TOXIC EFFECTS OF MOLYBDENUM ON FOREST SMALL MAMMALS AT THE ENDAKO MINE IN NORTH-CENTRAL BRITISH COLUMBIA

A Thesis Submitted to the College of
Graduate Studies and Research
In Partial Fulfilment of the Requirements
for the Degree of Master of Science
in the Department of Biology
University of Saskatchewan
Saskatoon

By
Susan F. Mathieu
Fall 1996

PERMISSION TO USE

In presenting this thesis in partial fulfilment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

Requests for permission to copy or to make other use of material in this thesis in whole or part should be addressed to:

Head of the Department of Biology
112 Science Place
University of Saskatchewan
Saskatoon, Saskatchewan S7N 5E2

ABSTRACT

Molybdenum is an essential element for both plants and animals but high dietary levels can result in molybdenum toxicity in some mammals. This study documented the spatial pattern of molybdenum in the environment surrounding an active molybdenum mine in north-central British Columbia, and also examined the potential toxic effects of molybdenum in small rodents, including the red-backed vole (*Clethrionomys gapperi*), the deermouse (*Peromyscus maniculatus*), and the meadow vole (*Microtus pennsylvanicus*).

Results from this study indicated that molybdenum mining increased molybdenum concentrations in the water and soils surrounding the mine site. Elevated molybdenum concentrations in soils were mainly found within the first 5 m (riparian zone) on either side of the streams at both mine-influenced (treatment) and control sites. In contrast, this study demonstrated no relationship between the concentration of molybdenum in the livers of herbivorous small mammals and the spatial pattern of molybdenum measured in soils. Molybdenum concentrations in small mammals were not higher in individuals captured in treatment areas than in those captured in control areas. Nor were liver molybdenum concentrations higher in small mammals captured within the riparian zone than in areas farther from the stream. Individuals with elevated concentrations of molybdenum in their livers were found in both treatment and control areas. Thus the effects of molybdenum on small mammals had to be investigated by comparing those individuals with low concentrations of molybdenum in their livers (<2 mg/kg) to individuals with high concentrations (>5 mg/kg), regardless of area of capture.

In this study symptoms of molybdenum toxicity were not observed in small mammals with elevated concentrations of molybdenum in their livers although these levels were similar to those in related species which exhibited toxic effects in laboratory tests.

Elevated concentrations of molybdenum in the liver of small mammals did not appear to reduce growth in body mass or cause abnormal long bone development. In males with elevated liver molybdenum concentrations, both the mass of reproductive organs and accessory glands, and sperm production were not affected. Similarly for females, the incidence of pregnancy, ovulation rates, litter size, and the incidence of resorption were unrelated to molybdenum concentration in their livers. It should be noted though, that there was a trend for reduced ovulation rates and litter sizes in *C. gapperi* with elevated molybdenum concentrations, but these trends were not statistically significant.

This study illustrates three critical points for conducting field toxicological studies. First, this study illustrates the importance of determining contaminant loading in individuals rather than composite sampling. Composite sampling may not be sensitive enough to determine the pattern of contaminant loading in wildlife. Secondly, it also illustrates the importance of avoiding the assumption that contaminant loading of individual animals will follow the overall pattern observed in the environment when designing field toxicity studies. Thirdly, there were no toxic effects observed although the concentrations of molybdenum in the livers of small mammals were similar to those in rats which exhibited toxic effects in controlled experiments. The lack of observed toxic effects may be because different species of rodents respond differently to the same exposure level of molybdenum. It may also be possible that the populations of small mammals found around the mine area have developed a resistance to the toxic levels of molybdenum over evolutionary time.

ACKNOWLEDGEMENTS

It feels like I have lived a lifetime during the $5^1/_2$ years it has taken me to complete this degree (and, yes, some would say it has been a lifetime). Throughout it all, an array of people have played significant roles in my research and in my life.

I would like to thank my advisor, Dr. Dick Neal, and his family (Jenny, Christine and Tim) for the numerous times they opened up their home and their hearts to me and my "boys" (Calvin & Hobbes - oh yeah, and Jake). A special thanks to Dick for being on-call even when busy, and for showing tough love when necessary. I would also like to thank the members of my committee, Drs. F. Messier and D.G. Irvine, for providing helpful comments and guidance on all aspects of this thesis, and who were so tolerant when dealing with their satellite graduate student.

I gratefully acknowledge the financial support of Placer Dome Inc., and the personal support of various Placer personnel, especially, T.C. Baker, Dr. M.B. Thorpe, K.D. Ferguson, J. Robertson, and G. Paspalas. Thanks for believing in me! A BIG THANKS goes out to both Twyla Yobb ("the little alien") for her dedication and hard work during my second field season, and Cathy Harview for her diligence in helping with the manuscript (especially the literature citations!!). I would also like to thank the support staff in the Department of Biology (E. Peters, C. Hartney, J. Ryan, K. Weiman, and D. Dick) for helping me finish things yesterday. And thanks to Moly, the warehouse cat, for being my most productive snap-trap.

I would like to thank special friends and fellow graduate students who were there for me during the various stages of my "lifetime", especially, Jim and Anja Whittaker, JDG, Bill Iko, Nikki & Mark, BFD, Amy Leis, Jim Woolsey, Mark & Lynne Neal, Ron Parker, Karla Guyn-DeVries, Stuart Slattery, Mark and Leanne Smith, and Scott Halpin.

I would not be the person I am today without sharing so much of life's experiences with them (so, you're all to blame!).

Last but not least, I would like to thank those people who have helped me during my real lifetime. Thanks Mom & Dad, Donald, Lisa and Carmen for your prayers and encouragement, especially during those many phone calls when I wanted to forget about my thesis and just work. Your faith in me, more than anything else, gave me the strength to persevere through the rough times. Thank you.

HEY! I'M FINISHED - THANK GOD!!

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

Placer Dome Canada Ltd., Endako Mines Division, is an open pit molybdenum mine in north-central British Columbia. Preliminary studies have indicated that molybdenum is elevated in the environment surrounding the mine. Although molybdenum is an essential element for both plants and animals, high dietary levels can result in molybdenum toxicity in mammals. The purpose of this chapter is to present background information on molybdenum in the environment, its role in animal nutrition, its potential toxicity, and the scope of the ensuing study.

1.1 Molybdenum in the Environment

Molybdenum is a trace element distributed widely in nature. The average concentration of molybdenum in the earth's crust varies from 1.0 to 2.3 mg/kg (Day 1963, Sandell 1946). Concentrations in individual rock types may, however, range over three orders of magnitude. Mineable deposits tend to have molybdenum concentrations on the order of 2000 to 3000 mg/kg (Chappell 1976).

The molybdenum content of soils is dependent on the molybdenum content of the parent material and the concentration is usually greater than that of the parent material (Massey and Bailey 1967). In terms of total molybdenum concentrations in soils, samples from the western United States had a median concentration of 6 mg/kg compared to 0.5 mg/kg for soil samples from the eastern United States, with a median for the whole of the United States of slightly more than 1 mg/kg (Kubota 1975). Swain (1986) has indicated that the typical range of molybdenum of soils in British Columbia is 0.6 to 3.5 mg/kg.

Molybdenum does not exist in nature in a pure metallic form but is always found in association with other elements such as sulphur, oxygen, lead, iron, and bismuth, forming important primary minerals (Jarrell *et al.* 1980). Molybdenum is fairly readily released from primary minerals by weathering and, compared with other metals, it remains relatively mobile as potentially soluble molybdates (Mo^{6+}) (Gupta and Lipsett 1981). Consequently, movement by leaching is likely.

Molybdenum is present in fresh waters in its dissolved, anionic form, molybdate (MoO_4^{2-}). Molybdate is highly soluble at higher pH, in contrast to other metals which are precipitated as hydroxides.

In general, molybdenum concentrations in many surface waters range from zero to a few μg/L of water (Jarrell *et al.* 1980). In a review of water quality data in North America, Chappell *et al.* (1976) suggested that background concentrations of molybdenum in fresh waters of the United States are in the order of 1μg/L, and that waters with more that 10 to 20 μg Mo/L are likely affected by human activities such as mining and agriculture. Bachmann and Goldman (1964) and Wetzel (1975) reported that the concentration of molybdenum in natural fresh waters in the United States ranges from 0.1 to 0.8 μg/L. The median concentration of molybdenum in surface waters of North America is 0.35 μg/L (Hem 1970). In a review of water quality in British Columbia, Swain (1986) reported molybdenum concentrations ranging from 0.5 to 1.1 μg/L in waters in the southern portion of the province to "as high as" 30 μg/L in the Nechako River near Vanderhoof (90 km east of the Endako Mine).

The entry of molybdenum into surface or ground waters may be elevated near ore bodies (Jackson *et al.* 1975) where concentrations may reach several mg/L. In contrast, Runnells *et al.* (1977) found that waters in streams draining from highly mineralized areas of Colorado rarely contained more that 1 to 2 μg/L. Voegeli and King (1969) cite molybdenum concentrations in Colorado streams ranging from 5 μg/L to 3800 μg/L. These researchers correlate molybdenum concentrations in stream waters exceeding 5 μg/L with occurrences of molybdenum mineralization, and concentrations exceeding 60 μg/L with mining activity.

Molybdenum in water and soil solution is available for uptake by plants which appear to absorb molybdenum in the form of the molybdate anion (Chesnin 1972 cited in Gupta and Lipsett 1981). The molybdenum content of plants varies with species, age, part of the plant, and plant-available molybdenum content in soils. Factors that increase plant-available molybdenum content in soils include parent material, soil pH, soil drainage, soil organic matter content, and competing ions (Barshad 1951).

Molybdenum is an essential trace element for plants and its most important functions in plants are associated with nitrogen metabolism (Spence 1976). Its role is primarily that of an enzyme activator for nitrogenase and nitrate reductase.

Plants appear to demonstrate a flexible capability for tolerating high concentrations of molybdenum in their tissues. Unlike all other micronutrients, molybdenum has never been shown to reach phytotoxic concentrations in field-grown plants (Jarrell *et al.* 1980). Plants can readily accumulate high levels of molybdenum in their tissues without exhibiting visible external symptoms or reduced growth.

1.2 Molybdenum in Animal Nutrition

Molybdenum was first established as an essential nutrient in 1953 (Rickert and Westerfield 1953 cited in Underwood 1977). In mammalian systems, molybdenum is an essential constituent of various enzymes which catalyze redox reactions: sulphite oxidase, xanthine oxidase and aldehyde oxidase (Rajagopalan 1987). Thus, molybdenum is required for growth, cellular oxidation, and purine metabolism.

The molybdenum requirements of mammals are remarkably low. For example, the rat is able to grow and reproduce normally when on a diet containing only 0.2 μg/g (Higgins *et al.* 1956). Molybdenum is ingested, transported, and excreted as molybdate. It is readily and rapidly absorbed from most diets and from most inorganic forms of the element (Underwood 1977). The urine is a major route of excretion of molybdenum in rats (Nielands *et al.* 1948) and other non-ruminants but apparently not in cattle or sheep, where it remains within the digestive tract and is excreted via fecal matter.

Molybdenum occurs in low concentrations in all the tissues and fluids of the body, and species differences in distribution tend to be small (Underwood 1977). Molybdenum concentrations in organs and tissues of the adult rat range between 0.06 and 1.8 mg/kg, with the lowest value seen in the muscle tissue and the highest value seen in the liver (Table 1.1).

Table 1.1 Normal molybdenum concentrations in rat organs and tissues (from Higgins *et al.* 1956)

	Molybdenum Concentration (mg/kg, dry matter basis)				
	Liver	Kidney	Spleen	Brain	Muscle
Adult rat	1.8	1.0	0.52	0.24	0.06

The molybdenum status of a mammal is best indicated by its concentration in the liver (Anke *et al.* 1985), where the highest molybdenum concentrations occur (Rajagopalan 1987). On normal diets, the molybdenum concentration in the liver of mammals mainly lies within the range of 2-4 mg/kg (Rajagopalan 1987). Similar liver molybdenum concentrations are observed in neonates indicating that molybdenum is not preferentially stored in the fetal liver during pregnancy. However, molybdenum can cross the placental barrier with the result that high levels of the element in the diet of the dam increase hepatic molybdenum in the newborn (Cunningham 1950, Cunningham and Hogan 1959).

Changes in dietary molybdenum intake are reflected in corresponding changes in the levels of the element in the tissues and hair or wool (Davis *et al.* 1960). However, many other dietary factors can influence the resulting tissue concentrations (Mills and Davis 1987 - see section 1.3). High concentrations of molybdenum in mammalian tissues return to normal within a few days when exposure to high dietary molybdenum levels ceases (Underwood 1977).

1.3 Molybdenum Toxicity

In mammals, molybdenum compounds, particularly molybdates, depress the availability of copper (Mason 1978). Ferguson and co-workers (1938) were the first to associate a scouring disease of cattle in certain areas of England with high molybdenum content of pastures. A molybdenum-copper interaction became apparent when large doses of copper demonstrated both preventative and curative effects. Further evidence of a molybdenum-copper interaction came from Australia when molybdenum, as molybdate, was found to be effective in the treatment of chronic copper poisoning in sheep (Dick and Bull 1945). These early studies demonstrated a physiological antagonism between molybdenum and copper, i.e., molybdenum interferes with the copper metabolism of the animal.

Further studies, however, have shown that the relationship is more complex. The effect of high dietary molybdenum intake depends on the species of animal, its age, the amount and chemical form of the ingested molybdenum, the copper status and copper intake of the animal, and the amount of sulphur in the diet. The literature on molybdenum toxicity is extensive. Some reports, particularly those written before the major influence of these variables were fully appreciated, are difficult to interpret except in light of the more recent, if still incomplete, understanding of the mode of action of such factors. The following generalizations, however, can be made.

Species differences in tolerance to molybdenum are substantial, the most obvious being the relative insensitivity of non-ruminants and the high sensitivity of ruminants to molybdenum. Cattle are by far the least tolerant, followed by sheep, while horses and pigs are the most tolerant of farm livestock (Underwood 1977). Rats, rabbits, and poultry are not so tolerant of molybdenum as pigs but are much more tolerant than cattle (Underwood 1977). In addition, there appear to be differences, as yet poorly understood, between different ruminants. For example, deer can tolerate at least ten times more dietary molybdenum than domestic ruminants (Nagy *et al.* 1975, Osman and Sykes 1989).

The clinical manifestations of molybdenosis vary among different species. In

cattle, it is characterized by severe scouring, unthriftiness, rapid loss of weight and condition, the development of harsh, discoloured coats, and if left to progress, ultimately death. Sterility in males and failure or delay of oestrus in females may also be associated with elevated dietary levels of molybdenum in cattle (Hornick and Baker 1976, Phillippo *et al.* 1987). Ataxia, or swayback, is a classic sign of copper deficiency in lambs and kids associated with pastures high in molybdenum, sulphur, and iron (Dick and Bull 1945).

Early attempts were made to study the copper-molybdenum interaction in non-ruminant animals such as the rat (Gray and Daniel 1954, Van Reen 1959), the chick (Arthur *et al.* 1958) and the rabbit (Arrington and Davis 1953). These studies indicated that the ingestion of food supplemented with molybdenum may cause inhibition of growth, failure of haemoglobin synthesis, and the production of skeletal abnormalities. Sterility of males and reproductive failure in females was also demonstrated in rats fed high levels of molybdenum (Jeter and Davis 1954, Fungwe *et al.* 1986, 1989, 1990).

Molybdenum also exerts different effects on the distribution of tissue copper in ruminants compared to rats. Under most conditions, molybdenum supplementation in ruminants usually depletes tissue copper reserves resulting in a secondary copper deficiency. In contrast, increased dietary molybdenum promotes the accumulation of biologically unavailable copper in the liver of the rat (Miller *et al.* 1956, Mills 1960).

The differences in sensitivity of ruminants and non-ruminants were initially not fully understood and could not be explained simply by differences in absorption. By the mid-1970's, it was clear that the antagonism did not arise from a simple two-way chemical interaction between copper and molybdenum. It was a result of a complex three-way interaction also involving sulphur compounds (Dick *et al.* 1975, Suttle 1974). Further studies showed that events in the rumen explained why the interaction had marked effects on ruminant species. Copper, molybdenum and sulphur (from organic or inorganic sources) could combine in the rumen to form an unabsorbable triple complex, cupric tetrathiomolybdate ($CuMoS_4$), which depleted the tissues of copper (Suttle 1974).

