Bioavailability and Toxicity of Lead Shot to Small Mammals and Soil Invertebrates from Canadian Prairie Shooting Ranges

A Thesis Submitted to the College of Graduate Studies and Research
In Partial Fulfillment of the Requirements for the
Degree of Master of Science
in the
Toxicology Graduate Program
University of Saskatchewan
Saskatoon, SK, Canada

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PREFACE

This thesis has been organized as a series of two manuscripts (Chapters 3 and 4) which will be submitted for publication in scientific journals. Some repetition of introductory and methodological material is unavoidable.

ABSTRACT

Environmental contamination by lead (Pb) from shot pellets is a common cause of poisoning in waterfowl, raptors, passerines, and other wildlife. In Canada and the United States, Pb shot has been banned for waterfowl hunting, but its continued use on shooting ranges contributes tonnes of Pb shot each year into the environment. This study assessed the uptake and toxicity of Pb shot in soil invertebrates, and native small mammals from three Canadian prairie trap and skeet shooting ranges.

Information about recreational shooting in the Canadian prairie provinces was obtained by distributing a questionnaire during the fall of 2000 to all identified gun clubs in Alberta, Saskatchewan and Manitoba. The survey return rate was 22%. Three trap and skeet shooting ranges located in Eastend Saskatchewan, Provost Alberta, and Vegreville Alberta were selected as study sites, based on results of the questionnaire. Field research was conducted at the chosen study sites during the summer of 2001.

The sublethal effects of Pb on *Eisenia fetida* were evaluated in the laboratory using freshly spiked soil and soil collected from the trap and skeet shooting ranges in the fall of 2002. Earthworm lysosomal neutral red retention time (NRRT) was reduced in soil spiked with Pb acetate in a concentration-dependent manner ($p < 0.001$), and was negatively correlated with earthworm body burdens ($r = -0.80$, $p<0.001$). After exposure to soil from the three trap and skeet shooting ranges, earthworm growth and fecundity measurements did not differ significantly between any of the skeet ranges and their reference sites. However, NRRT was significantly reduced in all three ranges compared with their reference sites ($p < 0.05$). Lysosomal NRRT was negatively correlated with

$Ca(NO_3)_2$-extractable Pb ($r = -0.80, p < 0.001$) and soil total Pb ($r = -0.73, p < 0.001$), and with earthworm Pb tissue levels ($r = -0.67, p < 0.002$).

Lead shot density was high in surface soil at all three sites, however soil total Pb (after removal of Pb pellets) and $Ca(NO_3)_2$-extractable Pb levels were remarkably low and ultimately determined the results for all biological endpoints measured. Small mammal (ground squirrels and deer mice) tissue Pb concentrations measured at all shooting ranges and reference sites fell within the range reflective of background exposure for both species and all tissue types (blood, liver, kidney, femur) measured. Blood Pb concentrations fell below the threshold associated with inhibition of the enzyme δ-aminolevulinic acid dehydratase (ALAD), and no correlation between blood Pb and ALAD activity was found.

Acknowledgements

First and foremost, I would like to thank my supervisor Dr. Mark Wickstrom for his guidance and encouragement throughout this project. He was always patient and available to address my many questions, thank you! To my advisory Committee, Dr. Barry Blakley and Dr. Trent Bollinger for their valuable contributions to this thesis, and Dr. Marc Cattet for graciously donating time to serve as an external examiner.

Sincere appreciation is extended to my friends and colleagues at the University of Saskatchewan, especially Tara George, Adriana Brown, and Vince Rogers.

Deep thanks to my parents for their years of care and love. Ja was kocham!

This research was funded by the Toxic Substances Research Initiative.

DEDICATION

This thesis is dedicated to my Mother, Irena Krzeszowiec and Father, Andrzej Palasz.

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ALA	aminolevulinic acid
δ-ALAD	delta aminolevulinic acid dehydratase
dw	dry weight
EC_{50}	median effects concentration
EDTA	ethylenediaminetetraacetic acid
NRRA	neutral red retention assay
NRRT	neutral red retention time
NTA	nitriloacetic acid
OC	organic carbon
OM	organic matter
Pb	lead
SOM	soil organic matter
ww	wet weight

Chapter 1. Introduction

1.1 Background to the Study

Lead (Pb) shot contamination of wetlands has resulted in the death of millions of waterfowl and other birds from Pb poisoning in North America. Although now banned for waterfowl hunting in the US and Canada, Pb shot continues to be used for recreational shooting and upland game bird and small mammal hunting in both countries. Relatively little is known about the bioavailability and ecological effects of Pb from Pb shot deposited in terrestrial habitats, especially dry grassland ecosystems. This study examined the uptake and biological effects of Pb from trap and skeet ranges in terrestrial food chains on the Canadian prairies.

1.2 Study Objectives

This study was undertaken to:

- Compile a list of active and historic shooting ranges on the Canadian prairies
- Measure Pb concentrations in soil and biota on several trap and skeet ranges
- Assess toxicity of soil from these ranges to earthworms, as representative soil fauna
- Assess Pb uptake and toxicity to native small mammals from these shooting ranges

1.3 Literature Review

1.3.1 Lead and Wildlife Lead Exposure

Lead is a naturally occuring toxic trace element that can be found in tissues of all living things and environmental media (Scheuhammer and Norris 1995; Goyer 1996; Pain 1996). It has been mined and smelted for centuries, but demand for this metal increased greatly following the Industrial Revolution with large numbers of applications. As a result of its malleability, density, low melting point, and ease of processing, Pb has been used as the preferred metal for munitions, including Pb shotshell ammunition for hundreds of years (Scheuhammer and Norris 1995; Pain 1996). As recently as 10 years ago, it was estimated that waterfowl, upland game bird and small mammal hunters deposited 1,500 tonnes of Pb as Pb-shot pellets into the Canadian environment every year (Scheuhammer and Norris 1996). However, heightened awareness of Pb's harmful environmental and health effects has resulted in recent bans or severe restrictions in its use in products such as gasoline, crystal ware, pencils, paints and pigments, and plumbing systems. Use of Pb shot for waterfowl hunting was banned in the United States in 1986 and in Canada in 1999. However, Pb shot is still commonly used for upland game bird and small mammal hunting, as well as target shooting. Of the total global anthropogenic contribution (4-6 million tonnes annually) of Pb to the environment, the production of Pb-shot and fishing sinkers accounts for <1% (Scheuhammer 1996), but in some habitats these sources of Pb still represent a significant risk to wildlife health.

Lead poisoning from Pb-shot is most commonly seen in wild birds, due in part to the pecularities of the avian digestive system, and consequent feeding habits. For example, foraging waterfowl may purposefully ingest Pb-shot as grit to aid in the

digestion process in the gizzard, or inadvertently when feeding on the bottom of contaminated ponds or marshes (Trost 1981). Since the first report of waterfowl Pb poisoning in 1894 (Grinell 1894; Hough 1894), hundreds of incidents of a similar nature have been documented throughout the world, including Australia (Kingsford *et al.* 1989), Britain (Mudge 1983), France (Pain 1990), the United States (Bellrose 1959), Japan (Honda 1990), and Canada (Scheuhammer and Dickson 1996). According to the United States Fish and Wildlife Service an estimated 1-3 million ducks and geese died annually as a result of Pb shot ingestion prior to its ban in 1986 (USFWS 1986). Birds of prey have also been shown to suffer from Pb poisoning as a result of consumption of prey animals that have ingested lead shot, or have had lead shot or bullet fragments directly embedded in their tissues (Pattee and Hennes 1983; Pain and Amiard-Triquet 1993; Kendall *et al.* 1996; Wayland and Bollinger 1999; Wayland *et al.* 1999).

The incidence of lead toxicosis among other terrestrial species has not been well documented, and has usually been associated with well defined and highly contaminated areas, such as those surrounding metal smelters (Beyer *et al.* 1985) and target shooting ranges (Ma 1989; Stansley and Roscoe 1996; Lewis *et al.* 2001). Terrestrial vertebrates are generally exposed to Pb through inhalation of aerosols and/or soil ingestion (Pain 1996). According to Beyer *et al.* (1994), intentional and inadvertent soil ingestion is a significant route of exposure to environmental contaminants, including Pb, with some species consuming up to 8% of their total diet as soil (dry matter basis). When silage harvested from a field previously used for clay target shooting, was fed to a group of 14 cattle one animal died, a second showed clinical signs of Pb poisoning, and all exhibited depressed δ-aminolevulinic acid dehydratase activity indicative of exposure (Rice *et*

*al.*1987). Braun *et al.* (1997) reported acute lead poisoning in five calves pastured in the target area of a shooting range. One calf was euthenized and the remainder died within several hours of the first occurrence of symptoms.

1.3.2 Levels in the Environment

Soil

Lead is a ubiquitous trace constituent in rocks, soil, water, plants, animals, and air (Eisler 2000). It is the most common toxic metal on a global scale (Goyer *et al.* 1995). The natural Pb content in soil is derived from weathering of Pb-containing minerals, especially galena (PbS). Background concentrations typically range from <10-30 μg/g soil, but they may vary greatly, depending on proximity to Pb-containing ore bodies, as well as deposition and accumulation from anthropogenic sources (ATSDR 1998).

Tables 1.1 and 1.2 list representative Pb concentrations in soil and plants respectively, from selected global sites.

Table 1.1 Lead concentrations in selected soils

Soil Type [a]	Location	Median Soil Pb (mg/kg)
World estimated average [a]		17
Agricultural Soil Ap Horizon [a]	Canada	14
Agricultural Soil –Top (0-25cm) [a]	Finland	7
Agricultural Soil – Bottom (50-75cm) [a]	Finland	<5
Topsoil (0-15cm) [a]	England & Wales	40
Soil near lead smelter [b]	Missouri USA	60,000
Soil [c]	Inner City Minneapolis/St. Paul USA	423
Soil [c]	Rural Minnesota	7

[a] Reimann and Caritat (1998).
[b] Palmer and Kucera (1980).
[c] ATSDR 1998.

Table 1.2 Lead concentrations in selected plants [a]

Plant Type	Location	Median Plant Pb (mg/kg)
Moss	Norway	9
Moss	Germany	8
Lichen	Northwest Territories/Canada	4[b]
Dandelion	Europe	2[c]
Spruce bark	Canada	349

[a] Modified from Reimann and Caritat (1998)
[b] Mean
[c] Geometric Mean

Table 1.3 lists Canadian soil quality guidelines for Pb for the protection of environmental and human health.

Table 1.3 Canadian soil quality guidelines for lead [a]

Land Use	Concentration (mg/kg)
Agricultural	70
Residential/Parkland	140
Commercial	260
Industrial	600

[a] Modified from CCME (1999).

In contrast to hunting activity, which typically results in Pb deposition over a large area in relatively low concentration (except, of course, in the case of certain wetlands), target-shooting deposits Pb in relatively small, potentially highly-concentrated areas. In Canada prior to the ban on use of Pb shot for waterfowl hunting, an estimated

1500 tons of lead shot were deposited annually into the environment (Scheuhammer and Norris 1996). No data exist to establish the amount of Pb deposited annually at Canadian shooting ranges. However, information from studies from other countries suggests remarkably high Pb loads at some trap and skeet shooting ranges. Tanskanen *et al.* (1991) reported the annual metal accumulation at one range in Finland to be 15 tons, with soil concentrations in some cases exceeding 10,000 mg/kg. Jorgensen and Willems (1987) reported annual Pb-loads deposited at three Danish shooting ranges to vary from 240 kg to 5000-6000 kg. The same authors noted that annual Pb deposition to the Danish environment from ammunition was three times greater than that resulting from Pb fuel additives. In Sweden, an estimated 500-600 tons of Pb are deposited annually from shotgun ammunition nationwide (Comet 1992). At one particular Swedish shooting range, Lin (1996) estimated the annual deposition of Pb at 2800 kg. Pellet distribution in The Netherlands from hunting and target shooting accounted for 400 tons of Pb released annually compared to 800 tons from emissions from motor vehicles (Ma 1989). According to Mukherjee (1993), an estimated 563 tons of Pb ammunition was discharged into the environment in Finland in 1990. The same author (1992) estimated that 1600 tons of Pb ammunition are spread throughout Finnish forests, fields, target shooting ranges and lake bottoms. Braun *et al.* (1997) reported soil Pb concentrations at a former military shooting facility in the Swiss Alps of 3900 mg/kg. Similar studies have been conducted at rifle/pistol shooting ranges. At a shooting facility in northern England, Mellor and McCartney (1994) found lead concentrations in soil ranging from 5000-10,620 mg/kg. In a comparable study, Rooney *et al.* (1999) reported soil Pb concentrations ranging between 4000 and 8300 mg/kg at a New Zealand target shooting

range. At least 30% of the shotfall area sampled exceeded the New Zealand and Australian guidelines of 300 mg Pb kg^{-1}. In the United States, Murray and Bazzi (1995) documented ≥1000 mg/kg of Pb at 6 of 8 shooting ranges in Michigan, Illinois and Connecticut. Murray *et al.* (1997) found soil Pb concentrations at Michigan shooting range 10-100 times greater than the background concentration of 23 mg/kg. Stansley and Roscoe (1996) reported total Pb concentrations of 75,000 mg/kg at a range in New Jersey, which was 10,000 times higher than background values. Another study in Maryland found shooting range soil Pb concentrations of 110 to 27,000 mg/kg (Vyas et al 2000).

Chen *et al.* (2002) investigated Pb deposition in soil at a Florida rifle and pistol range. The density of Pb bullets in the berm was 180 g/kg soil. Total soil lead concentration (after bullets were removed) varied at different points in the berm, and ranged from 1201 mg/kg to 17850 mg/kg. Astrup *et al.* (1999) conducted a comparable study of the mobility and distribution of Pb in a soil embankment used as bullet stop at a 31-year-old shooting range in Denmark. They recovered an estimated 60-70 kg of Pb bullets from a 0.2 m transect taken directly behind the target of a firing line. Total soil Pb concentrations along the same transect were reported to be as high as 96 g/kg.

1.3.3 Geochemical Processes Affecting Lead Mobility/Behaviour in Soils

Geochemical interactions are important in dictating the mobility and solubility of Pb in the environment. The fate of Pb-shot in soil, and the bioavailability of Pb in terrestrial ecosystems is largely dependent on several geochemical processes, including oxidation, precipitation, adsorption, and complexation.

The oxidation state of Pb determines the chemical form which will exist in the soil, and greatly influences potential mobility. Metallic Pb (Pb°) is thermodynamically unstable and overtime (albeit a long time) will be transformed to more stable forms. In most natural soil environments, including shooting ranges, Pb° will be oxidized to more soluble species (e.g. $Pb^{\circ} \rightarrow Pb^{2+}$, or Pb^{4+}). The rate at which this process occurs is highly site specific and dependent on various chemical and physical parameters. Important parameters affecting Pb transformation in soil include redox potential, pH, ionic strength, concentration of reducing agents, presence of reactants (e.g. acids, bases, sulfate, carbonate), inhibitory reactants (e.g. phosphate), surface area to mass ratio, infiltration of water into soil, and depth of burial (SAAMI 1996). According to Jorgensen and Willems (1987), 5-17% of metallic Pb at shooting range sites was transformed into more soluble species within 6-13 years, with transformation rates increasing in cultivated versus natural soils. Furthermore, rate of transformation was markedly reduced when soil pH and/or organic matter content was high. The same authors estimated that total transformation would occur within 100-300 years. According to Lin et al. (1995), an average of 4.8% of metallic Pb from Pb shot in shooting range soil was transformed to lead carbonate and lead sulfate within 20-25 years.

Precipitation is the chemical process by which components in solution combine to form solids. Dissolution is the process where solids are separated into individual soluble components. In the case of metals, dissolved forms are more mobile and available for uptake than precipitates. Various Pb-containing minerals, including lead oxides, phosphates, sulfates, sulfides, hydroxides, and carbonates are likely to form under the conditions present at shooting ranges (Jorgensen and Willems 1987, Lin *et al.* 1995,

SAAMI 1996). Jorgensen and Willems (1987), reported the presence of hydrocerussite (lead hydroxyl carbonate), cerussite (lead carbonate) and, to a lesser degree, anglesite (lead sulfate) and hydroanglesite (lead hydroxyl sulfate) at shooting ranges. Lin *et al.* (1995), found the predominant transformation products to be hydrocerussite, cerussite, and anglesite. Lead precipitated as Pb carbonate or Pb sulphate is retained by the soil and not available for uptake. According to SAAMI (1996), a number of different precipitates are likely to form at shooting ranges, such that only a fraction of the total Pb present will be in a mobile form. Ma *et al.* (1995) reported that phosphates greatly reduce lead mobility as well as bioavailability on Pb metal contaminated sites.

Adsorption is the process by which ions bind to the surface of solid particles, such as soil or sediment. This binding reduces the total amount of dissolved material (e.g. Pb) in the substrate. Desorption is the opposite process (SAAMI 1996). Cation exchange capacity is a measure of the ability of a soil or sediment to adsorb cations. Weakly adsorbed cations are exchanged for stronger ones. One result of this is the tendency for the majority of Pb in soil to exist in the bound, rather than soluble state. Soils possess a variety of potential adsorption sites, especially associated with clays or organic matter, which will adsorb to Pb and lessen its bioavailability, and therefore toxicity. Various factors such as pH, total Pb concentration, concentration of competing ions, and competing ions (Mg^{2+}, Ca^{2+}) already adsorbed will affect Pb adsorption. Low levels of competing ions, many sorption sites and neutral to slightly alkaline pH's are considered best conditions for adsorption of Pb (SAAMI 1996).

Complexation/chelation is the process where metal ions combine with other dissolved components to form new dissolved species, and potentially increase the dissolved

concentration of metals. Various Pb complexing agents have been identified, including ethylenediaminetetraacetic acid (EDTA), nitriloacetic acid (NTA), and fulvic acid. However, at shooting ranges, fulvic acid is the only compound likely to be encountered. The significance of this process is that complexion of Pb reduces bioavailabilty to various biota, and therefore decreases toxicity. This process also limits mobility through soil, as reported by Turner and Lambert. (1985).

1.3.4 Soil Characteristics Affecting Lead Bioavailability

Bioavailability can be defined as the total dose, administered by any route, which makes it to systemic circulation in an unchanged form. The bioavailability and uptake of heavy metals such as Pb in soils is not dependent solely on the level of contamination, but is related to a number of physical and chemical soil variables (Ma 1982,1983; Mielke and Heneghan 1991; Boekhold and Van der Zee 1992). In Pb-contaminated areas such as target shooting ranges, soil characteristics such as soil texture, pH, organic matter content, redox potential, cation exchange capacity, presence of other elements, and vegetation and microbial activity all influence Pb behaviour (Gambrel 1994; Turpeinen *et al.* 2000). Other soil chemistry variables that affect soil-Pb availability are concentrations of acid volatile sulfides, carbonate, iron and manganese oxides and phosphates, chloride, and hydroxide (Ruby *et al.* 1999). Consequently, the mobility and bioavailability of lead in soils is highly site-specific, and the extent to which the various dissolution, precipitation, adsorption and complexation processes occur will vary for different soil types (Ruby *et al.* 1999; Turpeinen 2000). Although a variety of soil

parameters influence sorption capacity and Pb bioavailability, pH and organic matter content are most critical (Ma 1982; 1983; Yuan and Lavkulich 1997).

Generally, lower pH (higher acidity) is associated with increased soil Pb bioavailability (Mielke and Heneghan 1991). The converse is also true, as an increase in pH will usually decrease the solubility, bioavailability, and toxicity of Pb in soils (Turpeinen *et al.* 2000). Decreased pH results in an increased desorption of metal cations from soil binding sites due to competition with H^+ ions (Ma 1982).

Organic matter is an important constituent which influences many physical and chemical soil properties (Brady 1974). In general terms, soil organic matter is made up of plant and animal remains in various stages of decomposition, ranging from non-decomposed tissue to humus. The latter is a complex mixture of brown to dark brown amorphous and colloidal substances, which make up the majority of soil organic matter (Schnitzer 1978). Humic substances serve a variety of important soil functions, including soil stabilization, atmospheric carbon fixation, and nitrogen storage. However, for the purpose of this discussion, the effect of humic substances on metal toxicity is most important. Humic substances may buffer the potential toxic effects of metals through various chemical interactions, including ion exchange, adsorption, chelation and complexation. Humic materials react with metals by providing large external surfaces for adsorption, in addition to firmer retention in internal spaces (Schnitzer 1978). Humic substances also interact with metal ions, oxides, and hydroxides to form metal organic associations because they contain many oxygen-containing functional groups, which can complex and dissolve metals, and thus decrease their bioavailability (Schnitzer 1978).

Consequently, soils rich in organic matter have a greater capacity to reduce the toxic potential of metals than soils with less organic matter.

Quantification of soil organic matter (SOM) is usually done indirectly by measuring organic carbon content, and assuming that organic carbon in SOM ranges from 50-58%. Organic carbon values are subsequently converted to SOM using, a conversion factor ranging from 1.72-2.00. However, recent work suggests that these conversion factors are not appropriate, because SOM is variable and no one factor is appropriate for all soils. Consequently, current recommendations are that organic carbon be determined and reported directly (Baldock and Nelson 2000).

1.3.5 Toxicological Effects of Lead

There is no biological requirement for Pb, and its ubiquitous distribution and relatively high intrinsic toxicity has resulted in reports of a variety of adverse effects in humans and many species of animals for over 2500 years (Scheuhammer and Norris 1995). The response of an organism to Pb is dependent on the severity and duration of exposure. Consequently, Pb intoxication is a continuum ranging from subtle psychological changes to severe pathological alterations (Mautino 1997). It is a nonspecific metabolic poison, that when absorbed modifies the normal functioning of many organ systems, including the kidney, bone, the haemopoetic system, and the central and peripheral nervous systems (Eisler 1988). Mautino (1997) broadly classified the major biochemical effects of Pb into three categories. First, due to Pb's affinity for sulfhydryl groups, it may inhibit the normal functioning of biologically essential sulfhydryl-dependent enzymes. Second, Pb competes with calcium in metabolic

processes, thereby deleteriously affecting mitochondrial respiration and neurological function. Lastly, Pb may alter genetic information by affecting the synthesis of DNA and RNA. Ultimately, the degree of pathological changes in animals is related mainly to the concentration of the toxicant in three tissues: the kidney, nervous system and bone marrow (Bankowska and Hine 1985).

1.3.5.1. Haemapoetic System

The first toxicological effects following Pb exposure are seen in the blood and the haemapoetic system (Pain 1996). Lead induces anemia as a result of: 1) reduced erythrocyte life span, due to increased fragility of the cell membrane from effects on Na^+, K^+ -ATPase, or the inhibition of the enzyme pyrimidine-5-nucleotidase (P5N) in red cells; and 2) impairment of heme synthesis (Goyer 1996). This is discussed in more detail in section 1.3.8.2. Lead-induced anemia results in red blood cells that are microcytic and hypochromic, coupled with an increase in circulating reticulocytes and stippled erythrocytes (Goyer 1996).

1.3.5.2 Bone

Lead is absorbed from the gastrointestinal tract into the blood plasma, where it is transported to one of three distinct compartments: 1) a rapid exchange compartment that consists of blood and well-vascularized organs, accounting for 4% of the total Pb body burden; 2) a second compartment of intermediate exchange rate consisting of soft tissue organs, comprising 2% of the body burden; and 3) a slow exchange compartment which consists of bone tissue. The skeleton harbours 90-94% of the total body burden of Pb

(Tamalge and Walton 1991; Bankowska and Hine 1995; Mautino 1997). Lead is able to replace calcium in bone, and it is continuously mobilized from the skeleton (Jacobs 1996). Bone-Pb concentrations are not a good indicator of recent Pb exposures, but they are representative of previous exposure (Pain 1996), and because excretion of Pb is slow, the total Pb body burden increases with time.

1.3.5.3 Liver and Kidney

Since the liver and kidney receive a significant amount of total cardiac output and total blood flow, they have the potential to be highly exposed to circulating toxicants such as Pb. Acute nephrotoxicity from Pb exposure resulting in functional and morphological changes in proximal tubular cells has been reported by Goyer *et al.* (1970a). Proximal tubular damage associated with acute toxicity may result in proteinuria, glucosuria, and decreased urine specific gravity (Goyer *et al.* 1970b; Gerhardsson and Skerfving 1996). Chronic exposure may result in interstitial nephritis, tubular atrophy, azotemia and end-stage renal failure (Gerhardsson and Skerfving 1996). The formation of a protein-Pb complex, manifesting as inclusion bodies, is a typical alteration appearing in renal tubular cells as a result of Pb exposure (Goyer 1996, Mouw and Hartung 1975). Goyer *et al.* (1970b) reported that the majority of lead in the kidney is concentrated in intranuclear inclusion bodies in renal tubular cells, rather than in the cytoplasm or mitochondria, thereby decreasing potential toxicity to cytoplasmic organelles. It has also been reported that Pb-exposed small mammals have increased average relative kidney weight relative to controls (Goyer *et al.* 1970b; Bankowska and Hine 1985; Ma 1989). In addition, Pb has also been reported to be a renal carcinogen in

rodents (Goyer 1996). Osweiler *et al.* (1985) reported the potential for liver degeneration and necrosis, with reduced hepatic protein production, in some cases of Pb exposure in cattle.

1.3.5.4 Neurotoxic Effects

Acute and chronic Pb poisoning in mammals is associated with encephalopathy, and peripheral neuropathy, respectively (Hunter and Wobeser 1980). Lead has been reported to affect the timing of cell-to-cell connections and migration of neurons during development. Lead competes with calcium and blocks voltage-dependent membrane channels, resulting in a reduction in neurotransmitter release. Lead will also bind to calcium-dependent 2nd messenger receptors, such as protein kinase C, which functions in the regulation of cell division and proliferation, cell-to-cell communication, and organization of the cytoskeleton (Goyer 1996). Neurologic symptoms of acute lead intoxication reported in cattle include blindness, continuous clonic convulsive activity, hyperesthesia, bellowing, frothing at the mouth, severe depression, and involuntary movements (Osweiler *et al.* 1985).

Peripheral neuropathy has been reported in guinea pigs (Fullerton 1966) and rats (Lampbert and Schochet 1968) due to chronic lead intoxication. Hunter and Wobeser (1980) reported segmental demyelination in Pb-shot dosed ducks, and noted that the peripheral nervous system was more vulnerable to Pb than the central nervous system. Peripheral neuropathy in humans with excessive exposure to occupational lead is a common syndrome that manifests as foot drop or wrist drop (Goyer 1996). There may be pain in the extremities, as well as paralysis, and nerve conduction velocity may be

reduced (Jacobs 1996). This is the result of segmental demyelination and possibly axonal degeneration (Jacobs 1996; Goyer 1996).

1.3.6 Study Species

According to Beardsley *et al.* (1978), criteria for an effective biomonitor species include abundance, ease of capture, small home range, year round residence, widespread distribution and generalized food habits. Small mammal species most commonly used for monitoring studies of contaminants in terrestrial habitats belong to one of three families: Soricidae (Shrews), Cricetidae (mice, rats, voles), and Muridae (Old World mice and rats) (Talmage and Walton 1991). Members of these families are good models for assessing heavy metal contamination, mobility, and toxicity because of their close relationship with soil, small home ranges, high reproductive rate, early sexual maturity, and short gestation periods (Talmage and Walton 1991). Food is considered the major source of contaminant exposure to small mammals at most contaminated sites, although inadvertent soil ingestion is also a potentially significant route of exposure (Beyer *et. al* 1994). Consequently, feeding strategy (i.e. insectivores vs. herbivores, granivores, and omnivores) is an important criterion in selecting a biomonitor species, with insectivores being the most useful sentinels for many contaminants. For example, the high food intake rate of shrews coupled with the tendency for Pb to accumulate in soil invertebrates, their primary food items, results in generally higher Pb body-burdens in insectivorous shrews than in herbivorous voles living on the same sites. However, it is often difficult to trap sufficient numbers of Soricidae on study sites to meet statistical power requirements (Talmage and Walton 1991). Stansley and Roscoe (1996)

demonstrated these points when they captured 1 shortail shrew (*Blarina brevicauda*) on a trap and skeet range in New Jersey, and reported tissue Pb concentrations 35 to 1038 times greater than those measured in shrews from the reference site. White-footed mice (*Peromyscus leucopus*) trapped in the same area showed tissue elevations of 5 to 64 fold, relative to animals from the reference site. Ma (1989) also found that shrews accumulated more Pb in tissues than bank voles or mice, and concluded that the difference was, in part, attributed to feeding habits.

