two-day period (ATSDR, 1994). However, there have been reports of teratogenic effects in sheep, and disrupted cholesterol metabolism in humans, both thought to be due to the adverse effects of high zinc concentrations on copper metabolism (Health Canada, 1989).

Dose-response Assessment

The lowest-observed-adverse-effect-level and the no-observed-adverse-effect-level values for humans from oral exposure to zinc are recorded in Table 7.

Table 7: Threshold levels for zinc

Exposure duration	NOAEL mg zinc/kg/day	LOAEL mg zinc/kg/day	Health effect
10 wks 2x/day		0.83	Decreased superoxide dismutase activity
once		0.5	Decreased serum cortisol level
once		6.7	GI distress; diarrhoea
12 wks 1x/day		0.71	Decreased serum HDL-cholesterol
5 wks 2x/day		2.3	Decreased serum HDL-cholesterol
8yrs 1x/day	2.0		Decreased RBC
6 wks 3x/day		2.0	Abd. cramps, vomiting, nausea
6 wks 2x/day		4.3	Impaired lymphocyte

Source: ATSDR, 1994

As indicated by Table 7, exposure to large amounts of zinc is required to reach the threshold levels of adverse effects in humans. For example, the amount of zinc required to reach the threshold level that would cause gastrointestinal distress in a 70-kg person would be 460 mg/day. This threshold value is 52 times the recommended daily intake for an adult.

The U.S. EPA has adopted an oral RfD of 0.3 mg/kg-day for zinc. This oral RfD is based on a clinical study by Yardick et al. (1989) which investigated the effects of oral zinc supplements on copper and iron balance. Yardick et al. (1989) conducted a 10-week study of zinc supplements in 18 healthy women who were given zinc supplements twice daily (1mg/kg-day). The daily oral supplements resulted in a significant decrease of erythrocyte superoxide dismutase (ESOD) activity at ten weeks. Reduction in ESOD, an antioxidant enzyme in red blood cells, may result in pathological conditions including renal diseases.

The RfD of 0.3mg/kg/day is computed using estimations from the FDA Total Diet Study for 1982-1986 plus the reported supplemental dose of the Yardick et al. (1989) study. For example, for the Yardick et al. (1989) study, the dose is based on 50 mg zinc supplement per day plus the average dietary intake of 9.72 mg zinc per day (estimated by FDA, Total Diet Study). The total of the above two sources equals 60 mg/day. This total is divided by the assumed average body weight of the participants (60 kg) to arrive at a dose of 1.0 mg/kg/day. An uncertainty factor of 3 was used in consideration of a substance that is an essential dietary unit. The resulting oral RfD is calculated accordingly (1mg/kg/day divided by 3) to give the resulting RfD of 0.3 mg/kg/day (ATSDR, 1993).

For an adult weighing 70 kg, the oral RfD in units of mg/day is computed as follows:
0.3 mg/kg-day X 70 kg = 21 mg/day inclusive of diet.
For a child weighing 10 kg, the oral RfD in units of mg/day is:
0.3 mg/kg-day X 10 kg = 3 mg/day inclusive of diet

The Yardick et al. (1989) clinical study that was used as the basis for the above oral RfD is supported by other studies which indicate that zinc supplementation can alter copper balance. For example, Fischer et al. (1984) demonstrated that zinc supplementation therapy with doses of 150 mg to 5 g/day, taken for 1-2 years has produced copper deficiency anemia. The effects on copper biochemistry are considered of concern since long-term copper deficiency could result in significant adverse health effects as described in the hazard identification section.

Exposure Assessment

For the purpose of this assessment, the main additional source of zinc that will be considered is from the consumption of crops (wheat and oats) grown on biosolids-amended soil. The data on the zinc content in crops as an additional source of oral zinc exposure were from the study conducted by the City of Winnipeg Water and Waste Department as explained in Section 6.2. The addition of biosolids which contains zinc, to

the soil has resulted in the absorption of the zinc metal by the crops grown on such soil. The amounts of zinc absorbed by the plants and transported to the edible grains have been determined by the City of Winnipeg, Laboratory Services using atomic absorption spectroscopy. The average concentrations of zinc in such crops has been calculated accordingly and reported in Table 8. The complete data set in which the means are derived is attached as Appendix A-2.

Table 8: Average zinc content in crops grown on biosolids-amended soil.

Sludge Application Rate (x1000 kg/ha)	Average (Avg.) Grain Zinc Content (mg/kg)							
	Wheat				Oats			
	Fertilized (biosolids and chemical)		Not fertilized (sludge only)		Fertilized (biosolids and chemical)		Not fertilized (sludge only)	
	Avg	Std. deviation	Avg.	Std. deviation	Avg.	Std. deviation	Avg.	Std. deviation
0	29.00	2.00	38.00	5.00	18.00	5.00	25.00	5.00
10	31.00	2.00	43.00	4.00	19.00	3.00	24.00	3.00
25	35.00	3.00	40.00	2.00	37.00	17.00	24.00	3.00
50	39.00	9.00	41.00	4.00	24.00	2.00	24.00	2.00
100	54.00	8.00	47.00	6.00	49.00	11.00	31.00	11.00

Comparison analysis of zinc content in crops grown on soil amended with different rates of biosolids:

One-way analysis of variance (ANOVA) statistical test was applied to zinc levels found in the crops grown by the City of Winnipeg on biosolids-amended soil. The purpose of applying ANOVA was to determine whether a significant difference exists between the levels of zinc in crops grown on soil amended with different biosolids application rates. Hence, this determined if there was an increased health risk of consuming crops grown on soil with different levels of biosolids.

One way analysis of variance was conducted on the following four different groups of plots:

1. Wheat grown on soil amended with biosolids and fertilizer
2. Wheat grown on soil amended with biosolids (no fertilizer)
3. Oats grown on soil amended with biosolids and fertilizer
4. Oats grown on soil amended with biosolids (no fertilizer)

The analysis of variance tests on the above plots revealed the following:

Group 1) Wheat grown on soil amended with biosolids and fertilizer:

- The zinc content in the wheat grown on soil amended with an application rate of 100000 kg (biosolids)/ha is significantly different (p=0.000004) from the zinc content in wheat grown on soil with 0, 10000, 25000 and 50000 kg (biosolids)/ha.
- The zinc content in wheat grown on soil amended with 0, 10000, 25000 and 50000 kg (biosolids)/ha are not significantly different. The null hypothesis of the four means (from the different biosolids application rates) of zinc content been equal is therefore accepted.

Detailed calculations of the analysis of variance for the above plot are attached as Appendix F.

Group 2) Wheat grown on soil amended with biosolids (no fertilizer):

- The zinc content in wheat grown on soil amended with 100000 kg (biosolids)/ha is significantly different (p= .0418) from the zinc content in wheat grown on soil free of biosolids.
- The zinc content in wheat grown on soil amended with 10000, 25000 and 50000 kg (biosolids)/ha are not significantly different. Thus, the null hypothesis of the three means (from the different biosolids application rates) of zinc content been equal is therefore accepted.
- The zinc content in wheat grown on soil amended with 10000, 25000 and 50000kg (biosolids)/ha are not significantly different from the zinc content grown on soil amended with 100000 kg of biosolids per hectare.

Detailed calculations of the analysis of variance for the above plots are attached as Appendix G.

Group 3) Oats grown on soil amended with biosolids and fertilizer:

- The zinc content in the oats grown on soil with an application rate of 25000 kg (biosolids)/ha is significantly different (p=0.000108) from the zinc content in oats grown on soil with 0 and 10000 kg (biosolids)/ha.
- The zinc content in the oats grown on soil with an application rate of 100000 kg (biosolids)/ha is significantly different (p=0.000108) from the zinc content in oats grown on soil with 0, 10000 and 50000 kg (biosolids)/ha.
- The zinc content in oats grown on soil amended with 0, 10000 and 50000 kg (biosolids)/ha are not significantly different from each other. Thus, the null hypothesis of the three means (from the different biosolids application rates) of zinc content been equal is therefore accepted.
- The zinc content in oats grown on soil amended with 100000 kg (biosolids)/ha is not significantly different from the zinc content in oats grown on soil amended with 25000 kg (biosolids)/ha. Thus, the null hypothesis of the two means (from the different biosolids application rates) of zinc content been equal is therefore accepted.

Detailed calculations of the analysis of variance for the above plot are attached as Appendix H.

Group 4) Oats grown on soil amended with biosolids (no fertilizer):

- The zinc content in the oats grown on soil amended with 0, 10000, 25000, 50000 and 100000 kg (biosolids)/ha are not significantly different. Thus, the null hypothesis of the five means (from the different biosolids application rates) of zinc content in oats been equal is therefore accepted.

Detailed calculations of the analysis of variance for the above plot are attached as Appendix I.

Risk characterisation

This risk analysis characterizes the risks associated with the oral exposure of zinc from crops grown on biosolids-amended soil. The risk characterization is based on average individuals weighing 70 kg (average adult) and 10 kg (child) with a RfD of 21.0 and 3.00 mg zinc per day, respectively (inclusive of diet).

The maximum amount of grain (harvested from crops grown on biosolids-amended soil) that can be consumed by humans per day for a lifetime without experiencing an increased risk of deleterious effects is computed using the following formula:

$$\textit{kg of grain that can be consumed per day during a lifetime} = \textit{RfD (mg / day)} \div \textit{zinc content in grain (mg / kg)}$$

As an example, the kg of wheat grown on soil amended with 10000 kg biosolids per hectare (without chemical fertilizer) that can be consumed per day by a 70-kg person during a lifetime is calculated as follows:

$$\textit{kg of grain that can be consumed per day during a lifetime}^{*} = \textit{RfD (mg / day)}^{**} \div \textit{zinc content in grain (mg / kg)}$$

$$= 21 \text{ mg/day} \div 43 \text{ mg/kg}$$

$$= 0.49 \text{ kg/day or } 0.007 \text{kg/kg-day}$$

* daily consumption for a 70 kg person

** reference dose for oral zinc = 0.3 mg zinc/kg-day. For a 70 kg person, the RfD converts to 21 mg/day.

The kilograms of grain that can be consumed per day during a lifetime for individuals weighing 70 and 10 kg without increased risk of deleterious effects are reported in Table 9.

Table 9: Maximum daily intake of grain grown on biosolids-amended soil for individuals weighing 70 and 10 kg without deleterious effects from zinc.

Sludge Application Rate (x1000 kg/ha)	Maximum Daily Intake of Grain (kg) for individuals weighing 10 and 70 kg							
	Wheat				**Oats**			
	Fertilized (biosolids and chemical)		Not fertilized (biosolids only)		Fertilized (biosolids and chemical)		Not fertilized (biosolids only)	
	10 kg	**70 kg**	**10 kg**	**70 kg**	**10 kg**	**70 kg**	**10 kg**	**70 kg**
0	0.10	0.70	0.08	0.56	0.17	1.19	0.12	0.84
10	0.10	0.70	0.07	0.49	0.16	1.12	0.13	0.91
25	0.09	0.63	0.08	0.56	0.08	0.56	0.13	0.91
50	0.08	0.56	0.07	0.49	0.13	0.91	0.13	0.91
100	0.06	0.42	0.06	0.42	0.06	0.42	0.10	0.70

To put these numbers in perspective, a similar example to that of Cd using 100% whole wheat bread will be used. For the purpose of this example, the 280 gram loaf of bread will be considered. Therefore, the same individuals can consume the following amounts of bread (280 gram loaf) per day (Table 10) made with wheat grown on biosolids-amended soil without increased risk of deleterious health effects:

Table 10: Maximum daily intake of 100% whole wheat bread made from wheat grown on biosolids-amended soil for individuals weighing 70 and 10 kg without increased risk of deleterious health effects from zinc.

Sludge Application Rate (x1000 kg/ha)	Maximum Daily Intake of wheat(kg) and number of 100% whole wheat loaves of bread for individuals weighing 10 and 70 kg							
	Wheat				**loaves of 100% whole wheat bread**			
	Fertilized (biosolids and chemical)		Not fertilized (biosolids only)		Fertilized (biosolids and chemical)		Not fertilized (biosolids only)	
	10 kg	**70 kg**	**10 kg**	**70 g**	**10 kg**	**70 kg**	**10 kg**	**70 kg**
50	0.08	0.56	0.07	0.49	0.6	3.8	0.5	3.5
100	0.06	0.42	0.06	0.42	0.4	2.8	0.4***	2.8

***Sample calculation for wheat grown on soil amended with 100000kg/ha of biosolids:

- Amount of wheat in a 280 g loaf of bread:

  (280 g of bread/loaf)x(51 g of wheat/100g of bread) = 143 g of wheat/loaf

- For a 10 Kg person, the maximum daily intake of wheat grown on soil amended with 100000 kg/ha of biosolids= 0.06 Kg or 60 grams
- Therefore, the number of loaves that can be consumed:

  (60 grams of wheat)/(143 grams of wheat/loaf) = 0.4 loaves

Using the same example but substituting oats for wheat, the same individual can consume the following amounts of bread (280 gram loaf containing 51%(w/w) oats) per day (Table 10-A) made with oats grown on biosolids-amended soil without increased risk of deleterious health effects:

Table 10-A: Maximum daily intake of bread made from oats grown on biosolids-amended soil for individuals weighing 70 and 10 kg without increased risk of deleterious health effects from zinc.