The forms in which sulphur compounds are found in tissues accumulating molybdenum influence molybdenum tolerance. The sulphate ion, whether originating from

the diet or from sulphur-amino acid breakdown, restricts molybdenum uptake, enhances its excretion, and thus increases tolerance. In contrast, generation of acid-labile sulphide within the gut and, probably within tissues, potentiates molybdenum toxicity in both ruminants and non-ruminants.

By the end of the 1970's, considerable support for the "copper thiomolybdate" hypothesis had accrued and a systemic dimension had been added to it: copper could be rendered unavailable pre- as well as post-absorptively by the highly reactive thiomolybdates (TMs) formed in the sulphide rich environment of the rumen (Mason 1981, Suttle 1980).

More recently, the interaction has become more complicated by the suggestion that molybdenum rather than copper may be the dominant interactant, exerting toxic effects on metabolism that copper can negate (Bremner *et al.* 1987, Phillippo *et al.* 1987). This means that the problem may be one of molybdenum excess rather than copper deficiency: molybdenosis not hypocuprosis. The case for the alternative "TM toxicity hypothesis" is unproven as of the early 1990's (Suttle 1991). Molybdenum toxicity in rodents will be examined further in Chapter 3.

1.4 Purpose and Scope of the Study

The preceding literature survey demonstrates that molybdenum concentrations in soils and water are elevated near actively mined molybdenum deposits. This molybdenum, in its soluble form molybdate, is absorbed by plants and may reach concentrations that are toxic to some mammals. The primary concern at Endako Mine is, therefore, that the mining of molybdenum may lead to toxic effects in exposed mammals. To assess this effect, small rodents were selected as suitable target animals for two reasons. First, elevated dietary molybdenum intakes are known to be toxic to experimental rats. Second, the relatively limited home ranges of small mammals allows for the comparison of populations living in adjacent watersheds that have differing molybdenum concentrations.

In Chapter 2, the pattern of distribution of molybdenum in the environment

surrounding the Endako Mine is documented as the basis for determining if the uptake of molybdenum of the various small mammal species, as measured by its concentration in their livers, follows the same pattern. The hypothesis is that molybdenum contamination of the area surrounding a mine is mainly through contaminated water draining from the mine site. Thus, small mammal populations within contaminated watersheds should have higher molybdenum concentrations in their livers than those found in watersheds with low concentrations of molybdenum. An extension of this hypothesis is that within contaminated watersheds, small mammals caught adjacent to the streams should have higher liver molybdenum concentrations than those caught farther from the streams.

In Chapter 3, the potential toxic effects of molybdenum in small rodents is examined by comparing various parameters in those individuals with low ("normal") molybdenum concentrations in their liver to those with high molybdenum concentrations in their livers. The parameters examined include diet, hepatic copper concentrations, body mass, skeletal structure, and reproduction. The hypothesis in this case is that small mammals with high concentrations of molybdenum in their livers should have stomach contents with higher molybdenum concentrations and a higher incidence of symptoms of molybdenum toxicity than those with normal concentrations of molybdenum in their livers.

2. DISTRIBUTION OF MOLYBDENUM IN THE ENVIRONMENT SURROUNDING A MINE AND ITS RELATIONSHIP TO LIVER CONCENTRATIONS IN SMALL MAMMALS

2.1 Introduction

Placer Dome Canada Ltd., Endako Mines Division, is an open pit molybdenum operation that has been in production since 1965. Molybdenum concentrations in seepage from the tailings impoundments range up to 15 mg/L. Surface water from a partially mined open pit contains molybdenum concentrations of up to 30 mg/L.

A preliminary investigation (Norecol 1990) of the influence of elevated levels of molybdenum in streams receiving effluent from the mine site was conducted in 1989. The study demonstrated a positive correlation among the molybdenum content of water, soil and vegetation (Norecol 1990). The highest soil molybdenum concentrations occurred near streams receiving drainage from the mine. Molybdenum concentrations farther (i.e., >5-10 m) from the stream were generally comparable to those in soils from outside of the mine's influence. The highest concentrations of molybdenum in vegetation were also generally found within 0-5 m of the streams receiving drainage from the mine. The influence of elevated levels of molybdenum in water on plant tissue levels was further demonstrated in vegetation having very high molybdenum concentrations when growing in areas of seepage 10-20 m from the stream. Based on this preliminary investigation, it seemed likely that increased molybdenum concentrations in streams receiving drainage from the mine caused increased molybdenum concentrations in soils within the riparian zone. This in turn was reflected in higher levels of molybdenum in vegetation growing in the riparian zone.

The preliminary study conducted by Norecol (1990) further suggested that animals feeding exclusively on vegetation within mine-influenced drainage basins would be exposed to between 2- to 11-fold increases of molybdenum in their diet when compared to animals feeding on vegetation growing in areas outside the mine's influence. Although measuring molybdenum levels in animals was beyond the scope of the Norecol study, it was recommended that small mammals could be sampled as indicators of environmental uptake of molybdenum. Measuring the molybdenum concentration in the livers of small mammals would provide an accurate indicator of molybdenum exposure through their diets. Studies have shown that molybdenum-supplemented diets greatly increase the molybdenum concentration of liver (Mills and Mitchell 1971, Nederbragt 1980).

The purpose of this chapter is to report the results of a more detailed investigation of the molybdenum levels in soils for mine-influenced and non-influenced drainage basins. It is predicted that, first, soils in mine-influenced drainages would have higher molybdenum concentrations than soils in non-influenced drainages. And second, it is predicted that the molybdenum concentration in soil will decrease with increasing distance from the streams. This chapter also determines the relationship between molybdenum concentrations in soil samples and the liver of small mammals. It is predicted that the molybdenum concentration in the liver of small mammals will show a strong correlation to the molybdenum concentration in the soil within the immediate area of capture.

2.2 Methods

2.2.1 Study Area

The Endako Mine property is located 10 km southwest of the town of Endako, approximately 160 km west of Prince George in north-central British Columbia (Figure 2.1). The Endako Mine is located within the Interior System of the Canadian Cordillera and, more specifically, within the physiographic subdivision referred to as the Nechako Plateau. The local terrain is gently rolling, with flat topped hills and broad valleys.

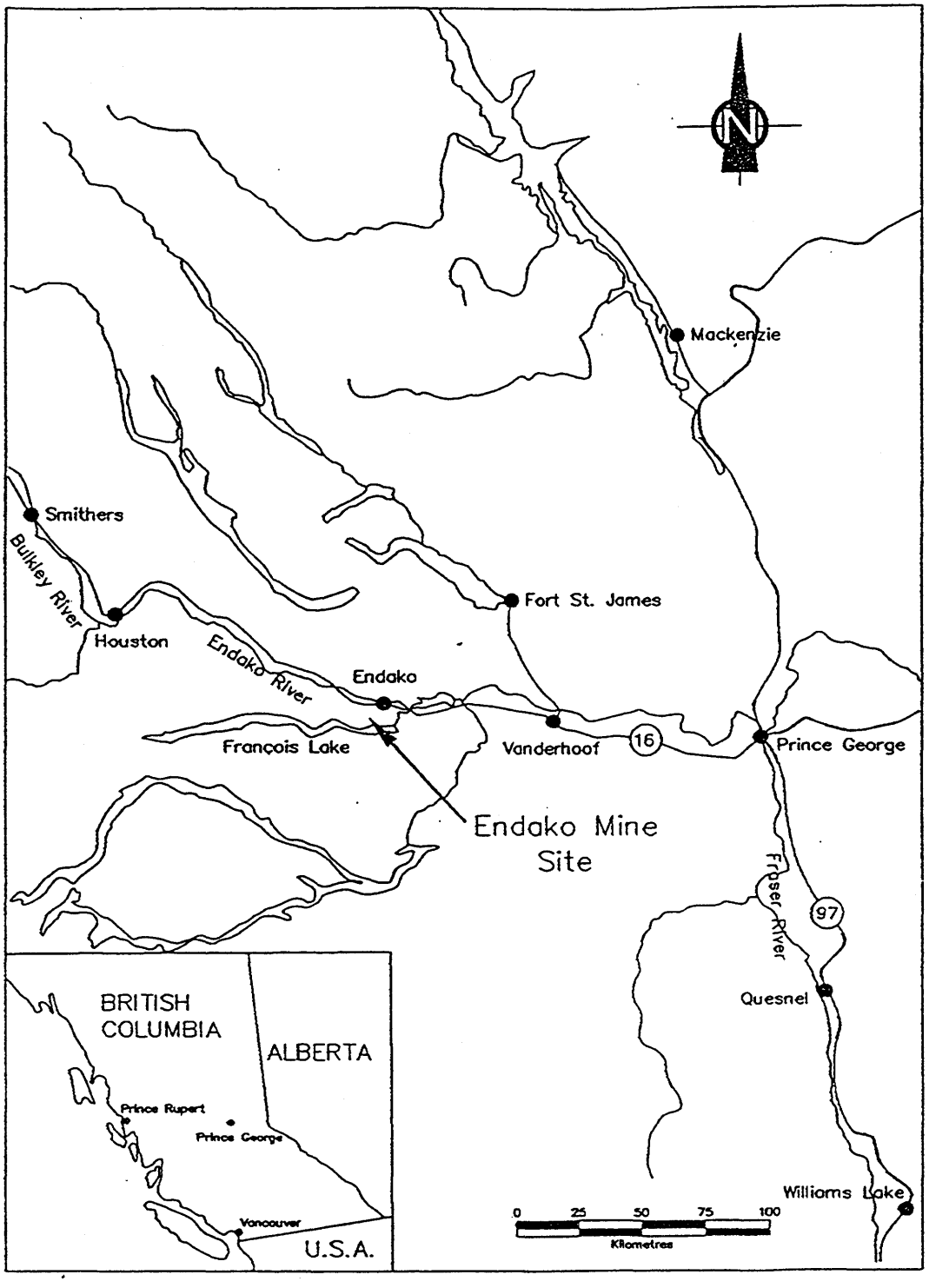

Figure 2.1 The location of Placer Dome Canada Ltd., Endako Mine Division in north-central British Columbia.

Topographic relief ranges from an elevation of 670 m asl at the town of Endako to 1070 m asl on the crest of the Endako open pit. Drainage systems for the mine and surrounding areas either flow north to the Endako River or south to François Lake.

The study area is dominated by upland coniferous forests, typical of the Sub-Boreal Biogeoclimatic Zone (Pojar and Nuszdorfer 1988). Hybrid spruce (*Picea engelmanni* x *P. glauca*), white spruce (*P. glauca*), and subalpine fir (*Abies lasiocarpa*) are the dominant tree species. Black spruce (*P. mariana*) occur occasionally. Seral stands are extensive in the mine area, and are dominated by lodgepole pine (*Pinus contorta*) and quaking aspen (*Populus tremuloides*). Paper birch (*Betula papyrifera*) is a pioneer species on moister sites, and is present along many of the streams in the area.

Understorey vegetation in the study area was dominated by dense stands of bracted honeysuckle (*Lonicer involucrata*) and green alder (*Alnus sinuata*), particularly adjacent to the streams. Saskatoon (*Amelanchier alnifolia*), wild red raspberry (*Rubus strigosus*), common wild roses (*Rosa woodsi*), and snowberry (*Symphoricarpos albus*) were found in the shrub layer. Representatives of the herb layer included cow parsnip (*Cicuta maculata*), nettles (*Utrica dioica, U. gracilis*), thimbleberry (*Rubus parvifloris*), baneberry (*Actaea rubra*), vetch (*Vicia americana*), and bunchberry (*Cornus canadensis*). Horsetail (*Equisetum sp.*), clubmoss (*Lycopodium sp.*) and various bryophytes were also present.

2.2.2 Study Design

The overall approach to this study involves comparing data from two different types of areas: 'affected' (treatment) and 'unaffected' (control) drainage systems. Affected drainage systems represented areas of high molybdenum concentrations in the waters, and presumably soils and vegetation, and thus, were used to assess potential treatment effects. These areas contained streams that receive direct molybdenum input from the mine, i.e. the molybdenum content is mine-influenced. Control drainage systems were located outside of the mine's influence, i.e. they did not receive input from mining activities.

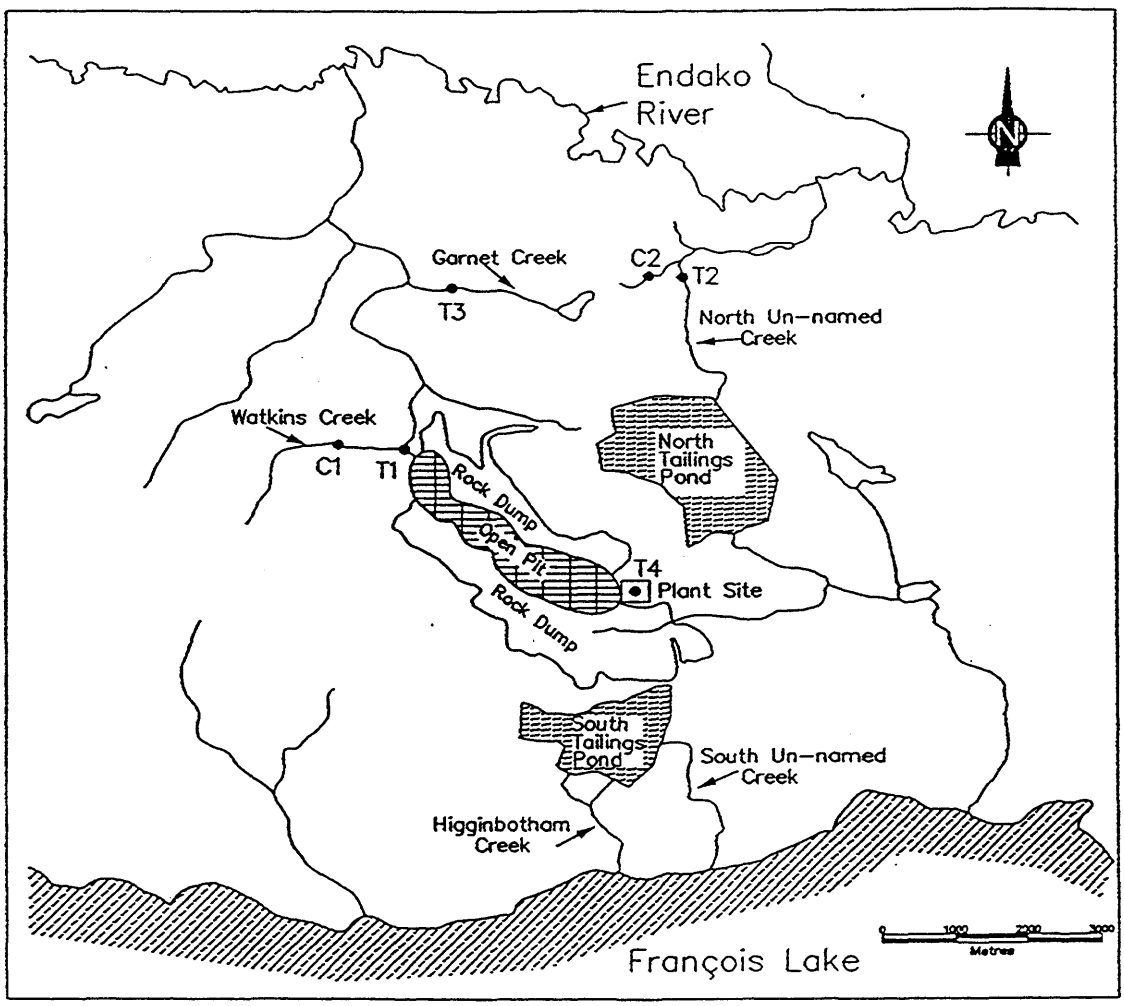

Figure 2.2 The location of study areas surrounding the Endako Mine Site. Study areas include two control areas (C1 and C2) and four treatment areas (T1, T2, T3 and T4).

The sites selected for study were based on dissolved molybdenum concentrations of streams (Figure 2.2). Control streams (C1 and C2) contained less than 1 mg Mo/L and treatment streams (T1 and T2) had greater than 5 mg Mo/L. Dissolved copper concentrations were similar among all streams and did not appear to be related to mining activities.

Control and treatment streams were matched according to the vegetation surrounding them in order to decrease the influence of forest type and structure on small mammal communities. Sample sites C1 and T1 were located west of the mine site in the Watkins Creek drainage basin (Figure 2.2). The two sites were separated by a hill and trapping of small mammals was designed to avoid collecting animals with home ranges extending into both sample sites. C2 and T2 were located north of the mine site, approximately 1.3 km apart.