Richardson's ground squirrels (*Spermophilus richardsonii*), thirteen-lined ground squirrels (*Spermophilus tridecemlineatus*) and deer mice (*Peromyscus maniculatus*) were selected as model species for this study. The Richardson's ground squirrel is widely distributed throughout the Canadian prairies, while the thirteen-lined ground squirrel is most often found in the southern parts of this region. The former species consumes a diet consisting of crickets, grasshoppers, and caterpillars, as well as seeds, leaves and stems of many kinds of plants. The latter species eats mainly caterpillars, grasshoppers, grass and weed seeds, and occasionally flesh from shrews and mice. The deer mouse has a very extensive range in both Canada and the United States. In prairie habitats deer mice rely on seeds of foxtail grass and wheat, as well as corn and other seeds, and caterpillars and other insects (Knopf 1998).

1.3.7 Small Mammals and Shooting Ranges

Since trap and skeet ranges often cover many hectares, it is reasonable to assume that most ground-dwelling small mammals trapped well within range boundaries will feed mostly on the grounds of the range. A relatively small number of recent studies

have examined the biological effects of Pb shot on small mammals inhabiting shooting ranges (Ma 1989; Stansley and Roscoe 1996; Lewis *et. al* 2001). Ma (1989) used small mammals to assess the bioavailability and toxicity of Pb shot ammunition on a shooting range with relatively acidic soil. Highly elevated tissue Pb concentrations were reported in wood mice (*Apodemus sylvaticus),* bank voles (*Clethrionomys glareolus*) and shrews (*Sorex araneus*). All of the shrews and 24% of the bank voles exhibited renal Pb concentrations > 25 μg/g dry weight, a threshold value diagnostic of Pb intoxication (Osweiler *et al.* 1985). All of the shrews also exceeded a higher critical renal-Pb value of 42μg/g (dry weight), with two specimens exceeding 1000μg/g. Shrews and bank voles also showed a significant increase in kidney-to-body weight ratios relative to controls. The author concluded that contamination of herbivorous and carnivorous small mammals indicated that Pb could enter terrestrial food chains via plant roots and soil fauna. Furthermore, it was concluded that metallic Pb from Pb shot was transformed into a more mobile, bioavailable species, probably Pb^{+2}, in the acidic soil of this shooting range. In contrast, Manninen and Tanskanen (1993), reported little plant-Pb uptake at a shooting range with soil pH of 5.6-5.9.

Stansley and Roscoe (1996) assessed the bioavailability and effects of lead in wildlife at a trap and skeet range that had been in continuous operation for at least 30 years. The average total soil Pb concentration in the shot fall zone was 75,000 mg/kg (dw). Mean tissue Pb concentrations observed in white-footed mice (*Peromyscus leucopus*) trapped in the shot-fall zone included 4.98, 34.9 and 245 μg/g (dw) for liver, kidney and femur, respectively, and were 5 to 64 times greater than concentrations in

mice from a reference site ($P<0.01$). A significant reduction in blood ALAD activity was also reported ($P=0.0384$).

1.3.8 Biomarkers of Lead Exposure and Effect

1.3.8.1 Lead Accumulation in Tissue

A biomarker is defined as a xenobiotically induced alteration in cellular or biochemical components or processes, structures, or functions that is measurable in a biological system or sample (Scott-Fordsmand and Weeks 2000). Biomarkers for Pb exposure have traditionally included the measurement of total Pb in biological samples such as fluids or tissues (ATSDR 1998). The best measure of recent Pb exposure is blood Pb concentration (Ma 1996). Under conditions of prolonged and repeated exposure, blood Pb concentrations reach a steady state in which Pb is bound to haemoglobin within erythrocytes (Ma 1996). According to Ma (1996), the half-life of Pb in blood is 2-4 weeks. Baars *et al.* (1990) as cited by Ma (1996) reported an average half-life in cattle of 10.5 days.

Blood samples for Pb analysis are easily obtained from live specimens, but because Pb cycles between blood and bone, a single sample does not accurately distinguish between low-level chronic or intermediate exposure and high-level acute exposure (ATSDR 1998). Furthermore, the relationship between Pb exposure and blood concentration is non-linear, such that the incremental increase in blood Pb concentration is less under conditions of high exposure than at low exposure (ATSDR 1998). Nevertheless, measurement of blood Pb concentration is a valuable tool to relate various biological effects to exposure (ATSDR 1998).

Other Pb exposure biomarkers used along with blood-Pb concentration include the measurement of Pb in bone, liver, kidney, and brain. The distribution of Pb in mammalian tissues generally reflects the following order: bone>kidneys>liver>brain>muscle (Ma 1996). In soft tissues, the highest Pb concentrations occur in the kidneys and liver (Ma 1996, Puls 1994), making them common diagnostic samples for analysis in cases of suspect poisoning.

1.3.8.2 Heme Synthesis Impairment

The heme biosynthesis pathway is a multi enzyme process which converts simple precursors to the complex tetrapyrrole protoporphyrin IX, which is then complexed with ferrous iron to form heme; an essential molecule for the function of all aerobic cells (Phillips and Kushner 1999). Lead interferes with several hematopoetic enzymes, resulting in increased excretion or elevation of intermediate compounds, and a decrease in heme synthesis. Lead's main adverse effects on hematopoiesis manifest through the suppression of several enzymes, including the cytoplasmic enzyme delta (δ)-aminolevulinic acid dehydratase (ALAD), and the mitochondrial enzymes ferrolochelatase and coprogenase. However, the inhibition of ALAD by Pb is considered the most sensitive biological indicator, and a standard bioassay of low-level Pb exposure as well as Pb poisoning in humans and animals.

Delta aminolevulinic acid dehydratase is a catalytic enzyme responsible for the condensation of two molecules of δ-aminolevulinic acid (ALA) to form porphobilinogen (Scheuhammer 1987a, 1987b; Fujita 1999). Normal ALAD activity is necessary to maintain hemoglobin content in erythrocytes, and to maintain sufficient amounts of heme

for incorporation into mitochondrial cytochromes (Dieter and Finley 1978). The inhibition of ALAD results in a decrease in heme production, interference in energy production through the electron transport chain, and a decrease in oxygen carrying capacity as well as erythrocyte malformation (Dieter and Finley 1978; Phillips and Kushner 1999). Lead-induced ALAD inhibition also results in an increase in ALA precursors in plasma and urine, which may be toxic to nerve cells (Phillips and Kushner 1999).

Delta aminolevulinic acid dehydratase is a sulfhydryl and zinc dependent enzyme expressed abundantly in liver and erythroid tissues, as well as spleen, kidney and brain. The activity of ALAD is reduced when sulfhydryl groups are inactivated through oxidation, Pb binding, or zinc displacement by Pb (ATSDR 1998; Phillips and Kushner 1999; Fujita 1999). Inhibition of ALAD can be measured by: 1) quantifying the amount of enzymatically synthesized porphobilinogen in a lysed blood sample (Fujita 1999); 2) directly measuring enzyme activity; or 3) comparing enzyme activity in the inhibited sample to a sample in which activity has been restored through the addition of Zn^{+2}, and/or –SH-reducing compounds such as dithiothreitol or reduced glutathione, or heat. The most accurate and reliable method depends on the measured difference in sample enzyme activity before and after restoration of full enzyme function. This approach eliminates potential sources of error as it takes into account, age, sex, dietary differences, and other confounding variables (Dieter and Finley 1979; Scheuhammer 1987b).

Lead-induced δ-ALAD inhibition has been reported in various species of freshwater and marine fishes, as well as birds and mammals (Eisler 2000). Mautino and Bell (1987) dosed mallard ducks (*Anas platyrhynchos*) with 2, # 4 lead shot pellets, and

concluded that a maximal 80% inhibition of blood δ-ALAD activity occurred at 1 week post dosing. Enzyme activity returned to normal by the seventh week post dosing. Other waterfowl oral dosing studies have shown that one shotgun pellet may cause 60-90% inhibition of δ-ALAD activity in erythrocytes within 24 hours of ingestion. This inhibition may last up to 3 months (Finley et al. 1976a; Dieter and Finley 1978). Dieter and Finley (1979) concluded that blood, brain and liver Pb levels, and the degree of δ-ALAD inhibition in brain and liver, were represented by blood δ-ALAD inhibition with reasonable accuracy. Mouw and Hartung (1975) assessed differences in Pb accumulation and toxicity in wild rats from urban and rural habitats, and demonstrated an inverse correlation between δ-ALAD activity and Pb concentrations in blood and kidney ($r = -0.60, p < 0.01$). The authors concluded at 50% inhibition of blood ALAD activity corresponded to about 0.3 ppm blood Pb and 5 ppm kidney Pb. Stansley and Roscoe (1996) reported a significant depression in blood δ-ALAD activity ($P < 0.01$) in white-footed mice (*Peromyscus leucopus*) from a trap and skeet range. They concluded that blood δ-ALAD activity was an indication of recent Pb exposure, but was not, in itself, an indication of toxic exposure, since δ-ALAD is not the rate-limiting enzyme in heme synthesis. Scheuhammer (1987c) came to the same conclusion, stating that δ-ALAD activity was a good indicator of recent Pb exposure, but could not be used for estimating Pb body burdens. Porru and Alessio (1996) reported that blood Pb concentrations ranging from 0.1-0.4 μg/dL were proportional to ALAD activity in humans. Erythrocyte ALAD activity in blood samples began to decrease rapidly when blood Pb concentrations reached 0.1 μg/ml, with almost complete inhibition being observed at 0.7-0.8 μg Pb/ml.

1.3.8.3 Interpreting Lead Concentrations in Mammalian Tissues

Improvements in analytical techniques have resulted in a steady decrease in detection limits for Pb in biological samples (ATSDR 1998). However, the use of tissue Pb concentrations to diagnose Pb toxicosis (sublethal or lethal) can be difficult, since toxic or lethal effects of Pb vary with species, sex, age, and diet (Eisler 1988).

Normal baseline blood Pb values have been reported to be 0.04 μg/ml in laboratory rats (Barret and Livesey 1985), 0.08 μg/ml in wood mice, and 0.04 μg/ml in bank voles living in uncontaminated areas (Ma 1996). Background levels of blood Pb may be 0.02-0.06 μg/ml higher in immature animals than in adults (Ma 1996). Neuman and Doollhopf (1992) reported background blood Pb levels in mature and immature cattle to be 0.05 and 0.07 μg/ml, respectively. The same study found that mature and immature cattle grazing in the vicinity of a lead smelter had blood Pb concentrations of 0.21 and 0.78 μg/ml, respectively.

Table 1.4 lists published blood Pb concentrations and associated health effects in humans and various domestic animals.

Table 1.4 Effect of blood lead concentrations in humans and domestic mammals

Blood Pb (μg/ml)	Effect	Species	Source
1.7	anemia, weight loss, renal necrosis	Dogs	Forbes and Sanderson 1978
0.4-0.5	decrease in peripheral nerve conduction velocity	Humans	WHO 1980
0.8	clinical Pb toxicity	Cattle	Osweiler and Van Gelder 1983
0.35	clinical Pb toxicity	Cattle and Horses	Osweiler and Van Gelder 1983, Baars *et al.* 1990

The lowest reported blood Pb levels that have been associated with clinical lead toxicosis in mammals are 0.35 μg/ml, in cattle and horses. This value has been used as a biomarker of acute toxic lead exposure in mammals (Beyer 1996).

Normal tissue Pb levels in cattle have been reported by Vreman *et al.* (1986) as 0.16 and 0.46 μg/g (ww) in liver and kidney respectively. Puls (1994) lists normal cattle liver and kidney Pb concentrations as 0.1-1.0 and 0.2-2.0 μg/g (ww), respectively. Published reports of normal tissue Pb concentrations in wild small mammals have varied widely (Ma 1996). The lower range of published normal liver and kidney levels are ≤ 1μg/g (dry weight) (Mierau and Favara 1975; Chmiel and Harrison 1981; Ma 1989). However, as mentioned earlier, insectivorous or carnivorous species such as shrews may have higher normal baseline values due to higher dietary exposures (Ma *et al.* 1991). Ma

(1989) reported liver and kidney Pb values in shrews from reference sites to be ≤ 2 μg/g and 5 μg/g (dw), respectively.

Because soil characteristics affect Pb bioavailability, normal background tissue Pb concentrations in small mammals may reflect soil type. For example, in areas with peat or clay soils, mean background kidney Pb concentrations were 0.2 – 0.6 μg/g (dw) in mice and voles, and 3 –11 μg (dw) in shrews. In sandy soils, mean background values were 0.4 – 1.5 μg/g (dw) in mice and voles, and 13 – 19 μg/g (dw) in shrews (Ma 1996). Background Pb concentrations in bone have been reported as 2 – 3 μg/g (dw) in mice and voles from reference sites (Jeffries and French 1972; Ma 1989).

In general, organ Pb concentrations associated with Pb toxicosis in various species are less well established than blood Pb thresholds (Ma 1996). Tables 1.5 and 1.6 list Pb concentrations in selected tissue from cattle and sheep, which are considered to represent normal, elevated, or toxic levels.

Table 1.5 Lead concentrations reported in cattle tissue (ppm)[a]

	Blood	Liver	Kidney	Bone
Normal	0.01-0.2	0.1-1	0.2-2	1-7
High	0.3-0.4	2-10	3-20	30-75
Toxic	0.35-32	5-300	5-700	30-100
	Wet weight	Wet weight	Wet weight	Dry weight

[a] Puls (1994)

Table 1.6 Lead concentrations reported in sheep tissue (ppm)[a]

	Blood	Liver	Kidney	Bone
Normal	0.02-.25	0.03-0.8	0.1-0.8	1-7
High	0.7-0.9	5-25	5-100	30-75
Toxic	1-5	10-100	5-200	30-100
	Wet weight	Wet weight	Wet weight	Dry weight

[a] Puls (1994)

Some researchers have attempted to establish general guidelines for the interpretation of tissue Pb concentrations. Lewis *et al.* (2001) categorized liver and kidney Pb levels (not a species specific categorization) as background at < 1 ppm (ww), elevated but indicative of subclinical exposure at 1-2 ppm (ww), and consistent with potential clinical Pb poisoning at ≥ 6 ppm (ww).

1.3.9 Earthworms and Soil Lead

Earthworms play many vital roles in soil ecosystems. The activity of earthworms significantly affects soil structure and health (Kula and Larink 1998). For example, the intensive mixing of different soil fractions with micro-organisms in their digestive tract results in the formation of casts. These casts are rich in nutrients and enzymatic activity. (Laskowski *et al.* 1998). Earthworms also form burrows lined with a mucus layer in which the majority of soil microbial activity is concentrated (Laskowski *et. al* 1998). They play a major role in breaking down soil organic matter and releasing it to soil microorganisms for further breakdown and incorporation into the soil matrix. Besides their benefits in organic material breakdown, earthworms function to enhance soil drainage and aeration (Laskowski *et al.* 1998). Because of their intimate relationship with soil as decomposers, their sensitivity to different contaminants, and their relative high biomass in soil (10-200 g fresh weight/m^2), earthworms are often used in soil toxicity tests or as biomonitors (Laskowski *et al.* 1998). Their relative abundance in soils means they are also an important food source for many predatory soil organisms, such as beetles and staphylinids, as well as various birds and mammals (Kula and Larink 1998; Laskowski *et al.* 1998).

As early as 1895, Hogg demonstrated that a *Lumbricus* species of earthworm living in discarded wood from a Pb works contained high levels of Pb in its tissues. Various authors have reported that Pb accumulates in different earthworm species, resulting in tissue Pb concentrations far exceeding Pb concentrations in surrounding soil (Ireland 1975 a,b; Ireland and Richards 1977).

Tissue metal concentration in earthworms is dependent on soil metal concentration, intrinsic rate of bioaccumulation, and organism metal tolerance (Ma 1982). Lead accumulation in earthworms, as with other biota, is also influenced by soil properties and worm-related factors. For example Pb uptake in *L. rubellus* and *A. caliginosa* was greater in sandy soils with low organic matter content than in loamy soils (Ma 1982; Ma *et al.*, 1983). Soils with lower pH were also associated with greater Pb accumulation in these species. In fact, soil pH and organic matter content have been shown to account for more than 70% of the variance in worm Pb content (Ma 1983). This same study reported that, when using growth and survival as endpoints, adult worms are more tolerant of Pb exposure than juveniles. Furthermore, the mechanisms by which different species of earthworms accumulate metals from the soil environment vary. Some species tolerate relatively high soil concentrations of a toxic metal by adjusting metal assimilation efficiency or binding capacity, by enhanced excretion efficiency, or by storing the metal in a non-toxic form (Ireland 1979; Marino and Morgan 1999). Different feeding strategies also account for variability in Pb uptake between species. Consequently, earthworms accumulate varying amounts of Pb from polluted sites, and tolerate a range of Pb body burdens due to species and other worm-related differences.

Numerous authors have reported increased Pb tissue concentrations in various earthworm species from polluted sites, such as sludge contaminated fields, roadways, smelting complexes and mining sites (Czarnowska and Jopkiewicz 1978; Ireland 1979; Beyer *et al.* 1985), and confirm the usefulness of these organisms as monitors of soil contamination. Ireland (1979) reported tissue concentrations as high as 3600 mg/kg (dw) in *L. rubellus* from a Pb mining site in Wales with total soil Pb concentration of 1300 mg/kg. Kennette *et al.* (2002) investigated Pb uptake and toxicity in the earthworm *L. terrestris* from Pb contaminated urban soils collected in Montreal, Canada. Despite the soils' high levels of contamination (between 30 and 7100 mg/kg), there was no significant mortality reported after a 56-day exposure period. However, the earthworms did accumulate Pb in their tissues, with concentrations being correlated with soil metal content. The relatively low metal bioavailability and toxicity observed in this study was attributed to soil characteristics, especially alkaline pH (mean = 7.5). In laboratory studies, Reinecke and Reinecke (1996) exposed two groups of *E.fetida* to 0.1% Pb nitrate solution for 90 or 120 days. Mean Pb body burdens were 35 ± 17 mg/kg (ww) and 108 ± 35 mg/kg (ww), respectively. These findings supported previous observations by Reinecke and Reinecke (1997) in which *E. fetida* were exposed to a similar concentration of Pb for 8 weeks, with mean tissue concentration of 41.8 mg/kg (ww). Terhivuo *et al.* (1994) sampled earthworms (*L. casteneus, L. rubellus, A.caliginosa, A. rosea*) at an abandoned Finnish smelting site, and from a nearby, less contaminated site. The authors reported that earthworm populations diminished with decreasing distance from the smelter. Total soil-Pb concentrations ranged from 412 to 79,963 mg/kg, and mean tissue Pb in *L. casteneus* was 75.6 ± 46 μg/g (dw). This is far less than the tissue burdens of

348 ± 72 μg/g Pb dry weight reported by Svendsen *et al.* (1996) for the same species living in an environment with soil Pb concentrations that did not exceed 200 mg/kg. These results demonstrate that measurement of total soil Pb does not accurately predict soil toxicity, due to various abiotic factors (i.e. pH, organic matter content) that influence bioavailability. Consequently, bioavailability can only be determined using living organisms. However, laboratory methods such as weak electrolyte extraction and ion-exchange membrane metal uptake are good surrogate methods that should be used in preference to approaches that only assess total metal content of soils (Conder and Lanno 2000, Conder *et al.* 2001).

Studies of Pb uptake and toxicity to earthworms at shooting ranges are few. Vyas *et al.* (2000) assessed Pb concentration in a composite sample of earthworms (*L. rubellis*) from a shooting range with soil Pb concentrations of 110-27,000 ppm. Earthworm mean tissue Pb concentrations were reported to be 840 ppm and 660 ppm for animals with purged and non-purged alimentary traits, respectively. These concentrations were deemed toxicologically significant by the authors.

1.3.10 Other Soil Invertebrates

Positive correlations between tissue Pb concentrations and distance from roadways have been observed with various other soil invertebrate species. For example, Chmiel and Harrison (1981) reported that woodlice living near a roadway in the UK had tissue Pb concentrations of 152 mg/kg (dw) compared to 19 mg/kg (dw) in controls. A similar study from the US found tissue Pb concentrations in the same species near a major highway ranged from 380 to 682 mg/kg, (dw) (Beyer and Moore 1980).

1.3.11 Biomarker Responses in Earthworms

Earthworms are ubiquitous and ecologically important soil organisms (Weeks and Svendsen 1996). As a consequence, they have frequently been used in ecotoxicology testing, and are considered to be valuable in-situ sentinels for use in assessing biological risks in ecosystems (Weeks and Svendsen 1996; Scott-Fordsmand and Weeks 2000).

Eisenia fetida is the standard test organism recommended for use in terrestrial ecotoxicology testing in the European Union (OECD 1984; Kula and Larink 1998). It is a litter dwelling species (epigeic) that usually occurs at sites rich in organic matter, such as dung heaps. This species is easily cultured in the laboratory in large quantities, and an extensive database on effects of all classes of chemicals is available. *E. fetida* is considered a good representative test species because it is neither more nor less sensitive to common soil contaminants than many indigenous species (Kula and Larink 1998).

In recent years, much attention has been focused on the use of biomarkers in earthworms to assess exposure or effects from a wide range of anthropogenic compounds, including polyclic aromatic hydrocarbons, pesticides and metals (Neuhauser *et al.*1985; Weeks and Svendsen 1996; Reinecke and Reinecke 1996; Tang *et al.* 1999; Conder et al. 2001). The most commonly used species in biomarker studies to date are *Eisenia fetida* and *Lumbricus terrestris* (Scott-Fordsman and Weeks 2000). The use of biomarker responses has been adopted due to the limitations of the classical approach to soil toxicity assessment, which relates environmental concentrations to adverse organism effects. In the classical approach, bioavailability and toxicity may differ between laboratory tests and field conditions. As monitors of exposure, biomarkers address the

question of toxicity by assessing only the bioavailable fraction of the pollutant or mixture of pollutants, and are applicable under laboratory and field conditions (Scott-Fordsman and Weeks 2000).

An increasing number of biomarkers have been applied in earthworm toxicity testing. These include DNA alterations, concentrations of metalothionein and other metal-binding proteins, cholinesterase activity, cytochrome P-450/mixed function oxidase activity, energy reserve responses, ALAD activity, lysosomal membrane integrity, neurological responses, behavioural responses, and others (Grelle and Descamps 1998; Scott-Fordsmand and Weeks 2000). Only those relevant to the present study will be discussed here.

The neutral red retention assay (NRRA), a measure of lysosomal membrane integrity, has been identified as a reliable, easily applied biomarker in earthworms, which responds to various stressors in a dose-related fashion (Svendsen and Weeks 1997). The lysosomal system is a target for various toxic chemicals and non-chemical stressors, including hypoxia, osmotic shock, dietary depletion, etc. As a result, the NRRA can be used as a biomarker for multiple stressors (Weeks and Svendsen 1996; Scott-Fordsman and Weeks 2000).

Lysosomes are a morphologically heterogenous group of membrane-bound subcellular organelles that contain acid hydrolases, among other substances. They serve to catabolize organelles and macromolecules. In stable lysosomes, hydrolases can not come into contact with cell cytoplasm due to the protection provide by an intact membrane. However, under conditions of stress, membrane integrity is compromised and permeability increases (Weeks and Svendsen 1996; Scott-Fordsman and Weeks

2000). The NRRA is used to quantify lysosomal integrity in earthworm ceolomocytes after neutral red dye is taken up into cells and lysosomes by membrane diffusion (Weeks and Svendsen 1996). The ability of the ceolomocyte lysosomes to retain the dye (the neutral red retention time [NRRT]) is an indication of lysosomal membrane integrity, and is related to environmental stress.

Lysosomal stability studies in earthworms have generally involved metals, especially copper, but the NRRA has also been applied to pesticides and organic pollutants (Svendsen *et al.* 1996; Scott-Fordsmand and Weeks 2000, Booth *et al.* 2000). The first published application of the NRRA in earthworms reported a decrease ($p <$ 0.001) in NRRT for the earthworm *L. rubellus* following exposure to a range of copper concentrations (20-320 mg/kg) in both laboratory and mesocosm studies (Weeks and Svendsen, 1996). Worms exposed to the most contaminated soils exhibited a tenfold decrease in NRRT compared to controls for both field and laboratory studies. Earthworm copper residues and NRRT were correlated in both laboratory and field studies ($r = 0.73$ and 0.75, respectively).

Following a 3-day fire at a plastics recycling factory in Thetford, Norfolk, UK that covered 1200 m^2 with a mixture of inorganic (Pb, Cd and Sb) and organic contaminants (dioxins), Svendsen *et al.* (1996) collected earthworms (*L. castaneus*) along a contamination gradient to measure lysosomal membrane permeability. A positive correlation was observed between NRRT and distance from the factory. Soil Pb concentrations and earthworm tissue Pb residues decreased with increasing distance from the factory. Although the worms were also exposed to organic pollutants, the NRRT varied with soil metal levels at the site in a dose-dependent fashion. It was concluded

that the NRRA could be successfully applied to field studies involving contaminant mixtures without results being overshadowed by natural field-related variables.

Other sub lethal life cycle and behavioral biomarker responses have been used to quantify toxicity of various metals to earthworms. Endpoints commonly measured include survival, growth, cocoon production and viability, hatched juveniles, and avoidance behaviour (Svendsen and Weeks 1997a,b; Reinecke and Reinecke 1998; Spurgeon *et al.* 2000). High soil concentrations of Pb (i.e. 10,000 mg/kg) have been shown to have a negative affect on cocoon production and viability, population density, growth, and sexual development of earthworms (Spurgeon and Hopkins 1995; Reinecke *et al.* 1997).

Spurgeon and Hopkins (1995) concluded that the addition of Pb to artificial soils increased mortality and reduced growth and cocoon production in *E. fetida*, with cocoon production being the most sensitive indicator. The EC_{50} values for mortality (after 14 days), and cocoon production and growth rate (after 21 days) were determined to be >10,000, 1629, and 2249 μg/g, respectively. They also reported values for no observed affect concentrations (NOEC) for the same endpoints as 4793, 608, and 1966 μg/g.

Reinecke and Reinecke (1997) reported contradictory results after exposing *E. fetida* to 0.2 % Pb nitrate for a period of 76 days. They observed that earthworms exposed to low concentrations of Pb grew better and produced more cocoons than control animals ($p < 0.001$). This observation agreed with a subsequent study by the same authors (Reinecke and Reinecke 1996). Cocoon viability was deleteriously affected by low concentrations of soil Pb, with hatching success of 52.6% observed in the exposed group compared to 71.4% in controls ($p < 0.001$). Reinecke and Reinecke (1996) exposed

E. fetida to 0.1% Pb nitrate in food for 8 weeks and reported no differences in growth, maturation, or cocoon production compared with controls. However, they also reported significantly lower (p <0.05) cocoon viability in the exposed group. The authors concluded that growth and cocoon production may not be sensitive indicators of Pb toxicity in *E. fetida*, but cocoon viability may be a useful endpoint (Reinecke and Reinecke 1996).

1.3.12 Lead Uptake by Plants

Lead is considered a non-essential element to plants, but it is freely absorbed and accumulated in plant roots (Rooney *et al.* 1999), especially in soils with low pH or low organic matter content (Eisler, 2000). It is also translocated to leaves, but to a lesser extent, due to precipitation of Pb in the cell walls of plant roots (Adriano 1986). Excessive amounts of Pb inhibit plant growth, reduce photosynthesis by blocking of protein sulfhydryl groups and by effects on phosphate levels, and inhibit mitosis and water uptake (Holl and Hampp 1975; Demayo *et al.* 1982).