Sludge Application Rate (x1000 kg/ha)	Maximum Daily Intake of oats (kg) and loaves of bread (280 gram loaf containing 51%(w/w) oats) for individuals weighing 10 and 70 kg							
	Oats				**loaves of bread**			
	Fertilized (biosolids and chemical)		Not fertilized (biosolids only)		Fertilized (biosolids and chemical)		Not fertilized (biosolids only)	
	10 kg	**70 kg**	**10 kg**	**70 g**	**10 kg**	**70 kg**	**10 kg**	**70 kg**
50	0.13	0.91	0.13	0.91	0.91	6.37	0.91	6.37
100	0.06	0.42	0.10	0.70	0.42	2.94	0.70**	4.90

**Sample calculation for oats grown on soil amended with 100000 kg/ha of biosolids:

- Amount of oats in a 280 g loaf of bread:

  (280 g of bread/loaf)x(51 g of oats/100g of bread) = 143 g of oats/loaf

- For a 10 Kg person, the maximum daily intake of oats grown on soil amended with 100000 kg/ha of biosolids= 0.10 Kg or 100 grams
- Therefore, the number of loaves that can be consumed:

  (100 grams of oats)/(143 grams of oats/loaf) = 0.70 loaves

The above data (Table 10 and Table 10-A) demonstrate that individuals weighing 10 kg (child) and 70 kg (adult) must consume a substantial amount of bread made with wheat and oats grown on soil amended with different application rates of biosolids to reach the derived oral zinc reference dose of 0.3 mg per kg-day. These daily oral exposures to zinc are likely to be without appreciable risk of deleterious health effects during the persons' lifetime.

7.5 Nickel and Copper:

Hazard Identification:

Nickel

Most of the data regarding the adverse health effects of nickel arise from inhalation or cutaneous contact with the element. Oral intake of nickel is associated with the lowest level of toxicological response compared to other trace metals (ATSDR, 1997). This is partly due to the small (<1%) extent of nickel absorption from the gastrointestinal tract (Sunderman et al., 1989). In addition, the absorbed nickel is always completely excreted in the urine within 4 or 5 days of ingestion (Sunderman et al., 1989).

Oral exposure of humans to high levels of nickel is extremely rare. Only one human death was reported following oral exposure to nickel. A two year old child accidentally ingested approximately 570 mg nickel/kg which led to cardiac arrest four hours after ingestion and death at eight hours after exposure (ATSDR, 1997). No other reports of oral (food) toxicity were reported.

Dose-response Assessment:

Since nickel is poorly absorbed from the GI tract (<1%) and the amount that is absorbed is excreted within 4 to 5 days after ingestion, the potential for toxicity is very low. There are no data on the adverse effects from oral exposure to nickel in humans. Since oral dose-response data are not available for nickel, benchmark or safe dietary levels have not been established. Therefore, based on the available literature, a dose-response assessment on the oral intake of nickel could not be conducted.

Exposure Assessment

The average concentrations of nickel in the wheat and oat grains grown by the City of Winnipeg are reported in Table 11.

Table 11: Average nickel content in crops grown on biosolids-amended soil.

Sludge Application Rate (x1000 kg/ha)	Average (Avg.) Grain Nickel Content (mg/kg)							
	Wheat				Oats			
	Fertilized (biosolids and chemical)		Not fertilized (biosolids only)		Fertilized (biosolids and chemical)		Not fertilized (biosolids only)	
	Avg	Std. deviation	Avg	Std. deviation	Avg.	Std. deviation	Avg.	Std. deviation
0	0.20	0.00	0.10	0.00	2.30	0.50	1.40	0.20
10	0.30	0.30	0.20	0.10	2.30	0.90	1.20	0.40
25	0.10	0.10	0.10	0.00	3.70	1.90	1.20	0.30
50	0.10	0.10	0.10	0.10	1.10	0.50	1.40	0.70
100	0.60	0.30	0.20	0.00	4.50	1.00	2.40	2.10

It is important to note that nickel has been identified as one of the principal phytotoxic (toxic to plants) elements applied to soil in biosolids (Schmidt, 1997). The maximum permissible soil concentration for nickel was established using sensitive crop species and therefore the limits apply to all crops grown on a range of soil types from phytotoxicity (U.S. EPA, 1992). For example, barley has a nickel phytotoxicity threshold of 11-19 mg nickel/kg of barley tissue (Beckett and Davis, 1977). The nickel concentrations found in the grains of oats and wheat grown by the City of Winnipeg on soil amended with biosolids were on the average 15 times lower than the nickel phytotoxic threshold for barley.

Note from Table 11 that the nickel content in crops is much lower than the nickel phytotoxic threshold of 11-19 mg nickel/kg of barley tissue. As such, nickel does not pose a dietary risk because it is subjected to the soil-plant barrier since toxic concentrations in plant tissue are much lower than the amounts which are potentially harmful to humans (Smith, 1996).

Risk Characterization

In this risk analysis, quantification of the health hazard due to the oral exposure to nickel in crops grown on biosolids-amended soil was not possible since the available literature has not identified nickel as a health hazard when consumed in the diet. In addition, an oral reference dose or other benchmark level for nickel is not available.

Copper

Hazard Identification:

Copper is an essential element that is involved in a variety of enzyme reactions. For example, copper is important to the function of lysyl oxidase and superoxide dismutase (SOD). Lysol oxidase is responsible for the cross-linking of collagen and elastin while SOD is the antioxidant in charge of breaking down the superoxide free radical (ATSDR, 1990).

Copper deficiencies can cause a variety of adverse effects in humans. Deficiency related problems include brain impairment and hindered immune function (NAS, 1977).

Literature with respect to oral toxicity of copper in humans is not available. Most reports involve the consumption of water with high levels of copper or suicide attempts using large amounts of copper sulfate. The primary toxicological effect of consuming high levels (.07-1421 mg/kg) of copper in humans is gastrointestinal irritation, manifested as vomiting, nausea, diarrhea and anorexia (ATSDR, 1990).

Humans and animals are known to develop tolerance to continued dosing of copper intake (ATSDR, 1990). Tolerance is defined as a state of decreased responsiveness to a chemical toxic effect, resulting from prior exposure to the chemical. Haywood (1985), reported an upper limit to the amount of copper that can be tolerated in rats at around 250 mg copper/kg/day.

Copper toxicity in humans is further prevented by copper homeostasis. After copper requirements are met, several body mechanisms act to prevent copper overload. The excess copper absorbed into the gastrointestinal mucosal cells bind to methallothionein. The bound copper is then excreted from the body. Because of the body's efficient means of blocking the absorption of excess copper, the most likely pathway for the entry of the toxic amounts of copper would be long term inhalation (ATSDR, 1990).

Dose-response Assessment

There are no data on adverse effects from oral exposure to copper in humans. The animal studies on oral exposure to copper have reported no adverse effects. One of the main reasons for copper being non-toxic to humans is because humans have a copper homeostasis mechanism that acts to prevent copper overload. Since oral dose-response data are not available for copper, benchmark or safe dietary levels have not been established. A dose-response assessment on the oral intake of copper could, therefore, not be conducted at this time.

Exposure Assessment

Oral exposure to copper was assessed based on the levels of copper found in crops grown on biosolids-amended soil. The copper concentrations in such crops were derived from the study conducted by the City of Winnipeg, Water and Waste Department, as explained in Chapter 6, Section 6.2.

In this risk analysis, an exposure assessment of the oral exposure to copper from crops grown on biosolids-amended soil was not necessary since copper, in addition to nickel, has also been identified as one of the main principal phytotoxic elements applied to soil in biosolids (Schmidt, 1997). The maximum permissible soil concentration for copper was established using sensitive crop species and therefore the limits protect all crops grown on a range of soil types from phytotoxicity (U.S. EPA, 1992). The copper concentrations found in the grains of oats and wheat grown by the City of Winnipeg on soil amended with biosolids were on the average 5 times lower than the copper phytotoxic threshold for barley (14-25 mg copper/kg). The average concentrations of copper in the wheat and oat grains grown by the City of Winnipeg are reported in Table 12.

Table 12: Average copper content in crops grown on biosolids-amended soil.

Sludge Application Rate (x1000 kg/ha)	Average (Avg.) Grain copper Content (mg/kg)							
	Wheat				Oats			
	Fertilized (biosolids and chemical)		Not fertilized (biosolids only)		Fertilized (biosolids and chemical)		Not fertilized (biosolids only)	
	Avg.	Std. deviation	Avg.	Std. deviation	Avg.	Std. deviation	Avg.	Std. deviation
0	4.00	0.00	5.00	0.00	3.00	1.00	4.00	1.00
10	4.00	0.00	5.00	0.00	4.00	1.00	3.00	0.00
25	4.00	0.00	5.00	0.00	4.00	1.00	3.00	0.00
50	4.00	1.00	5.00	0.00	3.00	0.00	3.00	0.00
100	6.00	1.00	6.00	1.00	5.00	1.00	4.00	1.00

Note from Table 12 that the copper contents in crops are much lower than the copper phytotoxic threshold of 14-25 mg nickel/kg of barley tissue. As such, copper does not pose a dietary risk because it is subjected to the soil-plant barrier since toxic concentrations in plant tissue are much lower than the amounts, which are potentially harmful to humans (Smith, 1996).

Risk Characterization

In this risk analysis, quantification of the health hazard due to oral exposure to copper in crops grown on biosolids-amended soil was not possible since copper has not identified as a health hazard when consumed in the diet. In addition, oral reference dose or other benchmark level for copper is not available.

Chapter 8: Discussion and Conclusion

8.1 Discussion

With the ever-increasing application of biosolids to agricultural land, a number of concerns have arisen regarding the potential adverse health effects of such a practice. To address a portion of this problem, this study was conducted to assess the potential human health risk posed from the ingestion of metals found in crops grown on biosolids-amended soil.

The scientific literature describing the impact of heavy metals found in biosolids on plant growth and more importantly, human health can be summarized as follows: The addition of biosolids to the soil improves the soil condition by enriching the soil with nutrients (phosphorus and nitrogen) and organic matter (Chaney, 1973). Addition of biosolids to agricultural land has resulted in greater crop yields while reducing the operating costs compared to growing similar crops using chemical fertilizers (Hinsley et al., 1982). However, existing studies have shown that crops, in addition to using the nutrients found in biosolids, also absorb heavy metals (Chaney, 1973; Page et al., 1989; Houda, 1987). The amount of metals taken up by the plants depends on the plant species, metal concentration in the soil, soil pH, soil texture and the soils' cation exchange capacity (Page et al., 1989). The metals, copper, zinc and nickel have been demonstrated to be phytotoxic to plants when present in sufficient quantities (Schmidt, 1997). As for human toxicity, some metals have the potential to pose a health hazard if consumed in sufficient quantities. Chromium (III), nickel and copper do not pose a known health hazard when consumed at levels typically found in a normal diet (Health Canada, 1989). However, cadmium, lead and zinc have been identified as potential health hazards when their concentration in the diet exceeds the benchmark levels identified in Chapter 7.

Of the three metals (Cd, Pb and Zn) that have the potential of posing a health hazard to humans, Cd appears to be of most concern to the scientific community. Based on the

available literature, cadmium appears to require careful consideration because it is present in significant amounts in biosolids, it is absorbed by many human crops and it is accumulated by the human kidney.

The uptake of cadmium by crops as shown in the City of Winnipeg study is similar to that reported by other studies (Hinsley et al., 1984 and Houda, 1987). In general, the average concentration of cadmium in wheat grains of both the fertilized and unfertilized plots in this study tended to increase with increasing application rates of biosolids. With higher application rates of biosolids to the soil, the cadmium concentrations in the soil increased and thus more was available and absorbed by the wheat. The exception in this study was the average cadmium content in wheat grown on soil free of biosolids and chemical fertilizer. The average wheat cadmium content grown on soil free of biosolids was noted to be higher than the average wheat cadmium content grown on soils amended with biosolids. This difference may have been due to variability within and between the plots where some soil regions will naturally have higher cadmium concentrations than others. Another possible explanation for this difference is sampling errors where potential soil contamination of the grain may have occurred. Finally, the difference may be due to human or instrumental error.

The average concentrations of cadmium in the oat grains from the fertilized plot amended with 0, 10000, 25000 and 50000 kg/ha of biosolids were not significantly different from each other. However, the average oat cadmium content grown on soil amended with 100000 kg/ha of biosolids was significantly different from the oat cadmium content grown on soil amended with 0, 10000, 25000 and 50000 kg/ha of biosolids. This difference is indicative of the significant cadmium contribution to the soil by the addition of 100000 kg/ha as compared to the addition of 10000, 25000 and 50000 kg/ha of biosolids i.e. in these conditions, one has to add 100000kg/ha before the difference can be detected.

The average concentrations of cadmium in the oat grains from the non-fertilized plot at all biosolids application rates were not significantly different from each other. However,

the oat cadmium content from the plot grown on soil free of biosolids is higher than the oat cadmium content grown on plots having biosolids. Again this difference may have been due to natural (variability within the plots where some soil regions will naturally have higher metal concentrations than other regions) and sampling errors.

The average cadmium content was generally higher in wheat than oats. These results are in general agreement with other studies (Chaney, 1990 and Houda, 1987) demonstrating that certain crops will absorb particular metals more than others.

The oral reference dose for cadmium, which is an estimate of a daily exposure to the metal that is likely to be without appreciable risk of deleterious effects during a lifetime is 0.84 ug/kg-day inclusive of all routes of exposure and background levels. Adjusting for the background levels (dietary), the RfD becomes reduced to 0.7 ug/kg-day. This benchmark level was derived using an epidemiological study by Buchet et al. (1990) in a Belgian population. Although the RfD provides an important safe reference point, it is only an estimate. This estimate can easily change if more sensitive critical health effect markers for cadmium are identified. In addition, the use of general population studies with larger cohorts and having a greater proportion of sensitive sub-populations may also yield different reference doses. Therefore, since the reference dose is only an estimate, it should not be categorically concluded that oral doses below the RfD are "acceptable" (risk free) and that all doses in excess of the oral RfD are "unacceptable" (will result in adverse health effects).