2.2.3 Water Sampling

Water samples were collected monthly from the control and treatment streams from May to August in 1991 and 1992.

Sampling Protocol: Water samples for dissolved molybdenum and copper analysis were collected in sterile, 250-ml plastic bottles. The bottles were rinsed three times in the stream prior to filling with water from the centre of the stream at mid-depth. Water was always sampled from the same location. The samples were vacuum filtered with 2 μm filter paper and preserved to pH <2 with nitric acid. Water samples were then shipped cold to the analytical laboratory.

Analytical Methods: Water samples, along with field blanks and laboratory standards, were analyzed for dissolved molybdenum and copper by Analytical Services Laboratory Ltd (ASL) by graphite furnace atomic absorption spectrophotometry (US EPA 1986, Method 7481). Results of water samping are reported as mg/L. Analytical detection limits are <0.001 mg/L for both metals. The recorded concentrations have been corrected for the addition of acid to the water sample.

Statistical Analysis: To determine if molybdenum and copper concentrations in streams are related to mining activities, independent nested ANOVAs (Balanced Design) were performed. Factors included in the analysis were year (1991 vs 1992), treatment (mine-influenced vs non-influenced) and individual study sites (C1 vs C2 and T1 vs T2) which were nested within treatment. Data were log-transformed prior to analysis to standardize the variance.

2.2.4 Soil Sampling

Soil samples were collected at control and treatment sites between 21 and 23 September 1991 to measure water-extractable molybdenum and copper concentrations. Water-extractable metal analysis provides a direct measurement of the total soil metal content that is available to plants.

Sampling Design: The design for soil sampling was influenced by the results from the Norecol (1990) study which indicated that the source of molybdenum in soils was from the mine drainage. Soils were collected at different distances from each stream because not all small mammals could be trapped adjacent to the stream. For each study site, soil sampling involved dividing the area along either side of the streams into three zones based on distance from the stream: Zone 1 was 0-5 m from the stream, Zone 2 was 5-15 m, and Zone 3 was 15-30 m. From each zone, five soil samples were randomly collected, two from one side of the stream, three from the other side, for a total of 15 samples per site.

Sampling Protocol: For each soil sample, the litter and humus were removed from a 20 x 20 cm area. The mineral soil fractions were collected, to a maximum depth of 10 cm, and mixed in a plastic bag. From this, a 250-g sample was removed and placed in a labelled plastic sample bag for analysis.

Analytical Methods: Soil samples were prepared and analyzed by ASL Ltd. The procedure involved leaching 5 g of sample in 50 ml of deionized/distilled water for 4 hours. The leachate was filtered through 0.45 μm filter paper. The resulting extracts were analyzed for total molybdenum and copper by graphite furnace atomic absorption

spectrophotometry (US EPA 1986, Method 7481). Soil sample results are reported as mg/kg on a dry weight basis. Detection limits were <0.01 mg/dry kg for both metals.

Statistical Analysis: To determine the influence of mining activities on the water-extractable (plant-available) molybdenum and copper concentrations in soils, independent nested ANOVAs (Balanced Design) were performed. Factors included treatment (mine-influenced vs non-influenced), individual study sites (C1 vs C2 and T1 vs T2) which were nested within treatments, and distance from the stream (Zones 1, 2, and 3). Data were log-transformed prior to analysis to standardize variance.

2.2.5 Small Mammal Sampling

Small mammals, voles and mice, were trapped at control and treatment sites in 1991 and 1992. Total molybdenum and copper concentrations in the liver were measured to assess contaminant levels for each individual.

Sample Design: At each sample site, every 10 m along the edge of the stream was marked with surveyor's flagging, extending 300-400 m downstream. For each small mammal sampling session in 1991, four newly-established transects were set perpendicular to the stream by placing a trap every 5 m starting at the stream's edge and extending 30 m outward on each side. The total number of traps per site for each trapping session was 56.

In 1992, the transect length was increased to extend 40 m outward from each side of the stream because by this time preliminary analyses suggested that contaminant levels in the small mammals were unrelated to the distance from the stream. Thus, for each sampling session, three newly-established transects were set perpendicular to the stream and extended outwards for 40 m to total 54 traps per site.

In 1991 and 1992, two sites (matched control and treatment) were sampled for small mammals before rotating to the other two sample sites. Transects were moved after each change in location resulting in a randomized design. It should be noted that selection of transects was not completely random in order to avoid over-trapping any one area within the grid.

Trapping Procedures: Small mammals were collected between mid-May and late-September in 1991 and 1992, using Standard Museum Special backbreak traps baited with peanut butter. Captured individuals were identified and sprung traps were freshly baited and re-set each day. Field-tagged specimens were dissected and processed at the village of Fraser Lake, approximately 20 km northeast of the mine, to avoid airborne molybdenum contamination of the tissues.

Sample Protocol: For each small mammal dissected, whole livers were removed and stored frozen in individual sample bags. Liver samples were flown to ASL Ltd at the completion of each field season for individual liver analyses.

Analytical Methods: Metal analysis of tissue was carried out using standardized procedures adapted from the Puget Sound Protocols (Tetra Tech. 1986). Liver tissue was mechanically homogenized prior to digestion with nitric acid, cooling, and further digestion with hydrogen peroxide. The resulting extract was bulked to volume with deionized/ distilled water and the digested portion of the sample was analyzed for total molybdenum and copper. The total molybdenum concentration was determined in the extract by graphite furnace atomic absorption spectrophotometry (US EPA 1986, Method 7481). The total copper concentration was determined in the extract by direct flame atomic absorption spectrophotometry (US EPA 1986, Method 7210). Results are reported as mg/kg on a dry weight basis. Analytical detection limits were <0.025 mg/dry kg for both metals.

An extensive quality assurance/quality control programme was incorporated with the sample analyses. This programme included the analysis of quality control samples to define precision and accuracy and to demonstrate contamination control for the type of samples and parameters under investigation. Quality control samples included method blanks (digestion blanks, extraction blanks) and standard reference material, which included bovine liver (NIST) and dogfish liver (NRCC) tissue certified for metals.

Statistical Analysis: To determine if molybdenum or copper concentrations in the livers of small mammals have a similar pattern to soil metal concentrations, a two-way ANOVA (GLM Unbalanced Design) was performed on the log-transformed liver concentrations for each species. The analyses independently tested for differences in liver

molybdenum or copper concentrations versus treatments (C1+ C2 and T1 + T2) and distance of capture from the stream captured (Zones 1, 2, and 3). Treatment areas were combined to provide an adequate sample for each zone within each treatment. In cases where sample size was adequate within an area (i.e., C1, C2, T1, T2), an independent, one-way ANOVA (GLM Unbalanced Design) was performed on the liver molybdenum concentrations versus distance from the stream.

2.3 Results

2.3.1 Molybdenum and Copper Concentrations in Streams

Water quality results for the 1991 and 1992 field seasons are presented in Table 2.1. For both years, average dissolved molybdenum concentrations in treatment (mine-influenced) streams were one to two orders of magnitude greater than control streams and these differences were extremely significant ($F_{1,24}$=971.4, $p<0.01$). There were also large, significant differences in mean molybdenum concentrations between replicates (i.e., C1 vs C2 and T1 vs T2) ($F_{2,24}$=33.9, $p<0.01$). However, a significant interaction was evident between years and the nested sites within treatments (replicates) suggesting that these differences were not consistent between all pairs in the two years ($F_{2,24}$=5.62, p=0.02). Table 2.1 shows that dissolved molybdenum concentrations in C2 decreased from 1991 to 1992 and increased during the same period in T1. Overall, though, elevated molybdenum concentrations are evident in the streams influenced by mining in comparison to streams outside of the influence of mining activities.

Dissolved copper concentrations were of the same order of magnitude in all four study streams during both field seasons (Table 2.1). Differences in mean copper concentrations between years and treatments were not significant ($F_{1,24}$=1.78, p=0.20, and $F_{1,24}$=1.83, p=0.19, respectively). However, differences in mean copper concentrations between replicates within treatment sites were significant ($F_{2,24}$=5.28, p=0.01). There were no significant interactions.

Table 2.1 Dissolved molybdenum and copper concentrations (mg/L) in study streams in 1991 and 1992. Study streams include two control areas (C1 and C2) and two treatment areas (T1 and T2). Sample size is four in each case.

		AREA			
		C1	C2	T1	T2
Dissolved Molybdenum					
1991					
	Mean	**0.24**	**0.72**	**22.7**	**7.1**
	Range	0.085-0.305	0.386-1.14	22.10-23.80	5.85-8.14
	LogMean (SEM)	-0.67(0.134)	-0.19(0.114)	1.36(0.007)	0.85(0.030)
1992					
	Mean	**0.26**	**0.25**	**30.0**	**7.1**
	Range	0.150-0.362	0.184-0.372	26.80-32.50	5.99-8.43
	LogMean (SEM)	-0.62(0.084)	-0.62(0.071)	1.48(0.020)	0.85(0.032)
Dissolved Copper					
1991					
	Mean	**0.007**	**0.004**	**0.008**	**0.005**
	Range	0.005-0.011	0.001-0.008	0.005-0.011	0.002-0.008
	LogMean (SEM)	-2.20(0.081)	-2.50(0.188)	-2.13(0.071)	-2.41(0.131)
1992					
	Mean	**0.006**	**0.005**	**0.014**	**0.005**
	Range	0.005-0.008	0.004-0.006	0.005-0.032	0.004-0.006
	LogMean (SEM)	-2.23(0.048)	-2.31(0.036)	-1.97(0.171)	-2.31(0.036)

2.3.2 Plant-Available Molybdenum and Copper Concentrations in Soils

The plant-available molybdenum content of soils was highly variable within both treatment and control sites (Table 2.2). However, mean molybdenum concentrations in soils of treatment areas were five to six times greater than in soils sampled from control areas and this difference was significant ($F_{1,48}$=6.24, p=0.02). There were no significant differences in mean molybdenum concentrations in soils within control and treatments, i.e., between replicates ($F_{2,48}$=0.61, p=0.55).

The plant-available molybdenum concentrations in soils varied significantly with distance from the stream ($F_{2,48}$=8.88, p<0.01). At all study sites, molybdenum concentrations were greatest within the first 5 m of the streams (Figure 2.3). After this distance, molybdenum concentrations decreased markedly within all study sites. There were no significant interactions.

The plant-available copper content of soils did not differ between treatment ($F_{1,48}$=3.15, p=0.08), replicates ($F_{2,48}$=1.81, p=0.18), or with distance from the stream ($F_{2,48}$=1.60, p=0.21) (Table 2.2). There were no significant interactions.

Table 2.2 Plant - available molybdenum concentrations (mg/kg on a dry weight basis) in soils relative to distance from the stream (Zones 1-3). The notation of area is the same as for Table 2.1. Sample size is five in each case.

DISTANCE FROM STREAM		AREA			
		C1	C2	T1	T2
Plant-Available Molybdenum					
ZONE 1	**Mean**	**1.6**	**1.4**	**8.9**	**8.7**
(0-5 m)	Range	0.35-3.26	0.40-3.79	0.19-60.90	0.36-64.8
	Log Mean (SEM)	0.21 (0.175)	0.14 (0.177)	0.95 (0.470)	0.94 (0.379)
ZONE 2	**Mean**	**0.3**	**0.4**	**0.8**	**1.7**
(5-15 m)	Range	0.02-1.83	0.10-3.90	0.08-30.0	0.43-18.0
	Log Mean (SEM)	-0.56 (0.348)	-0.37 (0.270)	-0.12 (0.475)	0.24 (0.345)
ZONE 3	**Mean**	**0.1**	**0.7**	**0.5**	**0.5**
(15-30 m)	Range	0.03-0.36	0.09-13.3	0.07-90.6	0.35-0.56
	Log Mean (SEM)	-0.95 (0.200)	-0.17 (0.384)	-0.31 (0.576)	-0.34 (0.033)
Plant-Available Copper					
ZONE 1	**Mean**	**0.1**	**0.2**	**0.2**	**0.1**
(0-5 m)	Range	0.04-0.13	0.06-1.35	0.05-0.59	0.03-0.39
	Log Mean (SEM)	-1.15 (0.083)	-0.77 (0.233)	-0.73 (0.202)	-1.03 (0.185)
ZONE 2	**Mean**	**0.1**	**0.1**	**0.1**	**0.1**
(5-15 m)	Range	0.04-0.09	0.04-0.22	0.04-0.26	0.05-0.25
	Log Mean (SEM)	-1.27 (0.066)	-1.11 (0.123)	-1.08 (0.159)	-1.02 (0.114)
ZONE 3	**Mean**	**0.04**	**0.1**	**0.1**	**0.1**
(15-30 m)	Range	0.02-0.05	0.02-0.21	0.02-2.04	0.05-0.44
	Log Mean (SEM)	-1.42 (0.081)	-1.14 (0.178)	-1.06 (0.362)	-0.84 (0.154)

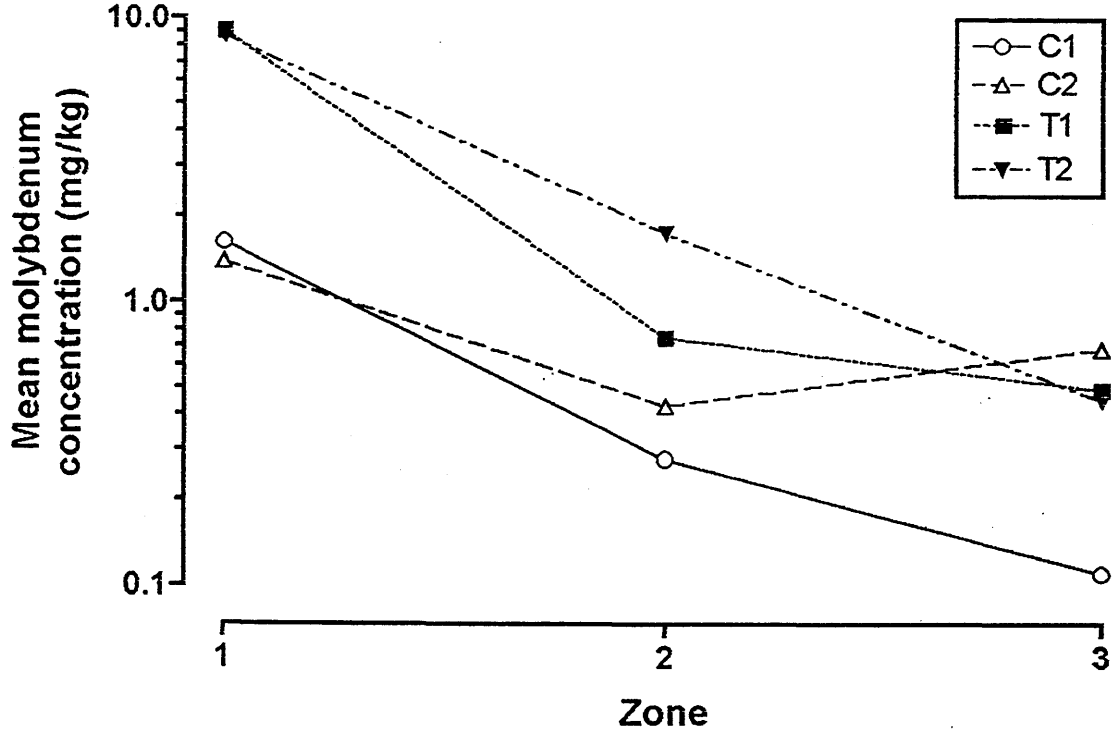

Figure 2.3 The plant-available molybdenum concentration (mg/kg on a dry weight basis) in soils in relation to distance from stream and study area. The notation for area and zone is the same as for Table 2.2.

2.3.3 Molybdenum and Copper Concentrations in the Livers of Small Mammals

A total of 266 small mammals were captured at C1, C2, T1 and T2 during the 1991 and 1992 field seasons. The total catch was comprised of *Peromyscus maniculatus* (n=148), *Clethrionomys gapperi* (n=59), and *Microtus pennsylvanicus* (n=59).

Table 2.3 summarizes the liver molybdenum concentrations in the three species of small mammals; treatment effects are represented by the rows and zone (distance from the stream) effects are represented by the columns.