According to Kabata-Pendias and Pendias (1992), global average background concentrations of Pb in grasses and clovers are 2.1 mg/kg and 2.5 mg/kg, respectively. Soil Pb concentrations associated with phytotoxicity have not been well established, due to variability imposed by the effects of soil characteristics on Pb uptake (Rooney et al. 1999). In general, soil concentrations of 100-500 mg/kg and plant concentrations of 200 mg/kg are considered to be toxic (Rooney *et al.* 1999). Reports suggest that Pb concentrations of this magnitude result in reduction of dry weight, photosynthesis, water absorption, phosphate concentration and root growth in most plant species (Koeppe

1977). Other reported effects of high soil Pb include reduced leaf size, chlorotic, reddish leaves with necrosis, and short black roots (Pahlsson 1989). Lead bioavailability to plants is influenced by the same soil characteristics as have been discussed previously. Consequently, uptake by plants and phytotoxic effects are very site specific.

The risk of Pb entry into terrestrial food chains is greater through soil fauna than plants due to relatively low uptake of Pb into the aerial parts of plants in most circumstances. A study by Rooney *et al.* (1999) reported that five plant species grown in Pb-contaminated soil had elevated root and leaf-Pb concentration. However, consistent with other studies (Davies 1990; Fergusson 1990; Kabata-Pendias and Pendias 1992; Mellor and McCartney 1994) they reported Pb concentrations in roots to be several orders of magnitude greater than in leaves. Nonetheless many of the leaf concentrations were well above the edible limit for foodstuffs set by the World Health Organization (Kabata-Pendias and Pendias 1992).

Chapter 2. Survey of Canadian Prairie Shooting Ranges

2.1 Introduction

Although banned in Canada and the USA for waterfowl hunting, lead shot continues to be used for recreational trap and skeet shooting, and for upland game bird and small mammal hunting in both countries. Prior to the waterfowl-hunting ban, it was estimated that hunters and recreational shooters in Canada were responsible for the deposition of 1500 tonnes of lead (Pb) as Pb-shot pellets (Scheuhammer and Norris 1996) in the environment every year. No data exist to describe how much of this total is deposited on Canadian shooting range sites. However, studies in other countries indicate that Pb deposition at some target shooting ranges is very significant, with yearly loadings being as high as 10-30 tonnes (Ordija 1993). At one Finnish range, Tanskanen et al. (1991) reported annual accumulations of 15 tonnes, while in Denmark, Jorgensen and Willems (1987) estimated Pb deposition at three separate shooting ranges to vary between 240 kg and 5000-6000 tonnes annually.

Trap and skeet are two distinct but related shooting sports in which shooters with shotguns fire at domed, disc-shaped clay targets launched by machines or using hand held devices. Trap shooting originated in England about 1750 as a way of practicing shooting skills. Live birds such as pigeons or quail were placed into a series of traps, cages or boxes, and were released on the shooter's command. Organized shooting events using live birds were common in the early 19th century, but the impracticality of using live birds lead to the search for a suitable substitute. Clay was first used to make targets in 1870. It was quickly abandoned in favour of other materials, but the name clay pigeon stuck (Missouri Conservationist Online [www.conservation.state.mo.us] 2001).

Skeet shooting was invented in Massachusetts in 1920. The name of the sport is derived from an old Scandinavian word (skjota) for "shooting". In skeet, shooters fire from eight separate stations around a semi-circle. The targets are released from either side of the shooters and cross in front them. At some stations, shooters fire at targets flying from the right, and at other times from the left, or from both directions at the same time. In trap, shooters stand in a narrower arc at one of five stations behind a single, hidden throwing house that launches targets at unpredictable angles. In both sports, a round consists of shooting at 25 targets. The typical shot size used in trap shooting is 7.5, while in skeet it is 9, representing a mass of 1.25 and 0.75 grams per pellet, respectively.

One of the objectives of this study was to compile a list of active and historic shooting ranges in the three Canadian prairie provinces. The purpose of this undertaking was to better understand the scope of recreational shooting in the prairies, and to identify and gain access to active shooting ranges to assess Pb contamination and uptake into wildlife food chains. Although by no means exhaustive, these data may be useful in establishing guidelines for the regulation of Pb-shot at gun clubs in Canada.

2.2 Materials and Methods

Information on shooting ranges in the Canadian prairies was gathered through correspondence with the Chief Firearms Officers of Alberta, Saskatchewan and Manitoba, the Alberta Trap Association, the Alberta Sporting Clays Association, the Alberta Federation of Shooting Sports, Saskatchewan Sport Inc., the Manitoba Trap and Skeet Shooting Association, and the Saskatoon Office of the Royal Canadian Mounted Police. A database was created by consolidating the information provided. The list

obtained was not exhaustive since smaller, more remote shooting ranges may operate without formal registration by any governing body or association. However, every effort was made to make it as comprehensive as possible.

Subsequently, a questionnaire inquiring about club history was drafted and sent to all identified shooting clubs in the three provinces (Fig. 2.1). Based on responses to this questionnaire and appropriateness of shooting activities, a subset of ranges was contacted to request permission to access the site to conduct field studies.

Fig. 2.1. Shooting range questionnaire

Please check or specify next to appropriate space provided.

1.) Name of Club:

2.) Location:

3.) Year established:

4.) Has the club been in continous use since it was established? Yes/No.
If no, please provide a brief history of its use.

5.) Membership size: 1-25 _____
26-50 _____
51-75 _____
76-100 _____
100-150 _____
Over 150 (please specify):_____

6.) What is the frequency of use of the club?:
a.) Year round (7 days/week_____ weekend only_____ other_____)
b.) Summer only (7 days/week_____ weekend only_____ other_____)
c.) Other:

7.) Range size: less than 1 acre_____
1-3 acres_____
3-5 acres_____
5-10 acres _____
Over 10 acres_____ (please specify):_____

8.) What type of sport(s) is the range used for (trap, skeet, etc.)?

9.) What is the predominant ammunition used?: Steel_____
Lead_____
Copper_____
Other_____

10.) What is the dominant vegetation on the range? (e.g. grasses, brush, tress, etc.)

11.) How would you characterize the landscape (e.g. flat, rolling, marshy, etc.)

2.3 Results

A total of 408 organized shooting ranges were identified, and subsequently sent questionnaires. Response to the questionnaire ranged from 19% (Saskatchewan) to 33% (Manitoba), with an average rate of 22% (Table 2.1).

Table 2.1 Canadian prairie shooting ranges

	Alberta	Saskatchewan	Manitoba
Total Identified Sites	156	182	70
Number responding	33	32	23
% Response	22	19	33

More than half of all the clubs responding had been in operation for less than 30 years, with approximately 40% falling into the 20-29 year category (Table 2.2).

Table 2.2 History of operation of shooting clubs in the Canadian prairies

Years in Operation	Alberta (n=33)	Saskatchewan (n=32)	Manitoba (n=23)
Uncertain	1	1	1
<10	1	4	1
10-19	3	5	6
20-29	13	15	7
30-39	6	3	1
40-49	5	2	6
50-59	3	1	0
≥60	1	1	1

Membership numbers varied greatly (Table 2.3). In all three provinces, small clubs (≤50 members) dominated, with the single most frequently reported size category being 1-25 members (42% of total ranges).

Table 2.3 Size of memberships of shooting clubs in the Canadian prairies

Membership Range	Alberta (n=33)	Saskatchewan (n=32)	Manitoba (n=23)
1-25	8	17	12
26-50	6	8	7
51-75	7	3	1
76-100	4	0	1
101-125	3	0	1
126-150	0	0	0
151-200	3	0	1
201-300	2	2	0
900-1000	0	1	0
1400-1500	0	1	0

Most of the clubs responding to the questionnaire were relatively small outdoor facilities (Table 2.4), with only 20% of the total respondents falling in the > 10 acre category.

Table 2.4 Area occupied by shooting clubs in the Canadian prairies

Acreage	Alberta (n=33)	Saskatchewan (n=32)	Manitoba (n=23)
<1	1	0	0
1-3	6	9	9
3-5	6	6	6
5-10	8	9	1
>10	9	7	2
Indoor	3	1	5

Trap and/or skeet shooting are practiced on 61, 50, and 39% of all responding ranges in Alberta, Saskatchewan and Manitoba, respectively. Lead ammunition was reported to be used at almost all of the shooting clubs that responded to the questionnaire. However, this does not mean that Pb is the only form of ballistics used on these ranges.

Table 2.5 Shooting sports and ammunition usage at shooting clubs in the Canadian prairies

	Trap/Skeet	No Trap/Skeet	Pb Ammunition
Alberta (n=33)	20	13	33
Saskatchewan (n=32)	16	16	29
Manitoba (n=23)	9	14	22

Frequency of range use was about evenly divided between year round and seasonal in all three provinces (Table 2.6). Most of the ranges responding to the questionnaire are used at least weekly, while an average of 43% of the total year-round and 33% of the seasonal ranges see daily use.

Table 2.6 Frequency of range use at shooting clubs in the Canadian prairies

Alberta (n = 33)	Year Round = 18			Seasonal = 15		
	Daily	Weekly	Occasional	Daily	Weekly	Occasional
	7	11	0	5	8	2
Saskatchewan (n = 32)	Year Round = 17			Seasonal = 15		
	Daily	Weekly	Occasional	Daily	Weekly	Occasional
	8	8	1	3	9	3
Manitoba (n = 23)	Year Round = 12			Seasonal = 11		
	Daily	Weekly	Occasional	Daily	Weekly	Occasional
	5	6	1	5	6	0

Based on survey results and subsequent correspondence, four shooting ranges were identified as sites to investigate Pb contamination and uptake into terrestrial food chains. These study sites were the Provost and District Fish and Game Association Silhouette

Range in Provost, Alberta, the Vegreville Wildlife Federation in Vegreville, Alberta, the Eastend Gun Club in Eastend, Saskatchewan, and The Pas Firearms Association Inc. in The Pas, Manitoba.

The Provost Silhouette range was established in 1970, and was in continuous use until its closure in 2000. It was located on a relatively small plot of land (3-5 acres) near a small wetland conservation area. The size of the shot-fall zone was approximately 70 x 90m, and included an ephemeral slough, which contained water in most years prior to the drought conditions of 2001 and 2002. The range was used solely for trap shooting, and Pb shot was the preferred munition.

The Vegreville Wildlife Federation gun club was established in the 1950's, and has been in continous use ever since. The membership consists of 76-100 people shooting year round in various disciplines including trap and skeet. The range covers 36 acres, which includes land that is not used for any shooting activity. There is a small creek running through the property, but it does not intersect the shot-fall zone, which extends between 105-160 m by 50 m from the shooting arc. Lead shot is used as the munition of choice at the trap range.

The Eastend Gun Club began operating in 1982, and currently reports 26-50 members that use the site for various shooting activities including trap and skeet. However, in recent years the club has seen dwindling interest and membership is declining. The property lies on 50 acres of dry, rocky soil with very little vegetation. The shot-fall zone is located 60-90 m from the shooting stations, and is approximately 60 m wide. The preferred munition was Pb.

The Pas Firearms Association Inc. range is approximately 20 km north of The Pas, Manitoba and has a membership of 26-50 individuals. It was established in 1982 and covers 110 acres of land in the Precambrian shield. Various shooting lanes (trap and skeet, rifle, etc.) are separated by rows of trees. Lead was the preferred munition at this range. The shot-fall zone for the trap and skeet portion of the range is large, extending from approximately 130 to 200 m from the shooting stations with a width of approximately 80 m. It is covered with a thick, moist layer of peat moss.

2.4 Discussion

Because of the resistance among some shooters to legislation banning Pb-shot for waterfowl hunting, as well as the high level of public resentment over the recent and on-going national gun registry initiative, it was rather difficult to obtain the cooperation from recreational shooters that was necessary to undertake this study. Many clubs and some associations did not want to reveal information regarding membership or the use of their ranges, and were reticent to allow access for the purpose of conducting the fieldwork. Some questioned the intentions of this study, and were concerned about potential closure of their clubs. However, a relatively small number of clubs were very forthright and willing to provide the information requested on the questionnaire, as well as to permit access to shooting sites. An average response rate to the survey of slightly over 20% was deemed satisfactory under the circumstances.

Survey results indicate that there is considerable interest in recreational shooting in the prairie provinces. However, it is not possible to extrapolate these results to the total population of shooters in the three provinces. Survey results would seem to indicate that

Saskatchewan has the greatest number of shooters, but this is possibly an artefact caused by the inclusion of data from two very large clubs with combined memberships of 2300. At the time the survey was taken (Fall 2000), total population of the prairie provinces was approximately 979,000, 1,142,000, and 2,800,000 for Saskatchewan, Manitoba and Alberta, respectively (Statistics Canada). Total identified shooting ranges numbered 182, 70 and 156, respectively, which would tend to suggest that Alberta should have the greatest number of recreational shooters, followed by Saskatchewan and Manitoba. The majority of clubs in the prairies are small (1-25 members). Alberta had the highest proportion of medium sized clubs (76-200 members) at 33%. Saskatchewan and Manitoba had very few clubs in this size range (0%, and 13%, respectively).

It is unclear whether the data generated by this survey is representative of the overall picture in each province. Alberta and Saskatchewan had a response rate of approximately 20%, with 123 and 150 identified clubs, respectively, not responding to the survey. It's not possible to determine if the 80% of non-responders represent clubs with the largest memberships, longest operation times, highest frequency of use, etc., or if they represent the lower end of the scale for each of those parameters. Consequently, these results should be viewed as a partial picture of recreational shooting. Furthermore, from the perspective of the larger study of the bioavailability of Pb shot on shooting ranges, 49% of the clubs responding to the questionnaire did not offer trap and skeet shooting. Some are indoor facilities, and many others are used for a variety of other shooting activities, such as handgun/rifle target shooting, .22 calibre, full bore, machine gun, etc. The significance of this is that although almost 100% of clubs permitted the use of Pb ammunition, much of the Pb used at these ranges would be confined to shooting

berms or even indoor shot stops. In these situations, Pb bullets and/or fragments would remain in a relatively confined area that would represent minimal risk of wildlife exposure.

More than 68% of clubs in all three provinces were between 1-10 acres in size. This represents the total acreage used for all shooting activities at the site. Of that, only a portion is used for trap and skeet shooting and the shot-fall zone represents only a fraction of that total. Most of the Pb-shot discharged during trap and skeet shooting lands between 115 to 235 m from the point of discharge on an area corresponding to roughly 4 acres (Shooting Range Stewardship 2001). Since only about 50% of all respondents engaged in trap and skeet shooting, the total land area heavily impacted by Pb deposition as a result of this sport in the prairie provinces would be on the order of 1000 acres, assuming the respondents are representative of all shooting ranges.

Future studies that attempt to characterize recreational shooting in the prairies should try to address more specific questions regarding trap and skeet shooting, since they represent the greatest potential for environmental effects. Information such as shot-fall zone size (rather than total size of range), frequency of use of trap and/or skeet range and the years of use, estimated Pb-loads based on membership involved in trap and skeet shooting, potential use of non-toxic shot vs. Pb shot, etc., could be valuable for a more comprehensive attempt at a risk assessment. In addition, it would be important to determine if shot-fall zones are in wetland areas, or areas occasionally flooded, since the risk to wildlife is increased under those circumstances.

Chapter 3. The Effect of Lead-Contaminated Soil from Prairie Skeet Ranges on the Neutral Red Retention Assay and Fecundity in the Earthworm *Eisenia fetida*

3.1 Introduction

Although recently banned in Canada for waterfowl hunting, lead (Pb) shot continues to be used routinely for small mammal and upland game bird hunting, as well as by recreational shooters at trap and skeet ranges. The deposition of Pb shot in terrestrial habitats represents a risk to wildlife and soil invertebrates. The release of Pb from shot pellets deposited at shooting ranges in particular may result in very high Pb levels in soil, with concentrations in the range of 1,000 to 75,000 mg/kg commonly reported in the United Sates and Europe (Jorgensen and Willems, 1987; Stansley and Roscoe, 1996; Vyas *et al.*, 2000). Soil invertebrates, especially earthworms, have been shown to accumulate Pb at contaminated industrial sites (Terhivuo *et al.* 1994), and this exposure may have adverse effects on soil community structure or function (Svendsen and Weeks 1997a). However, few studies have assessed Pb toxicity to soil invertebrates at shooting ranges. Due to the paucity of data on the toxicity of Pb to earthworms and other soil invertebrates at sites contaminated with Pb shot, it is important to increase our understanding of the bioavailability and toxicity of Pb to soil organisms at these types of sites using field collected soils.

Biomarkers have been used extensively to determine exposure and sublethal effects of environmental pollutants in earthworms (Weeks and Svendsen 1996). The lysosomal neutral red retention assay (NRRA) in particular has been shown to be a sensitive indicator of stress from exposure to various contaminants, including metals such as copper (Svendsen and Weeks 1997a, 1997b), nickel (Scott-Fordsmand and Weeks

2000), cadmium (Reinecke and Reinecke 1999), zinc (Reinecke and Reinecke 1999, Spurgeon *et al.* 2000) and lead (Reinecke and Reinecke 1999). The NRRA provides a measure of cell damage by evaluating lysosomal membrane stability. The underlying premise of this technique is that coelomocytes from healthy, unstressed earthworms are able to take up and retain the supravital dye, neutral red, within lysosomes. In contrast, the dye will leak out of lysosomes from stressed cells more rapidly, staining the cytoplasm, and resulting in lower neutral red retention time (NRRT). In the present study the NRRA was used to evaluate the sublethal effects of Pb in the earthworm *Eisenia fetida* using freshly spiked soil and soil collected from three Canadian prairie trap and skeet shooting ranges. The biomarker response was assessed in parallel with other parameters commonly used in earthworm toxicity tests, including mortality, growth, fecundity and contaminant body burden. Growth has frequently been shown to be a more sensitive indicator of exposure than mortality with many contaminants (Reinecke and Reinecke 1996) but apparently not in the case of Pb (Reinecke *et al.* 1997). Evaluation of reproductive effects relative to NRRA results may enable linkage of biomarker responses to important life cycle endpoints, and help establish the use of the assay as an early warning indicator of chronic effects at Pb contaminated sites.

Soil quality guidelines for evaluating toxicity at metal contaminated sites are usually based on total soil-Pb levels. However, this approach does not take into consideration the effects of modifying factors such as pH, organic matter content, clay content, and cation exchange capacity on metal bioavailability (Ma 1982). Furthermore, over time, the bioavailability as well as the toxicity of a soil contaminant to ecological receptors may be reduced (Conder and Lanno 2000). Soil metals are never 100%

bioavailable to organisms. The bioavailable fraction cannot be measured directly using chemical analyses, since only living organisms can determine bioavailability (Conder and Lanno 2000). Therefore, many researchers recommend that standard toxicity tests in which total metal concentrations are used should be abandoned in favour of approaches which assess biological responses to only the available fraction of the metal (Conder and Lanno 2000). Bioassays can be expensive and time consuming. Therefore, in order to assess bioavailability and toxicity, surrogate measures of bioavailability have been developed. A rapid, simple surrogate method that has been successfully used for measuring metal bioavailability is weak-electrolyte soil extraction. Electrolyte extractions using weak (<1 M) $CaCl_2$ or $Ca(NO_3)_2$ have shown potential as toxicity related measures in soil metal toxicity studies (Marinussen *et al.* 1997; Sloan *et al.* 1997; Conder and Lanno 2000) and are believed to extract only weakly bound metals which are deemed available to soil organisms such as earthworms.

The objective of this study was to measure earthworm biomarker responses, Pb tissue residues, and reproductive effects in earthworms, and to relate these variables to bioavailable (electrolyte-extractable) and total soil Pb concentrations in spiked soils and field collected soils from prairie shooting ranges.

3.2 Materials and Methods

Two experiments were conducted to evaluate the responses of adult earthworms to Pb-contaminated soil – a pilot study and a definitive study. In the pilot study, conducted in the fall summer of 2002, earthworms were exposed to soil freshly spiked with Pb, and NRRT and earthworm Pb body burdens were measured. This study was conducted in collaboration with the University of Guelph, Ontario, Canada. In the definitive study, conducted in the fall of 2002, earthworms were exposed to soil from three Canadian prairie trap and skeet ranges and matched reference sites, and NRRT, growth, fecundity, and earthworm Pb body burdens were measured. Soil total and $Ca(No_3)_2$-extractable Pb concentrations were correlated with these parameters. This study was conducted in collaboration with the Ohio State University, Columbus, Ohio, USA.

Earthworm culture and maintenance

For the freshly spiked soil experiments, adult *E. fetida* were obtained from a local vermiculturist and were maintained in plastic containers in a sandy silt soil (2.5% organic matter, pH 7.6). Shooting range experiments were conducted using adult specimens of *E. fetida* from a long-standing laboratory culture. Earthworms were maintained in plastic containers in separated dairy solids obtained from the Ohio Agriculture Research Development Center, Wooster, Ohio. All earthworms were maintained at 25°C in the dark.

Effect of soil freshly spiked with lead on neutral red retention time (NRRT) and body burdens

To determine the effect of Pb on NRRT in the laboratory, the sandy silt soil described above was spiked with increasing concentrations of lead acetate (Fisher Scientific, Pittsburgh PA, USA). Composted sheep manure was added to the soil as food for the earthworms. The soil and manure were mixed in a 4:1 ratio (80% soil to 20% manure, ww), resulting in an organic matter content of 5.6%. The desired amount of Pb was thoroughly mixed into soil as an aqueous solution to give six nominal exposure levels (0[control], 50, 100, 200, 500, and 1000 mg/kg substrate, selected based on pilot studies; Colin Darling, University of Guelph, Guelph, ON, Canada) (unpublished data), and 2 kg of prepared soil was placed into plastic containers. There were four replicates per treatment, and earthworms were acclimated in an unspiked soil mix for one week before experimentation. After the acclimation period, 30 to 40 worms were added to each container to ensure more than enough individuals for sampling. The start of the experiment was staggered and earthworms were placed into one replicate from each treatment on each of the first 4 d of the week. Earthworms were maintained in a controlled temperature room at 18°C on a 12:12-h light : dark photoperiod. Soil moisture was maintained at approximately 20%. Earthworms were exposed for four weeks, after which the NRRT was determined in five randomly selected earthworms from each replicate. After NRRT measurements, earthworms were placed into plastic Petri dishes containing wet filter paper to depurate along with remaining earthworms from each replicate. Earthworms were rinsed and placed on fresh filter paper daily for 4 d, then were frozen at -20°C until required for Pb body burden analysis.

Effect of soil from trap and skeet ranges on growth, fecundity, neutral red retention time (NRRT), and lead body burdens

Field soils were collected from three trap and skeet ranges and three matched reference sites that had similar soil characteristics (Table 3.1). At each trap and skeet range, the location of the shot fall zone was determined and a grid pattern was established in this zone. Each row and column was approximately 10 m apart, and a total of 12 samples were collected at each site. The same grid pattern and sample collection protocol was used in the reference areas. Soil samples were collected using a cylindrical soil corer (12-cm diameter) to a depth of 12 cm, including turf. Samples with even numbers were used for monitoring earthworm growth, fecundity, biomarker responses, Pb pellet counts, and total and $Ca(NO_3)_2$-extractable Pb determinations (i.e., a total of six replicate soil samples for each trap and skeet range and each paired reference area). Samples with odd numbers were used for measurement of pH and organic carbon content (Table 3.1).

Table 3.1. Soil characteristics of the Canadian prairie trap and skeet ranges and their paired reference sites.

N=6	pH (mean ± std. dev)	Organic Carbon (%) (mean ± std. dev.)	Soil Texture
Eastend Site	7.34 ± 0.21	1.11 ± 0.42	Sandy loam to clay loam
Eastend Control	7.06 ± 0.22	1.26 ± 0.53	Sandy loam to clay loam
Provost Site	6.38 ± 0.36	2.69 ± 0.45	Sandy loam to loamy sand (>50% sand, 5-10% clay)
Provost Control	7.13 ± 0.24	2.0 ± 0.91	Sandy loam to loamy sand (>50% sand, 5-10% clay)
Vegreville Site	5.51 ± 0.51	7.89 ± 3.34	Loam to clay loam (35-40% sand, 20-30% sand)
Vegreville Control	5.77 ± 0.62	6.62 ± 3.32	Loam to clay loam (35-40% sand, 20-30% sand)

Soil pH was measured in duplicate in $CaCl_2$ (10 g soil: 25 ml $CaCl_2$). Samples were thoroughly stirred, then allowed to sit for 30 min before measurement. Soil organic carbon was estimated using a LECO CR-12 Carbon Analyzer. Small amounts (0.2-0.4 g) of fine ground (40 mesh), air-dried soil was analyzed at a temperature of 840°C, an oxygen flow rate of 3.6 L/min, and a lancing flow rate of 1.0 l/min (Wang and Anderson 1998).

Laboratory exposures were conducted according to the American Society for Testing of Materials standard guidelines (ASTM 1997). Adult earthworms of 300 to 400 mg were acclimated in the reference site soil appropriate for each trap and skeet range for one week before experiments. Trap and skeet range and reference site soil samples were adjusted to 25% moisture content by weight. Two hundred grams (dw) of prepared soil was placed into 500-ml acid-rinsed glass jars, and 10 acclimated earthworms were weighed and added to each jar. Food (separated dairy solids) was supplied in a small

container on the soil surface. Three separate experiments were conducted, one for each trap and skeet range and its paired reference site. The start of each experiment was staggered and ten earthworms were weighed and placed into three replicate samples from each trap and skeet range and its paired reference site on each of the first 2 d of the week. Earthworms were maintained in an environmental chamber at 25°C in constant light. After four weeks, earthworms were removed from the soil, weighed, and the NRRT was determined in five randomly selected earthworms from each jar. Earthworms were then placed into plastic Petri dishes containing wet filter paper to depurate. Earthworms were rinsed and placed on fresh filter paper daily for 4 d, after which they were frozen at -20°C until required for Pb body burden analysis. The soil from which the earthworms were removed was hand sorted to remove cocoons. The cocoons were maintained at 25°C in constant light until hatching (four to six weeks) and cocoon viability, the number of juveniles produced, and the number of juveniles per cocoon calculated.

Neutral red retention (NRRT) assays

A neutral red (Sigma, St. Louis, MO, USA) working solution of 80 mg/ml was prepared in earthworm physiological ringer solution (Lockwood 1963). Coelomic fluid was collected from each earthworm by inserting a 25-gauge needle containing 50 μl of ringer into the coelomic cavity posterior to the clitellum and allowing it to fill by intracoelomic pressure and a gentle drawing action on the syringe. The coelomic fluid was then placed onto a clean glass slide and mixed with an equal volume of neutral red solution, before a cover slip was placed on top. Slides were scanned for 2 min at 5-min intervals, and the total number of stained and unstained cells per slide was counted.

Microscope slides were kept in a humidity chamber when not under observation. The cells were counted until 50% of the cells were red. This time was recorded by observers that were blinded as to the treatment and reported as the NRRT.

Analysis of earthworm lead body burdens

Earthworm body burden samples from the spiked soil experiment were freeze-dried, ground, and 0.2 g of sample placed into a 25ml plastic digestion vial with 5 ml of trace-metal grade nitric acid (HNO_3; Fisher Scientific, Pittsburgh, PA, USA). Samples were digested at room temperature for 1 h, followed by digestion in a heating block set at 100°C for 1 h. The samples were allowed to cool to room temperature, then made up to 10 ml with NANOpure® water (Millipore, Billerica, MA, USA). Samples were then analyzed by flame atomic absorption spectroscopy (FAAS) at 283 nm using a Perkin-Elmer Analyst 3300 flame atomic absorption spectrometer (Perkin-Elmer, Wellesley, MA, USA). Earthworm samples from the trap and skeet ranges and their reference sites were digested in HNO_3: perchloric acid (10:1.5), then made up to 25 ml with 1% HNO_3. Samples were then analyzed using a Varian graphite furnace atomic absorption spectrophotometer (110/220Z) with Zeeman background correction (Varian, Walnut Creek, CA, USA).

Analysis of soil total and $Ca(NO_3)_2$-extractable lead concentrations

Soil samples for determination of total Pb from the spiked soil experiment were dried and ground using a mortar and pestle. Three replicates of 0.5 g each per treatment were weighed into a 25-ml digestion tube, and samples were digested as for earthworm

body burden samples. After cooling, the samples were made up to 10 ml with NANOpure® water and analyzed by flame atomic absorption spectroscopy.