The results of this study in regards to the potential health risks from the ingestion of cadmium found in crops grown on biosolids-amended soil are consistent with results from other published studies (Chaney, 1990 and Page et al., 1989) indicating that the uptake of cadmium by the crops is too low to warrant any health concerns. To reach the maximum dietary oral exposure limit that is considered to be without appreciable risk of deleterious effects during a lifetime, an average 70-kg person would have to consume about 1.00 kg of wheat or about 4.62 kg of oats daily grown on soil amended with 100000 kg/ha of biosolids (based on City of Winnipeg data). Using 100% whole wheat

bread as an example, the 70-kg person can consume up to 7.1 loafs of bread (280 grams) per day without experiencing an increased risk of deleterious health effects. This allowable daily intake increases when lower application rates of biosolids are applied.

The health risk assessment of lead demonstrated that oral exposure to this metal could pose a health hazard and could be fatal. However, the concentration of lead in the crops grown on biosolids-amended soil was reported as having values of <0.1 mg-lead/kg-grain. These values can be interpreted as either the crops did not absorb lead or the capability of the spectroscoper was not sensitive enough to detect lead concentrations below 0.1 mg/kg. In the latter case, a quantitative assessment of the risk posed by the lead in the crops would not be possible since the concentration values are not absolute values.

Lead, as demonstrated by this study and other studies, does not appear to be a problem since available data suggest that lead is not appreciably taken up by plants.

Nickel, copper and chromium (III) have not been identified as potential health hazards when consumed in crops grown on biosolids-amended soil and therefore their presence in the biosolids does not appear at present to represent a hazard to humans.

Chromium (III) is frequently found in substantial amounts in biosolids. However, the findings of this study are in agreement with other studies, which demonstrated that plants do not accumulate trivalent chromium even when it is present in soil at high levels.

Nickel, also frequently found in substantial amounts in biosolids, is available to plants and may cause phytotoxicity. Fortunately, phytotoxicity occurs at concentrations lower than the levels which are potentially harmful to humans (Smith, 1996). In general, nickel does not represent any realistic hazard to human health because nickel is poorly absorbed from the GI tract, is readily excreted and is of low toxicity. The adverse health effects of nickel arise mainly from inhalation or cutaneous contact with the element.

Copper has also been identified as one of the main phytotoxic elements (Smith, 1996). Fortunately, human copper toxicity from crops is unlikely without severe phytotoxicity. In addition, copper does not represent any realistic hazard to human health because humans have a copper homeostasis mechanism that acts to prevent copper overload.

The element zinc has also been identified as a potential health hazard if consumed in sufficient quantities. Unlike cadmium and lead, zinc is considered an essential element in humans. Its presence in crops grown on biosolids-amended soil can significantly add to the dietary intake levels of zinc. One can argue that since zinc is an essential element, the addition of biosolids to the soil for crop production can prevent zinc deficiency health problems. However, since a RfD has been derived based on the most sensitive health effect (decrease in superoxide dismutase activity), consumption levels above this benchmark can increase the risk of deleterious health effects. Generally a 70-kg person can consume up to 0.68 kg of oats or about 0.45 kg of wheat grown on soil amended with 100000 kg/ha of biosolids (based on City of Winnipeg data). Using 100% whole wheat bread as an example, the 70-Kg person can consume up to 3.1 loaves of bread (280 grams) per day without experiencing an increased risk of deleterious health effects.

To protect the public from any adverse health effects associated with the consumption of trace metals, the daily intake of the crops must be limited by the metal that has the potential of causing an effect at the lowest level of consumption. In this study, zinc has been identified as the metal having the potential to produce an adverse health effect at the lowest levels. Therefore, to protect against any adverse health effects from the consumption of crops grown on biosolids-amended soil, the maximum daily oral exposure levels for zinc should be considered.

There is at present no literature addressing the synergistic effects of oral exposure to trace metals. For this reason, the combined effects of trace metals were not considered in this study. However, this assessment acknowledges that there might be a potential for the toxicity of a metal to increase or decrease by a simultaneous or consecutive exposure to another metal.

This study is the first to explicitly assess the human health risks associated with the consumption of trace metals in crops grown on biosolids-amended soil. Previous assessments of metals in crops from the application of biosolids to the soil have focused primarily on cadmium (Chaney, 1973; Hinsley, 1984). In these assessments, the main focus was not on the health implications of the metals but rather on how the metals interacted in the soil and were transported by the plants. This study illustrated how a health risk assessment model can help quantify the health risks associated with potential human hazards. Using the health risk assessment model in this study provided evidence as to the potential health risks associated with the consumption of crops grown on biosolids-amended soil.

Quantifying the health risk as described above was very important but just as important is how this risk is interpreted and communicated to the stakeholders, especially the public. Public perception of health risks usually differs widely from that of scientists. How the health risk is perceived and whether it is accepted by the public depends on many dimensions. Educating the public about the risks is one of many dimensions that affect risk perception and acceptance. Other dimensions that must be considered include familiarity of the risk, severity of the consequences and whether the exposure is voluntary or not. In light of these dimensions, the public has the right to be effectively informed if they are exposed to any potential hazards. Risk communication strategies that present the facts as they are and at a level understandable to the public are usually accepted and appreciated by the public (Covello, 1989).

8.2 Limitations

There are several potential limitations of this study. One limitation is inherent in the laboratory analytical methods used for analyzing metal content in the crops. The choice of equipment measuring the metal content may not have been adequate or sensitive enough to detect minute traces of the metals such as lead. In addition, the preparatory methods used to extract the metal from the crops may not have been the most efficient

method as the detection and quantification of the metal content in the crops relies heavily on the extraction methodology used.

A second potential study limitation is that the choice of crops tested was limited to wheat and oats. Since different crop species uptake metals and nutrients more efficiently than others, the results of this health risk assessment can thus only be generalized to wheat and oats.

A third potential limitation is the soil environment in which the study was conducted. Since the uptake of metals is influenced by pH, the results of this health risk assessment can thus only be generalized to wheat and oats grown on soils with pH of around 7.6.

A fourth limitation of this study deals with the derivation of the benchmark levels. Following is a list of the limitations associated with the benchmark levels:

- The experimental dose NOAEL is based on scientific judgement. As such, experimentally determined doses for a hazard producing a statistically significant adverse effect may differ amongst researchers resulting in different NOAELs for the same hazard.
- NOAEL is limited to the doses tested experimentally.
- Guidelines have not been developed to take into account the fact that some studies use larger number of animals and thus are more reliable than studies that use a lower number of test animals.
- Measuring techniques in laboratories have their limitations, which may skew the determination of the NOAELs.
- NOAELs for a specific substances may differ between animal species
- As scientific knowledge increases, questions about the selection of the appropriate health effect arise.
- Data relating to the upper and lower ends of the dose-response may be difficult to obtain because large exposures are relatively rare and low level exposures may be too small to detect.
- Safety factors used for calculating the RfD are arbitrary.

- Since the use of SF is a judgement call, different values for ADI, MRL and RfD may exist for the hazard.
- The term SF suggests the notion of absolute safety. In the majority of cases, a firm experimental basis for this notion does not exist.

8.3 Conclusion

This study was conducted to identify and quantify the health hazard associated with the consumption of heavy metals in crops grown on biosolids-amended soil. Based on the results of the health risk assessment on the six heavy metals (cadmium, lead, zinc, chromium, copper and nickel), the following conclusions can be made:

1. Of the six heavy metals in the biosolids that had the potential to be taken up by the crops, cadmium, lead and zinc have been identified to have the potential to pose a health risk to humans if consumed in sufficient amounts. To prevent incurring an increased risk of any adverse health effects from cadmium, an average person who weighs 70 kg can consume up to 0.98 kg of wheat (or 7.0 loaves of 100% whole wheat bread [280g/loaf]) or 4.62 kg of oats (or 32.34 loaves of bread [280 gram loaf containing 51% (w/w) oats]) per day that is grown on soil amended with 100000 kg of biosolids per hectare over a lifetime. Since there were no significant contributions of cadmium in oats due to the addition of biosolids to the soil (at all application rates of biosolids) compared to the soil free of biosolids, an individual consuming oats grown on biosolids-amended soil will not incur any increased risk of adverse health effects compared to the consumption of oats grown on soil free of biosolids.
2. To prevent incurring an increased risk of adverse health effects from zinc, an average person who weighs 70 kg can consume up to 0.45 kg of wheat (or 3.1 loaves of 100% whole wheat bread [280g/loaf]) or 0.70 kg of oats (or 4.90 loaves of bread [280 gram loaf containing 51% (w/w) oats]) per day that is grown on soil amended with 100000 kg of biosolids per hectare over a lifetime. This amount is considerably lower than that of cadmium. Therefore, to protect the public from any adverse health effects

associated with the consumption of trace metals in crops grown on biosolids-amended soil, the maximum daily oral exposure levels for zinc should be considered.

3. Lead has been identified as a potential health hazard if consumed. Quantification of the health hazard due to oral exposure of lead from crops grown on biosolids-amended soil was not possible due to the data set reporting all lead concentrations in the grains at <0.1 mg/kg.

Overall, the application of biosolids to agricultural land provides many benefits including reduced operating costs to the producer and greater crop yield. The metals found in biosolids are taken up by the crops but in such minute quantities that they do not pose a measurable increased risk of deleterious health effects. Quantifying the risk from oral exposure to these metals in such crops has demonstrated that a substantial amount of the crops must be consumed on a daily basis over ones' lifetime to reach a point where additional exposure may increase the risk of deleterious health effects.

References

American Public Health Association, American Waterworks Association and Water Environment Association. Standard Methods for the Examination of Water and Wastewater. 20th Edition. American public Health Association.

Anderson, R.A. (1986). Chromium Metabolism and its Role in Disease Process in Man. Clinical Physiology and Biochemistry, 4:31-41.

Anderson, R.A. (1995). Chromium and Parental Nutrition. Nutrition 11(Supplement 1): 83-86.

Anderson, R.A. Bryden, N.A. and Polansky, M.M. (1997). Lack of Toxicity of Chromium Chloride And Chromium Picolanate in Rats. Journal of the American College of Nutrition, 16(3): 273-279.

ATSDR (1990). Toxicological Profile for Copper. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Services, Agency for Toxic Substance and Disease Registry.

ATSDR (1994). Toxicological Profile for Zinc (Update). Atlanta, GA: U.S. Department of Health and Human Services, Public Health Services, Agency for Toxic Substance and Disease Registry.

ATSDR (1993). Toxicological Profile for Cadmium. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Services, Agency for Toxic Substance and Disease Registry.

ATSDR (1993a). Toxicological Profile for Chromium. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Services, Agency for Toxic Substance and Disease Registry.

ATSDR (1993b). Toxicological Profile for Lead (Update). Atlanta, GA: U.S. Department of Health and Human Services, Public Health Services, Agency for Toxic Substance and Disease Registry.

ATSDR (1997). Toxicological Profile for Nickel (Update). Atlanta, GA: U.S. Department of Health and Human Services, Public Health Services, Agency for Toxic Substance and Disease Registry.

Beckett, P.H.T. and Davis, R.D. (1977). Critical Levels of Toxic Elements in Plants. New Phytology, 79: 95-106.

Bernard, A.M., Roels, A., Cardenas, A. and Lauwerys R. (1990). Assessment Of Urinary Protein 1 and Transferrin as Early Markers of Cadmium Nephrotoxicity. British Journal of Medicine 47:559-565.

Bitton, G., Damron, B.L., Edds, G.T. and Davidson, J.M. (1980). Sludge-Health Risks of Land Application. Ann Harbor, Michigan: Ann Harbor Science Publishers.

Bolton, R.L. and Klein, L. (1971). Sewage Treatment: Basic Principles and Trends (2nd Ed.). Ann Arbor Michigan: Ann Arbor Publishers Inc.

Buchet, J.P., Lauwerys, R. and Nick, L. (1990). Renal Effects of Cadmium Body Burden of the General Population. Lancet, 336:699-702.

Buckler, H.M. (1986). Self-Poisoning with Oral Cadmium Chloride. British Medical Journal, 292: 1559-1560.

Buhler, D.R. (1985). Availability of Cadmium From Foods And Water. In: Inorganics in Drinking Water and Cardiovascular Disease. Ch. XXIV, Princeton Scientific Publication, Princeton.

Bush, J.A., Mahony, J.P. and Markowitz, H. (1955). Studies on Copper Metabolism. Journal of Clinical Investigations, 34: 1766-1778.

Campbell, J.K. and Mills, C.F. (1979). Toxicity of Zinc to Pregnant Sheep. Environmental Research, 20:1.

Cartwright, G.E. and Wintrobe, M.M. (1964). Copper Metabolism in Normal Subjects. American Journal of Clinical Nutrition, 14: 224232.

Chaney, R.L. (1990). Twenty Years of Land Application Research. Biocycle, September, 1990.

Chaney, R.L., (1973). Crop and Food Chain Effects of Toxic Elements in Sludges and Effluents. Washington, D.C.: National Associate State University and Land Grant Colleges, pp. 129-141.

Chandra, R.K. (1984). Excessive Intake of Zinc Impairs Immune Responses. JAMA 252:1443-1446.

Chobanian, S.J. (1981). Accidental Ingestion of Liquid Zinc Chloride: Local and Systemic Effects. Ann Energy Med 10: 91-93.

Chung, J., Nartley, N. and Cherian, M.G. (1986). Metallothionein Levels in Liver and Kidney of Canadians : A Potential Indicator of Environmental Exposure To Cadmium. Arch. Environmental Health 41(5): 319-323.

Chuttani, H.K., Gupta, P.S. and Gulati, S. (1965). Acute Copper Sulphate Poisoning. American Journal of Medicine, 39: 849-854.

Cools, A., Salle, H.J.A. and Verberg, M.M. (1976). Biochemical Response of Male Volunteers Ingesting Inorgainc Lead for 49 Days. International Arch of Occupational and Environmental Health, 38:129-139.

Cousins, R.J. (1985). Absorption, Transport and Hepatic Metabolism of Copper and Zinc: Special Reference to Metallothionein and Ceruloplasmin. Physiology Review 65: 238-309.