Small mammals captured in the treatment areas (T1 and T2) did not have correspondingly higher concentrations of molybdenum in their livers compared with those captured in control areas (Table 2.3). At the species level, significant differences were not evident in mean molybdenum concentrations in livers of individuals captured in drainage basins influenced by mining activities compared to control areas for *P. maniculatus* ($F_{1,142}$=0.79, p=0.38) or *C. gapperi* ($F_{1,53}$=1.76, p=0.19). Molybdenum concentrations were almost significantly higher in the livers of *M. pennsylvanicus* captured in the combined treatment sites than those from the combined control sites ($F_{1,53}$=3.84, p=0.06).

Small mammals captured in the riparian zone (Zone 1), in which molybdenum concentrations in the soils were the highest, did not have correspondingly higher concentrations of molybdenum in their livers compared to those from further from the stream (Table 2.3). Significant differences were not evident in mean molybdenum concentrations in livers of individuals from each zone for *C. gapperi* ($F_{2,53}$= 0.47, p=0.63) or *M. pennsylvanicus* ($F_{2,53}$=0.53, p=0.59). Distance from the stream was nearly significant for *P. maniculatus*, but, liver molybdenum concentrations were highest in Zone 2 ($F_{2,142}$=2.39, p=0.10).

Table 2.4 summarizes the liver copper concentrations in the small mammals. Similar to the trends with molybdenum, copper concentrations in the livers of small mammals did not differ significantly between combined control and treatment areas for *P. maniculatus* ($F_{2,142}$=0.86, p=0.36), *C. gapperi* ($F_{2,53}$=0.73, p=0.40), and *M. pennsylvanicus*

($F_{2,53}$=1.64, p=0.21). Although differences in concentrations of copper in livers were not significant between zones for *C. gapperi* ($F_{2,53}$=0.22, p=0.80) and *M. pennsylvanicus* ($F_{2,53}$=1.28, p=0.29), significant differences did exist in liver copper concentrations between zones for *P. maniculatus* ($F_{2,142}$=4.27, p=0.02). Copper concentrations in the liver of this species increased with increasing distance from the stream.

Table 2.3 The molybdenum concentration (mg/kg on a dry weight basis) in the livers of small mammals in relation to distace from the stream and study area. The notation for area and zone is the same as for Table 2.2.

P. maniculatus		DISTANCE FROM STREAM			
AREA		ZONE 1	ZONE 2	ZONE 3	p-value
C1	**Mean**	--	**2.34**	**1.91**	
	Log Mean (SEM)	--	0.369 (0.0365)	0.278 (0.0344)	0.21
	Sample Size	0	4	15	
C2	**Mean**	**0.72**	**1.66**	**1.66**	
	Log Mean (SEM)	-0.137 (0)	0.220 (0.0407)	0.224 (0.1383)	0.99
	Sample Size	1	3	10	
C1+C2	**Mean**	**0.72**	**2.02**	**1.8**	
	Log Mean (SEM)	-0.137 (0)	0.305 (0.0392)	0.0256 (0.0576)	--
	Sample Size	1	7	25	
T1	**Mean**	--	**1.95**	**1.86**	
	Log Mean (SEM)	--	0.295 (0.0552)	0.0274 (0.0417)	0.81
	Sample Size	0	9	34	
T2	**Mean**	**1.48**	**1.62**	**1.55**	
	Log Mean (SEM)	0.170 (0.0593)	0.241 (0.0274)	0.195 (0.0197)	0.83
	Sample Size	5	16	51	
T1+T2	**Mean**	**1.48**	**1.74**	**1.69**	
	Log Mean (SEM)	0.170 (0.0593)	0.241	0.227 (0.0207)	--
	Sample Size	5	25	85	

Table 2.3 (cont) The molybdenum concentration (mg/kg on a dry weight basis) in the livers of small mammals in relation to distace from the stream and study area. The notation for area and zone is the same as for Table 2.2.

C. gapperi		DISTANCE FROM STREAM			
AREA		ZONE 1	ZONE 2	ZONE 3	p-value
C1	**Mean**	**5.62**	**3.47**	**3.48**	
	Log Mean (SEM)	0.754 (0)	0.539 (0.1163)	0.595 (0.0797)	0.69
	Sample Size	1	6	7	
C2	**Mean**	**1.32**	**1.41**	**1.29**	
	Log Mean (SEM)	0.117 (0.0670)	0.155 (0.0484)	0.109 (0.0489)	0.82
	Sample Size	4	6	11	
C1+C2	**Mean**	**1.75**	**2.22**	**1.99**	
	Log Mean (SEM)	0.244 (0.1377)	0.347 (0.0834)	0.298 (0.0710)	--
	Sample Size	5	12	18	
T1	**Mean**	**3.47**	--	**0.38**	
	Log Mean (SEM)	0.544 (0)	--	-0.417 (0.6797)	--
	Sample Size	1	0	2	
T2	**Mean**	**1.41**	**1.17**	**1.23**	
	Log Mean (SEM)	0.152 (0)	0.070 (0.0681)	0.088 (0.0680)	0.89
	Sample Size	1	5	15	
T1+T2	**Mean**	**2.23**	**1.17**	**1.06**	
	Log Mean (SEM)	0.348 (0.1959)	0.070 (0.0681)	0.028 (0.0929)	--
	Sample Size	2	5	17	

Table 2.3 (cont) The molybdenum concentration (mg/kg on a dry weight basis) in the livers of small mammals in relation to distace from the stream and study area. The notation for area and zone is the same as for Table 2.2.

M. pennsylvanicus		DISTANCE FROM STREAM			
AREA		ZONE 1	ZONE 2	ZONE 3	p-value
C1	**Mean**	--	**1.48**	**1.45**	
	Log Mean (SEM)	--	0.166 (0.0355)	0.158 (0)	--
	Sample Size	0	2	1	
C2	**Mean**	**1.01**	**1.10**	**1.02**	
	Log Mean (SEM)	0.004 (0.0525)	0.038 (0.0623)	0.007 (0.0655)	0.23
	Sample Size	8	7	15	
C1+C2	**Mean**	**1.01**	**1.16**	**1.04**	
	Log Mean (SEM)	0.004 (0.0525)	0.066 (0.0515)	0.017 (0.0626)	--
	Sample Size	8	9	16	
T1	**Mean**	**3.98**	**1.26**	--	
	Log Mean (SEM)	0.0600 (0)	0.100 (0)	--	--
	Sample Size	1	1	0	
T2	**Mean**	**1.29**	**1.51**	**1.21**	
	Log Mean (SEM)	0.107 (0.0521)	0.290 (0.0404)	0.223 (0.0504)	0.54
	Sample Size	9	4	11	
T1+T2	**Mean**	**1.44**	**1.47**	**1.21**	
	Log Mean (SEM)	0.157 (0.0608)	0.166 (0.0349)	0.081 (0.0504)	--
	Sample Size		5	11	

Table 2.4 The copper concentration (mg/kg on a dry weight basis) in the livers of small mammals in relation to distance from the stream and study area. The notation of area and zone is the same as for Table 2.2.

P. maniculatus		DISTANCE FROM STREAM			
AREA		ZONE 1	ZONE 2	ZONE 3	p-value
C1	**Mean**	--	**6.60**	**7.35**	
	Log Mean (SEM)	--	0.818 (0.0165)	0.866 (0.0361)	0.51
	Sample Size	0	4	15	
C2	**Mean**	**4.47**	**6.78**	**7.21**	
	Log Mean (SEM)	0.650 (0)	0.832 (0.0523)	0.858 (0.0247)	0.63
	Sample Size	1	3	10	
C1+C2	**Mean**	**4.47**	**6.66**	**7.29**	
	Log Mean (SEM)	0.650 (0)	0.823 (0.0218)	0.863 (0.0234)	--
	Sample Size	1	7	25	
T1	**Mean**	--	**6.51**	**7.27**	
	Log Mean (SEM)	--	0.814 (0.0203)	0.861 (0.0219)	0.29
	Sample Size	0	9	34	
T2	**Mean**	**6.21**	**6.28**	**6.88**	
	Log Mean (SEM)	0.793 (0.0326)	0.798 (0.0207)	0.838 (0.0102)	0.12
	Sample Size	5	16	51	
T1+T2	**Mean**	**6.21**	**6.36**	**7.03**	
	Log Mean (SEM)	0.793 (0.0326)	0.804 (0.0150)	0.847 (0.0107)	--
	Sample Size	5	25	85	

Table 2.4 (cont) The copper concentration (mg/kg on a dry weight basis) in the livers of small mammals in relation to distance from the stream and study area. The notation of area and zone is the same as for Table 2.2.

C. gapperi		DISTANCE FROM STREAM			
AREA		ZONE 1	ZONE 2	ZONE 3	p-value
C1	**Mean**	**8.07**	**6.81**	**7.21**	
	Log Mean (SEM)	0.907 (0)	0.833 (0.0794)	0.858 (0.0334)	0.77
	Sample Size	1	6	7	
C2	**Mean**	**4.99**	**4.77**	**4.28**	
	Log Mean (SEM)	0.698 (0.0270)	0.679 (0.0632)	0.631 (0.0188)	0.42
	Sample Size	4	6	11	
C1+C2	**Mean**	**5.50**	**5.70**	**5.24**	
	Log Mean (SEM)	0.740 (0.0467)	0.756 (0.0537)	0.719 (0.0316)	--
	Sample Size	5	12	18	
T1	**Mean**	**6.45**	--	**5.73**	
	Log Mean (SEM)	0.810 (0)	--	0.758 (0.0106)	--
	Sample Size	1	0	2	
T2	**Mean**	**4.66**	**4.73**	**4.74**	
	Log Mean (SEM)	0.688 (0)	0.675 (0.0219)	0.676 (0.0250)	0.98
	Sample Size	1	5	15	
T1+T2	**Mean**	**5.48**	**4.73**	**4.85**	
	Log Mean (SEM)	0.739 (0.0705)	0.675 (0.0219)	0.685 (0.0229)	--
	Sample Size	2	5	17	

Table 2.4 (cont) The copper concentration (mg/kg on a dry weight basis) in the livers of small mammals in relation to distance from the stream and study area. The notation of area and zone is the same as for Table 2.2.

M. pennsylvanicus		DISTANCE FROM STREAM			
AREA		ZONE 1	ZONE 2	ZONE 3	p-value
C1	**Mean**	--	**4.43**	**4.59**	
	Log Mean (SEM)	--	0.646 (0.0098)	0.662 (0)	--
	Sample Size	0	2	1	
C2	**Mean**	**4.45**	**4.12**	**4.004**	
	Log Mean (SEM)	0.648 (0.0214)	0.614 (0.0243)	0.607 (0.0293)	0.59
	Sample Size	8	7	15	
C1+C2	**Mean**	**4.45**	**4.18**	**4.07**	
	Log Mean (SEM)	0.648 (0.0214)	0.621 (0.0192)	0.610 (0.0276)	--
	Sample Size	8	9	16	
T1	**Mean**	**9.07**	**4.53**	--	
	Log Mean (SEM)	0.958 (0)	0.656 (0)	--	--
	Sample Size	1	1	0	
T2	**Mean**	**4.57**	**4.42**	**4.36**	
	Log Mean (SEM)	0.660 (0.0183)	0.646 (0.0206)	0.639 (0.0301)	0.84
	Sample Size	9	4	11	
T1+T2	**Mean**	**4.89**	**4.44**	**4.36**	
	Log Mean (SEM)	0.690 (0.0340)	0.648 (0.0161)	0.639 (0.0301)	--
	Sample Size	10	5	11	

2.4 Discussion

The influence of molybdenum mining at the Endako Mine was evident in the surrounding environment. Dissolved molybdenum concentrations in streams influenced by mining activities were two to three orders of magnitude greater than streams found outside of the mine's influence. Dissolved molybdenum concentrations measured in streams for this study follow similar patterns compared with streams draining from highly mineralized areas of Colorado. Voegeli and King (1969) correlated molybdenum concentrations in stream waters exceeding 5 μg/L with occurrences of molybdenum mineralization, and concentrations exceeding 60 μg/L with mining activity.

Mine-influenced drainage basins also had higher concentrations of plant-available molybdenum in the soil than control basins. However, elevated molybdenum concentrations were mainly evident within the first 5 m of the streams and this was seen at both treatment and control areas. Presumably, the molybdenum enters the soil solution from the stream to an extent that may be related to the steepness of the stream bank. The higher concentrations of molybdenum in the riparian soils in both mine-influenced and non-influenced drainage basins measured in this study confirm the preliminary results observed in the Norecol (1990) study.

Molybdenum concentrations observed in the livers from individuals from all three species of small mammals from this study typically ranged between 0.5 and 3 mg/kg, with the majority of values ranging between 1 and 2 mg/kg (49%, 62%, and 63% for *C. gapperi*, *P. maniculatus*, and *M. pennsylvanicus*, respectively). These results are comparable to values reported for experiments with rats. For example, on normal diets, typical molybdenum concentrations in the liver of rats measured 1.8 mg/kg (Higgins *et al.* 1956) and 2.4 mg/kg (Fungwe *et al.* 1989). "Elevated" molybdenum concentrations in the livers of individuals from my study tended to be at least 2.5 times greater than the majority of the observed values, with few values falling between these two categories. Elevated values ranged from 5.2 to 12.1 mg/kg, with the molybdenum concentration in the liver of one individual measuring 26 mg/kg (*P. maniculatus* from control site C2).

This study measured the amount of molybdenum in soils that is available for uptake by vegetation (section 2.3.3). However, Norecol (1990) demonstrated that sites with the highest soil molybdenum concentrations generally had the highest molybdenum levels in the vegetation. Consumers of this vegetation would, therefore, be expected to have elevated concentrations of molybdenum in their diet and correspondingly elevated concentrations in their livers (Davis *et al.* 1960). This study, however, demonstrated a very poor relationship between the pattern of molybdenum in the livers of herbivorous small mammals and the pattern of molybdenum measured in soils. Molybdenum concentrations in small mammals were not higher in individuals captured in treatment areas than in those captured in control areas. Nor were liver molybdenum concentrations higher in small mammals captured within the riparian zone than in areas further from the stream.

There may be two reasons why small mammals do not show these general patterns of contaminant loadings. First, small mammals may be ranging over larger areas than assumed for the design of this study. In addition, the influence of the streams on environmental molybdenum levels is limited to a narrow band on either side of the streams. Small mammals may not be limited to foraging within this zone. Second, uptake of molybdenum varies among plant species. Studies on a limited number of plant species (primarily forage species for cattle) indicate legumes accumulate higher levels of molybdenum than grasses (Kubota 1975, Vlek and Lindsay 1977). The species of plants making up the bulk of the diet of small mammals may not contain high levels of molybdenum. In addition, laboratory experiments have shown that rats will learn to avoid diets high enough in molybdenum to cause diarrhea (Monty and Click 1961). It is possible that the small mammals in this area learn to associate a gastrointestinal disturbance with vegetation containing toxic levels of molybdenum and, therefore, avoid this vegetation when feeding. The level of molybdenum in the diets of small mammals will be examined in Chapter 3.

Overall, it is obvious that the effects of molybdenum in small mammals cannot be studied by comparing symptoms of toxicity in individuals caught in treatment areas versus those captured in control areas. Fortunately for this study, the molybdenum concentrations

in the liver of individual small mammals were determined, which allows the study to be re-designed. The effects of molybdenum in small mammals can be determined by comparing those individuals with low concentrations of molybdenum in their livers to individuals with high concentrations. The toxic effects of molybdenum in small mammals are presented in Chapter 3.

The results presented in Chapter 2 also illustrate the importance of determining contaminant loading in individuals rather than composite sampling. Composite sampling may not be sensitive enough to determine the pattern of contaminant loading in wildlife and may, therefore, obscure patterns. Chapter 2 also illustrates the importance of avoiding the assumption when designing field toxicity studies that contaminant loading of individual animals will follow the overall pattern observed in the environment (see Green 1979).

3. THE TOXIC EFFECTS OF MOLYBDENUM IN SMALL MAMMALS

3.1 Introduction

The literature survey in section 1.3 focused on three main points. First, a high dietary intake of molybdenum, as molybdate, is toxic to mammals, and this toxicity is primarily due to induced copper deficiency. Second, copper supplementation can, to a limited extent, negate these toxic effects. And third, research further demonstrated that the molybdenum-copper interaction can be strongly influenced by sulphur compounds. This interaction is particularly important in ruminants and accounts for their relatively high sensitivity to dietary molybdenum.