Total Pb content in soil from the trap and skeet ranges and the reference sites was determined by wet digestion of 1-g (dw) soil sample using 5 ml trace-metal grade HNO_3 after dry sieving through a 1.7mm mesh screen. Samples were digested as described for earthworms. The samples were cooled to room temperature, then made up to 15 ml with 0.5 M HNO_3, filtered through Whatman 540 filter paper (Maidstone, Kent, UK), made up to a final volume of 50 ml with 0.5 M HNO_3, and analyzed by flame atomic absorption spectroscopy.

Weak electrolyte-extractable Pb was measured using 0.1 M $Ca(NO_3)_2$ extraction (Conder and Lanno 2000). A 1-g (dw) soil sample was mixed with 20 ml 0.1 M $Ca(NO_3)_2$ and placed on a rotary mixer for 16 h at room temperature. Samples were then centrifuged at 2,500 g for 15 min, and the supernatant filtered through 0.45 μm polyvinylidene fluoride membrane filter and acidified with 1 ml of trace-metal grade HCL (Fisher Scientific). Samples were analyzed using a Perkin-Elmer Analyst 700 graphite atomic absorption spectrometer using L'vov platform tubes.

Statistical analysis

Data from the spiked soil experiment were analyzed using analysis of variance in Systat® (SPSS, Chicago, IL, USA), with post hoc comparisons of treatment means made using the Bonferroni procedure. The NRRT and earthworm body burden data were log transformed before analysis to satisfy assumptions of normality. Soil Pb levels were

correlated with earthworm Pb body burdens and NRRT. The significance of linear correlation coefficients was estimated using the Bonferroni multiple comparison tests.

Statistical analysis (Sanders *et al.* 2000) for earthworm growth, NRRT, cocoon production, juvenile production, number of juveniles per cocoon, and cocoon viability data were analyzed using a t-test ($p \leq 0.05$) to compare each trap and skeet range with its matched reference site. Growth data were log-transformed before analysis to satisfy assumptions of normality.

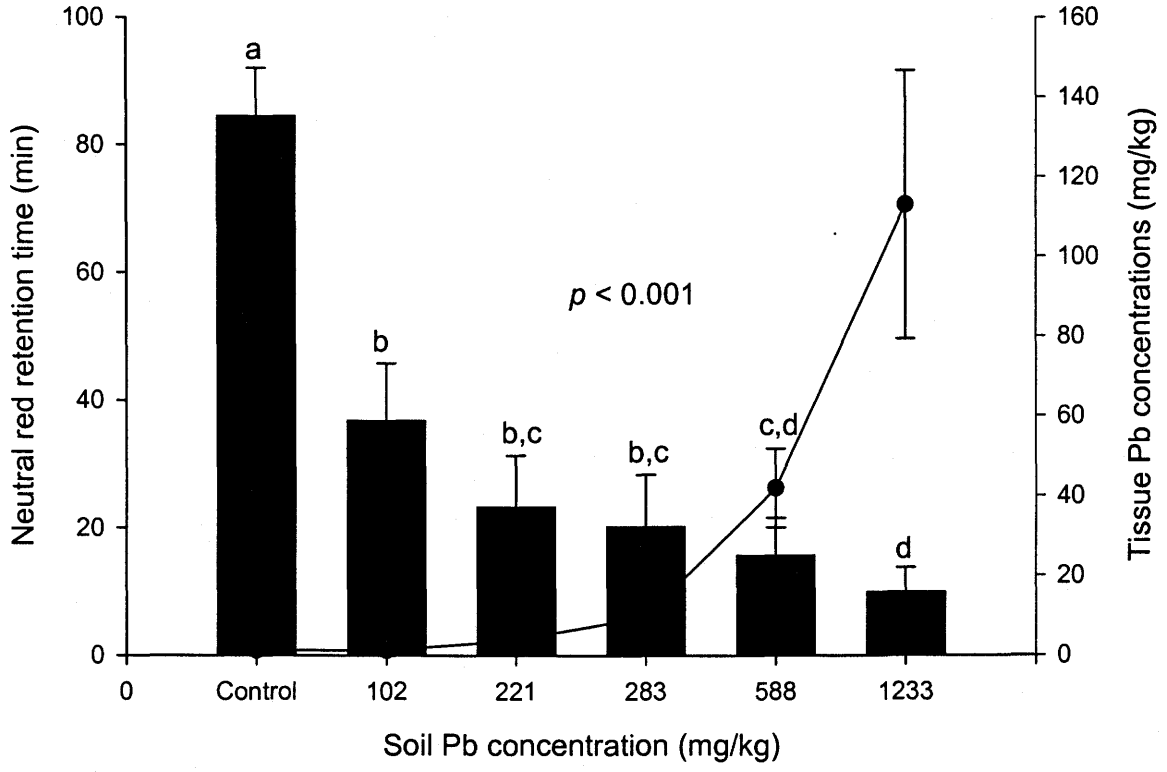
Soil total and $Ca(NO_3)_2$-extractable Pb levels were correlated with earthworm Pb body burdens and NRRT. Soil total and $Ca(NO_3)_2$-extractable Pb levels and earthworm body burden data were log-transformed before analysis to satisfy assumptions of normality. The significance of linear correlation coefficients was estimated using a post hoc Bonferroni multiple comparison test.

3.3 Results

Effect of soil freshly spiked with lead on neutral red retention time (NRRT), and lead body burdens

Neutral red retention time decreased with increasing soil Pb concentrations, and all treatments were significantly different from the control ($F_{5,18} = 11.412, p < 0.001$; Fig. 3.1).

Figure 3.1

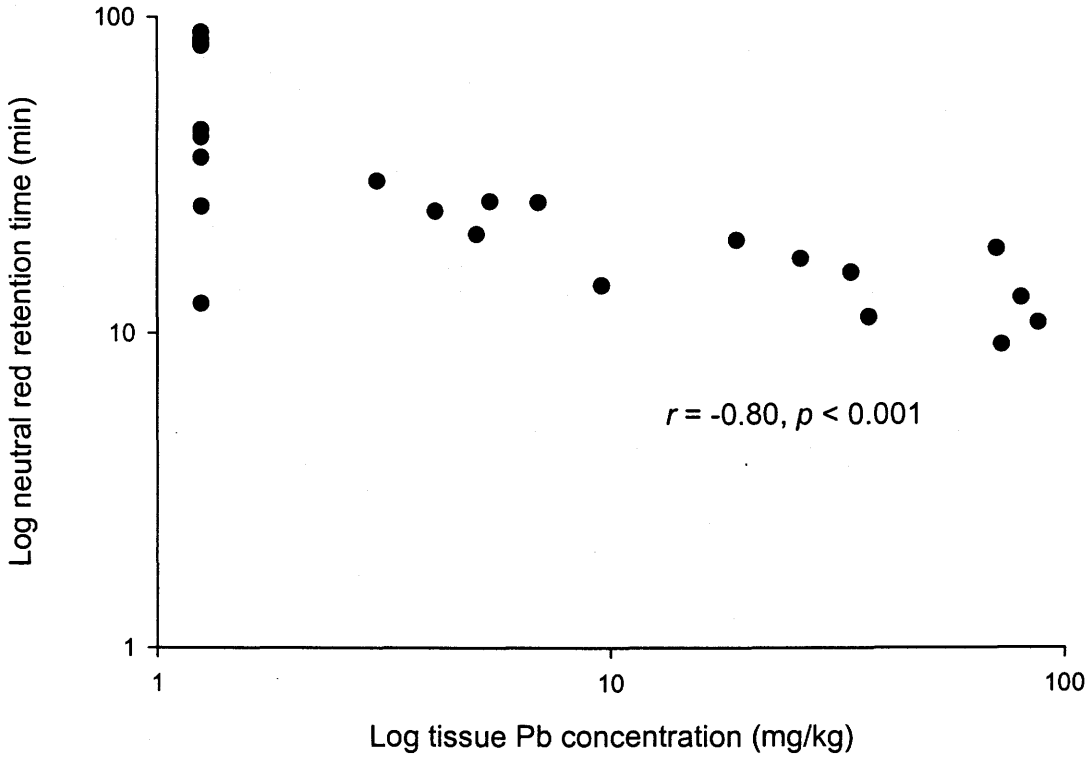


Pb = lead

Fig. 3.1 Effect of increasing concentrations of lead on neutral red retention time and lead body burden in *Eisenia fetida* exposed to freshly spiked soil for 28 d (mean ± SE). Means with the same letter are not significantly different (n = 18).

The background soil Pb concentration was 32.7 ± 0.2 mg/kg, and Pb-spiked soils ranged from 102 ± 0.7 mg/kg to 1,233 ± 45 mg/kg (nominal concentration 50-1,000 mg/kg). Earthworm Pb body burdens were significantly increased after exposure to Pb-spiked soil compared with the control earthworms ($F_{5,18} = 9.459, p < 0.015$; Fig. 3.1). Earthworm Pb body burdens were strongly correlated with soil total Pb concentrations ($r = 0.89, F_{1,22} = 87.47, p < 0.001$). Earthworm Pb body burdens were plotted against earthworm lysosomal NRRTs and showed a significant negative correlation ($r = -0.80, F_{1,22} = 39.59, p < 0.001$; Fig. 3.2).

Figure 3.2



Pb = lead

Fig. 3.2 Correlation of lead body burdens with lysosomal neutral red retention time in *Eisenia fetida* exposed to freshly lead-spiked soil for 28 d.

Effect of trap and skeet range soil on growth, fecundity, neutral red retention time(NRRT), and lead body burdens

There was no mortality in any of the trap and skeet range or reference soils. There were no significant differences between the trap and skeet ranges and their reference sites for growth or fecundity ($p > 0.05$ in all cases; Table 3.2).

The NRRT was significantly reduced in earthworms exposed to soil from all three trap and skeet ranges compared with their respective reference sites (Table 3.2). Exposure to soil from the Eastend pair reduced the NRRT from 54 min in the reference site to 46 min in the trap and skeet range soil ($F_{1,10} = 9.66, p = 0.011$). Exposure to soil from the Provost pair reduced the NRRT from 56 min in the reference site to 30 min in the trap and skeet range soil ($F_{1,10} = 20.5, p = 0.001$). Exposure to soil from the Vegreville pair reduced the NRRT from 56 min in the reference site to 40 min in the trap and skeet range soil ($F_{1,10} = 15.25, p = 0.003$).

Table 3.2 Effect of trap and skeet range and reference sites soils on growth, fecundity, and biomarker response in the earthworm *Eisenia fetida* (mean ± SE).

Endpoint	Eastend Range	Eastend Reference	Provost Range	Provost Reference	Vegreville Range	Vegreville Reference
Growth (% of start weight.)	-7.6 ± 4.1	-8.3 ± 2.1	-21.3 ± 1.7	-17.6 ± 1.8	9.8 ± 6.3	16.8 ± 6.7
Cocoon production	52.7 ± 5.7	53.5 ± 2.8	51.5 ± 2.8	55.8 ± 2.4	43.8 ± 4.2	45.5 ± 1.9
Number of juveniles	87.0 ± 3.7	83.2 ± 9.7	98.2 ± 7.2	96.8 ± 7.7	83.3 ± 10.5	85.8 ± 6.4
Cocoon viability (%)	88.4 ± 2.5	82.5 ± 7.3	93.0 ± 1.6	89.6 ± 3.6	92.5 ± 1.7	91.2 ± 0.8
Juveniles per cocoon	1.98 ± 0.20	1.88 ± 0.12	2.08 ± 0.18	1.93 ± 0.08	2.04 ± 0.15	2.08 ± 0.15
NRRT (min)	46 ± 6.0	54 ± 2.43	30 ±13.7	56 ± 4.0	40 ±10.1	56 ± 2.1

NRRT = neutral red retention time

Lead content in the trap and skeet range and reference site soils and correlation with earthworm neutral red retention time

Soil Pb concentrations in the trap and skeet ranges ranged from 17.5 to 292 and 0 to 6.9 mg/kg for total and $Ca(NO_3)_2$-extractable Pb, respectively (Table 3.3). Background total Pb in the reference sites ranged from 2.3 to 12.3 mg/kg, whereas $Ca(NO_3)_2$-extractable Pb was below the detection limit in all cases.

Soil total Pb concentrations were strongly correlated with $Ca(NO_3)_2$-extractable Pb concentrations ($r = 0.93$, $F_{1,15} = 98.6$, $p < 0.001$). The ratio of total Pb to $Ca(NO_3)_2$-extractable Pb was calculated for each pair (trap and skeet range and reference site), and the proportion of lead extracted by $Ca(NO_3)_2$ was significantly higher in soil from the Provost pair (1.06 ± 0.3%) than in soil from the Eastend pair (0.29 ± 0.08%, $p = 0.031$) and the Vegreville pair (0.39 ± 0.11%, $p = 0.048$). Total and $Ca(NO_3)_2$-extractable Pb concentrations for each soil sample were plotted against earthworm NRRT's and showed significant correlation for both extraction methods ($r = -0.73$, $F_{1,16} = 17.7$, $p = 0.001$ [Total], and $r = -0.80$, $F_{1,15} = 27.3$, $p < 0.001$ [$Ca(NO_3)_2$-extractable]; Fig. 3.3).

Earthworm lead body burdens and correlation with neutral red retention time and soil lead concentrations

Earthworm Pb body burdens ranged from 0.015 to 0.097 mg/kg in reference sites and 0.043 to 3.494 mg/kg in the trap and skeet range sites (Table 3.3). Soil total and $Ca(NO_3)_2$-extractable Pb levels were correlated with Pb body burdens ($r = -0.81$, $F_{1,16} = 31.3$, $p < 0.001$ [Total], $r = -0.82$, $F_{1,15} = 31.8$, $p < 0.001$ [$Ca(NO_3)_2$-extractable]; Fig.3.4). Earthworm Pb body burdens were also plotted against earthworm lysosomal

NRRTs and showed a significant negative correlation (r = -0.67, $F_{1,16}$ = 13.1, p = 0.002; Fig. 3.5).

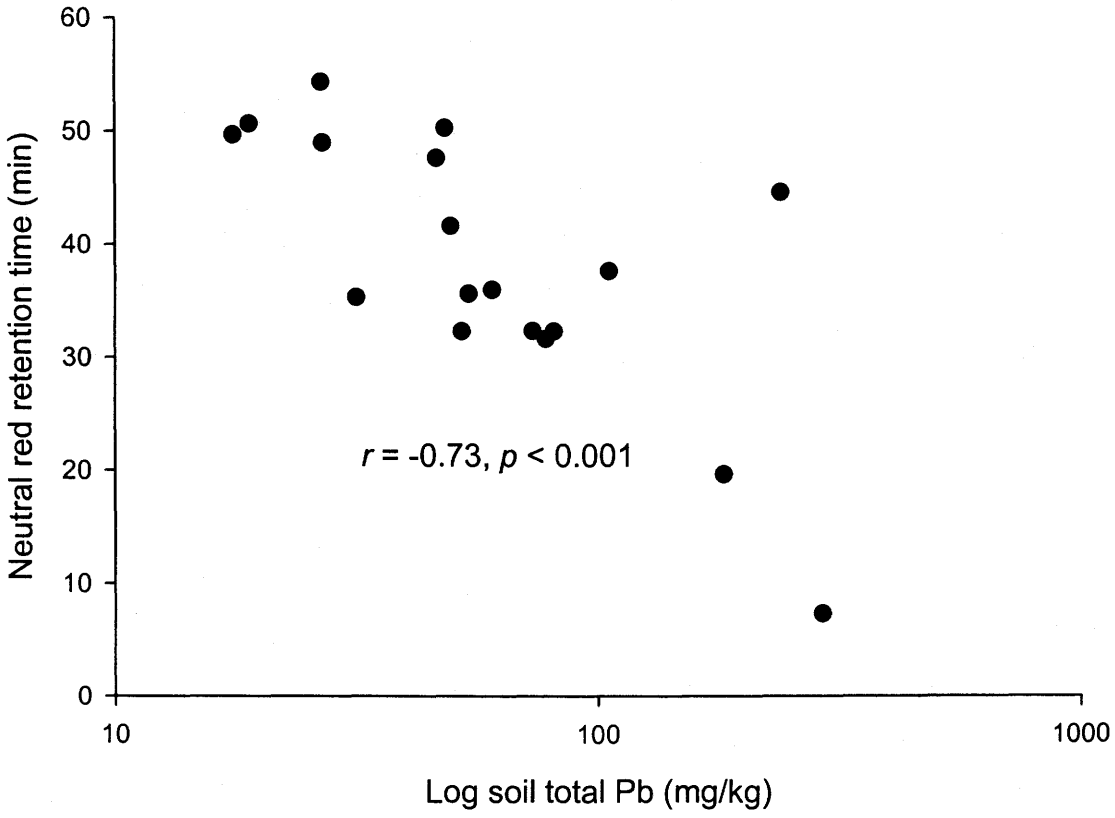
Table 3.3 Total and $Ca(No_3)_2$-extractable lead concentrations, and earthworm body burdens in trap and skeet range and reference soils, mean (range).

Site (N=6)	Total Pb (mg/kg)	$Ca(NO_3)_2$ - extractable Pb (mg/kg)	Pb tissue residues mg/kg
Eastend Site	31.79 (17.54 - 49.55)	0.1 (0.01 - 0.25)	0.106 (0.04 – 0.31)
Eastend Control	6.61 (4.33 – 8.00)	BDL [1]	0.034 (0.02 – 0.06)
Provost Site	159.61 (60.4 – 291.63)	2.12 (0.28 – 6.9)	1.39 (0.19 – 3.50)
Provost Control	3.50 (2.33 – 4.30)	BDL	0.021 (0.02 – 0.03)
Vegreville Site	55.95 (26.7 – 81.18)	0.23 (0 – 0.46)	1.09 (0.29 – 3.45)
Vegreville Control	7.66 (4.33 – 12.33)	BDL	0.076 (0.05 – 0.10)

[1]BDL = below detection limit (<0.005 mg/kg)
Pb = lead

Figure 3.3

(a)

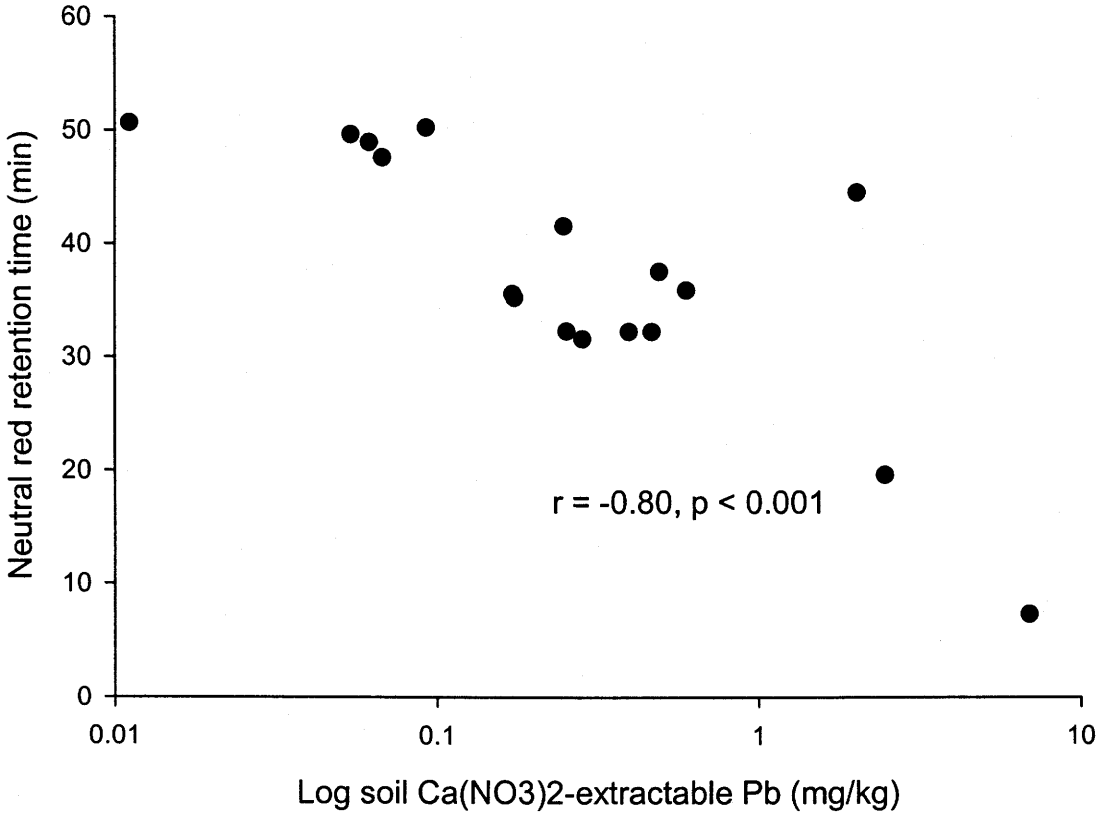


Pb = lead
NRRT = neutral red retention time

Fig. 3.3(a) Correlation of soil total lead with lysosomal neutral red retention time in *Eisenia fetida* exposed to lead-contaminated soil from Canadian prairie trap and skeet ranges for 28 d (n = 18).

Figure 3.3

(b)

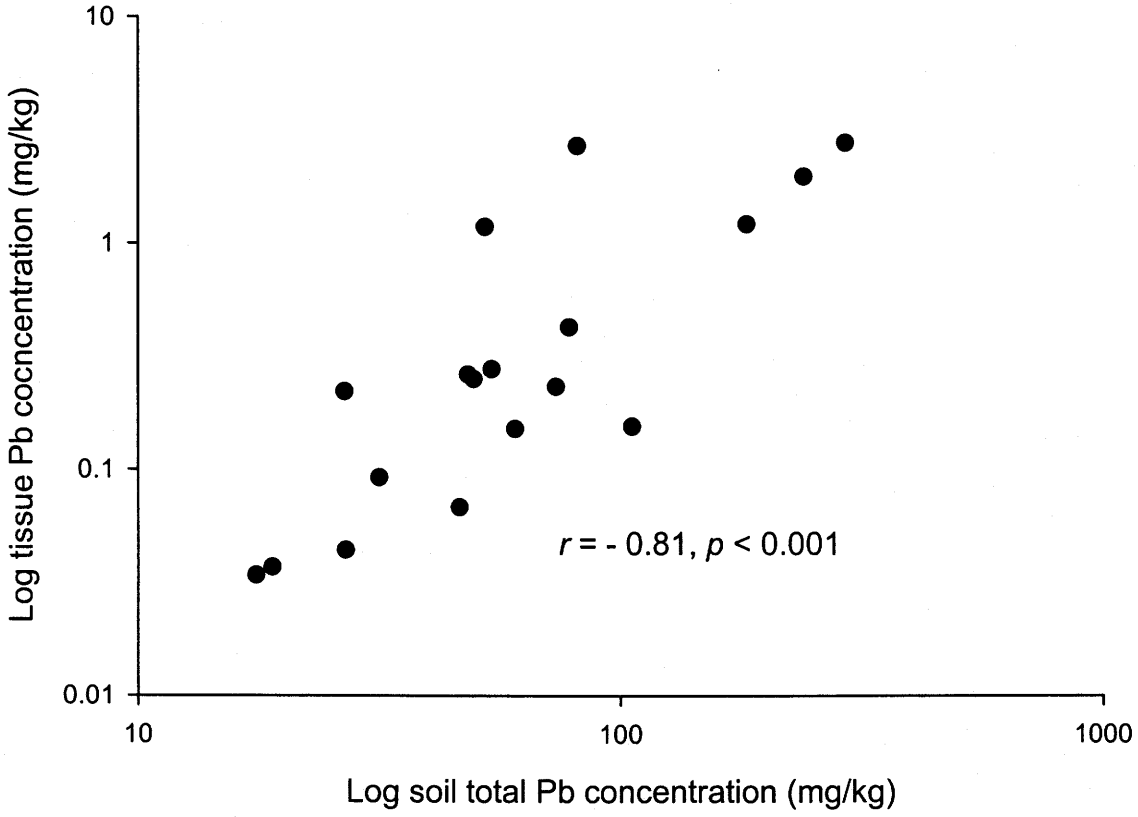


Pb = lead
NRRT = neutral red retention time

Fig. 3.3(b) Correlation of $Ca(NO_3)_2$-extractable lead with lysosomal neutral red retention time in *Eisenia fetida* exposed to lead-contaminated soil from Canadian prairie trap and skeet ranges for 28 d (n = 18).

Figure 3.4

(a)

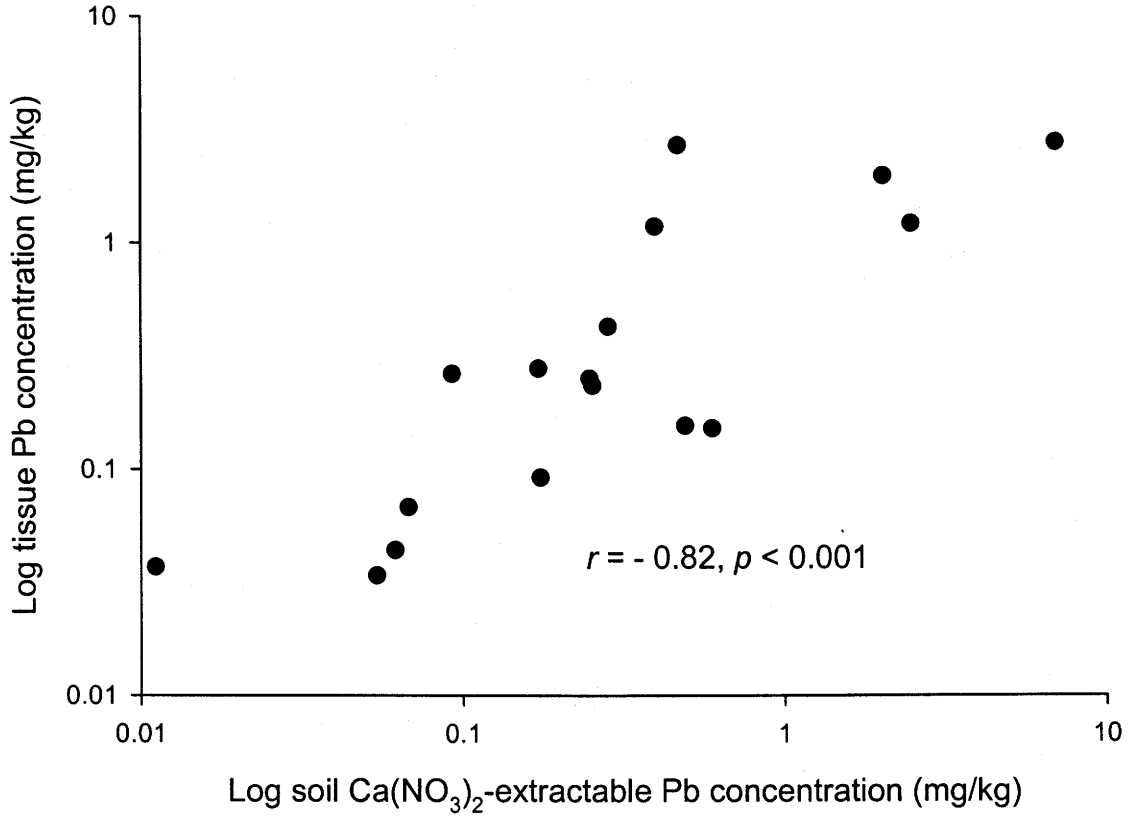


Pb = lead

Fig. 3.4(a) Correlation of soil total lead with lead body burdens in *Eisenia fetida* exposed to lead-contaminated soil from Canadian prairie trap and skeet ranges for 28 d (n = 18).