Covello, VT. (1989). Informing People About Risks from Chemicals, Radiation and Other Toxic Substances: Review of Obstacles to Public Understanding and Effective Risk Communications. In: Prospects and Problems in Risk Communications. Leiss W (ed). University of Waterloo Press, Ontario, Canada.

Dabbeka, R.W., Mckenzie, A.D. and Lacroix, G.M.A (1987). Dietary Intake of Lead, Cadmium, Arsenic and Fluoride by Canadian Adults: A 24 Hour Duplicate Diet Study. Food Additatives and Contaminants, 4(1): 89.

Delafuente, J.C. (1991). Nutrients and Immune Responses. Rheum Disease Clin North America 17(2): 203-212.

Doll, R., Mathews, J.D. and Morgan, L.G. (1977). Cancer of the Lungs and Nasal Sinuses in Nickel Workers. A Reassessment of The Period of Risk. Britain Journal Ind Med, 34: 102-105.

Donaldson, R.M. and Barreras, R.F. (1966). Intestinal Absorption of Trace Quantities of Chromium. Journal of Laboratory and Clinical Medicine, 68:484-493.

Dourson, M.L. and Stara, J.F. (1983). Regulatory Toxicology and Pharmocology. 3:224-238.

Ellbeticha, A. and Al-Hamood, M.H. (1997). Long Term Exposure of Male and Female Mice to Trivalent Chromium Compounds: Effect on Fertility. Toxicology, 116:39-47.

Ellen, G., Egmond, E., Vanloon, J.W. and Sahertian, E.T. (1990). Food Additives and Contaminants 7(2):207-221.

Environment Canada (1976). National Inventory of Sources and Emissions of Cadmium (1972). Report APCD 76-2, Air Pollution Control Directorate, Ottawa.

Environment Canada (1984). Manual For Land Application of Treated Municipal Wastewater and Sludge. Minister of Supply and Services Canada. Cat. No. En48-6/84-1E, Ottawa.

FDA (1993). Guidance Document for Cadmium in Shellfish, Center for Food Safety and Applied Nutrition, United States Food and Drug Administration, Washington DC. 20204.

Fischer, P., Giroux, A. and L'Abbe, A.R. (1984). Effect of Zinc Supplementation on Plasma High Density Lipoprotein and Zinc. American Journal of Clinical Nutrition, 40:743-746.

Fleischer, M. (1974). Environmental Impact of Cadmium: A Review By The Panel on Hazardous Trace Substances. Environmental Health Perspective, 7:253.

Friberg, L., Piscator, M., Nordberg, G.F. and Kjellstorm, T. (1974). Cadmium in the Environment. 2nd Edition. CRC Press, Ohio.

Fulkerson, W. and Goeller, H.E. (1973)Cadmium: The Dissipated Element. Report ORNL-NSF-EP-21.

Gauvin, M.J. (1986). Cadmium. In: Canadian Minerals Yearbook. Mineral Resources Branch, Energy, Mines and Resources Canada, Ottawa.

Goyer, R.A. (1996). Toxic Effects of Metals. In Casareett and Doull's Toxicology: The Basic Science of Poisons (Klassen, CD). 5th Edition. McGraw-Hill, New York.

Gras, N.S.B. (1925). A History of Agriculture. New York: F.S. Crafts and Company Publishers.

Hallenbeck, W.H. (1984). Human Health Effects of Exposure to Cadmium. Experientia, 40:136.

Hanninen, H., Mantere, P. and Kock, B. (1979). Subjective Symptoms In Low-Level Exposure to Lead. Neurotoxicology, 1:333.

Hastie, A.B. (1993). The Effect of Municipal Sewage Sludge and Inorganic Cadmium on Cadmium Bioavailability to Wheat and on The Chemistry of Soil Cadmium. M.Sc. Thesis. The University of Manitoba.

Hayano, M., Nogawa, K. and Honda, R. (1996). Dose-Response Relationship Between Urinary Cadmium Concentration and Beta-Microglobulinuria Using Logistic Regression Analysis. Arch. Environmental Health, 51(2):162-167.

Haywood, S. (1985). Copper Toxicosis and Tolerance in the Rat. Liver, 5:267-275.

Health Canada (1989). Guidelines For Canadian Drinking Water Quality. 4th Edition. Department of National Health and Welfare.

Health Canada (1993). Investigating Human Exposure to Contaminants in the Environment. A Handbook for Exposure Calculations (Draft). Great Lakes Health Effects Division, Health Protection Branch.

Hiatt, V. and Huff, J.E. (1975). The Environmental Impact of Cadmium: An Overview. International Journal of Environmental Studies, 7:277.

Hinsley, T.D., Redborg, K.E., Pietz, R.E. and Ziegler, E.L. (1984). Cadmium and Zinc Uptake by Corn With Repeated Applications of Sewage Sludge. Journal of Agriculture and Food Chemistry, 32, 155-163.

Hinsley, T.D., Redborg, D.E., Ziegler and Innes, I.H. (1982). Effects of Chemical and Physical Changes in Soil Amended with Sewage Sludge on the Uptake of Metals by Plants. University Park, PA: The Pennsylvania State University Press.

Houda, P.S. Mcnulty, D. and Alloway, D.J. (1987). Plant Availability of Heavy Metals In Soils Previously Amended With Heavy Application of Sewage Sludge. Journal of Science, Food and Agriculture, 73, 446-454.

Ivankovic, S. and Preussman, R. (1975). Absence of Toxic and Carcinogenic Effects After Administrations of High Doses of Chronic Oxide Pigment in Subacute and Long Term Feeding Experiments in Rats. Food Cosmet Toxicology, 13:347-351.

Juberg D.R., Kleiman, C.F. and Simona, C.K. (1997). Position Paper of the American Council on Science and Health: Lead and Human Health. Ecotoxicology and Environmental Safety, 38: 162-180.

Jung, K.M., Pergande, H.J., Fels, L.M., and H. Stole (1993). Urinary Proteins and Enzymes As Early Indicators of Renal Dysfunction in Chronic Exposure to Cadmium. Clinical Chemistry, 39(5): 757-765.

Kagamimori, S., Watanabi, M. and Nakagawa, H. (1986). Case-Control Study on Cardiovascular Function in Females With a History of Heavy Exposure to Cadmium. Environmental Contamination Toxicology, 36:484-490.

Kaufman, D.B., Dinicola, W., Mcintosh, R. (1970). Acute Potassium Dichromate Poisoning. American Journal of Diseases in Children, 119:374-376.

Kawai, M.K., Furkuda and Kimura, M. (1976). Effects and Dose Response Relationship of Toxic Metals. Amsterdam: Elsevier, Pp. 343-370.

Kehoe, R.A. (1961). The Metabolism of Lead in Man in Health and Disease. The Harben Lectures. J R Institute of Public Health And Hygiene, 24:177-203

Kido, T., Nogawa, K. and Honda, R. (1990). The Association Between Renal Dysfunction and Osteopenia in Environmental Cadmium Exposed Subjects. Environmental Research, 51:71-82.

Kitamura, M., Sumiro, K. & Kamatanni, N. (1970). Cadmium Concentrations in Livers, Kidneys and Bones of Human Bodies. Japanese Journal of Public Health, 17:507.

Kjellstrom, T. (1992). Cadmium and Health: A Toxicological and Epidemiological Appraisal. Vol 2, CRC Press, Boca Raton, Florida, pp. 111-158.

Kladivko, E.J. and Nelson, D.W. (1979). Changes in Soil Properties from Application of Anaerobic Sludge, Journal WPCF, Vol.51, No.2, February,1979.

Kotsonis, F.N. and Klassen, C.D. (1978). The Relationship of Metallothionin to the Toxicity of Cadmium After Prolonged Administration to Rats. Toxicological Application In Pharmacology, 46:39-54.

Kumar, S., Mehta, T. And Aggarwal, C.S. (1987). Encephalopathy Due to Inorganic Lead Exposure in Adults. Japan Journal of Medicine, 26:253-254.

Kusiak, R.A., Ritchie, A.C. and Springer, J. (1993). Mortality from Stomach Cancer in Ontario Miners. British Journal of Medicine, 50:117-126.

Laureys, R. (1978). Criteria for Cadmium. C.E.C., Pregamon Press, Oxford, Reference 39.

Lester, M.L., Horst, R.L. and Thatcher, R.W. (1986). Protective Effects of Zinc and Calcium Against Heavy Metal Impairment of Children's Cognitive Function. Nutritional Behavior, 3:145.

Lu, F.C. (1996). Basic Toxicology: Fundamentals, Target Organs and Risk Assessment. 3rd Edition. Taylor and Francis, Washington, DC.

Lymburner, D.B. (1974). Environmental Contaminants Inventory Study No. 2. The Production, Use and Distribution of Cadmium in Canada. Report Series No. 39, Canada Center for Inland Waters, Inland Waters Directorate, Ottawa.

Mackenzie, R.D., Byerrum, R.U. and Decker, C.F. (1958). Chronic Toxicity Studies. II. Hexavalent and Trivalent Chromium Administered in Drinking Water to Rats. American Medical Association Arch Ind Health, 18:232-234.

Mahaffey, K.R. (1990). Nutritional Factors in Lead Poisoning. Nutritional Review, 39:353.

Mahaffey, K.R., Rosen, J.F., Chesney, R.W. and Peeler, D.J. (1982). Association Between Age, Blood Lead Concentration and Serum Levels in Children. American Journal of Clinincal Nutrition, 35:1327.

Marceau, N., Aspin, N. and Kortask, A. (1970). Absorption of Copper From Gastrointestinal Tract of the Rat. American Journal of Physiology, 218: 377-383.

Mason, H.J. (1990). Occupational Cadmium Exposure and Testicular Endocrine Function. Human Experimental Toxicology, 9:91-94.

Mason, H.J. (1988). Relations Between Liver Cadmium, Cumulative Exposure and Renal Function in Cadmium Alloy Workers. British Journal of Industrial Health, 45:44-53.

Mertz, W., Roginski, E.E. and Reba, R.C. (1965). Biological Activity and Fate of Trace Quantities of Intravenous Chromium (III) In the Rat. American Journal of Physiology, 209:489-494.

Metcalf and Eddy (1991). Wastewater Engineering-Treatment, Disposal, and Reuse (3rd ed.). U.S.A.: Irwin/McGraw Hill.

Mushak, P. and Crocetti, A.F. (1989). Determination of Numbers of Lead Exposed Children as a Function of Lead Source. Environmental Research, 50:210.

National Academy of Science (NAS) (1972). Lead. National Academy of Science. Washignton DC: National Academy Press.

National Academy of Science (NAS) (1975). Nickel. National Academy of Science. Washignton DC: National Academy Press.

National Academy of Science (NAS) (1977). Copper. National Academy of Science. Washignton DC: National Academy Press.

Nogawa, K., Honda, R. and Hamaya, H. (1989). A Dose Response Analysis of Cadmium In the General Environment with Special Reference to Total Cadmium Intake Limit. Environmental Research, 48:7-16.

Nogawa, K. and Kido, T. (1993). Biological Monitoring of Cadmium Exposure in Itai-Itai Disease Epidemiology. Int. Arch. Occupational And Environmental Health, 65:S43-46.

Nordberg, G.F. (1974). Health Hazards of Environmental Cadmium Pollution. Ambio, 3:55.

Nordberg, G.F. (1985). Cadmium and Health: a Toxicological and Epidemiological Appraisal. Volume 1, Exposure, Dose and Metabolism. CRC press, Inc., Boca Raton, Florida. Pp 103-178.

NRC (National Research Council), (1989). Zinc. University Park Press, Baltimore MD.

Otto, D.A., Beningus, V.A. and Schroeder, S. (1982). Effects of Low to Moderate Lead Exposure of Slow Cortical Potentials in Young Children. Neurobehavioral Toxiciology, 4:733.

Owen, C.A. Jr. (1965). Metabolism of Radio in the Rat. American Journal of Physiology, 209: 900-904.

Page, A.L., Logan, T.J. and Ryan T.A. (1989). Land Application of Sludge. U.S.A.: Lewis Publishers.

Pennington, J.A.T. (1996). Intakes of Minerals From Diets and Foods: Is There a Need For Concern? American Institute of Nutrition. Supplement pp. 2304S-2308S

Prige, E. (1978). Early Signs of Oral and Inhalative Cadmium Uptake In Rats. Arch Toxicology, 40:231-247.

Rahola, T., Aaran, R.K. and Miettinen, J.K. (1971). Half Time Studies on Mercury and Cadmium by Whole Body Counting. In: IAEW/WHO Symposium on the Assessment of Radioactive Organ and Body Burdens, Stockholm.

Rezuke, R.N., Knight J.A. and Sunderman F.W. Jr. (1987). Reference Values for Nickel Concentrations in Human Tissues and Bile. American Journal Ind Med, 11: 419-426.

Riihimaki, V. (1972). Cadmium. In: Long Term Programme in Environmental Pollution Control in Europe. The Hazards to Health of Persistent Substances in Water. Annex to a Working Report . World Health Organization.

Rummo, J.H., Routh, D.K. and Rummo, N.J. (1979). Behavioral and Neurological Effects of Symptomatic and Asymptomatic Lead Exposure In Children. Arch Environmental Health, 34:120-125.

Ryu, J.E., Ziegler, E.E. and Formon, S. (1983). Dietary Intake of Lead and Blood Lead Concentrations in Early Infancy. American Journal of Diseases in Children, 137:886.

Samman S.R.D. (1987). The Effects of Zinc Supplements on Plasma Zinc And Copper Levels and The Reported Symptoms in Healthy Volunteers. Medical Journal Australia 146: 246-249.

Schmidt, J.P. (1997). Understanding Phytotoxicity Threshold for Trace Elements in Land-Applied Sewage Sludge. Journal of Environmental Quality, 26:4-10.