Research involving laboratory rats supports the hypothesis that copper becomes biologically unavailable in animals with high levels of molybdenum in their diets, because molybdenum toxicity in rats can result in symptoms that are similar to copper deficiency. Symptoms of molybdenum-induced copper deficiency include anaemia, bone deformation and reduced calcification, cerebral edema and cortical necrosis, achromotrichia, and fetal absorption (Buck 1978).

Not all manifestations of molybdenum intoxication, however, are attributable to an inhibition of copper-dependent processes (Mills *et al.* 1981). Reduction in the rate of growth of rats, given high molybdenum diets, is only partially alleviated by increases in dietary copper (Gray and Daniel 1954). In rats, histopathological features of molybdenum intoxication in liver and kidney, and concurrent declines in the activities of certain enzymes, are also not typical of copper deficiency (Mills and Davis 1987). Other features probably characteristic of molybdenosis *per se* in rats are the development of mandibular

exostoses (Ostrom *et al.* 1961, Van Reen 1959), testicular damage (Jeter and Davis 1954), irregular oestrus cycles (Fungwe *et al.* 1986, 1989, and 1990), and disorders of phosphorus metabolism (Miller and Engel 1960). The mechanisms behind some symptoms (diarrhea, anaemia and some fatalities) in rats may include abnormal accumulation of sulphide in tissues due to excessive molybdenum levels which have depressed sulphide oxidase activities (Halverson *et al.* 1960).

3.1.1 Changes of Metal Concentrations in Liver Tissue

Supplementation of dietary molybdenum has been reported to promote the accumulation of both molybdenum and copper in the liver of rats (Fungwe *et al.* 1989, Kulwich *et al.* 1953, Miller *et al.* 1956, Nederbragt 1980). The magnitude of this effect can be directly correlated with the dose of molybdenum in the diet (Compere *et al.* 1965). Fungwe and co-workers (1989) dosed female rats with water containing 0.025 (basal diet), 5, 10, 50, and 100 mg Mo/L for 42 days. Molybdenum concentrations in the liver increased significantly as the dose of molybdenum increased. Average molybdenum concentration in the liver ranged from 2.4 mg/kg in rats fed the basal diet, to 13.2 mg/kg in rats fed the highest molybdenum dose. The supplemental molybdenum also caused the retention of copper in the liver from "normal" levels of 101 mg/kg to 131.3 and 158.7 mg/kg at the 50 and 100 mg/L doses, respectively (Fungwe *et al.* 1989). The concomitant increase in liver copper with increased dietary molybdenum suggests that molybdenum prevents the physiological utilization of copper after the absorption of molybdenum into body tissues, particularly in the liver (Miller *et al.* 1956, Mills 1960).

3.1.2 Growth Inhibition

High molybdenum intake may cause growth retardation or loss of body weight in mammals. Several experiments have shown that relatively high dietary concentrations of molybdate (300-1200 mg/kg in the diet) markedly depressed growth in rats (Compere *et*

al. 1965, Gray and Daniel 1954, Neilands *et al.* 1948, Van Reen 1959). When the initial copper status of the rat was lowered, 80 mg/kg of molybdate in the diet was sufficient to cause pronounced growth retardation (Comar *et al.* 1949). Growth depression, however, is not related directly to a lack of copper as this effect is only partially alleviated by an increased intake of copper (Gray and Daniel 1954, Van Reen 1954).

Subsequent studies led researchers to propose that the weight loss of molybdenosis results from a voluntary rejection of the diets (Monty and Click 1961). These researchers suggested that the rats learn to associate a gastrointestinal disturbance with a sensory attribute of diets containing toxic levels of molybdenum. Later studies demonstrated that the growth retardation and loss of body weight were not completely due to reduction in food intake (H.L. Johnson and Miller 1961, R.H. Johnson *et al.* 1969). These studies supported an early hypothesis outlined by Gray and Daniel (1954) that elevated levels of dietary molybdenum reduced the efficiency of absorption of nutrients through the intestinal wall.

3.1.3 Skeletal Abnormalities

Another feature of molybdenum toxicity in rats is an interference with normal bone development. Rats fed 2 mg/kg copper and 80 mg/kg molybdate showed retarded skeletal growth and poor calcification (Comar *et al.* 1949). Rats fed 1200 mg/kg molybdate developed connective tissue changes which resulted in mandibular and maxillary exostoses (spurs) (Ostrom *et al.* 1961, Van Reen 1959). In rats fed 100 or 200 mg Mo/kg of diet, the humerus, femur, and tibia were shorter, with an increase in the shaft diameter (Lalich *et al.* 1965) and an enlargement of the articulating surfaces (Lalich *et al.* 1965, Miller *et al.* 1956).

Spence and co-workers (1980) conducted a sequential study of the skeletal abnormalities that develop in rats given 6 mg Mo/kg as ammonium tetrathiomolybdate (ATTM). The skeletal lesions that developed had a widespread distribution: exostoses were found at the end of long bones, mandibles and rib joints, growth plates widened and

thickened, and osteogenesis decreased. These skeletal deformities appeared after 5-7 days on the ATTM diet in comparison to 4-6 week on a diet containing molybdate (Spence *et al.* 1980).

It is postulated that the molybdenum-induced skeletal deformities are due to a deficiency of tissue copper (Lalich *et al.* 1965). However, the development of exostoses at relatively high dietary levels of molybdate appear to be due to molybdenum toxicity *per se* although the mechanism is unknown (Mills and Davis 1987). Studies of calcium kinetics in bone of rats when fed 100 mg Mo/kg as molybdate, indicated that net calcium transport into bone was significantly reduced (Solomons and Bekemans 1976).

3.1.4 Reproductive Effects

Both male and female reproductive functioning is adversely affected by high dietary levels of molybdenum. Male rats given high molybdenum diets (80-140 mg/kg) experienced sterility associated with some testicular degeneration (Jeter and Davis 1954). Males that died due to a copper deficient diet showed an increase of sloughed cells in the epididymes (Hall and Howell 1973). Degenerative changes were also observed in the testes consisting of sloughing of germinal cells into the lumen of some of the seminiferous tubules (Hall and Howell 1973).

Studies with female rats suggest that a low copper status and a high dietary molybdenum intake could exert independent effects on reproduction (Phillippo *et al.* 1985, 1987). Some studies have demonstrated that low dietary copper adversely affects late fetal development as evidenced by resorption of fetuses at the thirteenth day of pregnancy, but oestrus activity and conception rates were not affected (Dutt and Mills 1960, Hall and Howell 1969a). Oestrus recommenced after an interrupted pregnancy in copper deficient rats (Hall and Howell 1969b).

Other studies seem to indicate that a high level of molybdenum intake (80-140 mg/kg) has little effect on fetal development, but does cause irregular oestrus cycles (Jeter and Davis 1954, Winston 1981). Prolonged oestrus cycles were confirmed in a later study

resulting from molybdenum supplementation of 50-100 mg/kg, but there was no significant effect on conception rate (Fungwe *et al.* 1989). This research group later reported adverse embryonic effects that included inhibition of bone ossification (Fungwe *et al.* 1990). Jeter and Davis (1954) demonstrated that supplementary molybdate resulted in deficient lactation (measured as decreased milk yield) in female rats fed diets containing 80-140 mg Mo/kg.

3.1.5 Objectives

The first objective of this Chapter is to determine if diet of small mammals is the likely source of liver molybdenum. It is predicted that the molybdenum concentration in the liver of small mammals will show a strong correlation to the molybdenum concentration in their stomach contents.

The second objective is to determine the influence of age on both molybdenum and copper concentrations in the liver of small mammals. It is predicted that molybdenum concentrations in the liver will be independent, and copper concentrations will be dependent, of the age of small mammals.

The third objective of this chapter is to determine if molybdenum mining at Endako might lead to toxic effects in small mammals in the surrounding environment. Specific effects of molybdenum toxicity that were investigated included copper concentrations in the liver, body mass, long bone development, and reproduction. First, it is predicted that copper and molybdenum concentrations in the liver will be positively correlated. Second, it is predicted that individuals with elevated molybdenum concentrations in their liver will have reduced body mass and abnormal long bone development. Lastly, it is predicted that males with elevated molybdenum concentrations in their liver will have lower sperm production and smaller reproductive organs and accessory glands. Similarly, females with elevated molybdenum concentrations in their liver are predicted to have a lower incidence of pregnancy, lower ovulation rates, smaller litter sizes, and a higher incidence of resorption events.

3.2 Methods

3.2.1 General Approach of the Toxicological Investigation

For the toxicological investigation, small mammals from each species were separated into three categories based on liver molybdenum concentration. The categories included those with **Low** liver molybdenum concentrations (<2 mg/kg), those with **Intermediate** levels (2-4 mg/kg), and those with **High** concentrations (>4 mg/kg). These categories were based on literature values reported for similar species (i.e., <2 mg/kg molybdenum is 'normal' for laboratory rats) and natural divisions observed in the concentrations of molybdenum in the livers of the small mammals captured for this study (Chapter 2).

In order to increase the number of individuals with High molybdenum concentrations, two sample sites were added to the study for the second field season (Figure 2.1). The new sites included treatment site T3, located in a drainage basin north west of the mine site in an area of molybdenum mineralization, and treatment site T4, located on the Endako Mine plant-site where airborne molybdenum contaminates surrounding soils and vegetation.

3.2.2 Molybdenum and Copper in the Diet of Small Mammals

The molybdenum availability in food was determined by analyzing stomach contents for total molybdenum and copper concentrations. Analytical methods involved the same procedures as those detailed for liver tissue analysis (section 2.2.5). Composite sampling of the stomach contents was necessary to achieve a minimum of two grams per sample on a dry weight basis, because this is required for laboratory analysis. Stomach contents were composited according to individual liver molybdenum concentrations (Low vs High), reproductive class (Immature vs Mature), species, and site. Composite sampling resulted in 33 samples classified in the Low liver molybdenum group and seven samples

classified in the High liver molybdenum group. The number of individuals per composite sample varied from 1 to 13.

To determine if molybdenum or copper concentrations of the stomach contents differed between the Low and High liver molybdenum groups, two-tailed Student t-tests were performed. Data were log-transformed to standardize the variance.

To determine if a relationship existed between the molybdenum concentrations of the liver and the molybdenum content of the diet, a Model I linear regression was performed using log- transformed data. The data were "weighted" using the number of individuals for each composite stomach sample to account for the unequal number of individuals involved in each sample.

3.2.3 Liver Molybdenum and Copper Concentration in Relation to Age

The mass of the eye lens was used as an indicator of age for the small mammals (Gliwica 1983, Lord 1959). Normally, both lenses were weighed (+/- 0.1 mg), but when only a single lens was recovered, its mass was doubled.

To determine if molybdenum or copper accumulated in the liver of small mammals with age (i.e., potential environmental exposure), the concentration of the metals in the liver were plotted against the mass of the eye lenses. Data were first grouped according to species, gender, and site and independently analyzed by performing a Model I linear regression on the arithmetic data. If the sample size was less than five, the data were not analyzed.

3.2.4 Copper Concentrations in the Livers of Small Mammals

The methods for determining the copper concentration in livers from small mammals was detailed in section 2.2.5. To determine if a relationship between copper and molybdenum concentrations existed, a Model I linear regression was performed. Data were grouped according to species and site and were log-transformed to standardize the variance.

3.2.5 Influence of Molybdenum on Body Mass

The eviscerated weight (the stomach, intestine, and reproductive tract removed) was measured (± 0.1 g) for each specimen. Body mass is related to age as assessed by mass of the eye lens, and as indicated by preliminary data analyses, this relationship is linear (see figure 3.1 for an example). Therefore, to determine if molybdenum inhibited growth in mass, an ANCOVA (GLM Unbalanced Design) was performed on body mass versus gender, site, and the three liver molybdenum categories, using mass of the eye lens as a covariate. It should be noted that, each time an ANCOVA was performed (i.e., sections 3.2.5, 3.2.6, and 3.2.7), I assumed no interaction between the covariate (mass of the eye lens) and the main effect (Liver Molybdenum Category) due to analytical limitations with small sample size. Visual inspection of the data, however, indicated that there was no consistent difference between slopes relating to the molybdenum concentration of the livers.

Data for *C. gapperi* from all sites were combined to increase sample size because preliminary statistical analyses demonstrated that the body mass of individuals did not differ significantly with respect to site or gender ($F_{4,58}$=2.16, p=0.09 and $F_{1,58}$=0.99, p=0.33, respectively). In contrast, for *P. maniculatus*, there were significant effects on body mass due to site ($F_{5,173}$=3.24, p=0.01) and interactions between site and gender were significant ($F_{5,173}$=3.68, p<0.01). Therefore, separate ANCOVAs (GLM for Unbalanced Design) were performed for each site. Data for *M. pennsylvanicus* were not included in this investigation because liver molybdenum concentrations rarely exceeded 2 mg/kg (i.e., only 2 representatives in the Intermediate liver molybdenum category and only 1 representative in the High liver molybdenum category).

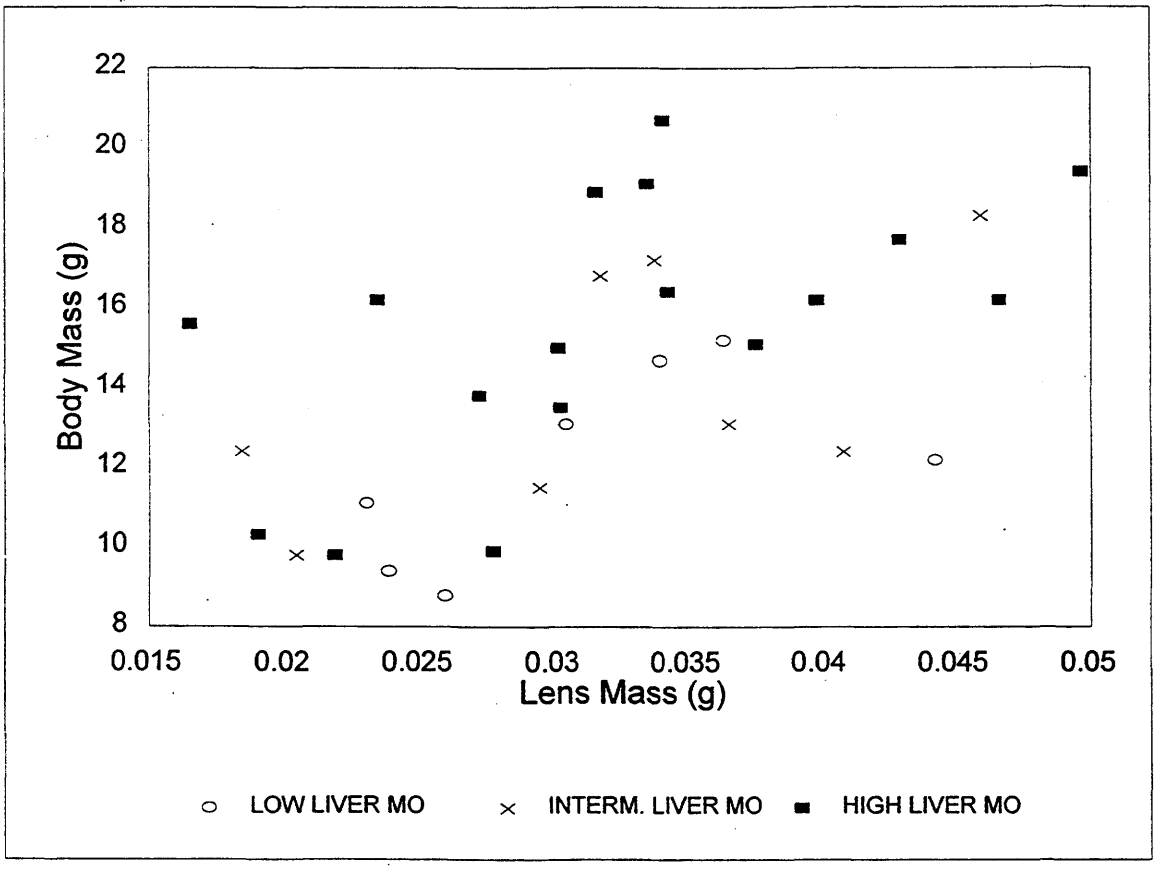

Figure 3.1 The linear relationship between age of the individual (mass of the eye lens) and body mass in *P. maniculatus* captured at treatment area T4. Molybdenum category separated individuals into those with Low (<2 mg/kg), Intermediate (2-4 mg/kg), and High (>4 mg/kg) molybdenum concentrations in their livers.