Figure 3.4

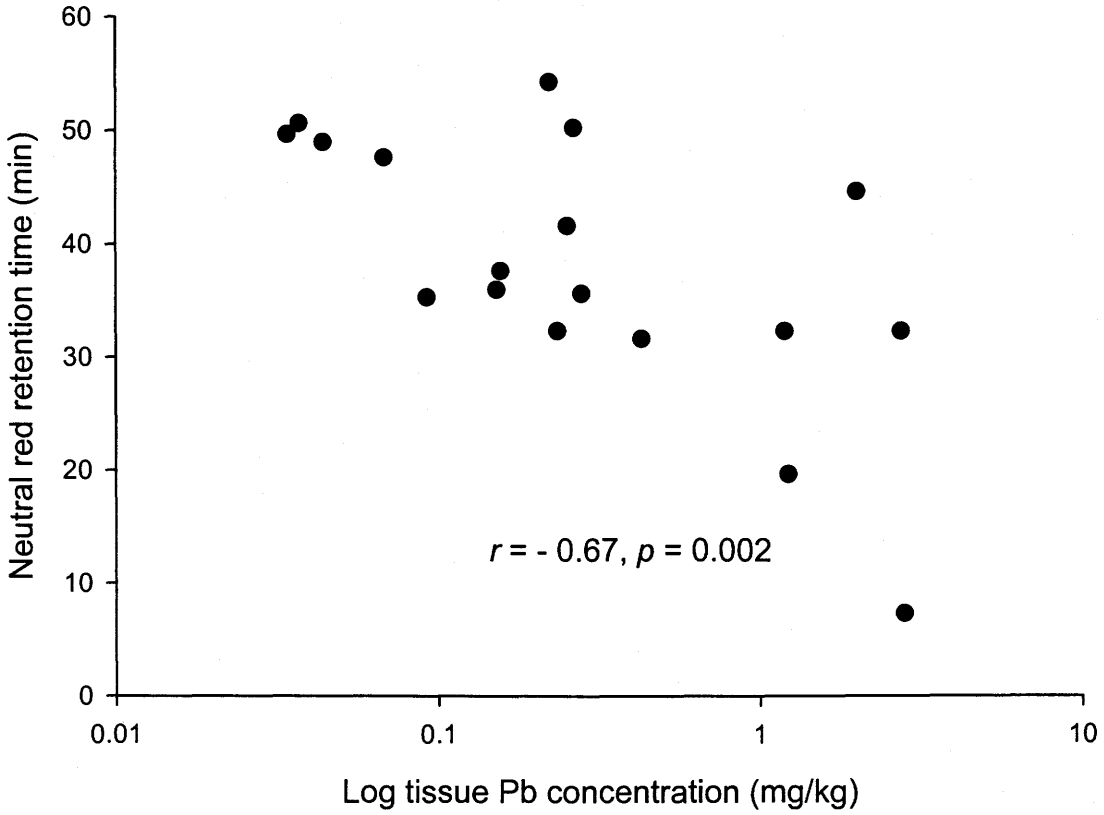
(b)



Pb = lead

Fig. 3.4(b) Correlation of soil $Ca(NO_3)_2$-extractable lead with lead body burdens in *Eisenia fetida* exposed to lead-contaminated soil from Canadian prairie trap and skeet ranges for 28 d (n = 18).

Figure 3.5



Pb = lead
NRRT = neutral red retention time

Fig. 3.5 Correlation of lead body burdens with lysosomal neutral red retention time in *Eisenia fetida* exposed to lead-contaminated soil from Canadian prairie trap and skeet ranges for 28 d (n = 18).

3.4 Discussion

To the authors' knowledge, only one previous study has collected earthworms from native, shooting range soils and assessed total body-Pb residues (Vyas *et al.* 2000), and no published reports have assessed biomarker responses in soil invertebrates from these sites. The goal of the present study was to measure an earthworm biomarker response (NRRT), Pb body residues, growth, and reproductive effects in relation to bioavailable and total soil Pb concentrations.

The effects of Pb on NRRT, Pb tissue residues, and measures of growth and fecundity were evaluated in the earthworm *E. fetida* using freshly spiked soil and soil containing aged Pb from three Canadian prairie shooting ranges. Neutral red retention time decreased in a concentration dependent fashion in earthworms exposed to Pb in freshly spiked soil (Fig. 3.1). This finding adds support to existing evidence that metals damage earthworm lysosomal membranes, making the NRRA a useful biomarker for detecting heavy metal exposure (Svendsen *et al.* 1996; Weeks and Svendsen 1996; Svendsen and Weeks 1997a; Svendsen and Weeks 1997b).

The three trap and skeet ranges used in the present study are likely to be representative of ranges in dry grassland ecosystems. Top soil in the shot fall zones of all three ranges was heavily contaminated with Pb shot pellets (shot densities averaged 5,308; 6,546; and 2,986 pellets/m^2 at the Eastend, Provost and Vegreville ranges, respectively) but soil total and bioavailable Pb concentrations did not reflect shot abundance (Table 3.3). The low levels of soil Pb observed may reflect low rainfall conditions and relatively neutral soil pH.

In spite of very low total and bioavailable soil Pb concentrations, the NRRT was also significantly reduced in all three trap and skeet shooting range soils compared to their respective reference sites (Table 3.3.). As with the spiked soil study (Fig. 3.2.), a good correlation was observed between NRRT and tissue Pb burdens (Fig. 3.5.). Although the NRRA appears to be a sensitive general biomarker for pollutant exposure in earthworms, with widespread applicability in terms of species and class of contaminant for both laboratory and field studies (Spurgeon *et al.* 2000; Svendsen *et al.* 1996; Weeks and Svendsen 1996), guidelines for risk assessment based on soil invertebrate biomarkers, such as NRRA, have not been developed. The adoption of the NRRA for risk assessment or routine screening of contaminated sites is dependent on establishing a clear link between the NRRT response and biologically important life cycle endpoints such as growth and fecundity. These endpoints are usually considered sensitive indicators of contaminant exposure. However, this study found no significant difference in growth or cocoon production in worms from any of the shooting range soils relative to reference sites (Table 3.3.). This finding is consistent with previous studies of Pb in earthworms. Reienecke and Reinecke (1996) reported that E. *fetida* exposed to 855.95 μg/g Pb in the medium, with a tissue Pb concentration of 41.81 μg/g, grew as well as controls, and produced as many cocoons. The authors concluded that growth, maturation and cocoon production may not be very sensitive indicators of Pb toxicity in E. *fetida*. Spurgeon *et al.* (1994) also reported that exposure to Pb at 2,000 mg/kg had no effect on growth or cocoon viability, but did reduce cocoon production. A study by Reinecke and Reinecke (1996), reported that E. *fetida* exposed to sublethal concentrations of 2000 μg/g Pb showed favourable growth and cocoon production relative to controls, while cocoon

viability was worse. Interestingly, the tissue Pb burden was 20.28 μg/g, which was roughly half of the previous study, even though the Pb concentration of the medium was considerably higher. The authors suggested that this variation was the result of the net effect of two opposing processes of uptake and depuration. The relative rate of these processes will depend on soil characteristics, including pH and organic matter. Consequently, the observed difference in tissue burdens between the two studies indicates that Pb concentration in earthworms may vary over time and does not always reliably represent exposure concentration. Since pH and organic matter vary greatly from site to site and sometimes from day to day within sites, such results represent a mere "snapshot" at the moment of sampling. Other contradictory findings support this conclusion. For example, Terhivuo *et al.* (1994) reported tissue Pb burdens of 75.6 ± 46 μg/g in the earthworm *L. casteneus* with soil concentrations of 412 to 79,963 mg/kg. These tissue burdens were far less than those reported by Svendsen *et al.* (1996), where the same species of worm had tissue Pb burdens of 348 ± 72 μg/g when exposed to soil containing no more than 200 mg/kg Pb. Bengtsson *et al.* (1996) reported that low metal concentrations can stimulate cocoon production, while Spurgeon and Hopkins (1995) concluded conversely that cocoon production was the most sensitive indicator of Pb toxicity. It is worth noting that all of the studies of Pb toxicity cited were conducted in artificial soil or cattle manure. The physical/chemical characteristics of artificial soil can increase the bioavailability of some contaminants compared to natural soil (Spurgeon and Hopkin 1995). In the present study using natural soil, it is clear that low level Pb exposure (Table 3.2) did not affect growth or fecundity in *E. fetida*. Obviously, the use of life cycle parameters in the assessment of soil invertebrate metal toxicity must be

accompanied by an adequate description of soil characteristics in order to be interpretable.

Soil characteristics affect the behaviour of persistent compounds such as metals, and may greatly alter metal bioavailability to organisms (Peijnenburg *et al.* 1999). Since only living organisms can determine bioavailability, the traditional total metal-based expression of exposure is inappropriate. Body residues are often better estimates of a chemical at the site of action in an organism (Peijnenburg *et al.* 1999; Conder and Lanno 2000). However, such measurements are costly and time consuming. The use of weak-electrolytic extraction as a surrogate measure of bioavailability is promising (Conder and Lanno 2000). Previous studies with other soil invertebrates and Zn have demonstrated the relationship between extractable metals and bioavailability (Marinussen *et al.* 1997; Van Gestel *et al.* 1998). Conder and Lanno (2000) and Conder *et al.* (2001) evaluated $Ca(NO_3)_2$ extraction as a surrogate for metal bioavailability to *E. fetida*, and reported that the $Ca(NO_3)_2$-extractable fraction was a better predictor of acute toxicity than soil total metal levels.

In this study, soil $Ca(NO_3)_2$-extractable Pb was strongly correlated with NRRT and earthworm Pb body burdens (Fig. 3.3b and 3.4b) but there were no obvious differences for either parameter when results were based on total or $Ca(NO_3)_2$-extractable Pb (i.e., similar correlation coefficients were found for both total and $Ca(NO_3)_2$-extractable Pb for both NRRT and body burdens). Consequently, the $Ca(NO_3)_2$-extractable fraction did not appear to be a better predictor of earthworm biomarker responses or body burden than total Pb levels. This is most likely due to the low Pb

levels recorded at the trap and skeet ranges and the significant correlation ($r = 0.93$) between total and extractable Pb levels for the three sites studied.

Neutral red retention time was significantly reduced by exposure to freshly spiked soil and aged Pb-contaminated soil, and was a more sensitive indicator of exposure than growth or fecundity. The NRRA may be a useful tool in predicting tissue Pb levels. However, due to the lack of adverse effects on growth or fecundity, the usefulness of the NRRA as a warning of long-term effects could not be determined in the present study. In the future, biomarker responses should be evaluated over a higher range of Pb concentrations to see if NRRT may be linked to important life cycle parameters.

Chapter 4. Bioavailability and Toxicity of Lead Shot Pellets to Small Mammals from Canadian Prairie Trap and Skeet Shooting Ranges

4.1 Introduction

Lead (Pb) poisoning is a well-documented cause of morbidity and mortality in wildlife, with waterfowl being most commonly affected. Both the United States and Canada have banned the use of Pb-shot for waterfowl hunting as a result of millions of Pb-induced deaths of many waterfowl species, as well as secondary poisonings of numerous raptorial birds (Sanderson and Bellrose 1986, Wayland and Bollinger 1999). However, Pb-shot continues to be used for recreational target shooting and upland game bird and small mammal hunting in both countries. The incidence of Pb toxicosis in birds and terrestrial mammals associated with these activities is less well known. Most cases of Pb exposure or poisoning among terrestrial wildlife appear to be associated with localized, highly contaminated areas, such as mining or smelting sites or trap and skeet shooting ranges (Ma 1989; Pascoe *et. al* 1994; Stansley and Roscoe 1996; Lewis *et. al* 2001).

The rise in the popularity of trap and skeet shooting in the United States and Canada has increased concern over potential ecological effects of spent Pb-shot in terrestrial habitats (Kendall *et. al* 1996). In the United States an estimated 12,800,000 people participate in recreational target shooting sports (http://www.nsga.org/guests/research/participation/participation.html). Prior to the ban on the use of Pb shot for waterfowl, hunters and recreational shooters were responsible for the annual deposition of 1,500 tonnes of Pb as Pb-shot pellets in the Canadian environment (Scheuhammer and Norris 1996). It is unclear what proportion of that total

was confined to Canadian shooting ranges, but studies in other countries indicate that the magnitude of environmental Pb contamination at some shooting ranges is staggering. At one Finnish range, Tanskanen *et al.* (1991) estimated Pb shot deposition at 15 tonnes per year, while in Denmark Jorgensen and Willems (1987) reported annual Pb loads at three separate shooting ranges to vary between 240 kg and 5000-6000 tonnes annually. Lin (1996) estimated 2800 kg of Pb shot had been deposited at one Swedish shooting range in one year. Studies in New Zealand, the United States, Switzerland and England have also reported large quantities of Pb shot deposition at trap and skeet shooting ranges (Mellor and McCartney 1994; Murray and Bazzi 1995; Braun *et al.* 1997; Murray *et al.* 1997; Rooney *et al.* 1999).

Once deposited into soil, Pb-shot pellets are slowly oxidized, resulting in the release of Pb compounds into the soil. Jorgensen and Willems (1997) noted that metallic Pb pellets in the soil environment are transformed into a crust-like material that is composed mainly of $Pb_3(Co_3)_2(OH)_2$ and $PbCO_3$. Depending on soil pH, this crust material may be rapidly dissolved, producing the more soluble and bioavailable Pb^{2+} species. Ma (1989) demonstrated that in acidic, sandy soil at a shooting range and smelter site, Pb was transformed to a more bioavailable form (probably Pb^{2+}), and readily entered the terrestrial food chain via uptake into soil invertebrates and to a lesser extent plants. Reports of Pb uptake by plants at trap and skeet ranges are few, and most studies of Pb behaviour have indicated minimal translocation of Pb from roots into leaves (Adriano 1986). Consequently, insectivorous or carnivorous small mammals from Pb contaminated sites tend to have higher tissue Pb concentrations than related herbivorous species (Ma *et al.* 1991). However, even if dietary transfer is low, herbivores may be

exposed to free soil Pb or Pb pellets through incidental soil ingestion while feeding or grooming. For example, strictly herbivorous bank voles (*Clethrionomys glareolus*) demonstrated elevated kidney and bone Pb concentrations at an inactive skeet shooting range in The Netherlands (Ma 1989).

The objectives of this study were to assess the bioavailability and biological effects of Pb from aged Pb shot pellets deposited in dry-land prairie soil, at three different trap and skeet ranges in western Canada. In spite of the popularity of recreational shooting, no studies have examined the behaviour and toxicity of Pb in this ecosystem. Bioavailability and potential food chain transfer were determined by measuring Pb in soil, plants, insects and small mammals. Potential adverse effects of Pb at the trap and skeet ranges were evaluated by measuring δ-aminolevulinic acid dehydratase (ALAD) activity in small mammals living in the shot fall zone.

4.2 Materials and Methods

Study site selection

Study sites were identified as outlined in Chapter 2. Trap and skeet ranges at Eastend, Saskatchewan, and at Provost and Vegreville, Alberta, were selected for this investigation and field work was subsequently conducted in the summer of 2001. Matched reference sites for each shooting range were also identified based on similar plant community composition and soil characteristics.

Soil and biota sample collection

A visual survey was conducted at each trap and skeet shooting range to determine the location of the shot-fall zone. The total area of the shot-fall zone was measured, and the area of greatest shot density was determined. A grid pattern was established for collection of soil and plant samples within the area of greatest shot density. Each row and column was approximately 10 m apart. Soil samples were collected using a cylindrical soil corer (12-cm diameter), to a depth of 12 cm, including turf. A total of 24 soil samples were collected at each shooting range and its paired reference site. Samples with even numbers were used for Pb pellet counts, and to measure total and $Ca(NO_3)_2$-extractable Pb concentrations. Samples with odd numbers were used for measurement of soil pH and organic carbon content. Plant specimens were collected for analysis of Pb uptake by clipping all plants within a 1 m radius of each soil sampling location. Soil sample locations with even numbers were used for broadleaf plant collection, while locations with odd numbers were used for grasses. Plants were clipped at the soil surface, placed in plastic bags, and frozen at -20°C for subsequent Pb analysis

An attempt was made at each shooting range to capture surface dwelling soil invertebrates using pit-fall traps. However, due to the record drought conditions across the Canadian prairies in the summer of 2001, sample size was inadequate for analysis. Consequently, a decision was taken to sample grasshoppers as a component of the food chain, and as the only macroinvertebrate family present in sufficient quantities at all sites. Grasshoppers were collected using sweep nets within the shot-fall zone of each shooting range and at each matched reference site. Twelve samples consisting of several

individual grasshoppers each were collected at each site, and were frozen in separate plastic bags at -20°C for subsequent Pb analysis.

Ground squirrels (*Spermophilus richardsonii*, *Spermophilus tridecemlineatus*) and deer mice (*Peromyscus maniculatus*) were captured at the Eastend and Provost shooting ranges and their matched reference sites using live traps (Tomahawk LiveTrap Company, Tomahawk, Wisconsin, USA). Traps were baited with a mixture of peanut butter and oatmeal, and were placed within the perimeter of the shot-fall zone at each range. The ground squirrel traps were checked every 2-3 hours during the day. Mousetraps were set only at night, and were checked every morning.

Captured ground squirrels and mice were anesthetised with halothane gas (MTC Pharmaceuticals, Cambridge, ON, Canada) followed by peritoneal injection of a mixture of 90 mg/ml ketamine (Vetrepharm Canada Inc., Belleville, ON, Canada) and 2 mg/ml xylazine (Bayer Inc., Toronto, ON, Canada) at 1.4 ml/kg (based on personal communication with Dr. Nigel Caulkett, University of Saskatchewan, Western College of Veterinary Medicine, Saskatoon, SK, Canada). Blood samples were collected from the posterior venae cava, and the animals were euthanized by exsanguination. A portion of the total volume collected was stored in heparanized Microtainer® tubes (Becton Dickinson Vacutainer Systems, Franklin Lakes, New Jersey, USA) for blood Pb analysis. These samples were shipped on ice to the Toxicology Laboratory, Prairie Diagnostic Services, Saskatoon SK, Canada and were analyzed for blood Pb within 2-3 days of collection. The remainder of each blood sample was transferred to cryovials and frozen in liquid nitrogen for subsequent δ-aminolevulinic acid dehydratase activity analysis. The liver, kidneys and femur were excised, placed in Whirlpac® bags (VWR

International, Mississauga, ON, Canada) and frozen at -20°C for tissue Pb analysis. Small mammal body weight and kidney weight were measured using a Sartorius® GE701 (Sartorius Inc, Bradford MA, USA) portable scale for kidney to body weight determinations.

Analysis of soil total lead; $Ca(No_3)_2$-extractable lead, and soil characteristics

Lead shot pellets were removed from all soil samples by dry sieving (ASTM Standard Test Sieve. No.12., Tyler, Texas, USA.). Pellets were counted individually and the results used to estimate pellet density in the shot fall zone. The total mass of Pb pellets per square metre was also determined to estimate Pb loads at each shooting range.

Unless otherwise indicated, laboratory disposables and reagents were obtained from VWR International (Mississauga, ON, Canada). Total Pb concentrations in soil from the trap and skeet ranges and reference sites was determined in triplicate by wet digestion of a 0.5 g (dw) soil sample using a mixture of ACS grade hydrochloric, nitric, and perchloric acids ($HCl:HNO_3:HCLO_4$ [15:5:3 ml]). The soil-acid mixture was digested for 24 hours at room temperature, followed by digestion on a hot plate at 100°C for approximately 3 hours. The digest was brought to volume in 1% HNO_3 in 50 ml volumetric flasks and left overnight to allow particulates to settle. The supernatant was filtered through a 0.45 μm Gelman® (Pall Gelman Laboratory Products, Ann Arbor, MI, USA) nylon membrane syringe tip filter into 8 ml Nalgene® sample vials. The samples were stored at 4°C for subsequent analysis using a Varian graphite furnace atomic absorption spectrophotometer (110/220Z) with Zeeman background correction (Varian, Walnut Creek, CA, USA).

Weak electrolyte-extractable Pb was measured using 0.1M $Ca(NO_3)_2$ extraction (Marino and Morgan 1999). A 1 g (dw) soil sample was mixed with 20 ml of 0.1M $Ca(NO_3)_2$ and placed on a rotary mixer for 16 hours at room temperature. Samples were then centrifuged at 2500 g for 15 minutes and filtered through 0.45 μm polyvinylidene fluoride membrane filter. The final extract was acidified with 1 ml of concentrated trace-metal grade HCL (Fisher Scientific Ltd, Nepean, ON, Canada). Samples were analyzed using a Perkin-Elmer Analyst 700 graphite furnace atomic absorption spectrometer using L'vov platform tubes.

Soil pH was measured in duplicate by thoroughly mixing 10 g (dw) of soil with 25 ml 0.01 M $CaCl_2$ solution, then allowing the suspension to sit for 30 min before measurement. Soil organic carbon was measured using a LECO CR-12 Carbon Analyzer (LECO, St. Joseph, MI, USA). A 0.2-0.4 g sample of finely ground (40 mesh), air-dried soil was analyzed at a temperature of 840°C, with a measured oxygen flow of 3.6 L/min, and a lancing flow of 1.0 L/min (Wang and Anderson 1998).

Analysis of plant total lead concentrations

Plant species collected at each site were identified at the University of Saskatchewan Department of Agriculture Herbarium, Saskatoon, SK, Canada. Samples were then oven dried for 24 hours at 80°C in paper bags, and ground with a mortar and pestle. Digestions were done in triplicate by adding 0.5 g of dried, ground sample to 20 ml of analytical grade HNO_3 and 3 ml $HClO_4$. Samples were digested at room temperature overnight, followed by digestion on a hot plate at 100°C for approximately 3 hours. The digests were brought to volume in 25 ml volumetric flasks using 1% HNO_3,

and treated as described for soil Pb analysis. A Varian graphite furnace atomic absorption spectrophotometer (110/220Z) with Zeeman background correction (Walnut Creek, CA, USA) was used to measure Pb concentration.

Analysis of grasshopper lead body burdens

Grasshopper specimens were identified to species by Mr. Murray Braun at Agriculture and Agri-Food Canada, Saskatoon SK, Canada. A random sub sample was taken from each of the 12 bags collected at each site and pooled to obtain a 0.3 g (dw) sample for Pb analysis. Grasshopper sub samples were digested and analyzed as described for plants.

Analysis of small mammal blood and tissue lead concentrations

Blood Pb concentrations were measured in fresh blood samples collected from ground squirrels and mice using a model 3010A ESA trace metal analyzer (ESA Inc., Chelmsford, Massachusetts, USA) at the Toxicology Laboratory, Prairie Diagnostic Service, Saskatoon, SK, Canada.

Ground squirrel tissue samples were treated as follows: 1 g (ww) of liver was added to 20 ml analytical grade HNO_3 and 3 ml $HCLO_4$; 0.5 g (ww) kidney was added to 10 ml HNO_3 and 1.5 ml $HCLO_4$; individual femur weights were recorded, and the entire sample was added to 20 ml of HNO_3 and 3 ml of $HCLO_4$ acid. Mouse tissue samples were treated as follows: 0.5 g of liver (unless sample size was too small, in which case the actual liver weight was recorded) was added to 10 ml HNO_3 and 1.5 ml $HCLO_4$;

individual kidney and femur weights were recorded, and the entire sample was added to 10 ml HNO_3 and 3 ml $HCLO_4$.

Ground squirrel and mouse tissues for Pb body burden measurement were subsequently digested in 50 ml beakers on a hotplate at 100°C for approximately 2-3 hours. The digests were brought to volume in 25 ml volumetric flasks using 1% HNO_3, and the samples were treated as described for soil Pb analysis. Lead concentration was measured using a Varian graphite furnace atomic absorption spectrophotometer (110/220Z) with Zeeman background correction.

δ -aminolevulinic acid dehydratase (ALAD) activity assays

The activity of the enzyme δ -aminolevulinic acid dehydratase in small mammal erythrocytes was determined using the method described by Fujita *et al.* (1981). The assay was conducted in duplicate in 1.5 ml microcentrifuge tubes on ice. Each tube contained 50 μl erythrocyte lysate (blood sample stored in liquid nitrogen), and 238 μl of ice-cold 100 mM Tris-acetate buffer (pH 7.2). After mixing, 12 μl of 100 mM aminolevulinic acid-HCl was added, and the tubes were incubated at 37°C for 60 min. The reaction was terminated by adding 300 μl of ice cold 10% trichloroacetic acid (TCA)/0.1 M $HgCl_2$ and mixing well. The mixture was subsequently centrifuged at 1000g for 5 min at room temperature. In a fresh tube, 500 μl of supernatant was combined with 500 μl of modified Ehrlich's reagent (1 g of *p*-dimethylaminobenzaldehyde [Sigma-Aldrich Canada, Oakville, ON, Canada] in 30 ml glacial acetic acid and 16 ml 70 % perchloric acid brought to 50 ml volume with acetic acid). The reagent blank consisted of equal volumes of 10% TCA/0.1 mM $HgCl_2$ and

modified Ehrlich's reagent. Samples were allowed to sit for 5 to 10 min at room temperature, and the absorbance at 555 nm was measured in 1-ml semimicro cuvettes using a Beckman Coulter DU 640 spectrophotometer (Beckman Coulter Inc., Fullerton, CA, USA).

The amount of enzymatically synthesized porphobilinogen (PBG) was estimated as an Ehrlich's-PBG colour salt with a molar absorption coefficient of 6.2×10^4 at 555 nm (Mauzerall and Granick, 1956). One unit (U) of enzyme activity is defined as 1 μmol of PBG formed per hr at 37°C. Enzyme activity in erythrocytes is expressed as U/ml packed cells, and calculated according to the following equation:

(A_{555}/extinction coefficient) × (assay volume/sample volume) × (dilution with TCA/$HgCl_2$) × (dilution with Ehrlich's reagent) × (60/15) × (1/1000) × 1000

Statistical analysis

Statistical analysis (Sanders *et al.* 2000) for lead concentrations in grasses, broadleaf plants, and grasshopper tissues were compared using the Mann-Whitney rank sum test ($p \leq 0.05$), since the data did not meet the assumptions of normality. Soil total Pb levels were correlated with tissue Pb concentration using Pearson product moment correlation in SigmaStat® after data were log transformed.

Small mammal blood and tissue Pb concentration data did not meet assumptions of normality and were analyzed using a Mann Whitney-rank sum test ($p \leq 0.05$). Comparisons of blood ALAD activity in ground squirrels from the Eastend shooting range and reference site were made using a t-test ($p \leq 0.05$). Eastend mouse and Provost ground squirrel blood ALAD activity data were analyzed using Mann-Whitney rank sum

order ($p \leq 0.05$), since data were not normally distributed. Kidney to body weight ratios were analyzed using a t-test ($p \leq 0.05$).

Correlation of small mammal blood Pb concentration with ALAD activity and tissue Pb concentration were performed in SigmaStat® using the Pearson product moment.

4.3. Results

Lead concentrations in shooting range and reference site soils, and grasses and broadleaf plants

Results of soil pH, organic carbon concentration and soil texture analyses are listed in Table 4.1. The Eastend and Provost sites were relatively similar with sandy loam soils with low organic matter and neutral pH. The Vegreville site was characterized by high clay and organic carbon content, and was more acidic. Soil characteristics were similar between shooting ranges and their respective reference sites. Mean shot pellet mass in the surface soil at the three shooting ranges ranged from 287.44 - 467.09 g/m^2 (Table 4.2). Mean soil Pb concentrations at the shooting ranges ranged from 27.5 to 171 mg/kg, and 0.1 to 2.12 mg/kg for total and $Ca(NO_3)_2$-extractable Pb, respectively (Table 4.2). Background total Pb in the reference sites ranged from 3.53 to 9.78 mg/kg, whereas $Ca(NO_3)_2$-extractable Pb was below detection limits in all cases (Table 4.2).

Table 4.1. Soil characteristics of Canadian prairie trap and skeet ranges and matched reference sites.

N=12	pH (mean ± std. dev)	Organic Carbon (%) (mean ± std. dev.)	Soil Texture
Eastend Site	7.34 ± 0.21	1.11 ± 0.42	Sandy loam to clay loam
Eastend Control	7.06 ± 0.22	1.26 ± 0.53	Sandy loam to clay loam
Provost Site	6.38 ± 0.36	2.69 ± 0.45	Sandy loam to loamy sand (>50% sand, 5-10% clay)
Provost Control	7.13 ± 0.24	2.00 ± 0.91	Sandy loam to loamy sand (>50% sand, 5-10% clay)
Vegreville Site	5.51 ± 0.51	7.89 ± 3.34	Loam to clay loam (35-40% sand, 20-30% sand)
Vegreville Control	5.77 ± 0.62	6.62 ± 3.32	Loam to clay loam (35-40% sand, 20-30% sand)

Table 4.2. Total and $Ca(No_3)_2$-extractable soil lead concentrations and lead shot distribution at trap and skeet ranges and matched reference sites.