Schroader, H.A., Nasan, A.P. and Tipton, I.H. (1966). Essential Trace Metals in Man: Copper. Journal of Chronic Diseases, 19: 1007-1034.

Schwartz, J., Landrigan, P.J., Feldman, R.G. and Baker, E.L. (1988). Threshold Effect in Lead-Induced Peripheral Neuropathy. Journal of Pediatrics, 112:12.

Seppalainen, A.M., Hernberg, S. and Vesento, R. (1983). Early Neurotoxic Effects of Occupational Lead Exposure: A Prospective Study. Neurotoxicology, 4:181.

Sherlock, J.C. (1984). Cadmium in Foods and Diets. Experientia, 40:152.

Smith, M., Delvis, T. and Lansdown, R. (1983). The Effects of Lead Exposure on Urban Children. Developmental Medicine and Child Neurology, 25(Supplement 47).

Smith, S.R. (1996). Agricultural Recycling of Sewage Sludge and the Environment. CAB (Centre for Agriculture and Biosciences) International, U.K.: Pp. 261-273.

Strickland, G.T., Beckner, W.M. and Leu, M.L. (1972). Absorption of Copper in Homozygotes and Heterozygotes For Wilson's Disease and Controls: Isotope Tracer Studies. Clinical Science, 43: 617-625.

Stuick, E.J. (1974). Biological Response of Male and Female Volunteers To Inorganic Lead. International Arch Arbietsmed, 33:83-97.

Sunderman F.W., Hopfer, S.M. and Sweeny K.R. (1989). Nickel Absorption and Kinetics in Human Volunteers. Proc Soc Exp Biol Med, 191:5-11.

U.S. EPA (1987). Risk Assessment Guidelines of 1986 (Epa/600/8-87/045, Dated August 1987).

U.S. EPA (1986). Urban Lead Soil Abatement Demostration Project. Vol. I (EPA/600/P-93/001AF).

U.S. EPA (1986a). Air Quality Criteria for Lead. Report No. EPA/6008-83/028F).

U.S. EPA (1992). Technical Support Document for Land Application of Sewage Sludge. Prepared for Office of Waer, US EPA, Eastern Research Corp, Lexington MA.

Visek, W.J. Whitney, I.B. and Kuhn, U.S.G. (1953). Metablosim of CR-51 By Animals as Influenced By Chemical State. Proc Soc Exp Biol Med, 84:610-615.

Valberg, L.S., Flanagan, P.R. and Chamberlain, M.J. (1984). Effects of Iron, Tin and Copper on Zinc Absorptions in Humans. The American Journal of Food Nutrition. September 1984, pp. 536-541.

Webber, M.D. (1984). Land Utilization Of Sewage Sludge - A Discussion Paper, Expert Committee on Soil and Water Management, Agriculture Canada,1984.

World Health Organization (1962). Evaluation of the toxicity of a number of antimicrobials and antioxidants. Sixth report. WHO technical report serial No. 228. Geneva:WHO.

World Health Organization (1974). Environmental Health Criteria for Cadmium. Geneva:WHO.

Yadrick, M.K., Kenney, M.A., Winterfeld, E.A. (1989). Iron, Copper and Zinc Status. American Journal of Clinical Nutrition, 49:145-150.

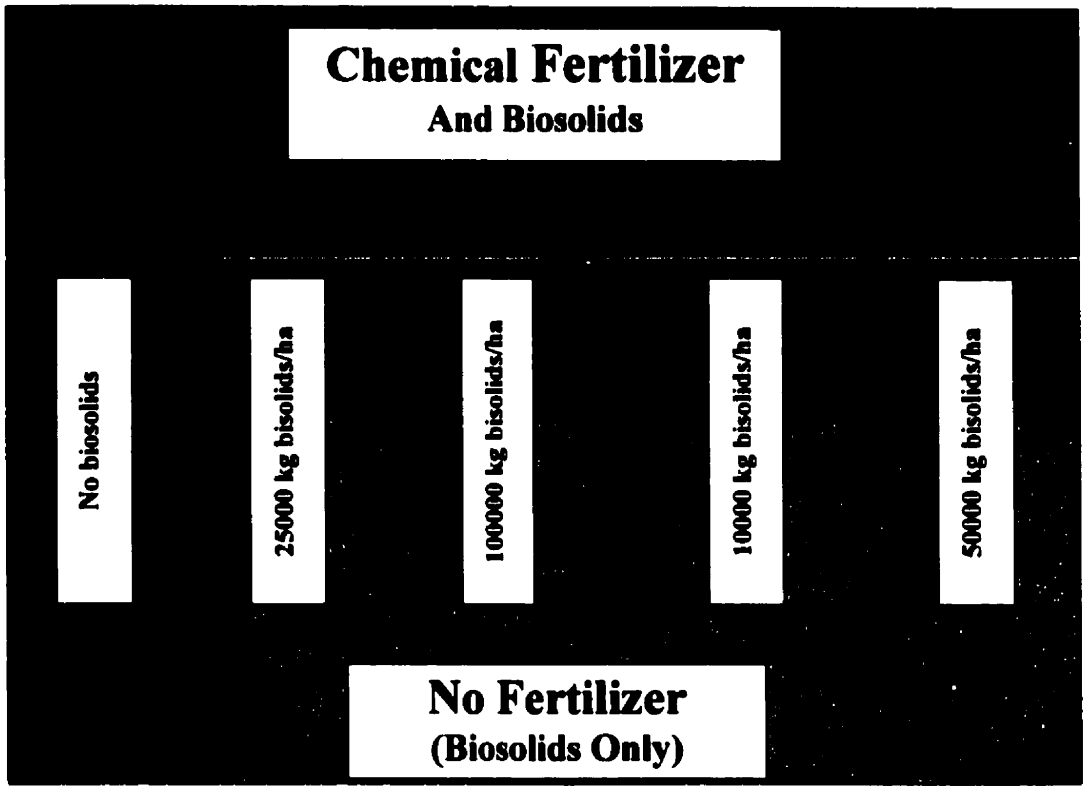
Yamagata, N. (1974). Absorption of Cadmium Via the Gastrointestinal Tract in a Normal Man. Summary Report for a Meeting on Studies of Cadmium Poisoning.

Ziegler, E.E., Edwards, B.B. and Jensen, R.L. (1978). Absorption and Retention of Lead By Infants. Pediatric Research, 12:29.

APPENDIX A

Experimental Plot Design

Test Plot Configuration



In total, there are 10 sub-plots in each of the 5.6 hectare lots for a total of 20 sub-plots. One 5.6 hectare plot was used to grow wheat while the other was used to grow oats.

APPENDIX A-1

METHODOLOGY APPLIED TO THE ANALYSIS OF METAL CONTENT IN CROPS GROWN ON BIOSOLIDS-AMENDED SOIL

Analytical Methods for Heavy Metals

1. Oven-dry an aliquot of plant sample at 103 C for at least 2 hours. Desicate

2. Weigh approximately 0.5g sample into a teflon digestion vessel. Record actual weight to two decimal places.

3. Include a reagent blank and a plant tissue standard reference material with every set of 10 samples

4. Add 6.0 ml concentrated HNO_3 . Seal vessels tightly and connect to microwave.

5. Digest using the following settings

Stages:	1	2	3	4	5
Power	50%	60%	65%	70%	50%
Pressure (psi)	120	140	160	170	180
Run time (min)	10	10	10	10	20
Time at pressure (min)	8	8	8	8	15

6. remove vessels from microwave. Open carefully. Transfer digestate into 50 ml mixing cylinder. Rinse digestion vessel several times with deionized water and dilute sample to 50 ml

7. analyze on AA spectrophotometer ***

*** The sample extracts (digestates) are analyzed for Cd, Pb, Ni, Cr on a Perkin-Elmer Model 5000 atomic absorption spectrophotometer and HGA furnace atomizer using Perkin Elmer's STPF protocols. Zn an Cu are analyzed on a Perkin Elmer Model 3100 Atomic Absorption Spectrophotometer.

(American Public Health Association et al., 1998)

APPENDIX A-2

ANALYTICAL RESULTS OF HEAVY METAL CONTENT IN CROPS (City of Winnipeg Data)

CITY OF WINNIPEG SOIL RESEARCH PROGRAM
ANALYTICAL RESULTS
WHEAT
FERTILIZED

	NUTRIENTS (mg.kg)			*TOTAL HEAVY METALS (mg/kg)*					
PPLICATION RATE	TKN	NO3-N	total P	CADMIUM	COPPER	LEAD	ZINC	NICKEL	HROMIUM
0	26000	n/a	4500	0.017	4	<0.1	31	0.3	<0.1
onnes/ha	25000	n/a	4200	0.013	3	<0.1	28	0.2	<0.1
	25000	n/a	4200	0.015	4	<0.1	29	0.2	<0.1
	27000	n/a	4400	0.016	4	<0.1	27	0.2	<0.1
	26000	n/a	4200	0.010	4	<0.1	28	0.2	<0.1
MEDIAN									
MEAN	25800		4300	0.014	4	<0.1	29	0.2	<0.1
STD. DEVIATION	837		141	0.003	0		2	0.0	
std. dev. as % of m	3		3	20	12		5	20	
10	27000	n/a	4600	0.022	5	<0.1	30	0.2	<0.1
onnes/ha	27000	n/a	5000	0.027	4	<0.1	35	0.7	<0.1
	26000	n/a	4300	0.021	4	<0.1	30	0.2	<0.1
	25000	n/a	4600	0.020	4	<0.1	31	0.1	<0.1
	25000	n/a	4900	0.025	4	<0.1	30	0.1	<0.1
MEDIAN									
MEAN	26000		4680	0.023	4	<0.1	31	0.3	<0.1
STD. DEVIATION	1000		277	0.003	0		2	0.3	
std. dev. as % of m	4		6	13	11		7	97	
25	24000	n/a	4200	0.022	4	<0.1	34	0.1	<0.1
onnes/ha	28000	n/a	5200	0.017	4	<0.1	34	0.1	<0.1
	30000	n/a	5200	0.016	3	<0.1	37	0.1	<0.1
	29000	n/a	4900	0.011	4	<0.1	33	0.1	<0.1
	26000	n/a	5400	0.019	4	<0.1	39	<0.1	<0.1
MEDIAN									
MEAN	27400		4980	0.017	4	<0.1	35	0.1	<0.1
STD. DEVIATION	2408		471	0.004	0		3	0.0	
std. dev. as % of m	9		9	24	12		7	0	
50	29000	n/a	5600	0.038	5	<0.1	46	<0.1	<0.1
onnes/ha	25000	n/a	5000	0.015	4	<0.1	35	<0.1	<0.1
	27000	n/a	5500	0.029	5	<0.1	44	<0.1	<0.1
	20000	n/a	3600	0.025	5	<0.1	43	<0.1	<0.1
	24000	n/a	4600	0.012	3	<0.1	25	0.1	<0.1
MEDIAN									
MEAN	25000		4860	0.024	4	<0.1	39	0.1	<0.1
STD. DEVIATION	3391		811	0.011	1		9		
std. dev. as % of m	14		17	45	20		23		
100	31000	n/a	5800	0.091	7	<0.1	67	0.2	<0.1
onnes/ha	32000	n/a	6100	0.029	6	<0.1	50	0.9	<0.1
	30000	n/a	5000	0.055	7	<0.1	51	0.9	<0.1
	28000	n/a	4700	0.051	6	<0.1	49	0.6	<0.1
	28000	n/a	5000	0.039	6	<0.1	51	0.6	<0.1
MEDIAN									
MEAN	29800		5320	0.053	6	<0.1	54	0.6	<0.1
STD. DEVIATION	1789		597	0.024	1		8	0.3	
std. dev. as % of m	6		11	44	9		14	45	

CITY OF WINNIPEG SOIL RESEARCH PROGRAM
ANALYTICAL RESULTS
WHEAT
UNFERTILIZED

	NUTRIENTS (mg.kg)			*TOTAL HEAVY METALS (mg/kg)*					
PPLICATION RATE	TKN	NO3-N	total P	CADMIUM	COPPER	LEAD	ZINC	NICKEL	HROMIU
0	31000	n/a	5300	0.015	5	<0.1	40	0.2	<0.1
onnes/ha	22000	n/a	5400	0.011	5	<0.1	40	0.1	<0.1
	23000	n/a	5000	0.027	5	<0.1	30	0.1	<0.1
	20000	n/a	5100	0.140	4	0.2	37	0.1	<0.1
	23000	n/a	5200	0.016	5	<0.1	42	0.1	<0.1
MEDIAN									
MEAN	23800		5200	0.042	5	0.2	38	0.1	<0.1
STD. DEVIATION	4207		158	0.055	0		5	0.0	
std. dev. as % of m	18		3	132	9		13	37	
10	10000	n/a	n/a	0.022	5	<0.1	36	<0.1	<0.1
onnes/ha	21000	n/a	5700	0.020	5	<0.1	42	0.1	<0.1
	20000	n/a	4800	0.010	5	<0.1	44	0.2	<0.1
	20000	n/a	5200	0.012	5	<0.1	44	0.3	<0.1
	21000	n/a	5000	0.025	6	<0.1	47	0.1	<0.1
MEDIAN									
MEAN	18400		5175	0.018	5	<0.1	43	0.2	<0.1
STD. DEVIATION	4722		386	0.006	0		4	0.1	
std. dev. as % of m	26		7	36	9		10	55	
25	25000	n/a	5600	0.029	5	<0.1	41	0.1	<0.1
onnes/ha	24000	n/a	4600	0.029	5	<0.1	39	0.1	<0.1
	25000	n/a	5100	0.029	5	0.6	37	0.2	0.2
	25000	n/a	4600	0.030	5	<0.1	41	0.1	<0.1
	24000	n/a	5200	0.017	5	<0.1	41	0.1	<0.1
MEDIAN									
MEAN	24600		5020	0.027	5	0.6	40	0.1	0.2
STD. DEVIATION	548		427	0.005	0		2	0.0	
std. dev. as % of m	2		8	21	0		4	37	
50	25000	n/a	5200	0.041	5	<0.1	45	0.1	<0.1
onnes/ha	24000	n/a	5100	0.021	5	<0.1	41	0.1	<0.1
	22000	n/a	5400	0.019	5	<0.1	40	0.1	<0.1
	25000	n/a	4800	0.038	5	0.2	44	0.2	<0.1
	23000	n/a	4400	0.022	4	<0.1	34	<0.1	<0.1
MEDIAN									
MEAN	23800		4980	0.028	5	0.2	41	0.1	<0.1
STD. DEVIATION	1304		390	0.010	0		4	0.1	
std. dev. as % of m	5		8	37	9		11	40	
100	27000	n/a	4800	0.058	7	<0.1	55	0.2	<0.1
onnes/ha	24000	n/a	4800	0.077	6	<0.1	51	0.2	<0.1
	28000	n/a	5000	0.042	6	<0.1	44	0.2	<0.1
	26000	n/a	4600	0.036	6	<0.1	42	0.2	<0.1
	26000	n/a	4600	0.031	5	<0.1	42	0.2	<0.1
MEDIAN									
MEAN	26200		4760	0.049	6	<0.1	47	0.2	<0.1
STD. DEVIATION	1483		167	0.019	1		6	0.0	
std. dev. as % of m	6		4	38	10		13	8	