A more detailed analysis of the effect of molybdenum on body mass was conducted using all the *P. maniculatus* from treatment site T4. The investigation involved only this group because a relatively large range of molybdenum concentrations (i.e., Low, Intermediate, and High) was represented by the group, thus making comparisons possible. An additional ANCOVA (GLM Unbalanced Design) was carried out with the *P. maniculatus* at treatment site T4, in which the liver molybdenum categories were changed to determine if an effect could be detected at extremely high molybdenum concentrations as measured in individuals from this site. For this analysis only, the liver molybdenum categories were Low (**<5 mg/kg**), Intermediate (**5-10 mg/kg**), and High (**>10 mg/kg**).

3.2.6 The Effects of Molybdenum on Bone Development

The effects of molybdenum on bone development were investigated using the length and radius measurements for the femur and humerus of *C. gapperi* from C1 and *P. maniculatus* from T4. This investigation involved these two groups because a relatively large range of molybdenum concentrations were represented within each group, making comparisons possible. Each parameter was measured twice with digital calipers to reduce measurement error. The average of the two values was then used to calculate length/radius ratios for each bone type.

To determine the influence of molybdenum on long bone development, an ANCOVA (GLM Unbalanced Design) was performed using the ratio of each bone type versus gender and the three liver molybdenum categories. Mass of the eye lens was used as a covariate to determine if the age of the individual influenced indices of bone development. The relationship between the length/radius ratios of the long bones and the mass of the eye lens was linear (see figure 3.2 for example).

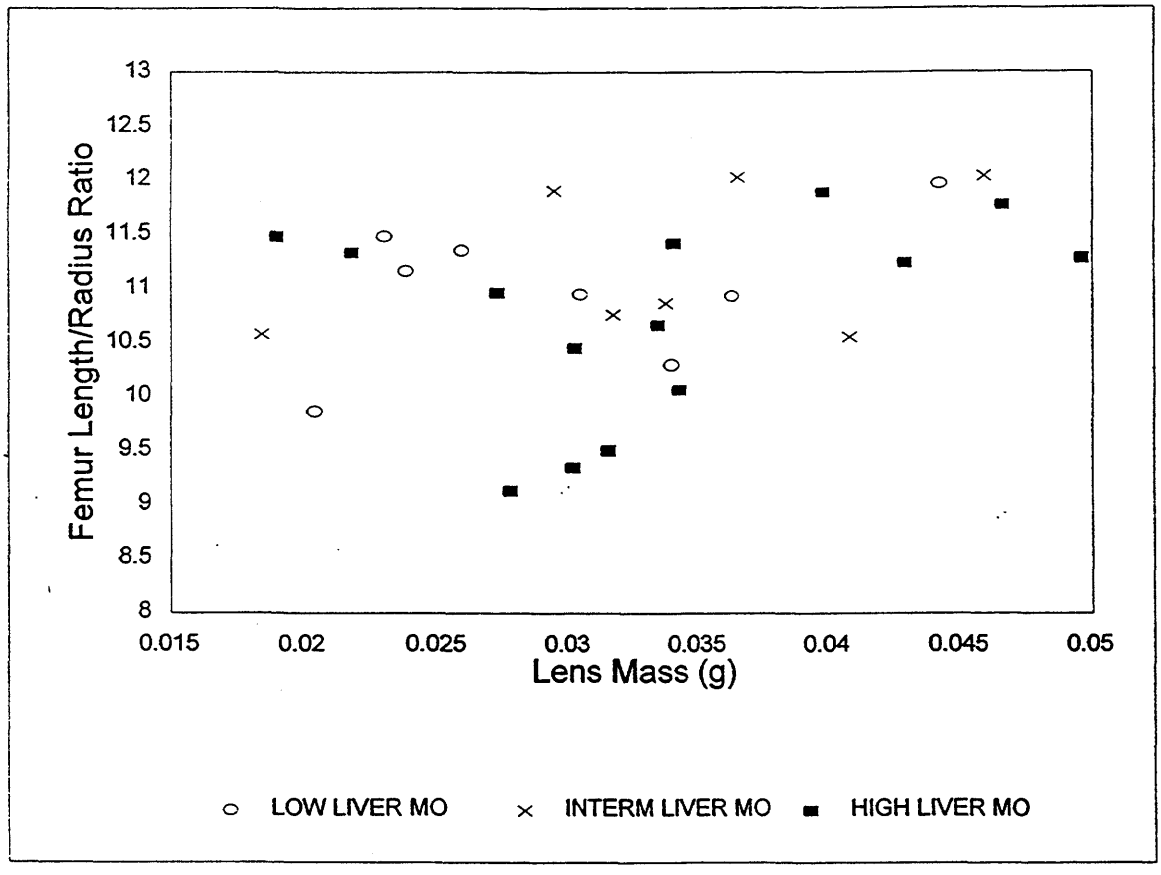

Figure 3.2 The relationship between age of the individual (mass of the eye lens) and the femur length/radius ratio in *P. maniculatus* from treatment area T4. The notation for liver molybdenum category is the same as for Figure 3.1.

3.2.7 The Reproductive Effects of Molybdenum in Male Rodents

Male rats fed diets high in molybdenum (80-140 mg/kg) experienced sterility and testicular degeneration. For this investigation, the influence of molybdenum on male reproduction involved both the mass of the testes and seminal vesicles, and an evaluation of sperm maturation.

The first part of this investigation involved measuring the mass of the testes (± 0.1 g) and seminal vesicles (either ± 0.1 or 0.0001 g, depending on size). The mass of testes and seminal vesicles are related to age, as assessed by the mass of the eye lens. To determine if molybdenum caused a decrease in the mass of the reproductive tissues, separate ANCOVAs (GLM Unbalanced Design) were performed on the mass of the testes or seminal vesicle mass, versus site and the three categories of molybdenum concentrations as specified in section 3.2.1, using mass of the eye lens as a covariate. The relationships between the mass of the reproductive organs and the mass of the eye lens were linear (see figure 3.3 for example).

Data for *C. gapperi* from all the sites were combined to increase sample size because preliminary statistical analyses demonstrated that neither the mass of the testes nor the seminal vesicles differed significantly between sites ($F_{2,26}$=1.61, p=0.20 and $F_{4,26}$=1.23, p=0.33, respectively).

In the case of *P. maniculatus*, there were significant effects on the mass of the testes due to site ($F_{5,84}$=2.64, p=0.03), but not on the mass of the seminal vesicles ($F_{5,67}$=0.91, p=0.48). Therefore, it was possible to combine data on the mass of seminal vesicles from each site. However, independent one-way ANCOVAs (GLM Unbalanced Design) for each site, except for control site C2 and treatment site T3 which did not have enough individuals for analysis, were performed on the mass of the testes. Two additional one-way ANCOVAs (GLM Unbalanced Design) were performed independently on the mass of the testes and the seminal vesicles of *P. maniculatus* from treatment site T4, versus three new categories of liver molybdenum concentrations, using mass of the eye lens as a covariate. For this analysis, the liver molybdenum categories were Low (**<5 mg/kg**), Intermediate

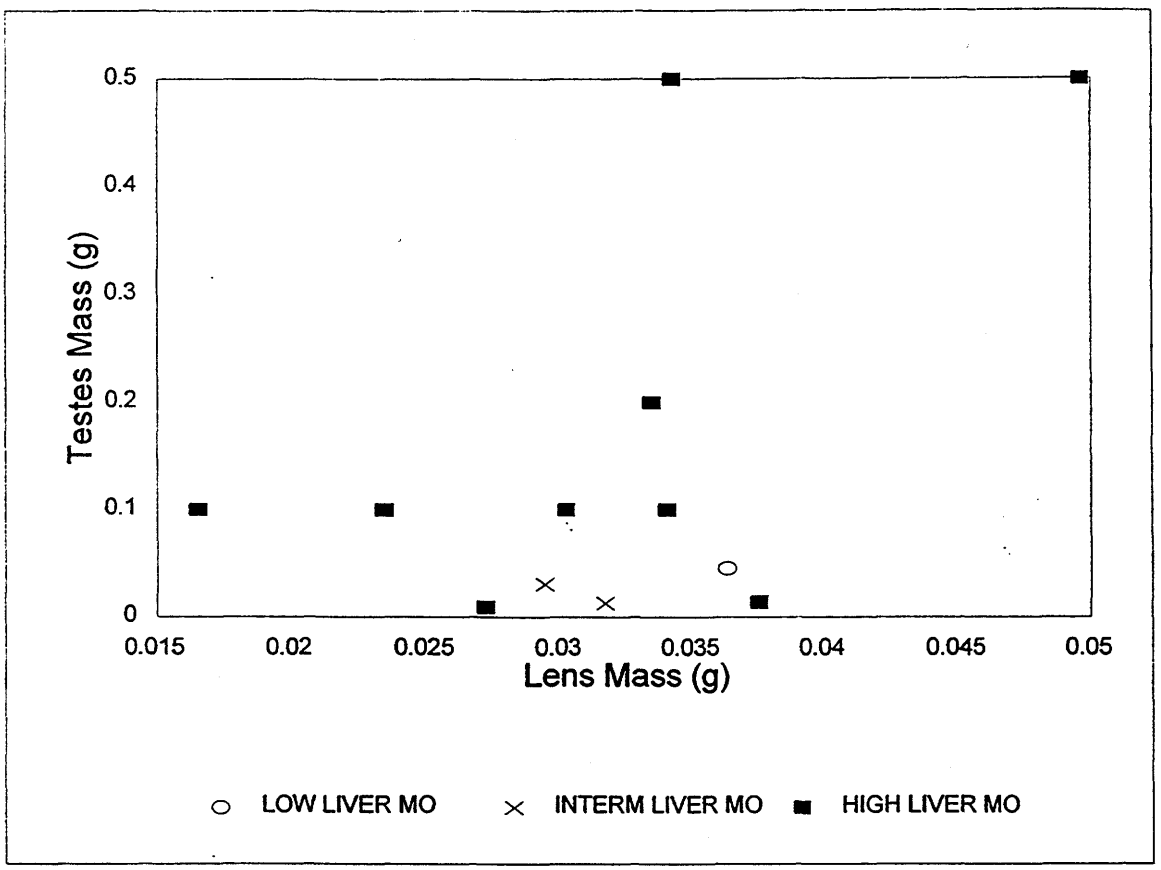

Figure 3.3 The relationship between age of the individuals (mass of the eye lens) and the mass of their testes for *P. maniculatus* from treatment area T4. The notation of liver molybdenum category is the same as for Figure 3.1.

(**5-10 mg/kg**), and High (**>10 mg/kg**) as described in section 3.2.5. The molybdenum categories were changed to determine if an effect could be detected at extremely high liver molybdenum concentrations measured in males from this site.

Data for *M. pennsylvanicus* were not included in this investigation because liver molybdenum concentrations rarely exceeded 2 mg/kg in this species.

The second part of this investigation, examined the relationship between the reproductive class of the males and the mass of their testes. The reproductive class for each male was determined from sperm samples collected from the epididymes and smeared on microscopic slides. Under 10X or 100X power of a microscope, the individual was classified according to the following criteria:

Immature: No sperm visible.

Sub-adult: Very few sperm visible among numerous and massive fat globules, or scattered sperm among fat globules.

Adult: Numerous sperm visible with some fatty material still present, or dense masses of sperm with no other recognizable material present.

The relationship between reproductive class and the mass of the testes was determined for *C. gapperi* and *P. maniculatus* using the Spearman Rank-Order Correlation Coefficient for non-parametric data. The molybdenum concentration in the livers of individuals deviating greatly from this overall trend were examined to see if the molybdenum concentrations in their livers were unusually Low or High.

3.2.8 The Reproductive Effects of Molybdenum in Female Rodents

The reproduction of female rats is adversely affected by high dietary levels of molybdenum. This study investigated the influence of molybdenum on the incidence of pregnancy, ovulation rates, litter size, and reproductive success in female small mammals.

The reproductive investigation involved only mature females which included those who were either reproductively active (corpora lutea visible), pregnant (embryos macroscopically visible), or non-pregnant. The reproductive tract of each female specimen

was dissected and examined for evidence of reproductive activity. The number of corpora lutea, embryos, and resorption events were recorded for each mature female. Data on mature females from all sites were combined for *C. gapperi* and *P. maniculatus* to increase sample size for the analyses. Data from *M. pennsylvanicus* were not included in this investigation because liver molybdenum concentrations rarely exceeded 2 mg/kg in this species.

To determine if molybdenum influenced the proportion of pregnant females, a Fisher's Exact Test was conducted for each species on the number of pregnant females versus the number of non-pregnant females, within the Low and High liver molybdenum categories (2x2 Contingency Table), as defined in section 3.2.1. Representation in the Intermediate category was inadequate for analysis. To account for any influence on the incidence of pregnancy due to season and year, the data were separated by season (May/June and July/August) for each year (1991 and 1992).

To determine if molybdenum influenced ovulation rates, females from each species were classified according to the number of corpora lutea counted. The classes were 1-3, 4-6, or 7-9 corpora lutea. Data were combined into these classes to increase the number of samples per classification. A Chi-squared Test for Independence was performed independently on the counts for each species versus liver molybdenum category (Low vs High).

A similar analysis was performed on the embryo counts for each species. Females were classified according to the number of embryos. The classes were 1-3, 4-6, or 7-9 embryos. A Chi-squared Test for Independence was performed independently on the embryo counts for each species versus liver molybdenum category (Low vs High).

Lastly, to determine if molybdenum influenced the incidence of resorption events, a Fisher's Exact Test was conducted for each species on the proportion of females that had evidence of resorption events versus the proportion of females that did not, within the Low and High liver molybdenum categories (2x2 Contingency Table).

3.3 Results

3.3.1 Small Mammal Trapping Results

Table 3.1 summarizes the number of individuals captured in each liver molybdenum category for the three small mammals species. *P. maniculatus*, the most abundant species, was captured at all the sites and individuals with High liver molybdenum concentrations were captured in all sites except C1 and T3. *C. gapperi* was found at all the sites except T4 and individuals with High liver molybdenum concentrations were captured only at C1.*M. pennsylvanicus* was represented by large numbers only at sites C2 and T2. Liver molybdenum concentrations rarely exceeded 2 mg/kg in this species.

3.3.2 Molybdenum and Copper in the Diet of Small Mammals

The source of molybdenum to small mammals is likely through their diet. Consequently, stomach contents were analyzed for molybdenum and copper to determine the amount of these metals available to small mammals through their diet.

Molybdenum concentrations in the stomach contents from small mammals with Low liver molybdenum concentrations averaged 23.4 mg/kg compared to 288.4 mg/kg in stomach contents from those with High liver molybdenum concentrations and this difference was significant (t_{38}=4.6, $p<0.01$, Table 3.2). The molybdenum concentration in the diet of the High molybdenum group is within the range in which adverse growth, skeletal, and reproductive effects have been observed. The copper concentrations in the stomach contents of the two groups did not differ significantly between individuals from Low and High liver molybdenum categories (t_{38}=0.98, p=0.33, Table 3.2).

There was a strong, positive relationship between the molybdenum concentration in the liver of small mammals and the molybdenum concentration of the stomach contents. The results of the linear regression showed that the molybdenum concentration in the liver is significantly related to the concentration of molybdenum measured in the stomach

Table 3.1 The number of small mammals for each species captured in each liver molybdenum category. Low is <2 mg/kg, Intermediate is 2-4 mg/kg, and High is >4 mg/kg. Study areas include two controls (C1 & C2) and four treatments (T1, T2, T3 & T4).

	P. maniculatus			*C. gapperi*			*M. pennsylvanicus*		
	Liver Molybdenum Category								
AREA	Low	Interm	High	Low	Interm	High	Low	Interm	High
C1									
Male	5	7	-	1	2	4	3	-	-
Female	6	1	-	2	2	3	-	-	-
C2									
Male	7	-	1	9	-	-	10	1	-
Female	6	-	-	11	1		18	1	-
T1									
Male	15	10	-	1	-		1	-	1
Female	11	4	3	2	-	-	-	-	-
T2									
Male	28	6	1	10	2	-	5	-	-
Female	32	5	-	9	-	-	19	-	-
T3									
Male	3	-	-	4	-	-	1	-	-
Female	6	-	-	6	-	-	-	-	-
T4									
Male	1	2	9	-	-	-	-	-	-
Female	7	5	8	-	-	-	-	-	-
Total	127	40	22	55	7	7	57	2	1

contents (b=1.5, p<0.01). Approximately, 49% of the variation in the liver molybdenum concentration is explained by the variation in the molybdenum concentration of the stomach contents.