Site	Total Pb (mg/kg) (n = 12)	$Ca(NO_3)_2$ - extractable Pb (mg/kg) (n = 6)	Mean pellet density (pellets/m^2) (n = 12)	Mean pellet mass (g/m^2) (n = 12)
Eastend Site	28 ± 21[a] (7 – 71)[b]	0.1 (0.01 - 0.25)	5308	467.09
Eastend Control	7 ± 1 (4 - 8)	BDL [c]	0	0
Provost Site	171 ± 85 (68 – 323)	2.12 (0.3 – 6.9)	6546	437.00
Provost Control	4 ± 1 (2 – 5)	BDL	0	0
Vegreville Site	50 ± 48 (7 – 136)	0.23 (0 – 0.5)	2424	287.44
Vegreville Control	10 ± 10 (3 – 38)	BDL [c]	0	0

[a] = mean ± std. dev.
[b] = range
[c]BDL = below detection limit (<0.005 mg/kg)
Pb = lead

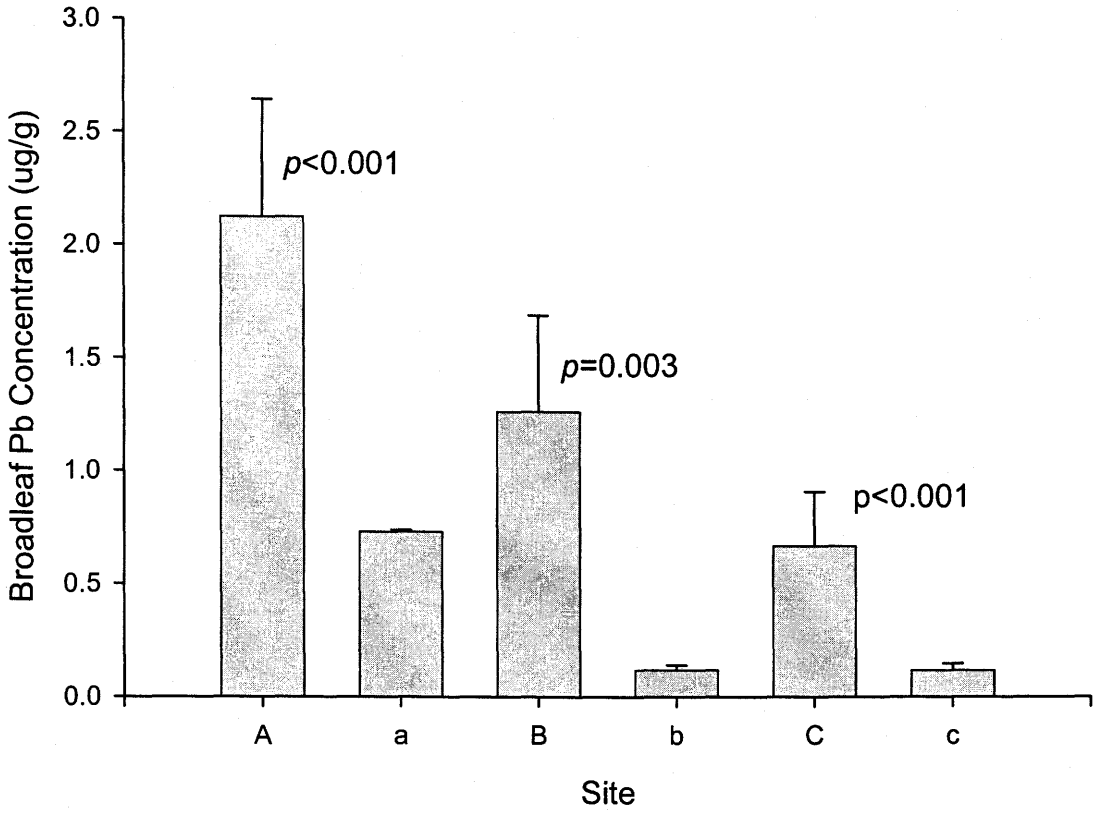
Table 4.3. Plant species identified at each shooting site and its matched reference site.

Site	Grasses	Broadleaf plants
Eastend Site	*Agropyron dasystachyum* *Artemisia frigida* *Bouteloua gracilis* *Malvastrum coccineum*	*Artemisia cana*
Eastend Control	*Agropyron sibricum*	*Artemisia cana*
Provost Site	*Agropyron cristalium* *Agropyron trachycaulum* *Bromis Inermis* *Poa pratensis*	NA
Provost Control	*Agropyron repens* *Bromus inermis*	NA
Vegreville Site	*Deschampsia caespitose* *Juncus balticus* *Poa pratensis*	NA
Vegreville Control	*Agropyron trachycaulum* *Distichlis stricta* *Poa pratensis*	NA

NA = samples were not available for collection

Lead concentration in grasses was significantly greater at the Eastend ($p<0.001$), Provost ($p=0.003$), and Vegreville ($p<0.001$) shooting ranges than at their matched reference sites (Fig. 4.1). Lead concentration in *Artemisia cana*, the only common broadleaf plant identified, was also greater at the Eastend shooting range than at the reference site ($p<0.001$) (Fig. 4.2).

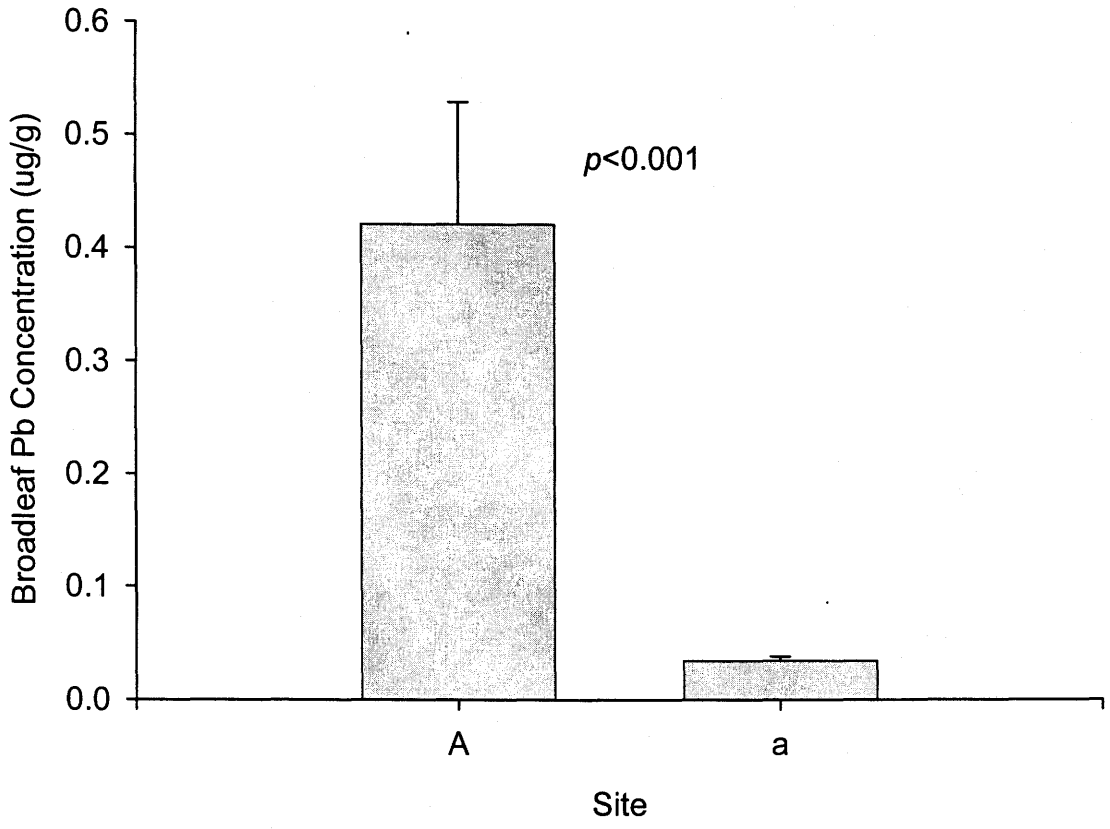
Fig. 4.1



A- Eastend shooting range
a- Eastend reference site
B- Provost shooting range
b- Provost reference site
C- Vegreville shooting range
c- Vegreville reference site
Pb = lead

Fig. 4.1. Lead concentrations in grasses (mean ± std. dev.) from prairie shooting ranges and their paired reference sites.

Fig. 4.2



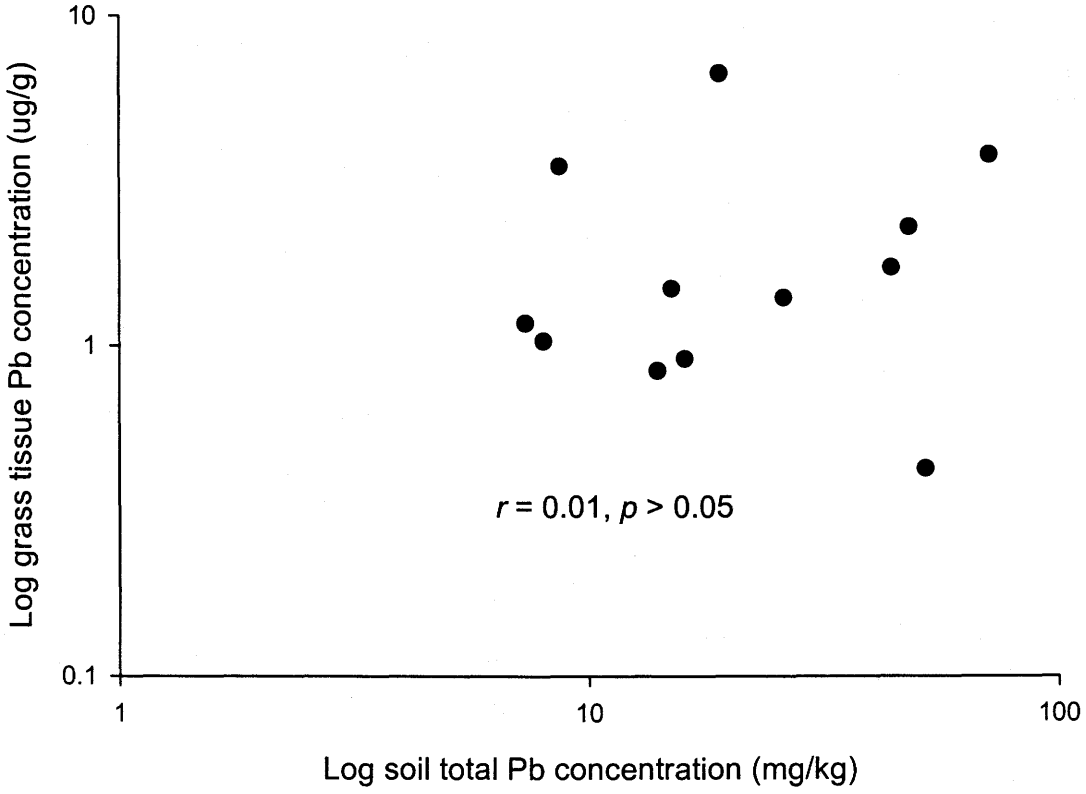
A- Eastend shooting range
a- Eastend reference site
Pb = lead

Figure 4.2. Lead concentration in *Artemisia cana* (mean ± std. dev.) from the Eastend shooting range and its reference site.

Soil total Pb concentration was not strongly correlated with grass tissue Pb concentration at the Eastend (r=0.01, p>0.05), Provost (r=0.38, p>0.05), or Vegreville (r=0.17, p>0.05) shooting ranges (Fig. 4.3a, 4.3b, 4.3c, respectively), or with Pb concentration in *Artemisia cana* at the Eastend shooting range (r=0.5, p>0.05) (Fig. 4.4).

Figure 4.3

(a)

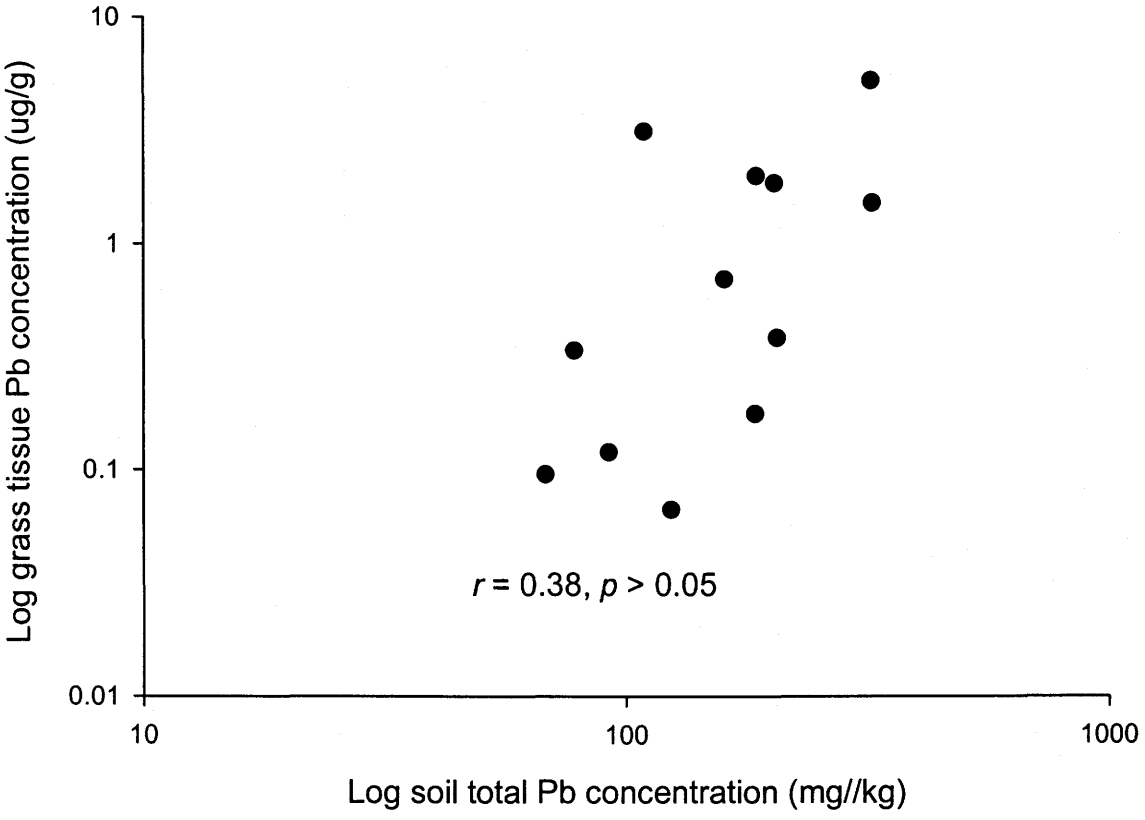


Pb = lead

Fig. 4.3 (a). Correlation of soil total lead concentration and lead concentration in grasses from the Eastend shooting range (n = 12).

Figure 4.3

(b)

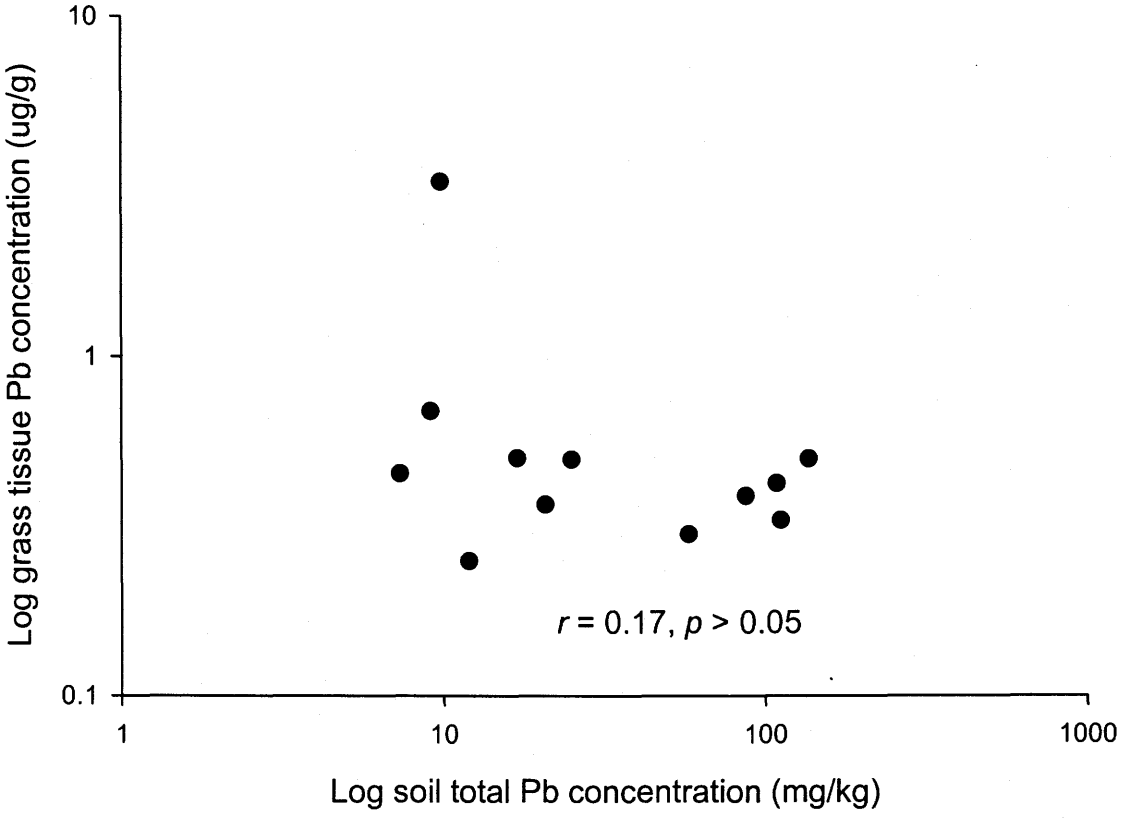


Pb = lead

Fig. 4.3 (b). Correlation of soil total lead concentration and lead concentration in grasses from the Provost shooting range (n = 12).

Figure 4.3

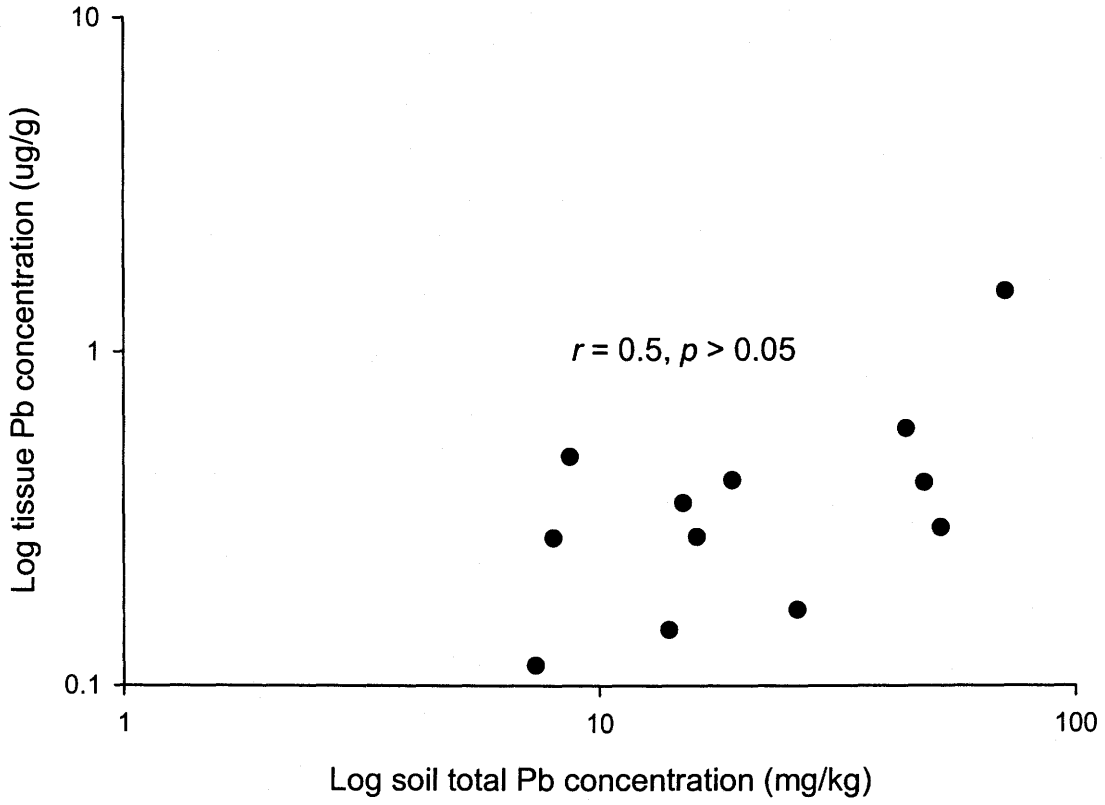
(c)



Pb = lead

Fig. 4.3 (c). Correlation of soil total lead concentration and lead concentration in grasses from the Vegreville shooting range (n = 12).

Figure 4.4



Pb = lead

Fig. 4.4. Correlation of soil total lead concentration and lead concentration in *Artemisia cana* at the Eastend shooting range (n = 12).

Lead concentration in grasshoppers

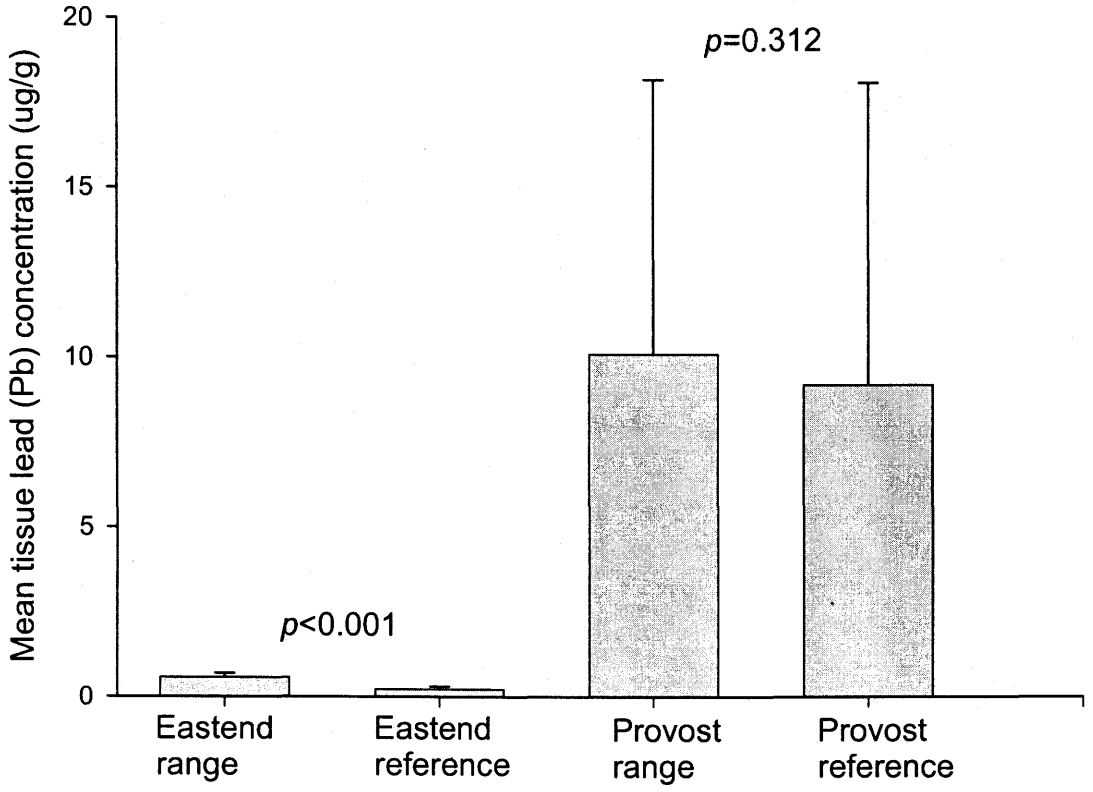
Table 4.4 lists grasshopper species identified at the Eastend and Provost shooting ranges, and their matched reference sites. Lead body burdens in grasshoppers collected on the shot fall zone at the Eastend shooting range were higher than at the reference site (p<0.001) (Fig. 4.5). However, there was no difference in Pb concentrations in grasshoppers from the Provost shooting range compared to its reference site (p=0.312).

Table 4.4. Grasshopper species identified at each shooting range and its matched reference site.

Eastend range	Eastend reference	Provost range	Provost reference
Ageneotettix deorum	*Ageneotettix deorum*	*Camnula pellucida*	*Camnula pellucida*
Encoptolophus costalis	*Encoptolophus costalis*	*Chorthippus curtipennis*	*Aeropedellus clavatus*
Hesperotettix viridis pratensis	*Camnula pellucida*	*Melanoplus bivittatus*	*Chorthippus curtipennis*
Melanoplus flavidus	*Melanoplus flavidus*	*Melanoplus confusus*	
Melanoplus gladstoni	*Melanoplus gladstoni*	*Melanoplus infantalis*	*Melanoplus infantalis*
Melanoplus infantalis	*Melanoplus infantalis*	*Melanoplus packardi*	*Melanoplus packardi*
Melanoplus kennicotti kennicotti	*Chorthippus curtipennis*	*Melanoplus sanguinipes*	*Melanoplus sanguinipes*
Melanoplus packardi	*Melanoplus packardi*		
Melanoplus sanguinipes	*Melanoplus sanguinipes*		
Phoetaliotes nebrascensis	*Melanoplus dawsonii*		
Stenobothrus brunneus	*Stenobothrus brunneus*		

Both Provost and reference site grasshoppers had tissue concentrations that were much greater than those from the Eastend sites.

Fig. 4.5



Pb = lead

Fig. 4.5. Lead concentration in grasshoppers (mean ± std. dev.) at two prairie shooting ranges and their matched reference sites.

Blood and tissue lead concentration in small mammals trapped at two prairie trap and skeet shooting ranges

Median blood Pb concentrations for all small mammals at the Eastend and Provost shooting ranges ranged from 0.004 to 0.014μg/ml, and from 0.001 to 0.003μg/ml

at the reference sites (Table 4.5). In spite of relatively low blood Pb concentrations in the exposed animals, both ground squirrels and mice from the Eastend shooting range had elevated values compared with controls (p=0.048 and 0.043, respectively). No difference was observed in blood Pb concentrations from ground squirrels at the Provost range compared with the reference site (p=0.087), perhaps due to relatively smaller sample sizes.

No difference was observed in kidney to body weight ratios in both ground squirrels and mice from the Eastend shooting range compared with controls (p>0.05 and p>0.05, respectively) and ground squirrels from the Provost site compared with controls (p>0.05) (Table 4.5).

Table 4.5. Kidney to body weight ratios in small mammals trapped at Canadian shooting ranges and matched reference sites. Values are means.