CITY OF WINNIPEG SOIL RESEARCH PROGRAM
ANALYTICAL RESULTS
OATS
FERTILIZED

	NUTRIENTS (mg.kg)			*TOTAL HEAVY METALS (mg/kg)*					
PPLICATION RATE	TKN	NO3-N	total P	CADMIUM	COPPER	LEAD	ZINC	NICKEL	HROMIUM
0	24000	n/a	4100	<0.005	3	<0.1	15	2.4	<0.1
onnes/ha	21000	n/a	3700	0.008	4	<0.1	25	3.0	<0.1
	21000	n/a	3900	<0.005	4	<0.1	22	2.4	<0.1
	22000	n/a	4000	<0.005	3	<0.1	15	1.9	<0.1
	18000	n/a	3500	<0.005	3	<0.1	15	1.6	<0.1
MEDIAN									
MEAN	21200		3840	0.008	3	<0.1	18	2.3	<0.1
STD. DEVIATION	2168		241	#DIV/0!	1	#DIV/0!	5	0.5	#DIV/0!
std. dev. as % of m	10		6	#DIV/0!	16	#DIV/0!	26	24	#DIV/0!
10	20000	n/a	4200	0.007	3	<0.1	17	1.9	<0.1
onnes/ha	20000	n/a	4200	<0.005	3	<0.1	18	1.6	<0.1
	22000	n/a	3600	0.006	5	<0.1	24	3.7	<0.1
	20000	n/a	3700	0.006	4	<0.1	18	2.6	<0.1
	19000	n/a	3800	0.007	3	<0.1	16	1.5	<0.1
MEDIAN									
MEAN	20200		3900	0.007	4	<0.1	19	2.3	<0.1
STD. DEVIATION	1095		283	0.001	1	#DIV/0!	3	0.9	#DIV/0!
std. dev. as % of m	5		7	9	25	#DIV/0!	17	40	#DIV/0!
25	38000	n/a	4000	0.007	5	<0.1	45	4.9	<0.1
onnes/ha	39000	n/a	3900	0.013	6	<0.1	62	5.4	<0.1
	20000	n/a	3900	0.006	4	<0.1	21	1.5	<0.1
	20000	n/a	4000	0.005	3	<0.1	25	1.8	<0.1
	26000	n/a	4600	0.005	4	<0.1	32	4.7	<0.1
MEDIAN									
MEAN	28600		4080	0.007	4	<0.1	37	3.7	<0.1
STD. DEVIATION	9370		295	0.003	1	#DIV/0!	17	1.9	#DIV/0!
std. dev. as % of m	33		7	46	26	#DIV/0!	45	51	#DIV/0!
50	17000	n/a	4000	0.010	3	<0.1	24	0.9	<0.1
onnes/ha	14000	n/a	3600	<0.005	3	<0.1	23	1.0	<0.1
	15000	n/a	3600	0.005	2	<0.1	20	0.9	<0.1
	20000	n/a	3800	0.006	3	<0.1	25	1.9	<0.1
	15000	n/a	3700	<0.005	3	<0.1	26	0.8	<0.1
MEDIAN									
MEAN	16200		3740	0.007	3	<0.1	24	1.1	<0.1
STD. DEVIATION	2387		167	0.002	0	#DIV/0!	2	0.5	#DIV/0!
std. dev. as % of m	15		4	35	16	#DIV/0!	10	41	#DIV/0!
100	24000	n/a	4300	0.016	5	<0.1	45	4.7	<0.1
onnes/ha	24000	n/a	4500	0.013	6	<0.1	52	5.9	<0.1
	24000	n/a	4400	0.009	4	<0.1	37	3.0	<0.1
	26000	n/a	4200	0.014	5	<0.1	46	4.5	<0.1
	16000	n/a	4700	0.033	6	<0.1	66	4.2	<0.1
MEDIAN									
MEAN	22800		4420	0.017	5	<0.1	49	4.5	<0.1
STD. DEVIATION	3899		192	0.009	1	#DIV/0!	11	1.0	#DIV/0!
std. dev. as % of m	17		4	55	16	#DIV/0!	22	23	#DIV/0!

CITY OF WINNIPEG SOIL RESEARCH PROGRAM
ANALYTICAL RESULTS
OATS
UNFERTILIZED

	NUTRIENTS (mg.kg)			*TOTAL HEAVY METALS (mg/kg)*					
PPLICATION RATE	TKN	NO3-N	total P	CADMIUM	COPPER	LEAD	ZINC	NICKEL	HROMIU
0	19000	n/a	4200	0.005	4	<0.1	31	1.7	<0.1
onnes/ha	20000	n/a	4100	<0.005	4	<0.1	27	1.5	<0.1
	18000	n/a	3900	<0.005	4	<0.1	27	1.6	<0.1
	18000	n/a	4000	0.082	3	<0.1	21	1.2	<0.1
	17000	n/a	3800	<0.005	3	<0.1	19	1.2	<0.1
MEDIAN									
MEAN	18400		4000	0.044	4	<0.1	25	1.4	<0.1
STD. DEVIATION	1140		158	0.054	1	#DIV/0!	5	0.2	#DIV/0!
std. dev. as % of m	6		4	125	14	#DIV/0!	20	16	#DIV/0!
10	16000	n/a	3600	<0.005	3	<0.1	25	1.2	<0.1
onnes/ha	18000	n/a	3900	<0.005	3	<0.1	22	1.3	<0.1
	17000	n/a	4000	<0.005	4	<0.1	29	1.8	<0.1
	15000	n/a	3800	<0.005	3	<0.1	25	0.9	<0.1
	15000	n/a	3700	<0.005	3	<0.1	20	0.9	<0.1
MEDIAN									
MEAN	16200		3800	<0.005	3	<0.1	24	1.2	<0.1
STD. DEVIATION	1304		158	#DIV/0!	0	#DIV/0!	3	0.4	#DIV/0!
std. dev. as % of m	8		4	#DIV/0!	14	#DIV/0!	14	30	#DIV/0!
25	16000	n/a	3900	0.006	3	<0.1	21	1.2	<0.1
onnes/ha	14000	n/a	3800	0.007	3	<0.1	26	0.8	<0.1
	16000	n/a	3900	0.006	3	0.1	27	1.6	<0.1
	14000	n/a	3800	0.005	3	<0.1	26	1.1	<0.1
	16000	n/a	3800	0.006	3	0.3	20	1.2	<0.1
MEDIAN									
MEAN	15200		3840	0.006	3	0.2	24	1.2	<0.1
STD. DEVIATION	1095		55	0.001	0	0.1	3	0.3	#DIV/0!
std. dev. as % of m	7		1	12	0	70.7	14	24	#DIV/0!
50	14000	n/a	3700	<0.005	3	<0.1	24	0.9	<0.1
onnes/ha	14000	n/a	3500	0.005	3	<0.1	21	0.9	<0.1
	13000	n/a	3800	<0.005	3	<0.1	24	0.9	<0.1
	18000	n/a	4100	0.005	3	<0.1	27	1.8	<0.1
	22000	n/a	4200	0.009	4	<0.1	23	2.3	<0.1
MEDIAN									
MEAN	16200		3860	0.006	3	<0.1	24	1.4	<0.1
STD. DEVIATION	3768		288	0.002	0	#DIV/0!	2	0.7	#DIV/0!
std. dev. as % of m	23		7	36	14	#DIV/0!	9	48	#DIV/0!
100	26000	n/a	4800	0.021	6	<0.1	50	6.2	<0.1
onnes/ha	20000	n/a	4300	0.012	4	<0.1	24	1.1	<0.1
	21000	n/a	3800	0.009	4	<0.1	28	1.6	<0.1
	19000	n/a	4000	0.006	4	<0.1	29	1.8	<0.1
	16000	n/a	3600	<0.005	3	<0.1	23	1.5	<0.1
MEDIAN									
MEAN	20400		4100	0.012	4	<0.1	31	2.4	<0.1
STD. DEVIATION	3647		469	0.007	1	#DIV/0!	11	2.1	#DIV/0!
std. dev. as % of m	18		11	55	23	#DIV/0!	36	87	#DIV/0!

APPENDIX B

ANALYSIS OF VARIANCE REPORT FOR CADMIUM CONTENT IN WHEAT GROWN ON SOIL AMENDED WITH BIOSOLIDS AND FERTILIZER

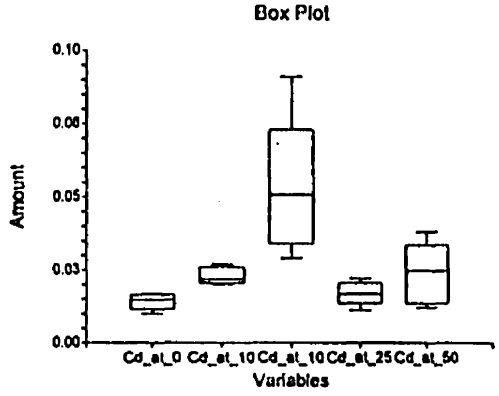
Analysis of Variance Report

Page/Date/Time 1 05-05-1999 15:07:00
Database A:\wheatfert.S0
Response Cd_at_0,Cd_at_10,Cd_at_100,Cd_at_25,Cd_at_50

Tests of Assumptions Section

Assumption	Test Value	Prob Level	Decision (0.05)
Skewness Normality of Residuals	2.7614	0.005756	Reject
Kurtosis Normality of Residuals	3.3140	0.000920	Reject
Omnibus Normality of Residuals	18.6078	0.000091	Reject
Modified-Levene Equal-Variance Test	2.8100	0.053181	Accept

Box Plot Section



Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A (...)	4	0.0048144	0.0012036	8.59	0.000332*	0.993506
S(A)	20	0.0028016	1.4008E-04			
Total (Adjusted)	24	0.007616				
Total	25					

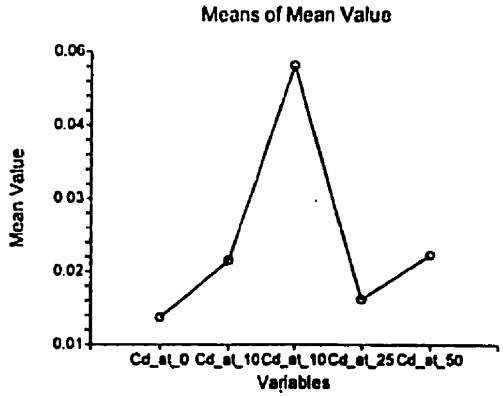
* Term significant at alpha = 0.05

Means and Effects Section

Term	Count	Mean	Standard Error	Effect
All	25	0.0262		0.0262
A:				
Cd_at_0	5	0.0142	5.293014E-03	-0.012
Cd_at_10	5	0.023	5.293014E-03	-0.0032
Cd_at_100	5	0.053	5.293014E-03	0.0268
Cd_at_25	5	0.017	5.293014E-03	-0.0092
Cd_at_50	5	0.0238	5.293014E-03	-0.0024

Page/Date/Time 2 05-05-1999 15:07:00
Database A:\wheatfert.S0
Response Cd_at_0,Cd_at_10,Cd_at_100,Cd_at_25,Cd_at_50

Plots of Means Section



Tukey-Kramer Multiple-Comparison Test

Response: Cd_at_0,Cd_at_10,Cd_at_100,Cd_at_25,Cd_at_50
Term A:

Alpha=0.050 Error Term=S(A) DF=20 MSE=1.4008E-04 Critical Value=4.231883

Group	Count	Mean	Different From Groups
Cd_at_0	5	0.0142	Cd_at_100
Cd_at_25	5	0.017	Cd_at_100
Cd_at_10	5	0.023	Cd_at_100
Cd_at_50	5	0.0238	Cd_at_100
Cd_at_100	5	0.053	Cd_at_0, Cd_at_25, Cd_at_10, Cd_at_50