3.3.3 The Relation of Age to Molybdenum and Copper Concentration in the Liver of Small Mammals

The concentration of molybdenum in the liver of small mammals was not related to age. Regression analyses indicate that none of the slopes were significantly different from zero (Table 3.3). Molybdenum did not appear to accumulate in the liver of small mammals with increasing age and/or environmental exposure time. Thus, for future statistical analyses, individuals from the same species and sex can be grouped together regardless of age (i.e., "age" does not have to be a co-variable unless some other parameter is related to age).

Table 3.4 summarizes the results of the regression analyses performed on liver copper concentration in relation to age. Copper concentration in the liver of small mammals was not related to age of the specimen for all species in all sites except for male *P. maniculatus* at sites T2 (b=37.4, p=0.02) and T4 (b=201.3, p=0.01) and female *C. gapperi* at site T2 (b=101.2, p<0.01). The latter results support literature which indicates that copper concentration in the liver of mammals is related to age but not gender. However, numerous other dietary factors can influence the copper status of the animal, making these differences difficult to detect (Davis and Mertz 1987).

Table 3.2 Total molybdenum and copper concentrations (mg/kg) in stomach contents of small mammals in Low (<2 mg/kg) and High (>4 mg/kg) liver molybdenum categories.

		Liver Molybdenum Category		
		Low	High	p-value
Molybdenum				
	Mean	**23.4**	**288.4**	**<0.01**
	Range	1.83-99.0	25.7-1240	
	Log Mean (SEM)	1.37(0.100)	2.46(0.221)	
	Sample Size	33	7	
Copper				
	Mean	**12.6**	**17.8**	**0.33**
	Range	2.22-403	4.89-46.8	
	Log Mean (SEM)	1.10(0.067)	1.25(0.154)	
	Sample Size	33	7	

Table 3.3 Results of linear regression of liver molybdenum concentration in relation to the age of the specimen (n = sample size, and if n<5 regression analysis was not performed). The notation of area is the same as for Table 3.1.

	AREA					
	C1	C2	T1	T2	T3	T4
P. maniculatus						
Males						
r^2 value	**0.03**	**0.02**	**0.02**	**0.02**	-	**<0.01**
p-value	0.57	0.77	0.51	0.46	-	0.85
n	12	6	24	35	3	12
Females						
r^2 value	**<0.01**	**<0.01**	**0.01**	**0.04**	**0.25**	**0.01**
p-value	0.96	0.92	0.69	0.22	0.31	0.66
n	7	6	17	37	6	20
C. gapperi						
Males						
r^2 value	**0.12**	**0.04**	-	**0.07**	-	-
p-value	0.49	0.61	-	0.41	-	-
n	6	9	1	12	4	0
Females						
r^2 value	**0.04**	**0.03**	-	**<0.01**	**0.30**	-
p-value	0.66	0.61	-	0.90	0.26	-
n	7	12	2	9	6	0
M. pennsylvanicus						
Males						
r^2 value	-	**0.15**	-	**0.10**	-	-
p-value	-	0.23	-	0.61	-	-
n	3	11	2	5	1	0
Females						
r^2 value	-	**0.02**	-	**<0.01**	-	-
p-value	-	0.58	-	0.76	-	-
n	0	19	0	19	0	0

Table 3.4 Results of linear regression of liver copper concentration in relation to the age of the specimen (n = sample size, and if n<5 regression analysis was not performed). The notation of area is the same as for Table 3.1.

	AREA					
	C1	C2	T1	T2	T3	T4
P. maniculatus						
Males						
r^2 value	**0.11**	**0.21**	**0.08**	**0.15**	**-**	**0.47**
p-value	0.28	0.36	0.17	0.02	-	0.01
n	12	6	24	35	3	12
Females						
r^2 value	**8E-05**	**0.04**	**0.05**	**0.02**	**0.64**	**0.17**
p-value	0.98	0.83	0.41	0.38	0.06	0.07
n	7	6	17	37	6	20
C. gapperi						
Males						
r^2 value	**0.03**	**0.20**	**-**	**<0.01**	**-**	**-**
p-value	0.74	0.23	-	0.92	-	-
n	6	9	1	12	4	0
Females						
r^2 value	**0.10**	**<0.01**	**-**	**0.74**	**0.23**	**-**
p-value	0.50	0.79	-	<0.01	0.33	-
n	7	12	2	9	6	0
M. pennsylvanicus						
Males						
r^2 value	**-**	**0.05**	**-**	**0.46**	**-**	**-**
p-value	-	0.52	-	0.21	-	-
n	3	11	2	5	1	0
Females						
r^2 value	**-**	**0.01**	**-**	**<0.01**	**-**	**-**
p-value	-	0.62	-	0.69	-	-
n	0	19	0	19	3	0

3.3.4 Copper and Molybdenum Concentrations in the Liver of Small Mammals

Copper concentrations in the liver tend to increase with increasing molybdenum concentrations in the liver, however, this trend is generally evident only when a relatively large range of liver-molybdenum values (x-values) are available. When liver molybdenum concentrations were normal (i.e., <2 mg/kg), no relationship existed between copper and molybdenum concentrations in the liver (Table 3.5). However, when liver molybdenum concentrations were elevated (i.e., >4 mg/kg), a positive relationship existed. This relationship is significant, with the p-values ranging between 0.05 and 0.001, depending on the species (Table 3.5).

3.3.5 Influence of Molybdenum on Body Mass

Body mass of the small mammals does increase with age, as indicated by mass of the eye lens, and this relationship was highly significant ($p<0.01$) for all species from all sites. Thus, it was necessary to statistically control for age in all subsequent analyses investigating the effects of molybdenum on the growth of body mass.

Results from this study suggested that growth in mass was not reduced by elevated concentrations of molybdenum in small mammals. Table 3.6 shows that significant differences in body mass were not evident in *C. gapperi* between the three liver molybdenum categories when all sites were combined ($F_{2,63}=0.92$, $p=0.40$). The gender of the individual did not significantly influence body mass of *C. gapperi* ($F_{1,58}=0.99$, $p=0.33$ for the combined sites.

For *P. maniculatus*, a preliminary analysis indicated that there were significant differences in body mass between the study sites ($F_{5,173}=3.24$, $p=0.01$). Thus, the influence of liver molybdenum concentration on body mass was analyzed separately for each site, except for treatment site T3 which did not have enough individuals for an analysis. Results, presented in Table 3.7, indicate that there were no significant differences in body

Table 3.5 Relationship between molybdenum and copper concentrations (mg/kg) in the liver of small mammals. The notation of area is the same as Table 3.1. Asterisks refer to level of significance (* = $p<0.05$, ** = $p<0.01$, and *** = $p<0.001$).

	AREA					
	C1	C2	T1	T2	T3	T4
P. maniculatus						
Max Liver Mo Concn	4	26	12	2.5	-	25
r^2 value	0.46**	0.16	0.85***	0.04	-	0.23***
b-value	0.13	1.87	0.53	0.011	-	0.73
Sample Size	19	14	43	72	-	32
C. gapperi						
Max Liver Mo Concn	8	3	-	2	2	-
r^2 value	0.89***	0.25*	-	0.001	0.0003	-
b-value	0.84	0.18	-	-0.02	-0.01	-
Sample Size	14	21	-	21	10	-
M. pennsylvanicus						
Max Liver Mo Concn	-	2	-	2	-	-
r^2 value	-	0.06	-	0.09	-	-
b-value	-	0.16	-	-0.17	-	-
Sample Size	-	30	-	24	-	-

Table 3.6 The influence of molybdenum on body mass (g) of *C. gapperi*. Means have been adjusted for age by ANCOVA (see text).

	Liver Molybdenum Category			
	Low (<2 mg/kg)	Interm. (2-4 mg/kg)	High (>4 mg/kg)	p-value
Adjusted Mean Body Mass	**15.8**	**16.3**	**13.0**	**0.40**
SD (pooled)	0.78	1.56	2.06	
Sample Size	55	6	7	

mass among the three molybdenum categories in *P. maniculatus* from control sites C1 ($F_{1,14}$=0.814, p=0.81; Note: no individuals within the High liver molybdenum category) and C2 ($F_{2,8}$=1.99, p=0.20), and treatment sites T2 ($F_{2,68}$=0.41, p=0.67) and T4 ($F_{2,25}$=1.53, p=0.24). At treatment site T1, however, there were significant differences in the body mass of *P. maniculatus* with differing levels of liver molybdenum ($F_{2,37}$=3.64, p=0.04). Significant differences were also evident in the body mass of *P. maniculatus* from treatment site T4 ($F_{2,28}$=3.99, p=0.03) after the liver molybdenum categories were changed to represent the extremely high liver molybdenum concentrations observed in this site (Table 3.7). However, in both of these latter cases, the individuals showed increases in body mass with increasing concentrations of molybdenum in their livers.

3.3.6 The Effects of Molybdenum on Long Bone Development

The effect of molybdenum on long bone development was investigated using the length/radius ratios for the femur and humerus of *C. gapperi* from control site C1 and *P. maniculatus* from treatment site T4. The results of each ANCOVA (Table 3.8) indicate that, first, skeletal development was not related to the age of the small mammals. Second, bone development did not differ between sexes for either species. And third, significant differences in length/radius ratios were not evident among the three liver molybdenum categories for *C. gapperi* or *P. maniculatus* (Table 3.9). Thus, molybdenum did not appear to influence the development of the long bones of small mammals because there was no evidence of clubbing in individuals with elevated molybdenum concentrations in their livers.

Table 3.7 The influence of molybdenum on body mass (g) of *P. maniculatus*. Means have been adjusted for age by ANCOVA (see text). Notation for area is the same as for Table 3.1. Treatment area T3 was not included in the analysis (see text).

	AREA					
	C1	C2	T1	T2	T4	T4†
Liver Mo Category	Adj Mean (SD)	Adj Mean (SD)	Adj Mean (SD)	Adj Mean (SD)	Adj Mean (SD)	Adj Mean (SD)
Low	**16.3** (1.15)	**12.5** (0.84)	**14.2** (0.52)	**14.1** (0.34)	**13.1** (1.29)	**13.1** (0.64)
Sample Size	11	11	25	60	8	16
Interm.	**15.9** (1.29)	**16.7** (1.88)	**15.2** (0.68)	**14.7** (0.65)	**14.2** (1.01)	**15.5** (0.78)
Sample Size	8	0	13	11	7	11
High	-	**13.8** (2.48)	**18.3** (1.45)	**13.2** (2.51)	**15.4** (0.59)	**15.9** (1.15)
Sample Size	0	1	3	1	17	5
p-value	0.81	0.20	0.04*	0.67	0.24	0.03*

† Liver molybdenum categories were changed to Low (0-5 mg/kg), Intermediate (5-10 mg/kg), and High (>10 mg/kg) for this analysis.

* $p<0.05$, significant

Table 3.8 Statistical summary of the investigation of age, gender, and liver molybdenum concentration on long bone development, expressed as length/radius ratios, in *C. gapperi* from control area C1 and *P. maniculatus* from treatment area T4. Molybdenum category separated individuals into those with Low (<2 mg/kg), Intermediate (2-4 mg/kg), and High (>4 mg/kg) molybdenum concentrations in their livers.

FACTORS	***C. gapperi* at C1**		***P. maniculatus* at T4**	
	FEMUR	HUMERUS	FEMUR	HUMERUS
Age	$F_{1,7} = <0.01$	$F_{1,7} = 1.03$	$F_{1,22} = 3.33$	$F_{1,22} = 0.43$
	p=0.98	p=0.35	p=0.08	p=0.52
Gender	$F_{1,8} = <0.01$	$F_{1,8} = 1.22$	$F_{1,22} = <0.01$	$F_{1,22} = 1.76$
	p=0.95	p=0.30	p=0.97	p=0.20
Molybdenum	$F_{2,7} = 1.89$	$F_{2,7} = 3.03$	$F_{2,22} = 0.91$	$F_{2,22} = 0.85$
Category	p=0.22	p=0.11	p=0.42	p=0.44
Sample Size	13	11	31	29

Table 3.9 The influence of molybdenum on the length/radius ratios of the humerus and femur in *C. gapperi* from control area C1 and *P. maniculatus* from treatment area T4. Liver molybdenum categories and notation for area is the same as Table 3.1. Means have been adjusted for age by ANCOVA (see text).

	Liver Molybdenum Category			
	Low	Interm	High	p-value
Length/Radius Ratio for Humerus				
C. gapperi				
Adj Mean	**9.84**	**13.08**	**10.83**	**0.11**
SD (pooled)	0.85	0.97	0.54	
Sample Size	3	2	6	
P. maniculatus				
Adj Mean	**9.07**	**8.92**	**9.38**	**0.44**
SD (pooled)	0.40	0.31	0.20	
Sample Size	8	7	14	
Length/ Radius Ratio for Femur				
C. gapperi				
Adj Mean	**10.53**	**11.26**	**10.07**	**0.22**
SD (pooled)	0.49	0.56	0.31	
Sample Size	3	4	6	
P. maniculatus				
Adj Mean	**10.97**	**11.27**	**10.72**	**0.42**
SD (pooled)	0.44	0.34	0.22	
Sample Size	8	7	16	

3.3.7 The Reproductive Effects of Molybdenum in Male Rodents

The mass of the testes in small mammals does increase with age, as indicated by mass of the eye lens, and this relationship was highly significant for both species (*C. gapperi*: b=22.8, p<0.01; *P. maniculatus*: b=11.9, p<0.01). The mass of the seminal vesicles also increased with age in *P. maniculatus* (b=7.99, p<0.01), however, this relationship was not quite significant in *C. gapperi* (b=10.9, p=0.070) probable due to the small sample size. Nonetheless, it was deemed necessary to statistically control for age in all subsequent analyses investigating the effects of molybdenum on the mass of reproductive tissues in males.

Table 3.10 shows that significant differences in testes mass were not evident in *C. gapperi* between the three liver molybdenum categories when all sites were combined ($F_{2,28}$=0.72, p=0.50). Similarly, significant differences in seminal vesical mass of *C. gapperi* were not evident between the three liver molybdenum categories ($F_{2,26}$=2.11, p=0.14).

A preliminary analysis involving *P. maniculatus* indicated that there were significant differences between study sites in testes mass ($F_{5,84}$=2.64, p=0.029), but not for the mass of seminal vesicles ($F_{5,67}$=0.91, p=0.481). Thus, the influence of liver molybdenum concentration on testes mass was analyzed separately for each site, except for control site C2 and treatment site T3 which did not have enough individuals for analysis. At control site C1, there were no representatives in the High liver molybdenum category (see Table 3.11). Results from the analyses indicate that significant differences in testes mass were not evident between the three liver molybdenum categories in *P. maniculatus* from any of the study sites (Control Site C1: $F_{1,9}$=0.18, p=0.69; Treatment Sites T1: $F_{2,19}$=0.55, p=0.59, T2: $F_{2,31}$=0.24, p=0.79, and T4: $F_{2,8}$=1.21, p=0.35). Results from the analysis on the mass of seminal vesicles of *P. maniculatus* from all sites combined, also indicate that significant differences in mass were not evident between the three liver molybdenum categories ($F_{2,70}$=1.43, p=0.25).

Table 3.10 The influence of molybdenum on the mass (g) of testes and seminal vesicles in *C. gapperi* from all areas combined. Liver molybdenum categories are the same as Table 3.1. Means have been adjusted for age by ANCOVA (see text).

	Liver Molybdenum Category			
	Low	Interm.	High	p-value
Mass of Testes				
Adj Mean	**0.22**	**0.33**	**0.29**	**0.50**
SD (pooled)	0.04	0.09	0.11	
Sample Size	25	3	4	
Mass of Seminal Vesicles				
Adj Mean	**0.10**	**0.25**	**0.19**	**0.14**
SD (pooled)	0.03	0.07	0.08	
Sample Size	21	2	4	

When the liver molybdenum categories were changed for *P. maniculatus* at treatment site T4 to represent the extremely high liver molybdenum concentrations observed in this site, no significant differences were observed in testes mass ($F_{2,8}$=1.44, p=0.29) and seminal vesicle mass ($F_{2,4}$=0.36, p=0.72) (Table 3.11).