Site	Species and (n)	Kidney to Body Weight Ratio (%)	p=(0.05)
Eastend Site	*Spermophilus richardsonii* (n = 18)	0.01	>0.05
Eastend Control	*Spermophilus richardsonii* (n = 20)	0.01	
Eastend Site	*Peromyscus maniculatus* (n = 19)	0.01	>0.05
Eastend Control	*Peromyscus maniculatus* (n = 12)	0.01	
Provost Site	*Spermophilus tridecemlineatus* (n = 14)	0.01	>0.05
Provost Control	*Spermophilus tridecemlineatus* (n = 10)	0.01	

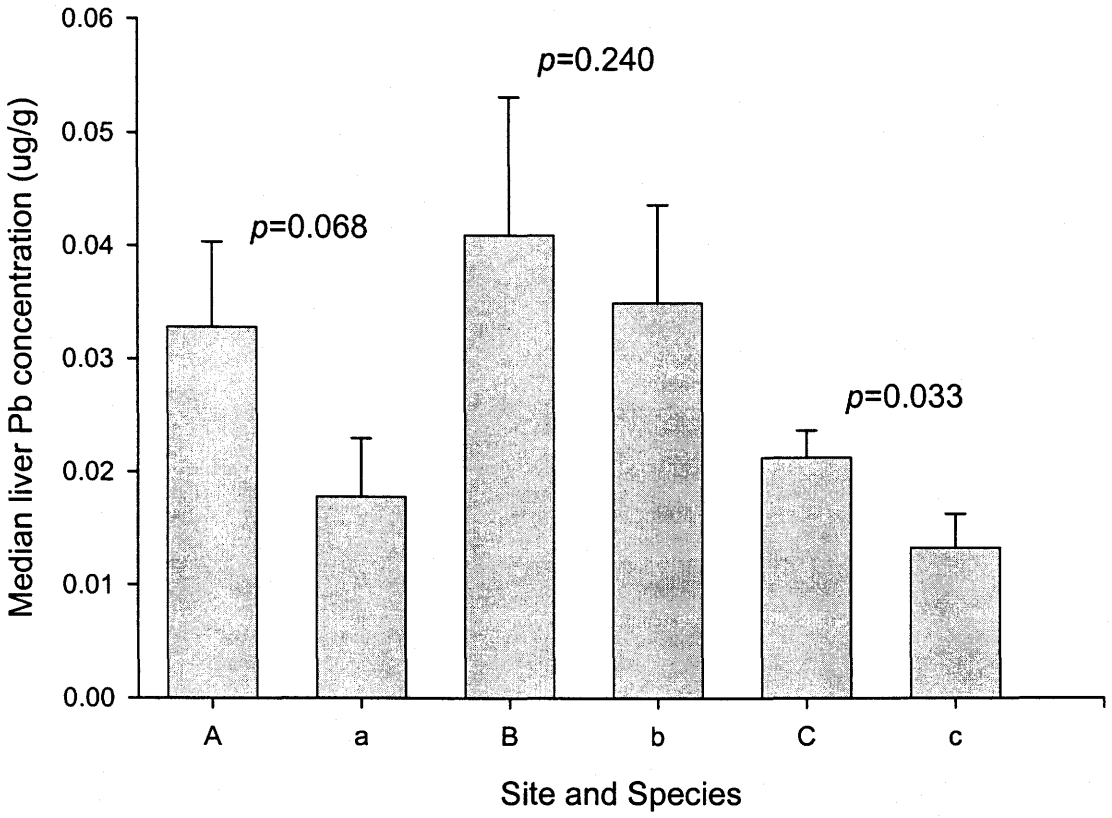
Table 4.6. Blood-lead concentrations in small mammals trapped at Canadian prairie shooting ranges and matched reference sites. Values are medians, with the 25th and 75th percentiles also presented.

Site	Species and (n)	Median Blood Pb Concentration (μg/ml) and (25%, 75%)	p=(0.05)
Eastend Site	*Spermophilus richardsonii* (n = 18)	0.004 (0.001, 0.015)	0.048
Eastend Control	*Spermophilus richardsonii* (n = 20)	0.001 (0, 0.005)	
Eastend Site	*Peromyscus maniculatus* (n = 19)	0.008 (0.005, 0.035)	0.043
Eastend Control	*Peromyscus maniculatus* (n = 12)	0.005 (0, 0.018)	
Provost Site	*Spermophilus tridecemlineatus* (n = 9)	0.014 (0.0095, 0.02)	0.087
Provost Control	*Spermophilus tridecemlineatus* (n = 9)	0.003 (0.00075, 0.009)	

Pb = Lead

Liver Pb concentrations ranged from 0.0213 to 0.041μg/g (ww) at shooting range sites and 0.0178 to 0.035μg/g at the reference sites (Fig. 4.6). The highest liver concentrations were found in Eastend shooting range mice, but they were not statistically different from reference site mice (p=0.240). Ground squirrel liver concentrations were not statistically different for the Eastend ground squirrels compared to control animals (p=0.068). Only ground squirrels trapped on the Provost range shot fall zone exhibited higher liver Pb concentrations than control animals (p=0.033).

Fig. 4.6



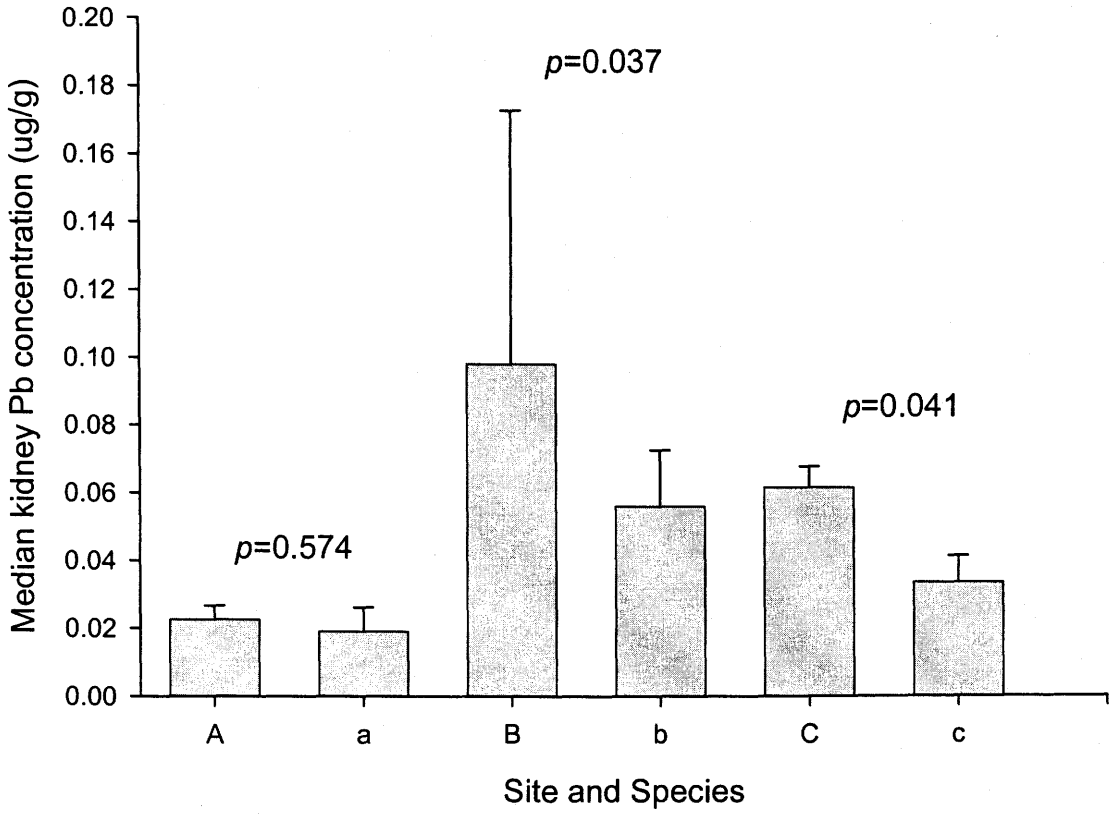
A- Eastend shooting range ground squirrels (n=19)
a- Eastend reference site ground squirrels (n=20)
B- Eastend shooting range mice (n=19)
b- Eastend reference site mice (n=12)
C- Provost shooting range ground squirrels (n=15)
c- Provost reference site ground squirrels (n=11)
Pb = Lead

Fig. 4.6. Median liver lead concentration (± standard error) in small mammals trapped on prairie shooting ranges and their matched reference sites.

Kidney Pb concentrations measured in mice from the Eastend shooting range and in ground squirrels from the Provost shooting range were significantly elevated compared with control kidneys (p=0.037 and 0.041, respectively) (Fig. 4.7). However, ground

squirrels from the Eastend range were not different than animals from the reference site (p=0.574).

Fig. 4.7



A- Eastend shooting range ground squirrels (n=19)
a- Eastend reference site ground squirrels (n=20)
B- Eastend shooting range mice (n=19)
b- Eastend reference site mice (n=12)
C- Provost shooting range ground squirrels (n=15)
c- Provost reference site ground squirrels (n=11)
Pb = lead

Fig. 4.7. Median kidney Pb concentration (± standard error) in small mammals trapped on prairie shooting ranges and their matched reference sites.

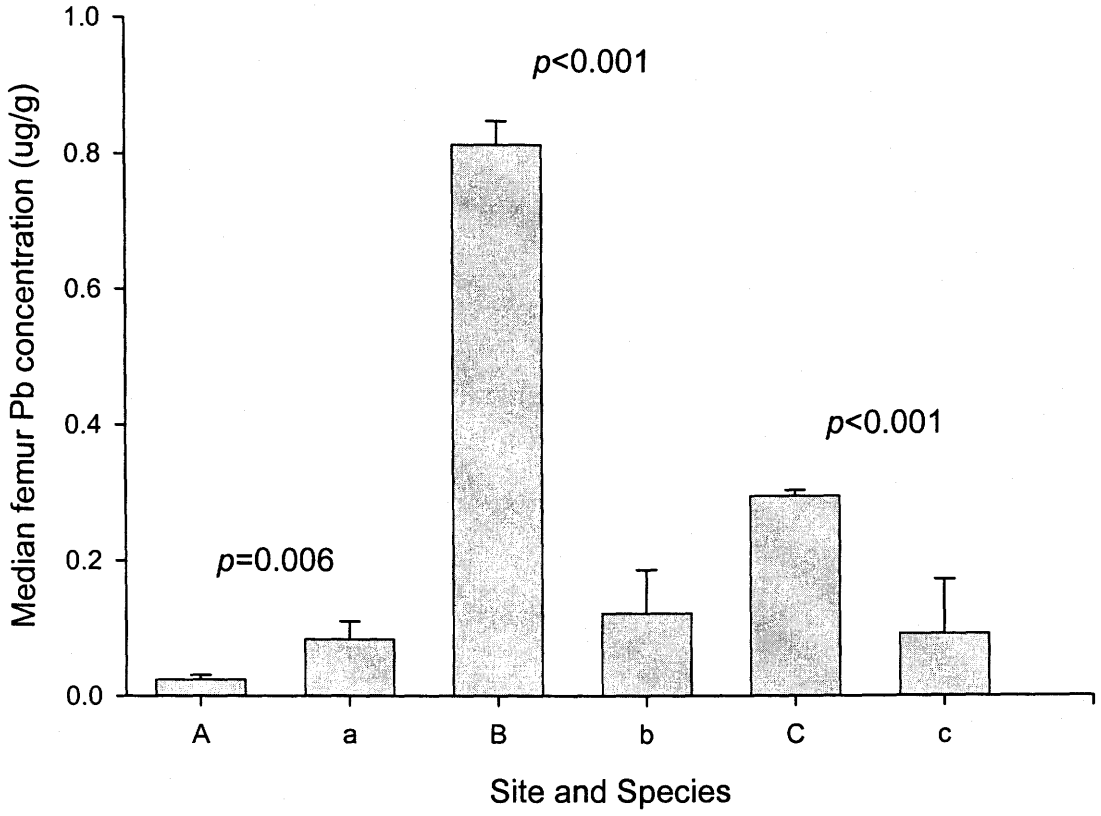
Femur Pb concentrations ranged from 0.024 to 0.813μg/g for small mammals trapped on shooting ranges, and 0.092 to 0.122μg/g for small mammals from the reference sites (Fig. 4.8). The highest concentrations were found in Eastend shooting range mice and were significantly greater than values measured in mice from the reference site (p<0.001). Provost ground squirrel femur concentrations were also greater than controls (p<0.001). There was also a difference in femur Pb concentration in ground squirrels from the Eastend sites, but the shooting range values were apparently less than those from the reference site animals (p=0.006).

None of the tissue or blood Pb concentrations observed in any of the animals trapped in this study were within the range (0.3-0.4 μg/ml-blood; 2-10 μg/ml-liver; 3-20-kidney in cattle) associated with Pb toxicosis (Puls 1994).

δ-aminolevulinic acid activity and correlation with blood lead concentrations

Blood ALAD activity measured in ground squirrels from the Eastend shooting ranges was not different than activity in reference site animals (p=0.111). However, ALAD activity appeared to be elevated in erythrocytes from Eastend shooting range mice (p=0.034) and Provost ground squirrels (p=0.012) compared with animals from their matched reference sites (Fig. 4.9).

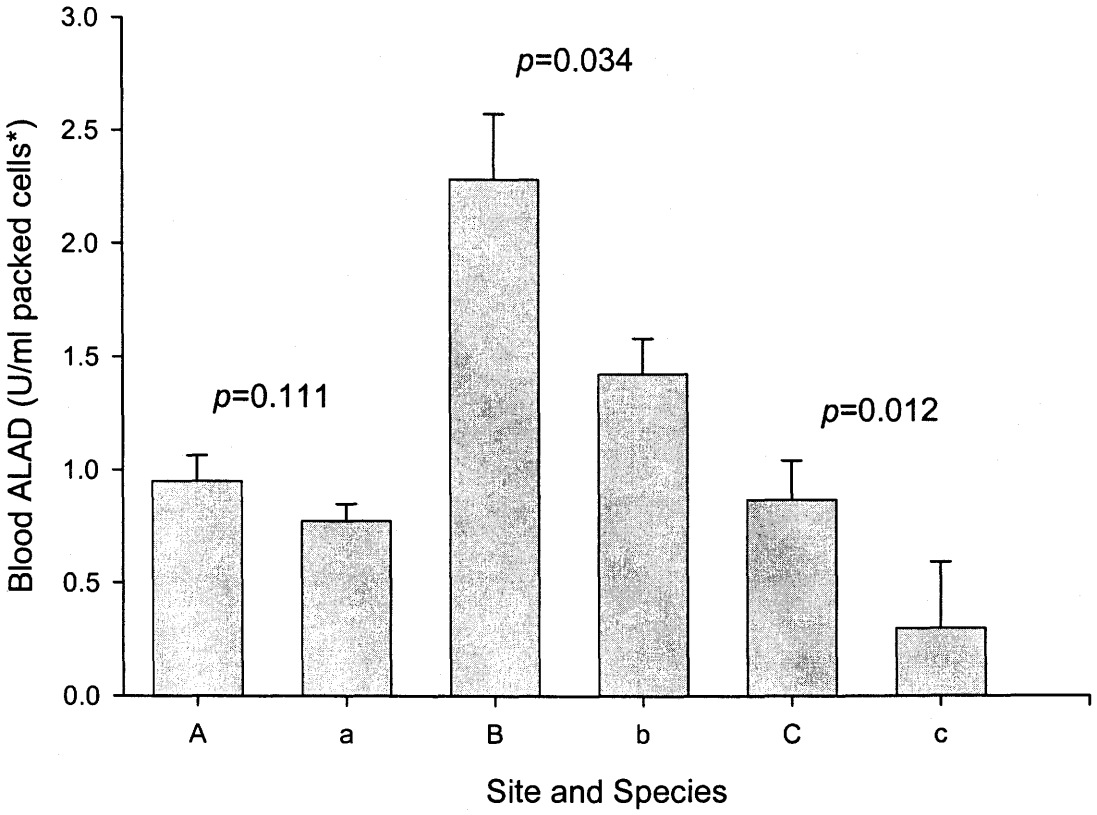
Fig. 4.8



A- Eastend shooting range ground squirrels (n=19)
a- Eastend reference site ground squirrels (n=20)
B- Eastend shooting range mice (n=19)
b- Eastend reference site mice (n=12)
C- Provost shooting range ground squirrels (n=15)
c- Provost reference site ground squirrels (n=11)
Pb = lead

Fig. 4.8. Median femur lead concentration (± standard error) in small mammals trapped on prairie shooting ranges and their matched reference sites.

Figure 4.9



A- Eastend shooting range ground squirrels (n=19)
a- Eastend reference site ground squirrels (n=20)
B- Eastend shooting range mice (n=18)
b- Eastend reference site mice (n=12)
C- Provost shooting range ground squirrels (n=12)
c- Provost reference ground squirrels (n=10)
* One unit (U) of enzyme activity = 1μmole of porphobilinogen/hr at 37°C
ALAD: aminolevulinic acid dehydratase

Fig. 4.9. Median blood aminolevulinic acid dehydratase activity (± standard error) in small mammals trapped on prairie shooting ranges and their paired reference sites.

There was no correlation between blood Pb concentration and blood ALAD activity for any small mammals trapped at the Eastend or Provost shooting ranges or at

the reference sites (Fig. 4.10, 4.11, and 4.12). Correlation coefficients for the Eastend shooting range and reference site ground squirrels, the Eastend shooting range and reference site mice, and the Provost shooting range and reference site ground squirrels were: r=0.12, p>0.05; r=0.16, p>0.05; r=0.07, p>0.05; r=0.036, p>0.05; r=0.047, p>0.05; r=0.068 and p>0.05, respectively.

Fig. 4.10

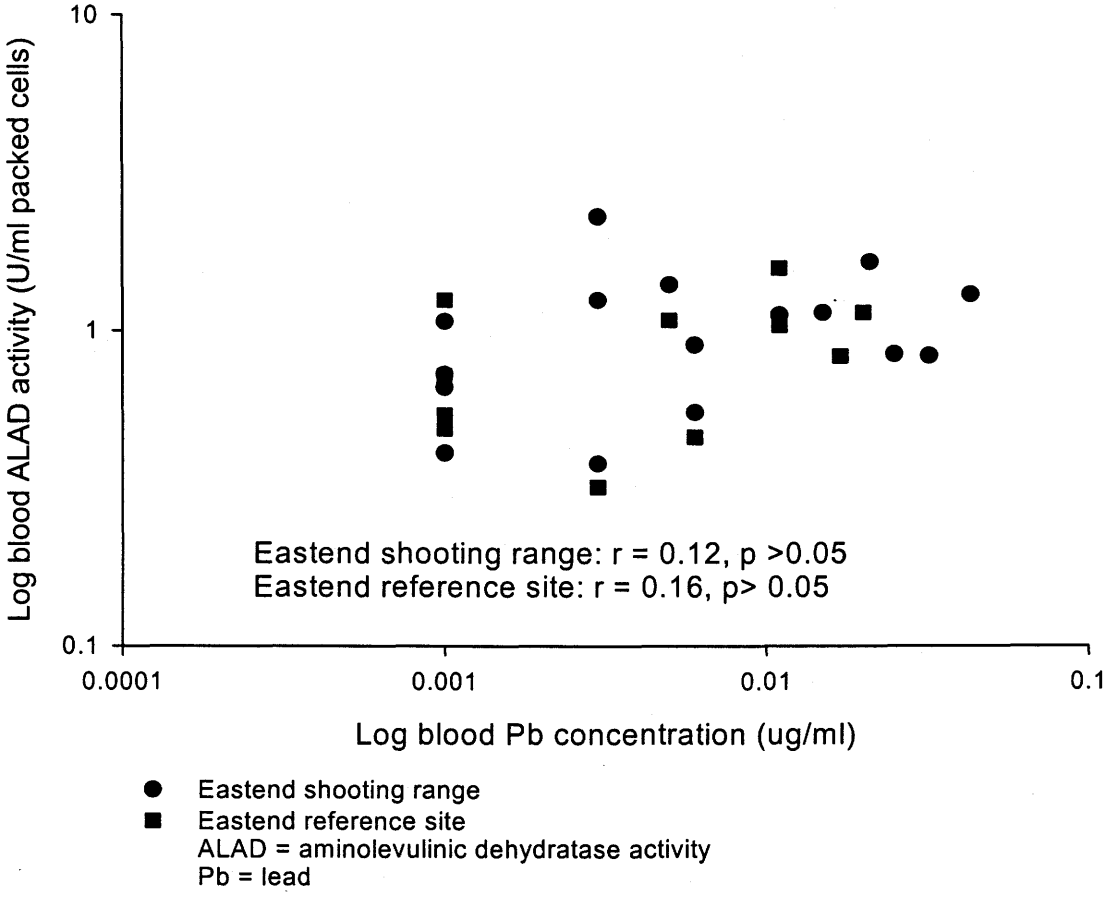


Fig. 4.10. Correlation of blood aminolevulinic acid dehydratase activity and blood lead concentration in ground squirrels from the Eastend shooting range and reference site.

Fig. 4.11

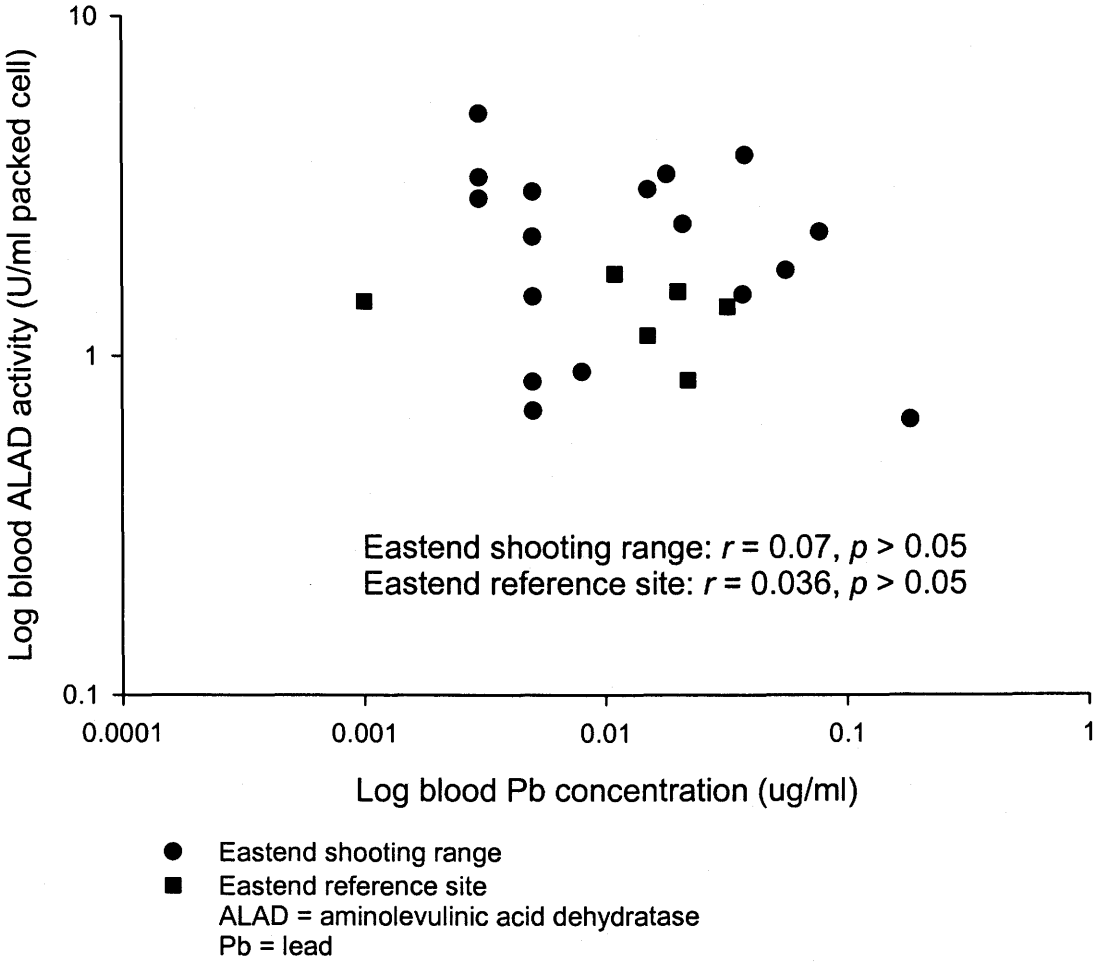


Fig. 4.11. Correlation of blood aminolevulinic acid dehydratase activity and blood lead concentration in mice from the Eastend shooting range and reference site.

Fig. 4.12

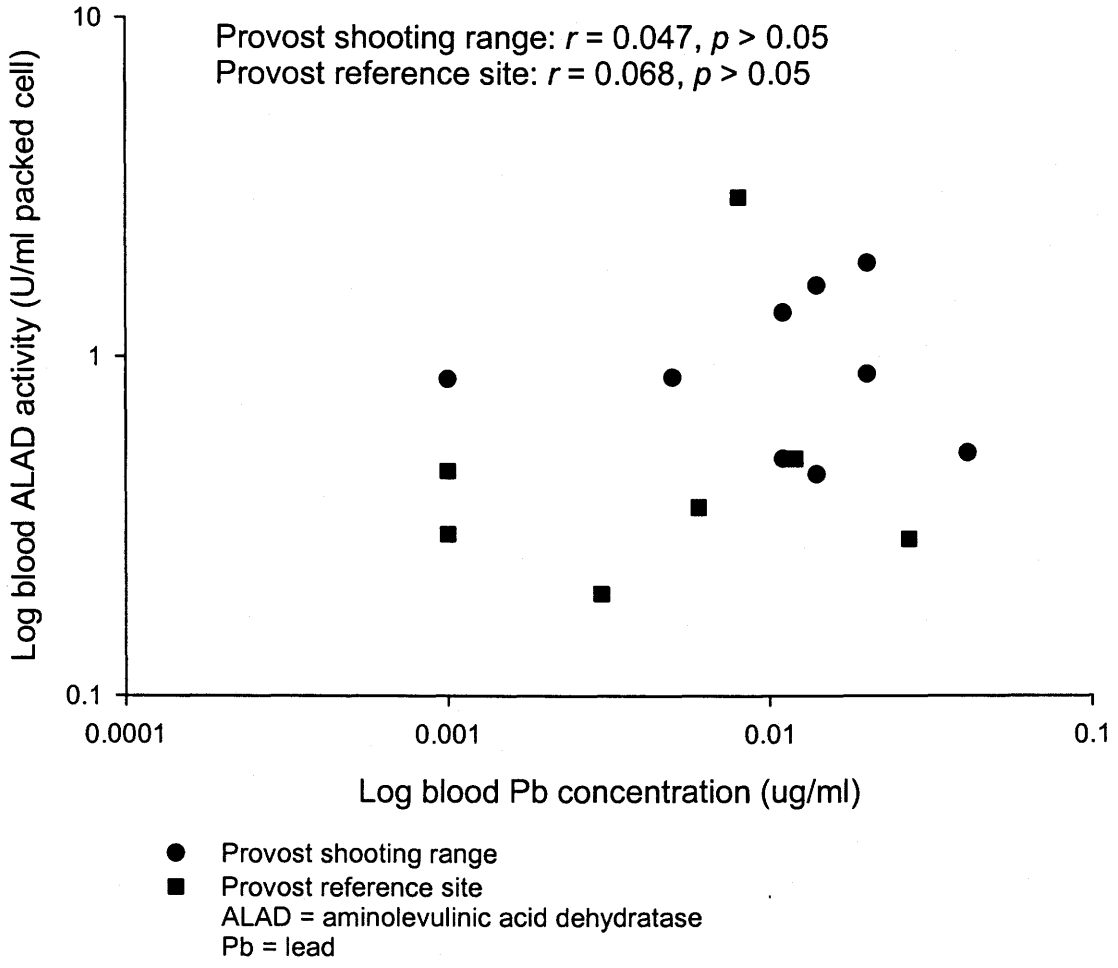


Fig. 4.12. Correlation of blood aminolevulinic acid dehydratase activity and blood lead concentration in ground squirrels from the Provost shooting range and reference site.

Correlation of blood and tissue lead concentrations in small mammals trapped at two Canadian prairie trap and skeet shooting ranges

Blood and tissue Pb concentrations for any tissues in all small mammals trapped at the two trap and skeet shooting ranges, with the exception of liver, kidney and femur Pb concentrations from mice at the Eastend range were not statistically different (Fig. 4.13, 4.14, 4.15). Correlation coefficients for liver, kidney and femur from ground squirrels at the Eastend range were r=0.095, $p>0.05$; r=0.19, $p>0.05$; and r=0.20, $p>0.05$, respectively. Correlation coefficients for liver, kidney and femur from mice at the Eastend range were r=0.59, $p<0.05$; r=0.77, $p<0.05$; and r=0.59, $p<0.05$, respectively. The Provost shooting range ground squirrel correlation coefficients for liver, kidney and femur were r=0.01, $p>0.05$; r=0.05, $p>0.05$; r=0.08 and $p<0.05$.