APPENDIX C

ANALYSIS OF VARIANCE REPORT FOR CADMIUM CONTENT IN WHEAT GROWN ON SOIL AMENDED WITH BIOSOLIDS

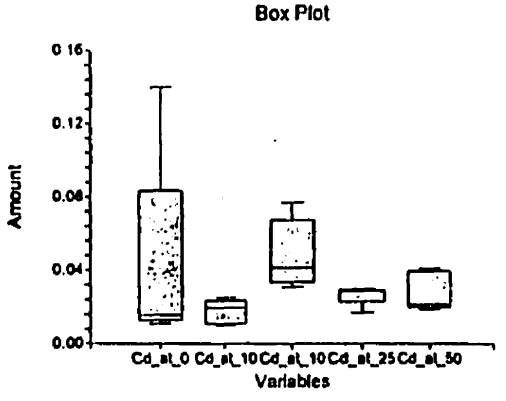
Analysis of Variance Report

Page/Date/Time 1 05-05-1999 15:09:27
Database A:\wheatunfer.S0
Response Cd_at_0,Cd_at_10,Cd_at_100,Cd_at_25,Cd_at_50

Tests of Assumptions Section

Assumption	Test Value	Prob Level	Decision (0.05)
Skewness Normality of Residuals	4.4222	0.000010	Reject
Kurtosis Normality of Residuals	4.0265	0.000057	Reject
Omnibus Normality of Residuals	35.7680	0.000000	Reject
Modified-Levene Equal-Variance Test	0.8197	0.527773	Accept

Box Plot Section



Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A (...)	4	3.09544E-03	7.7386E-04	1.08	0.392603	0.277831
S(A)	20	0.014326	0.0007163			
Total (Adjusted)	24	1.742144E-02				
Total	25					

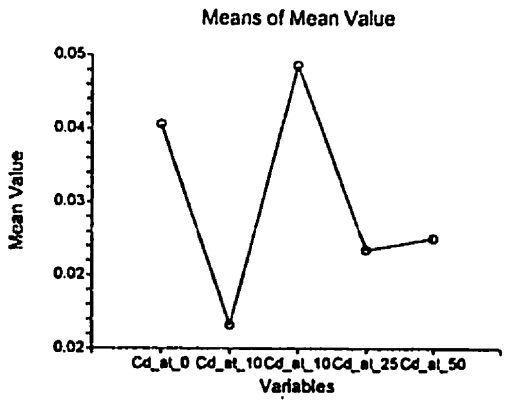
* Term significant at alpha = 0.05

Means and Effects Section

Term	Count	Mean	Standard Error	Effect
All	25	0.03268		0.03268
A:				
Cd_at_0	5	0.0418	1.196913E-02	0.00912
Cd_at_10	5	0.0178	1.196913E-02	-0.01488
Cd_at_100	5	0.0488	1.196913E-02	0.01612
Cd_at_25	5	0.0268	1.196913E-02	-0.00588
Cd_at_50	5	0.0282	1.196913E-02	-0.00448

Page/Date/Time 2 05-05-1999 15:09:28
Database A:\wheatunfer.S0
Response Cd_at_0,Cd_at_10,Cd_at_100,Cd_at_25,Cd_at_50

Plots of Means Section



Tukey-Kramer Multiple-Comparison Test

Response: Cd_at_0,Cd_at_10,Cd_at_100,Cd_at_25,Cd_at_50
Term A:

Alpha=0.050 Error Term=S(A) DF=20 MSE=0.0007163 Critical Value=4.231883

Group	Count	Mean	Different From Groups
Cd_at_10	5	0.0178	
Cd_at_25	5	0.0268	
Cd_at_50	5	0.0282	
Cd_at_0	5	0.0418	
Cd_at_100	5	0.0488	

APPENDIX D

ANALYSIS OF VARIANCE REPORT FOR CADMIUM CONTENT IN OATS GROWN ON SOIL AMENDED WITH BIOSOLIDS AND FERTILIZER

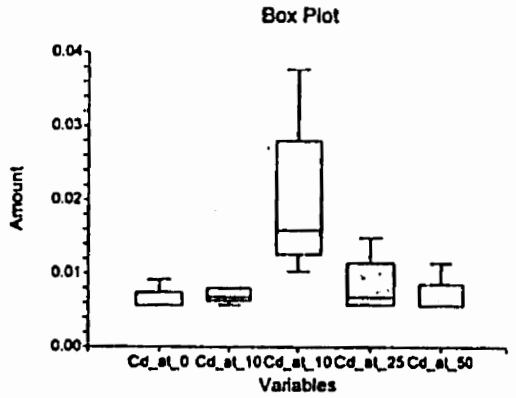
Analysis of Variance Report

Page/Date/Time 1 05-05-1999 14:49:37
Database A:\oatfert.S0
Response Cd_at_0,Cd_at_10,Cd_at_100,Cd_at_25,Cd_at_50

Tests of Assumptions Section

Assumption	Test Value	Prob Level	Decision (0.05)
Skewness Normality of Residuals	3.9418	0.000081	Reject
Kurtosis Normality of Residuals	3.7627	0.000168	Reject
Omnibus Normality of Residuals	29.6956	0.000000	Reject
Modified-Levene Equal-Variance Test	1.3455	0.287890	Accept

Box Plot Section



Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A (...)	4	0.0004692	0.0001173	5.58	0.003502*	0.937294
S(A)	20	0.0004208	2.104E-05			
Total (Adjusted)	24	0.00089				
Total	25					

* Term significant at alpha = 0.05

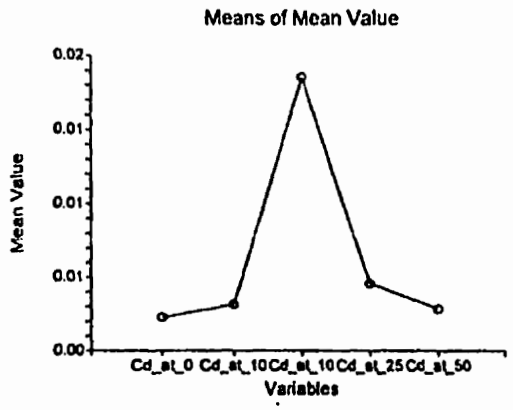
Means and Effects Section

Term	Count	Mean	Standard Error	Effect
All	25	0.0084		0.0084
A:				
Cd_at_0	5	0.0056	2.051341E-03	-0.0028
Cd_at_10	5	0.0062	2.051341E-03	-0.0022
Cd_at_100	5	0.017	2.051341E-03	0.0086
Cd_at_25	5	0.0072	2.051341E-03	-0.0012
Cd_at_50	5	0.006	2.051341E-03	-0.0024

Analysis of Variance Report

Page/Date/Time 2 05-05-1999 14:49:37
Database A:\oatfert.S0
Response Cd_at_0,Cd_at_10,Cd_at_100,Cd_at_25,Cd_at_50

Plots of Means Section



Tukey-Kramer Multiple-Comparison Test

Response: Cd_at_0,Cd_at_10,Cd_at_100,Cd_at_25,Cd_at_50
Term A:

Alpha=0.050 Error Term=S(A) DF=20 MSE=2.104E-05 Critical Value=4.231883

Group	Count	Mean	Different From Groups
Cd_at_0	5	0.0056	Cd_at_100
Cd_at_50	5	0.006	Cd_at_100
Cd_at_10	5	0.0062	Cd_at_100
Cd_at_25	5	0.0072	Cd_at_100
Cd_at_100	5	0.017	Cd_at_0, Cd_at_50, Cd_at_10, Cd_at_25

APPENDIX E

ANALYSIS OF VARIANCE REPORT FOR CADMIUM CONTENT IN OATS GROWN ON SOIL AMENDED WITH BIOSOLIDS

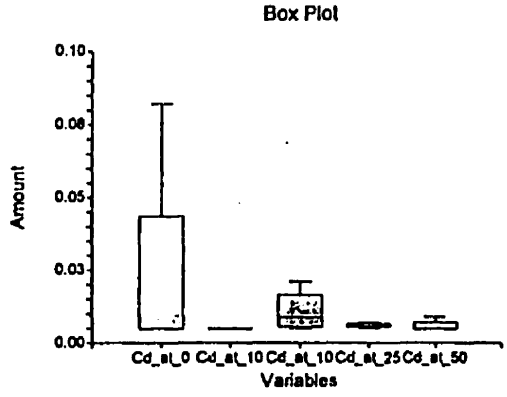
Analysis of Variance Report

Page/Date/Time 1 05-05-1999 15:03:53
Database A:\oatunfert1.S0
Response Cd_at_0,Cd_at_10,Cd_at_100,Cd_at_25,Cd_at_50

Tests of Assumptions Section

Assumption	Test Value	Prob Level	Decision (0.05)
Skewness Normality of Residuals	4.9575	0.000001	Reject
Kurtosis Normality of Residuals	4.4572	0.000008	Reject
Omnibus Normality of Residuals	44.4434	0.000000	Reject
Modified-Levene Equal-Variance Test	0.8738	0.496904	Accept

Box Plot Section



Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A (...)	4	8.3096E-04	2.0774E-04	0.84	0.513777	0.221494
S(A)	20	0.0049232	2.4616E-04			
Total (Adjusted)	24	5.75416E-03				
Total	25					

* Term significant at alpha = 0.05

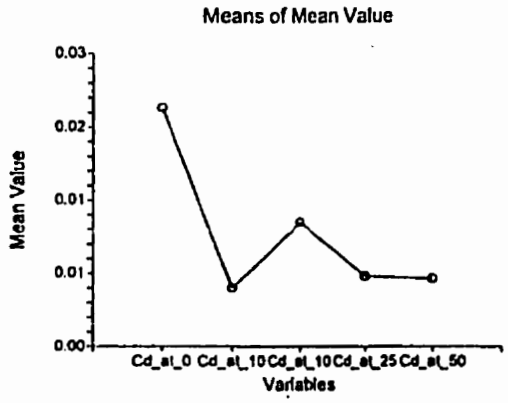
Means and Effects Section

Term	Count	Mean	Standard Error	Effect
All	25	0.00956		0.00956
A:				
Cd_at_0	5	0.0204	7.016552E-03	0.01084
Cd_at_10	5	0.005	7.016552E-03	-0.00456
Cd_at_100	5	0.0106	7.016552E-03	0.00104
Cd_at_25	5	0.006	7.016552E-03	-0.00356
Cd_at_50	5	0.0058	7.016552E-03	-0.00376

Analysis of Variance Report

Page/Date/Time 2 05-05-1999 15:03:53
Database A:\oatunfert1.S0
Response Cd_at_0,Cd_at_10,Cd_at_100,Cd_at_25,Cd_at_50

Plots of Means Section



Tukey-Kramer Multiple-Comparison Test

Response: Cd_at_0,Cd_at_10,Cd_at_100,Cd_at_25,Cd_at_50
Term A:

Alpha=0.050 Error Term=S(A) DF=20 MSE=2.4616E-04 Critical Value=4.231883

Group	Count	Mean	Different From Groups
Cd_at_10	5	0.005	
Cd_at_50	5	0.0058	
Cd_at_25	5	0.006	
Cd_at_100	5	0.0106	
Cd_at_0	5	0.0204	

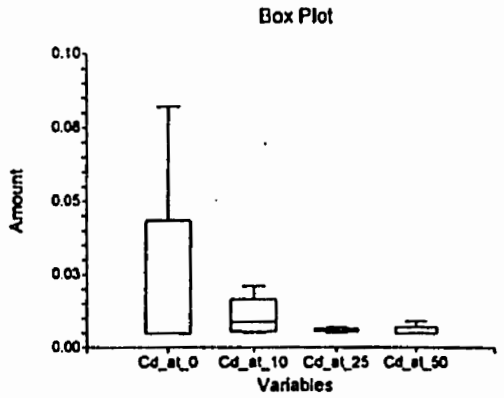
Analysis of Variance Report

Page/Date/Time 1 05-05-1999 15:01:59
Database A:\oatunfert1.S0
Response Cd_at_0,Cd_at_100,Cd_at_25,Cd_at_50

Tests of Assumptions Section

Assumption	Test Value	Prob Level	Decision (0.05)
Skewness Normality of Residuals	4.4392	0.000009	Reject
Kurtosis Normality of Residuals	4.0492	0.000051	Reject
Omnibus Normality of Residuals	36.1025	0.000000	Reject
Modified-Levene Equal-Variance Test	0.8106	0.506443	Accept

Box Plot Section



Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A (...)	3	0.000701	2.336667E-04	0.76	0.533119	0.176507
S(A)	16	0.0049232	0.0003077			
Total (Adjusted)	19	0.0056242				
Total	20					

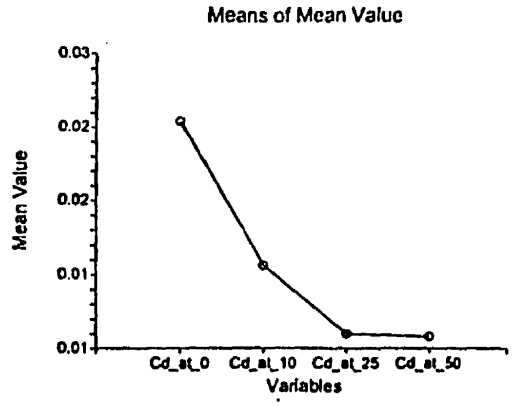
* Term significant at alpha = 0.05

Means and Effects Section

Term	Count	Mean	Standard Error	Effect
All	20	0.0107		0.0107
A:				
Cd_at_0	5	0.0204	7.844743E-03	0.0097
Cd_at_100	5	0.0106	7.844743E-03	-0.0001
Cd_at_25	5	0.006	7.844743E-03	-0.0047
Cd_at_50	5	0.0058	7.844743E-03	-0.0049

Page/Date/Time 2 05-05-1999 15:01:59
Database A:\oatunfert1.S0
Response Cd_at_0,Cd_at_100,Cd_at_25,Cd_at_50

Plots of Means Section



Tukey-Kramer Multiple-Comparison Test

Response: Cd_at_0,Cd_at_100,Cd_at_25,Cd_at_50
Term A:

Alpha=0.050 Error Term=S(A) DF=16 MSE=0.0003077 Critical Value=4.046122

Group	Count	Mean	Different From Groups
Cd_at_50	5	0.0058	
Cd_at_25	5	0.006	
Cd_at_100	5	0.0106	
Cd_at_0	5	0.0204	