Fig. 4.13

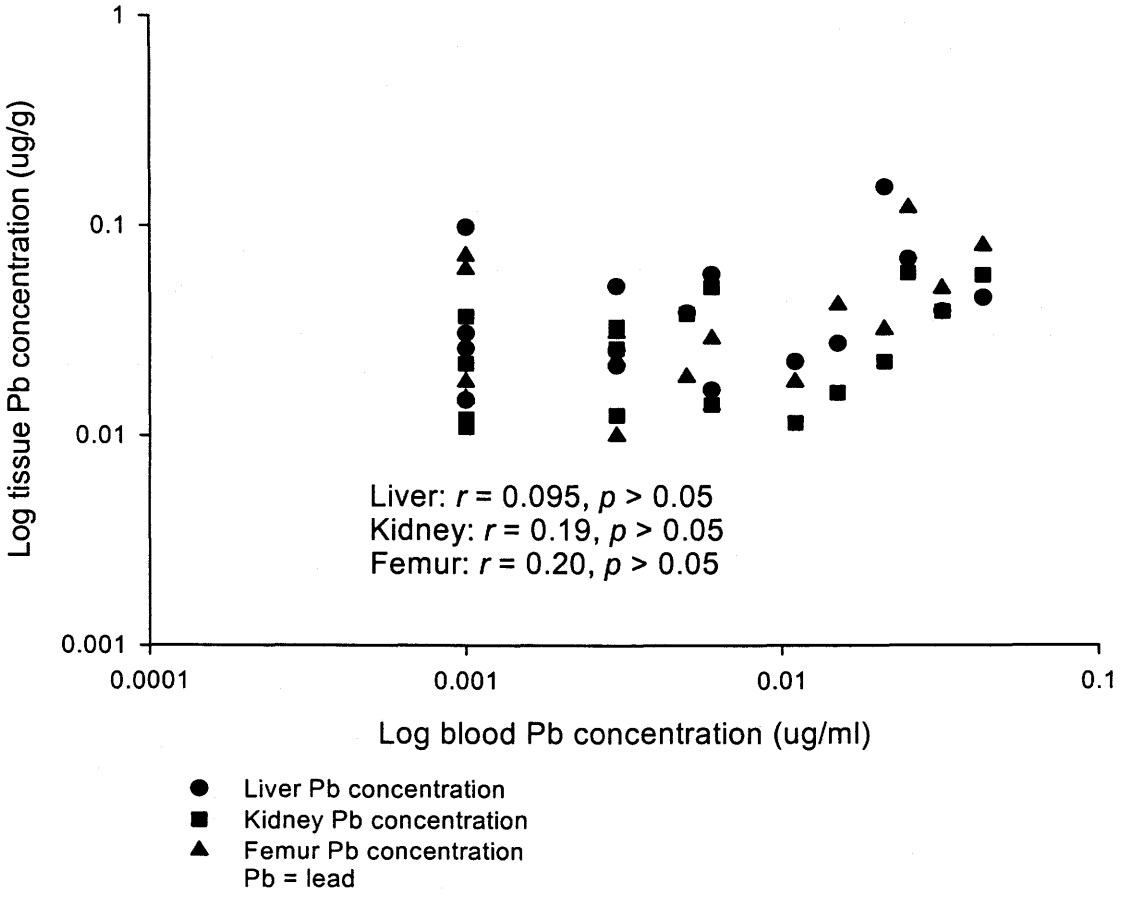


Fig. 4.13. Correlation of liver, kidney and femur tissue lead concentrations and blood lead concentration in Eastend shooting range ground squirrels.

Fig. 4.14

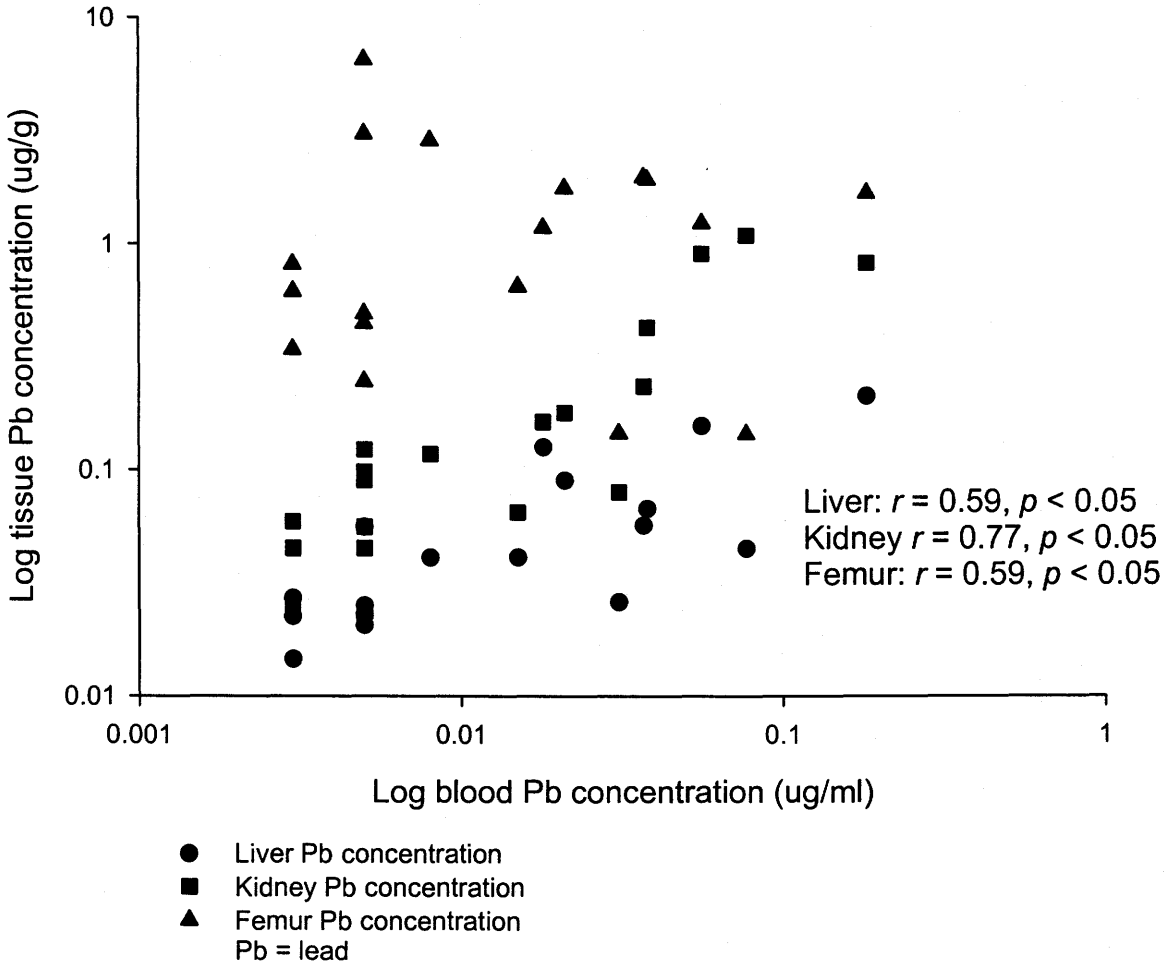


Fig. 4.14. Correlation of liver, kidney, and femur tissue lead concentrations and blood lead concentration in Eastend shooting range mice.

Fig. 4.15

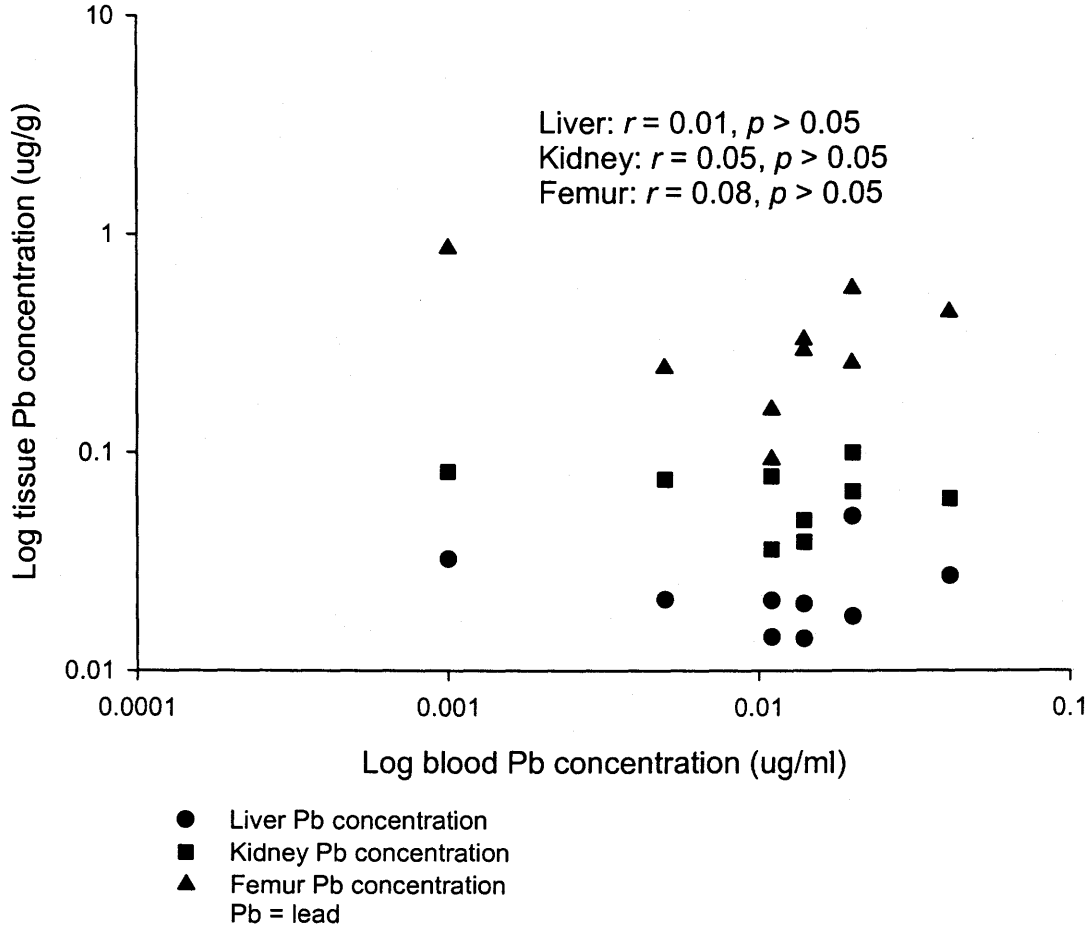


Fig. 4.15. Correlation of liver, kidney, and femur tissue lead concentrations and blood lead concentration in Provost shooting range ground squirrels.

4.4 Discussion

The shooting ranges used in the present study were heavily contaminated with Pb shot pellets in the shot fall zones. Lead loads in surface soil as determined from pellet counts ranged from 2874 kg/hectare at the Vegreville site to 4671 kg/hectare at the Eastend site (Table 4.2.). Data were also gathered on a fourth shooting range located at The Pas, Manitoba, but results were not included in this study because the site is located in the boreal forest ecoregion, and is therefore not comparable to the three grassland study sites. Pellet density at this range averaged 23,415/m^2, which is equivalent to 22759 kg Pb/hectare. Stansley and Roscoe (1996), estimated Pb loads at a range in New Jersey to be approximated at 266,000 kg Pb shot/hectare. In the present study only The Pas site had Pb loads comparable to those found in the Stansley and Roscoe (1996) study, with the other three study sites being an order of magnitude lower. It is important to note that extrapolation of Pb deposition to large areas, although impressive, may over estimate Pb contamination at these sites. In this study the shot fall zones at all shooting ranges were considerably smaller than the entire range, so it was more appropriate to present Pb distribution data on a g/m^2 basis. Nonetheless, the degree of Pb deposition in the surface soil of three prairie shooting ranges was very high in terms of Pb pellet density, and in some cases was higher than other studies in which total soil Pb levels were considerably greater than reported here (Vyas *et al.* 2000). Various comparable studies have reported total soil Pb levels ranging from 10,000 mg/kg to as high as 206,600 mg/kg (Manninen and Tanskanen 1993; Stansley and Roscoe 1996; Rooney and Mclaren 2000; Vyas *et al.* 2000). The latter extreme values are difficult to imagine if all metallic Pb pellets and fragments were actually removed from the soil before analysis. An interesting aspect of

the present study is that, in spite of relatively high Pb shot density, soil total Pb and $Ca(NO_3)_2$-extractable Pb levels were remarkably low (Table 4.2), with the exception of The Pas site where total Pb averaged 3298±2034 mg/kg. The observed levels in the shot fall zone were lower than background Pb concentrations reported from more urban control sites in some studies (Stansley and Roscoe 1996). A possible explanation for this low rate of Pb release into soil from Pb pellets may be variation in soil characteristics and precipitation. Lin *et al.* (1995) reported high variability in total soil Pb in a study in Sweden, and suggested that the dissolution of Pb shot in soil is site specific, and determined by soil conditions such as pH and organic matter content. In particular, acidity in the range of pH 3.03-4.55 appeared to enhance Pb release from Pb pellets, causing high total soil Pb concentrations. These findings were corroborated by Bruell *et al.* (1999) who reported an increase in dissolved Pb at pH of 3-4. None of the soils in the present study had soil pH levels this low (Table 4.1) with most sites having relatively neutral pH's. However, acidic conditions are not always required for Pb release. Stansley and Roscoe (1996) reported a soil Pb concentration of 75,000 mg/kg at a shooting range with soil pH of 6.3. Both the Provost and Vegreville sites had pH levels similar to this latter study, as well as high Pb loads, but total soil Pb concentrations were barely above typical background concentrations of 10-30 mg/kg (Rooney et al. 1999). Manninen and Tanskanen (1993) reported that most Pb found at shooting ranges was attached to soil organic matter, and that about 90% of the total Pb was EDTA extractable. Adsorption of Pb to organic matter often accounts for decreased bioavailability to soil invertebrates or small mammals, however it does not address why total soil Pb levels may be unexpectedly low. Low total soil Pb concentrations observed in this study likely

resulted from a complex interaction of soil pH, organic matter content, moisture, cation exchange capacity, redox potential and other soil factors whose characterization was beyond the scope of the present investigation.

Numerous reports have shown that soil Pb is poorly absorbed into aerial tissues of most plants (Menzel 1965; McLean *et al.* 1969; Keller and Zuber 1975). Plant roots uptake Pb to a limited degree, but translocation to the leaves is limited under most conditions (Kabata-Pendias and Pendias 1992; Mellor and McCartney 1994). In some cases, if bioavailable soil Pb levels are high (but not phytotoxic) plant uptake will occur. For example, Mellor and McCartney (1994), and Rooney *et al.* (1999) found that plants grown in soil collected from a trap and skeet shooting range had tissue Pb concentrations well above the allowable limit for foodstuffs. Results of the present study show little Pb uptake into plants from any of the sites (Figure 4.1 and 4.2). The highest plant-Pb concentration was measured in plant samples collected from soil with the lowest total Pb concentration (Figure 4.1), which supports the assertion that total Pb concentration is often a poor measure of risk. There was no statistically significant relationship between soil Pb and plant Pb concentrations at any of the sites. However, these results were expected in light of the relatively low soil total and $Ca(NO_3)_2$-extractable metal concentrations. Little can be concluded from this study about the ability of Pb to translocate from the root zone to stems or leaves, as root concentrations were not measured because they did not represent an important food source for the small mammals trapped on these ranges.

Studies investigating the effects of Pb contaminated soils to soil invertebrates have largely been restricted to earthworms, with only a few reports on other

species. Stone *et al.* (2002) reported that Carabid beetles (*Pterostichus oblongopunctatus F.*) living in soils contaminated with 136 to 2600 mg/kg total soil Pb at a smelting site had tissue concentrations ranging from 0.174 to 8.66 μg/g. Positive correlations between tissue Pb concentrations and distance from roadways have been observed with various other soil invertebrate species. Chmiel and Harrison (1981) reported that woodlice living near a roadway in the UK had tissue Pb concentrations of 152 mg/kg (dw) compared to 19 mg/kg (dw) in control woodlice. A similar study from the US found tissue Pb concentrations in the same species near a major highway ranged from 380 to 682 mg/kg, (dw) (Beyer and Moore 1980). No studies were found on Pb uptake into grasshoppers feeding on plants from Pb contaminated soils.

The majority of grasshopper species identified in Table 4.4 are mixed feeders with preference for grasses, with some preferring forbs. Results from this study indicated that there were no differences in grasshopper tissue concentrations between range and reference sites, and that overall tissue Pb concentrations were low (Fig. 4.5). This result was expected, based on tissue Pb concentrations in vegetation (Fig. 4.1, 4.2). Grasshoppers from the Provost shooting range and reference site had considerably higher tissue Pb concentrations than those from the Eastend range and reference sites. However, tissue Pb concentrations in grasses were higher at the Eastend sites compared to the Provost sites (Table 4.1). It is difficult to conclusively determine the reason for this result, but natural variability between populations may be a factor.

As previously discussed, numerous reports have shown that soil Pb is poorly absorbed into aerial tissues of most plants and translocation to the leaves is limited under most conditions (Kabata-Pendias and Pendias 1992; Mellor and McCartney 1994). For

this reason grasshoppers are probably not the best test species for this type of study. Furthermore, grasshoppers often migrate in search of food and would not be exclusively feeding on plants within the shot fall zone. Consequently, it is difficult to correlate, with any certainty, plant tissue Pb and grasshopper tissue Pb concentrations.

Metallic Pb pellets in contact with soil form oxidized Pb compounds on their surface that are subsequently released into soil, and may become available to soil organisms (Jorgensen and Willems 1987). Ma (1989) reported highly elevated Pb concentrations in both herbivorous and carnivorous small mammals at a shooting range in The Netherlands and concluded that Pb was mobilized in acidic soil and was made available to small mammals through uptake by both plants and soil fauna. Lackey *et al.* (1985) concluded that white-footed mice were exposed to Pb through consumption of Pb contaminated plants. Conversely, Manninen and Tanskanen (1993) concluded that plant Pb uptake was an unlikely source of Pb entering into terrestrial food chains. Incidental soil ingestion during feeding or grooming may account for a considerable portion of contaminant exposure to some species. Beyer *et al.* (1994) modelled soil exposure pathways and predicted that soil ingestion may account for the total estimated body burden in situations where soil intake amounts to <0.1% of the diet. This is important, since not all species consume equal amounts of soil. For example, in white-footed mice, soil intake may contribute <2% of the diet, while the shrew diet, which often consists of earthworms, may be 20-30% soil. Consequently, incidental ingestion of soil by small mammals in the shot-fall area may be a significant route of exposure, even in animals that typically ingest relatively little soil (Stansley and Roscoe 1996). In addition to soil ingestion, the ingestion of intact small metallic Pb shot pellets or shot fragments may be a

significant route of Pb exposure to wildlife on shooting ranges. This exposure is likely usually accidental, but in the case of some birds, Pb shot may be ingested purposely as grit (Scheuhammer and Dickson 1996).

Lead body burdens measured in small mammal tissues collected from contaminated sites show considerable variation among species, and often reflect dietary habits in addition to soil concentration and characteristics (Ma 1989; Clark et al. 1992). Normal background liver and kidney concentrations observed in mice and voles from sites with sandy soils range from 0.4- 1.5μg/g (dw) (Ma 1996). As expected, slightly lower body burdens were reported in animals of the same species collected from areas with peat or clay soils (0.2 – 0.6μg/g dw), due to decreased metal bioavailability. Lead in hard tissues represents long term, cumulative exposure with normal background concentrations of bone Pb in mice and voles reported as 2 – 3 μg/g (dw) (Ma 1996). Small mammal tissue Pb concentrations measured at all shooting ranges and reference sites in the present study fell within the range reflective of background exposure for both species and all tissue types measured (after adjustment for wet weight). Liver, kidney and bone (femur) Pb concentrations were elevated in ground squirrels from the Provost shooting range compared to the reference site (Fig. 4.6, 4.7, 4.8). Kidney and bone Pb were also increased in mice from the Eastend shooting range. However, ground squirrels from the Eastend site had lower concentrations of bone Pb than animals from the matched reference site. This discrepancy may simply reflect a difference in age between individuals trapped at these two sites. Lead accumulates over time in bone tissue, such that older individuals would have slightly higher body burdens. No attempt was made during this study to estimate the age of trapped mammals at any of the sites. However,

since all tissue concentrations are well within normal background levels, no adverse effects on animal health or fitness would be expected in any case.

There was no observed difference in kidney weight relative to body weight in any species trapped at the two shooting ranges relative to their matched reference sites. This result was expected as increases in kidney to body weight ratios have been reported at sites with considerably higher total Pb levels than those found in the present study (Bankowska and Hine 1985; Ma 1989).

Blood Pb levels are generally considered to be the best measure of current Pb exposure, especially under conditions of long term exposure where blood Pb concentration reaches a steady-state (Ma 1996). This is the situation that would be expected in resident small mammal populations at shooting range sites. Blood Pb results observed in the present study were consistent with reported background levels for wild small mammal species (Ma 1996). This result was expected in light of low total soil Pb levels. No correlation was observed between blood Pb and tissue Pb concentrations at any of the sites, with the exception of blood and liver, kidney and femur Pb concentrations in mice from the Eastend shooting range (Fig. 4.13, 4.14, 4.15). Because tissue levels fell well within normal background concentrations, and did not even approach values expected to cause clinical toxicosis (i.e. cattle: 0.3-0.4 μg/ml-blood; 2-10 μg/g-liver; 3-20 μg/g-kidney) (Puls 1994), individual variation may account for this lack of correlation as the relatively small amount of Pb in circulation may be handled differently from individual to individual.

The most sensitive biochemical biomarker of Pb exposure is inhibition of the cytosolic enzyme δ-aminolevulinic acid dehydratase (ALAD) (Ma 1996). The threshold

of blood Pb concentration at which ALAD begins to be inhibited in most species is 0.05-0.1 μg/ml. Results of blood Pb analysis in the present study revealed concentrations below this threshold for all shooting ranges and reference sites, and correlations between the blood Pb and ALAD activity were not strong. Enzyme activity was greater in small mammal blood samples collected on shooting ranges than control sites. In light of the weak correlation between blood Pb and ALAD activity, this finding is more likely the result of natural variation between populations than indicative of a previously undescribed stimulatory effect of low level Pb exposure.

The very low concentrations of soil total and $Ca(NO_3)_2$-extractable Pb observed on these shooting range sites were unexpected, and ultimately determined the results for all biological endpoints measured. Subsequent studies of these ranges would benefit by including a more comprehensive evaluation of soil characteristics that may explain low Pb mobility in the face of high shot density in surface soil. From the perspective of gun clubs and groups concerned with environmental Pb contamination, these findings demonstrate that Pb shot may not always pose as great a threat to terrestrial wildlife as is often assumed. Under some environmental conditions, the rate of Pb shot dissolution is apparently very slow. However, due to the site-specific nature of Pb behaviour in soils, it would not be prudent to conclude that this is the case in all grassland or prairie habitats. In addition, this study did not assess risks to avian wildlife that may be exposed to Pb by direct ingestion of pellets on the soil surface, or when low-lying sections of shot fall zones are seasonally flooded and attractive to waterfowl (Scheuhammer and Dickson 1996).

Chapter 5. Regulatory considerations for Pb shot

Evidence of Pb toxicity from Pb shot at trap and skeet shooting ranges to soil invertebrates and small mammals has been documented in the United Sates and Europe. However, no studies of this kind had been undertaken in Canada, and more specifically, in the dry grasslands of the Canadian prairies. Consequently, an objective of this study was to identify the number of trap and skeet shooting gun clubs in the Canadian prairies and the popularity of trap and skeet sport shooting. Representative shooting ranges and comparable control sites were then used to measure Pb concentrations in soil and biota, assess Pb uptake and toxicity to native small mammals, and assess toxicity of soil from these ranges to earthworms, as representative soil fauna.

There is considerable interest in recreational shooting in the prairie provinces with over 400 identified gun clubs in the three provinces. The shooting ranges identified as study sites were heavily contaminated with Pb shot, however total and $Ca(No_3)_2$-extractable –soil Pb levels remained low. This accounted for the limited uptake into plants and small mammals as well as the limited effect on blood ALAD activity measured in small mammal erythrocytes. Earthworm Pb concentrations were elevated and correlated well with NRRT, which was significantly reduced relative to earthworms from matched reference sites, however, there was a lack of adverse effects on growth or fecundity.

The ban on Pb-shot use for waterfowl hunting in the USA and Canada resulted from growing evidence of devastating annual loss, and it has undoubtedly reduced lead poisoning of waterfowl and their predators. However, environmental Pb deposition was recently estimated to be at 66% of the pre-ban rate, due to continued use of Pb shot for

upland game bird and small mammal hunting, and clay target shooting (Environment Canada http://www.cws-scf.ec). Some of that Pb shot use undoubtedly occurs over wetland habitats at shooting ranges. For these reasons, some countries (e.g. Finland and Sweden) have banned the use of Pb in wetland habitats, rather than imposing a ban targeted specifically at waterfowl hunting. Canadian legislation did not ban Pb-shot use in wetland habitats, but rather established non-toxic shot zones for waterfowl hunting and a national ban on Pb-shot for all migratory game bird hunting. Consequently, although it appears that the shot-fall zones at the shooting ranges in the Canadian prairie provinces may only cover a relatively small area of land, it is not known how many of these shot fall zones include wetland habitats. In the United States, 12 ranges, 6 of which were located in wetland areas, have been closed indefinitely or forced to switch to non-toxic shot use (SAAMI 1993). Furthermore, the OECD Working Group on the Development of a Lead Council Act, which met in Toronto in October of 1994, agreed that the use of Pb-shot in wetlands should be reduced, with the ultimate goal of a total phase out. This is in agreement with the aims of the Convention on Wetlands of International Importance Especially as Waterfowl Habitat (the Ramsar convention), of which Canada is a signatory nation, and has designated over 13 million hectares in Ramsar sites. A habitat-based policy makes more sense than a species-based one, and would be more protective, since clay target shooting over wetlands poses a risk of Pb-shot ingestion and poisoning to waterfowl that is akin to that from waterfowl hunting (Environment Canada http://www.cws-scf.ec).

Over and above the ban on Pb-shot use over wetland areas, some European countries (e.g. Norway and Sweden) have in place agreements with respective hunting

and target shooting associations for a voluntary phase out of Pb shot (Nordic Council of Ministers 1994). Such measures would include use at most shooting areas. Any attempts to duplicate this type of agreement in Canada or the US may be difficult to initiate. Hunters and recreational shooters prefer Pb for its intrinsic physical properties and ballistic performance, which are generally superior to the non-toxic alternatives. It is also generally cheaper than alternatives, and has been used for centuries resulting in a well established sales and distribution network (Environment Canada http://www.cws-scf.ec). In the context of trap and skeet shooting specifically, another factor is that international rules governing Olympic and other trap shooting competitions require the use of Pb ammunition. The official position of the International Shooting Union, as well as the International Olympic Committee, is that target shooting does not contribute significantly to an environmental Pb shot problem.

Future attempts to address policy governing Pb shot use in North America should include protection of wetland habitat. However, neither the Canadian Wildlife Service nor any provincial or territorial body have the resources to effectively assess all shooting ranges to determine if Pb shot use is appropriate, based on habitat type. In light of Canada's immense geographical area, the remoteness of many shooting ranges, limited resources available for monitoring, ammunition use, and resistant attitudes among some recreational shooters, it is likely that Pb shot will continue to be used at most shooting ranges.

Darling and Thomas (2003) used an indirect approach to assess ecological risk of Pb shot deposition in terrestrial habitats that was based on using maps of soil characteristic known to affect Pb bioavailability. This type of approach may be a good

screening tool for high risk sites, and does not require permission from the shooting ranges. However, the authors noted that, in the province of Ontario, pH maps existed for only 30% of all identified shooting ranges, and organic matter maps existed for only 28%. The complex nature of soil chemistry may often yield unexpected results as seen in this study. An approach to estimating Pb availability based on soil pH and organic matter content may accurately predict the risk only a fraction of the time. If applied to this study, an indirect approach would have erroneously identified each of the study sites as potential risks with the exception of The Pas site. This approach also does not take into account factors such as membership size, age and frequency of use, which would determine the Pb deposition (Darling and Thomas 2003). Cooperation by shooting clubs is required to obtain this latter information, as well as critical details on the distribution of wetland habitat on range property.

It is difficult to make general predictions or conclusions concerning the risks of Pb shot in terrestrial ecosystems. The findings of this study indicate that, under some environmental conditions, the risks to resident organisms are low. However, only with detailed soil chemistry and meteorological data from specific sites can the behaviour of Pb be predicted with any degree of certainty.

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