APPENDIX F

ANALYSIS OF VARIANCE REPORT FOR ZINC CONTENT IN WHEAT GROWN ON SOIL AMENDED WITH BIOSOLIDS AND FERTILIZER

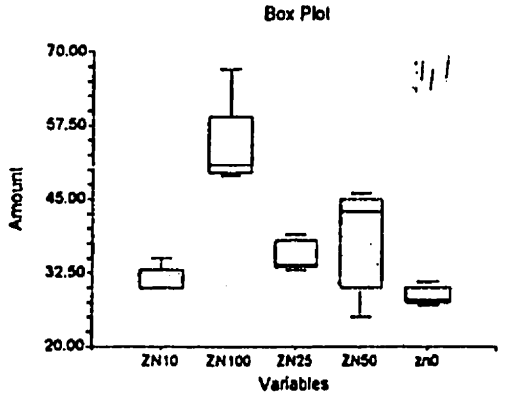
Analysis of Variance Report (WHEAT-FERTILIZED PLOT)

Page/Date/Time	1 05-25-1999 20:08:24
Database	WHEAT (FERTILIZED)
Response	ZN10,ZN100,ZN25,ZN50,zn0

Tests of Assumptions Section

Assumption	Test Value	Prob Level	Decision (0.05)
Skewness Normality of Residuals	0.3501	0.726284	Accept
Kurtosis Normality of Residuals	2.3545	0.018545	Reject
Omnibus Normality of Residuals	5.6664	0.058823	Accept
Modified-Levene Equal-Variance Test	1.0009	0.430243	Accept

Box Plot Section



Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A (...)	4	1918.64	479.66	16.49	0.000004*	0.999994
S(A)	20	581.6	29.08			
Total (Adjusted)	24	2500.24				
Total	25					

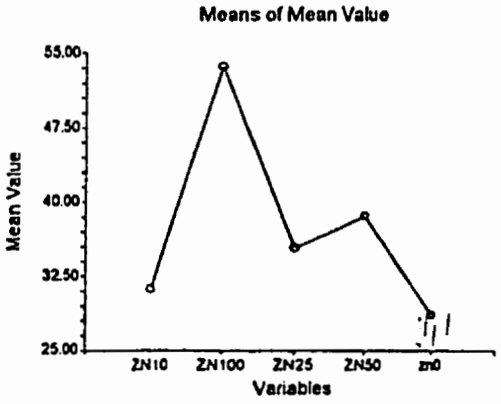
* Term significant at alpha = 0.05

Means and Effects Section

Term	Count	Mean	Standard Error	Effect
All	25	37.48		37.48
A:				
ZN10	5	31.2	2.411638	-6.28
ZN100	5	53.6	2.411638	16.12
ZN25	5	35.4	2.411638	-2.08
ZN50	5	38.6	2.411638	1.12
zn0	5	28.6	2.411638	-8.88

Page/Date/Time 2 05-25-1999 20:08:24
Database
Response ZN10,ZN100,ZN25,ZN50,zn0

Plots of Means Section



Tukey-Kramer Multiple-Comparison Test

Response: ZN10,ZN100,ZN25,ZN50,zn0
Term A:

Alpha=0.050 Error Term=S(A) DF=20 MSE=29.08 Critical Value=4.231883

Group	Count	Mean	Different From Groups
zn0	5	28.6	ZN100
ZN10	5	31.2	ZN100
ZN25	5	35.4	ZN100
ZN50	5	38.6	ZN100
ZN100	5	53.6	zn0, ZN10, ZN25, ZN50

APPENDIX G

ANALYSIS OF VARIANCE REPORT FOR ZINC CONTENT IN WHEAT GROWN ON SOIL AMENDED WITH BIOSOLIDS

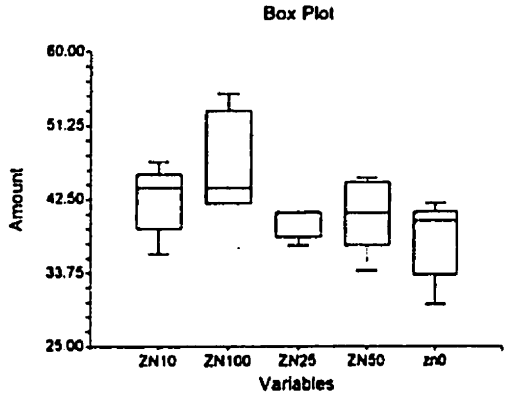
Analysis of Variance Report
ZINC CONTENT IN WHEAT (UNFERTILIZED PLOT)

Page/Date/Time 1 05-25-1999 20:18:28
Database WHEAT (UNFERTILIZED)
Response ZN10,ZN100,ZN25,ZN50,zn0

Tests of Assumptions Section

Assumption	Test Value	Prob Level	Decision (0.05)
Skewness Normality of Residuals	-0.6790	0.497138	Accept
Kurtosis Normality of Residuals	-0.1186	0.905601	Accept
Omnibus Normality of Residuals	0.4751	0.788556	Accept
Modified-Levene Equal-Variance Test	0.5561	0.696966	Accept

Box Plot Section



Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A (...)	4	231.76	57.94	3.03	0.041803*	0.695892
S(A)	20	382.4	19.12			
Total (Adjusted)	24	614.16				
Total	25					

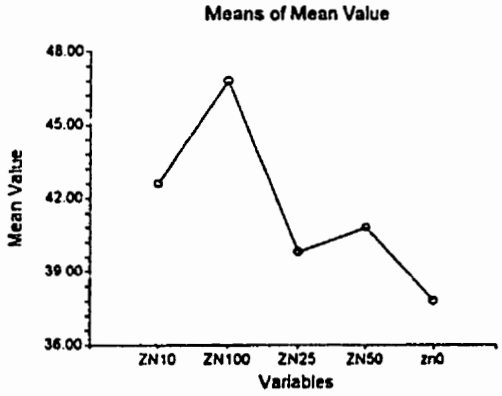
* Term significant at alpha = 0.05

Means and Effects Section

Term	Count	Mean	Standard Error	Effect
All	25	41.56		41.56
A:				
ZN10	5	42.6	1.955505	1.04
ZN100	5	46.8	1.955505	5.24
ZN25	5	39.8	1.955505	-1.76
ZN50	5	40.8	1.955505	-0.76
zn0	5	37.8	1.955505	-3.76

Page/Date/Time 2 05-25-1999 20:18:28
Database
Response ZN10,ZN100,ZN25,ZN50,zn0

Plots of Means Section



Tukey-Kramer Multiple-Comparison Test

Response: ZN10,ZN100,ZN25,ZN50,zn0
Term A:

Alpha=0.050 Error Term=S(A) DF=20 MSE=19.12 Critical Value=4.231883

Group	Count	Mean	Different From Groups
zn0	5	37.8	ZN100
ZN25	5	39.8	
ZN50	5	40.8	
ZN10	5	42.6	
ZN100	5	46.8	zn0

APPENDIX H

ANALYSIS OF VARIANCE REPORT FOR ZINC CONTENT IN OATS GROWN ON SOIL AMENDED WITH BIOSOLIDS AND FERTILIZER

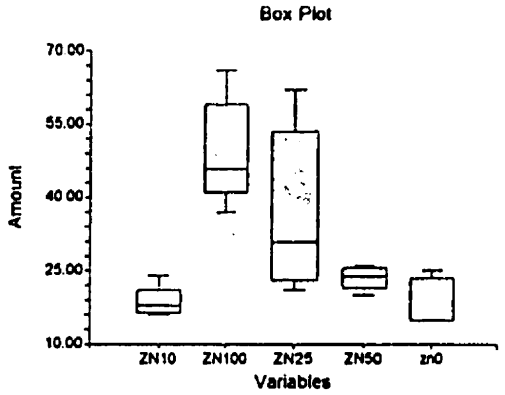
Analysis of Variance Report
ZINC CONTENT IN OATS (FERTILIZED PLOT)

Page/Date/Time 1 05-25-1999 20:23:16
Database ZINC CONTENT IN OATS (FERTILIZED PLOT)
Response ZN10,ZN100,ZN25,ZN50,zn0

Tests of Assumptions Section

Assumption	Test Value	Prob Level	Decision (0.05)
Skewness Normality of Residuals	2.1369	0.032605	Reject
Kurtosis Normality of Residuals	2.1386	0.032471	Reject
Omnibus Normality of Residuals	9.1399	0.010359	Reject
Modified-Levene Equal-Variance Test	2.1642	0.110236	Accept

Box Plot Section



Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A (...)	4	3590.24	897.56	10.30	0.000108*	0.998412
S(A)	20	1743.2	87.16			
Total (Adjusted)	24	5333.44				
Total	25					

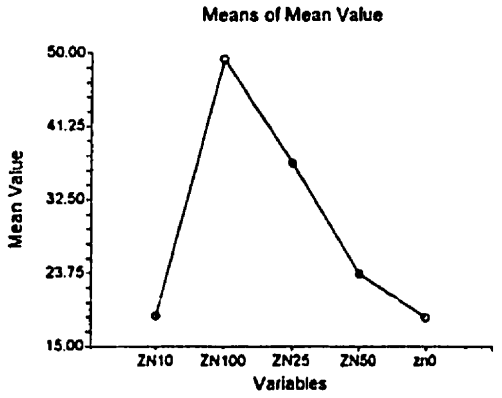
* Term significant at alpha = 0.05

Means and Effects Section

Term	Count	Mean	Standard Error	Effect
All	25	29.32		29.32
A:				
ZN10	5	18.6	4.175165	-10.72
ZN100	5	49.2	4.175165	19.88
ZN25	5	36.8	4.175165	7.48
ZN50	5	23.6	4.175165	-5.72
zn0	5	18.4	4.175165	-10.92

Page/Date/Time 2 05-25-1999 20:23:16
Database ZINC CONTENT IN OATS (FERTILIZED PLOT)
Response ZN10,ZN100,ZN25,ZN50,zn0

Plots of Means Section



Tukey-Kramer Multiple-Comparison Test

Response: ZN10,ZN100,ZN25,ZN50,zn0
Term A:

Alpha=0.050 Error Term=S(A) DF=20 MSE=87.16 Critical Value=4.231883

Group	Count	Mean	Different From Groups
zn0	5	18.4	ZN25, ZN100
ZN10	5	18.6	ZN25, ZN100
ZN50	5	23.6	ZN100
ZN25	5	36.8	zn0, ZN10
ZN100	5	49.2	zn0, ZN10, ZN50

APPENDIX I

ANALYSIS OF VARIANCE REPORT FOR ZINC CONTENT IN OATS GROWN ON SOIL AMENDED WITH BIOSOLIDS

Analysis of Variance Report
ZINC CONTENT IN OATS (UNFERTILIZED PLOT)

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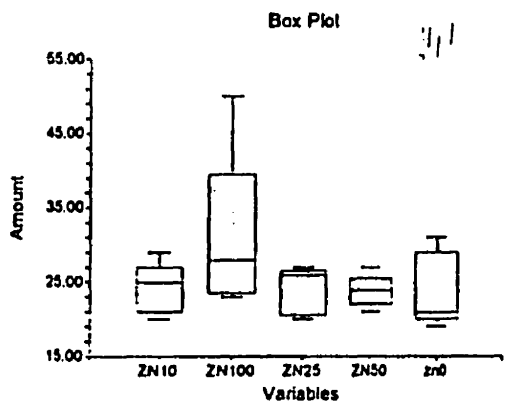
Database	ZINC CONTENT IN OATS (UNFERTILIZED PLOT)
Response	ZN10,ZN100,ZN25,ZN50,zn0

Tests of Assumptions Section

Assumption	Test Value	Prob Level	Decision (0.05)
Skewness Normality of Residuals	3.4498	0.000561	Reject
Kurtosis Normality of Residuals	3.1916	0.001415	Reject
Omnibus Normality of Residuals	22.0870	0.000016	Reject
Modified-Levene Equal-Variance Test	0.8104	0.533189	Accept

Box Plot Section



Analysis of Variance Table

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A (...)	4	188.24	47.06	1.35	0.285087	0.344054
S(A)	20	695.2	34.76			
Total (Adjusted)	24	883.44				
Total	25					

* Term significant at alpha = 0.05

Means and Effects Section

Term	Count	Mean	Standard Error	Effect
All	25	25.32		25.32
A:				
ZN10	5	24.2	2.636665	-1.12
ZN100	5	30.8	2.636665	5.48
ZN25	5	24	2.636665	-1.32
ZN50	5	23.8	2.636665	-1.52
zn0	5	23.8	2.636665	-1.52

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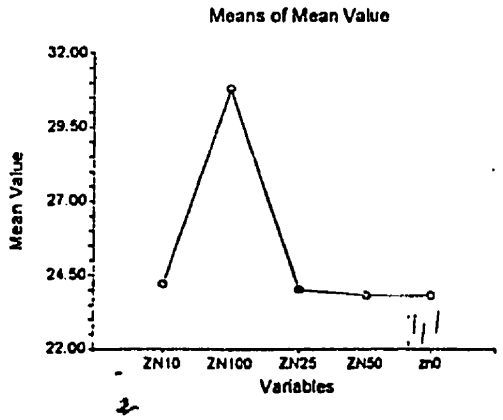
Database ZINC CONTENT IN OATS (UNFERTILIZED PLOT)
Response ZN10,ZN100,ZN25,ZN50,zn0

Plots of Means Section



Tukey-Kramer Multiple-Comparison Test

Response: ZN10,ZN100,ZN25,ZN50,zn0
Term A:

Alpha=0.050 Error Term=S(A) DF=20 MSE=34.76 Critical Value=4.231883

Group	Count	Mean	Different From Groups
zn0	5	23.8	
ZN50	5	23.8	
ZN25	5	24	
ZN10	5	24.2	
ZN100	5	30.8	