Reproductive class, based on sperm-smear ratings, was positively correlated with the mass of the testes and this relationship was highly significant in both species (*C. gapperi*: r=0.86, p<0.01; and *P. maniculatus*: r=0.83, p<0.01). For male *C. gapperi*, all testes weighing less than 0.06 g had sperm samples rated as Immature, except for one male (see below). All males with testes weighing greater than 0.2 g had sperm samples rated as Adult. The testes from sub-adult males were intermediate in size, weighing between 0.1 and 0.2 g. The testes from one male weighed 0.1 g (i.e. Sub-Adult or Adult), however, no sperm were visible in the sperm-smear and was rated as immature. This male had typical molybdenum concentrations in the liver (1.83 mg/kg).

Results were similar for *P. maniculatus*. All testes weighing less than 0.08 g had sperm samples rated as Immature, except for five males (see below). All testes weighing greater than 0.2 g were rated as Adult except for 2 males. The testes from Sub-Adult males generally weighed between 0.09 and 0.4 g. There were five Immature males with large testes (0.1 to 0.3 g), however, no sperm were visible in their sperm-smears. Two of the males had Low molybdenum concentrations in the livers (0.97 and 1.07 mg/kg), two males had Intermediate molybdenum concentrations in the livers (2.25 and 3.16 mg/kg), and one male had High molybdenum concentrations in the liver (10.1 mg/kg). There were also two Adult males with very small testes (i.e., <0.1 g), however, numerous sperm and some fatty material were visible in their sperm-smears. Both males had Intermediate molybdenum concentrations in their livers (2.47 and 2.48 mg/kg).

Table 3.11 The influence of molybdenum on the mass (g) of testes and seminal vesicles in *P. maniculatus*. Liver molybdenum categories and notation of area are the same as Table 3.1. Means have been adjusted for age by ANCOVA (see text).

	AREA				
	C1	T1	T2	T4	T4*
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Mass of Testes					
Low	**0.22**(0.08)	**0.09**(0.04)	**0.21**(0.03)	**-0.01**(0.16)	**0.02**(0.09)
Sample Size	5	15	28	1	4
Interm	**0.17**(0.07)	**0.15**(0.07)	**0.24**(0.06)	**0.04**(0.11)	**0.16**(0.06)
Sample Size	7	8	6	2	5
High	-	**0.16**(0.09)	**0.11**(0.17)	**0.18**(0.05)	**0.23**(0.09)
Sample Size	0	0	1	9	3
p-value	**0.69**	**0.59**	**0.79**	**0.35**	**0.29**

	ALL SITES COMBINED
	Mean (SD)
Mass of Seminal Vesicles	
Low	**0.10** (0.02)
Sample Size	46
Interm	**0.15** (0.03)
Sample Size	20
High	**0.14** (0.04)
Sample Size	9
p-value	**0.25**

*Liver molybdenum categories were changed to Low (<5 mg/kg), Intermediate (5-10 mg/kg), and High (>10 mg/kg) for this analysis.

3.3.8 The Reproductive Effects of Molybdenum in Female Rodents

Table 3.12 shows that the ratio of pregnant to non-pregnant females in the Low and High liver molybdenum categories were not significantly different from one another in *C. gapperi* and *P. maniculatus*, when season and year are taken into account. For *P. maniculatus*, there were no significant differences in ovulation rates (X^2_2=0.7, p=0.72) and litter size (X^2_2=1.6, p=0.46) between females with different concentrations of molybdenum in their livers (Table 3.13). The apparent inverse relationships between the concentration of molybdenum in the liver, and ovulation rate and litter size in C. gapperi (Table 3.13) were not significant (corpora lutea X^2_2=1.6, p=0.44; embryos X^2_2 =1.7, p=0.44). In addition, the proportion of females resorbing embryos were similar in females in both Low and High molybdenum categories in both species (Table 3.14).

Table 3.12 The number of mature females from each species that were pregnant compared to the number that were not pregnant in relation to the molybdenum concentration in their livers. Liver molybdenum categories on included Low (<2 mg/kg) and High (>4 mg/kg). All sites combined.

SEASON		**May/June 1991**			**May/June 1992**		
	Liver Mo Category	Pregnant	Non-Pregnant	p-value	Pregnant	Non-Pregnant	p-value
C. gapperi							
	Low	3	0	--	1	0	1.0
	High	0	0		0	1	
		July/August 1991			**July/August 1992**		
	Liver Mo Category	Pregnant	Non-Pregnant	p-value	Pregnant	Non-Pregnant	p-value
C. gapperi							
	Low	8	2	1.0	7	1	--
	High	1	0		0	0	
SEASON		**May/June 1991**			**May/June 1992**		
	Liver Mo Category	Pregnant	Non-Pregnant	p-value	Pregnant	Non-Pregnant	p-value
P. maniculatus							
	Low	8	1	0.35	7	1	0.44
	High	1	1		2	1	
SEASON		**July/August 1991**			**July/August 1992**		
	Liver Mo Category	Pregnant	Non-Pregnant	p-value	Pregnant	Non-Pregnant	p-value
P. maniculatus							
	Low	3	4	0.49	2	5	--
	High	2	0		0	0	

Table 3.13 The influence of molybdenum on the number of corpora lutea (ovulation rates) and embryos (litter size) of mature *C. gapperi* and *P. maniculatus*. Data from all sites are combined.

	Liver Molybdenum Category		
	Low	Interm	High
C. gapperi			
Number of Corpora Lutea			
Median	**6**	**5**	**4**
Range	2-8	3-6	2-4
Sample Size	19	3	3
Number of Embryos			
Median	**6**	**4.5**	**4**
Range	3-8	3-7	4
Sample Size	19	4	2
P. maniculatus			
Number of Corpora Lutea			
Median	**6**	**6**	**6**
Range	3-9	2-8	5-9
Sample Size	25	12	7
Number of Embryos			
Median	**5**	**5**	**6**
Range	3-7	4-6	5-9
Sample Size	19	5	5

Table 3.14 The number of females from each species that were resorbing embryos compared to the number that were not resorbing embryos in relation to the molybdenum concentration in their livers. Liver molybdenum categories included Low (<2 mg/kg) and High (>4 mg/kg). All sites combined.

Species	Liver Mo Category	# Females Resorbing Embryos	# Females Not Resorbing Embryos	p-value
C. gapperi				
	Low	4	13	1.0
	High	0	2	
P. maniculatus				
	Low	8	10	0.61
	High	1	4	

3.4 Discussion

The potential toxic effects of molybdenum on small mammals was examined by comparing animals with different concentrations of molybdenum in their livers. Measuring the molybdenum concentration in the liver of small mammals provides a reliable indicator of molybdenum uptake through their diets (Anke *et al.* 1985, Davis *et al.* 1960).

The molybdenum concentration measured in the stomach contents of some small mammals captured in this study ranged between 26 to 1200 mg/kg. The higher levels are similar to those in experimental diets which caused toxic effects in rats. For example, diets supplemented with 200 to 1200 mg/kg of molybdenum significantly reduced growth (Compere *et al.* 1965, Gray and Daniel 1954), caused skeletal abnormalities (Lalich *et al.* 1965, Miller *et al.* 1956, Ostrom *et al.* 1961), and interfered with reproduction in both male (Jeter and Davis 1954) and female rats (Fungwe *et al.* 1989 and 1990, Jeter and Davis 1954).

An increase of molybdenum in the diet of small mammals leads to an increase in the molybdenum concentration of the liver i.e., the two are positively correlated. This study supports the results of laboratory experiments with rats which showed that increased levels of molybdenum in the diet promotes the accumulation of molybdenum in the liver (Fungwe *et al.* 1989, Nederbragt 1980), and which showed that the concentration of molybdenum in the liver is directly correlated with the dose of molybdenum in the diet (Compere *et al.* 1965). In addition, the concentration of molybdenum that was observed in the livers of small mammals in the High liver molybdenum category (i.e., >4 mg/kg) is similar to those of rats which exhibit toxic effects in controlled experiments (Fungwe *et al.* 1989, Miller *et al.* 1956, Nederbragt 1980).

Copper concentration in the liver of small mammals also tends to increase with increasing molybdenum concentration in the liver. This study, therefore, supports studies that showed supplemented dietary molybdenum causes the retention of copper in the liver in rats (i.e., Fungwe *et al.* 1989, Miller *et al.* 1956). The concomitant increase in liver copper with increased dietary molybdenum suggests that molybdenum prevents the

physiological utilization of copper after the absorption of molybdenum into body tissues, particularly in the liver (Miller *et al.* 1956, Mills 1960).

Specific symptoms of molybdenum toxicity were investigated to determine if molybdenum mining at Endako might lead to toxic effects in small mammals in the surrounding environment. These included the influence of molybdenum on body mass, long bone development, and reproduction. The potential for toxic effects of molybdenum was examined by comparing these parameters in small mammals with low ("normal") molybdenum concentrations in their liver (i.e., <2 mg/kg) to those with high molybdenum concentrations (i.e., >4 mg/kg). These categories were based on literature values reported for similar species (Higgins *et al.* 1956, Fungwe *et al.* 1989) and natural divisions observed in the concentrations of molybdenum in the livers of small mammals captured for this study (see Chapter 2).

In this study, elevated concentrations of molybdenum in the diet, and consequently the liver of small mammals, however, did not appear to reduce growth in body mass or cause abnormal long bone development. In males with elevated liver molybdenum concentrations, both the mass of reproductive organs and accessory glands, and sperm production were not affected. Similarly for females, the incidence of pregnancy, ovulation rates, litter size, and the incidence of resorption was unrelated to molybdenum concentration in their livers. It should be noted though that there was a trend for reduced ovulation rates and litter sizes in *C. gapperi* with elevated molybdenum concentrations, but these trends were not statistically significant. Female reproductive results must be interpreted cautiously because the small number of females captured in this study prevented adjusting the data to account for confounding factors such as age and size of the individual, and season captured.

Results from this field toxicological investigation illustrate some of the difficulties encountered when applying laboratory toxicity tests to field studies. First, it was difficult to achieve sufficient sample size for some aspects of the study when species, gender, area, and season, were taken into account. Small sample size reduces the power of the investigation and may lead the researcher to reject the alternate hypothesis when it is

actually true.

Secondly, it is difficult to predict ecological effects based on laboratory experiments because species, and populations within species, react differently to the same exposure to a contaminant, for both genetic and environmental reasons. Species can differ appreciably in the way that they take in, accumulate, distribute, and get rid of contaminants (Chapman 1995, Moriarty 1983). In addition, certain populations within a species may have developed a resistance to the environmental contaminant in question. Resistance means a genetically-based decrease in response of a population to a contaminant as a result of exposure to the contaminant (Moriarty 1983).

The development of resistance or tolerance to toxic substances by populations is common. For example, drug resistance in bacteria and pesticide resistance in insects are common occurrences. The resistance to heavy metals in certain plants growing on contaminated soils has evolved many times (for example, Antonovics *et al.* 1971) The development of resistance to Warfarin, a widely used rodenticide, is common (Ford 1975), and demonstrates the ability of small rodents to evolve resistance to toxic substances over the course of a few generations.

The study conducted at Endako Mine may illustrate the phenomenon of evolved resistance to molybdenum toxicity. By virtue of their purpose, mines exist in geologically anomalous areas where elevated metals are a common feature of the surrounding area. In Chapter 2, results showed that exposure to elevated environmental concentrations of molybdenum was not restricted to areas influenced by mining activities. Small mammals with elevated concentrations of molybdenum in their livers were also captured in control sites. These areas are likely to contain significant amounts of molybdenum mineralization. Thus, small mammal populations in this study may have developed a resistance to molybdenum due to their highly mineralized environment. The development of resistance demonstrates the selection pressure an environmental contaminant such a molybdenum can exert on a population, if there is a suitable range of genetic variation within a species, so that a small number of slightly resistant individuals will act as a nucleus upon which a more complete resistant population can be built up.

4. SYNTHESIS

Placer Dome Canada Ltd., Endako Mines Division is an open pit molybdenum mine in north-central British Columbia. A preliminary study (Norecol 1990) indicated that molybdenum is elevated in the environment surrounding the mine. Although molybdenum is an essential element for both plants and animals, high dietary levels can result in molybdenum toxicity in some mammals (Mills and Davis 1987). The primary concern at the mine, therefore, is that the mining of molybdenum may lead to toxic effects in exposed mammals.

This study examined the potential for toxic effects of molybdenum in small rodents, which were selected as suitable target animals for two reasons. First, elevated dietary molybdenum intakes are known to be toxic to experimental rats (for example, Buck 1978, Gray and Daniel 1954, Jeter and Davis 1954). Second, the relatively limited home ranges of small mammals allows for the comparison of populations living in adjacent watersheds that have differing environmental concentrations of molybdenum. The concentration of molybdenum in the livers of small rodents was used as a reliable indicator of the uptake of molybdenum through their diets (Mills and Mitchell 1971, Nederbragt 1980).

The purpose of this study was two-fold. First, the pattern of distribution of molybdenum in the environment surrounding the mine was documented to determine if the uptake of molybdenum by the populations of various small mammal species, as measured by their liver concentrations, follows the same pattern. Second, the potential toxic effects of molybdenum in small rodents was examined by comparing small mammals with different concentrations of molybdenum in their livers. The specific symptoms of molybdenum toxicity that were investigated included the influence of molybdenum on

body mass, long bone development, and reproduction.

Results from this study indicated that the influence of molybdenum mining is evident in the surrounding environment. Dissolved molybdenum concentrations in streams influenced by mining activities were two to three orders of magnitude greater than streams found outside of the mine's influence. Furthermore, the concentrations of plant-available molybdenum in the soil were approximately ten times higher in mine-influenced drainage basins than in control drainages. However, elevated molybdenum concentrations were mainly evident within the first 5 m on either side of the streams and this was seen at both mine-influenced and control sites. The higher concentrations of molybdenum in the riparian soils of both mine-influenced and non-influenced drainage basins measured in this study confirm the preliminary results observed in the Norecol (1990) study.

This study measured the amount of molybdenum in soils that is available for uptake by vegetation. Soils with the highest molybdenum concentrations generally have the highest molybdenum levels in the vegetation (Kubota 1975). Consumers of this vegetation would, therefore, have elevated concentrations of molybdenum in their diet and correspondingly elevated concentrations in their livers. This study, however, demonstrated a very poor relationship between the pattern of molybdenum in the livers of herbivorous small mammals and the pattern of molybdenum measured in soils. Molybdenum concentrations in small mammals were not higher in individuals captured in treatment areas than in those captured in control areas. Nor were liver molybdenum concentrations higher in small mammals captured within the riparian zone than in areas further from the stream.

Molybdenum concentrations observed in the livers from individuals from all three species of small mammals from this study typically ranged between 0.5 and 3 mg/kg, with the majority of values ranging between 1 and 2 mg/kg. These results are comparable to values reported for experiments with rats (Fungwe *et al.* 1989, Higgins *et al.* 1956). "Elevated" molybdenum concentrations in the livers of small rodents from this study tended to be at least 2.5 times greater than the majority of the observed values, with few values falling between these two categories. Elevated values ranged from 5.2 to 12.1 mg/kg, with the molybdenum concentration in the liver of one individual measuring 26

mg/kg (*P. maniculatus* from control site C2).

This study also showed that an increase of molybdenum in the diet of small mammals leads to an increase in the molybdenum concentration of the liver. This observation supports laboratory experiments with rats that reported supplemented dietary molybdenum promotes the accumulation of molybdenum in the liver (Compere *et al.* 1965, Fungwe *et al.* 1989, Nederbragt 1980). In addition, elevated concentrations of molybdenum that were observed in the livers of small mammals are similar to those of rats which exhibit toxic effects in controlled experiments (Fungwe *et al.* 1989, Miller *et al.* 1956, Nederbragt 1980).

Copper concentration in the liver of small mammals also tends to increase with increasing molybdenum concentration in the liver. This study, therefore, supports studies that showed supplemented dietary molybdenum causes the retention of copper in the liver in rats (i.e., Fungwe *et al.* 1989, Miller *et al.* 1956). The concomitant increase in liver copper with increased dietary molybdenum suggests that molybdenum prevents the physiological utilization of copper after the absorption of molybdenum into body tissues, particularly in the liver (Miller and Engel 1960, Mills 1960).

In this study, elevated concentrations of molybdenum in the diet, and consequently, the liver of small mammals did not appear to reduce growth in body mass or cause abnormal long bone development. In males with elevated liver molybdenum concentrations, both the mass of reproductive organs and accessory glands, and sperm production were not affected. Similarly, females with elevated molybdenum concentrations in their livers did not have significantly lower incidence of pregnancy, lower ovulation rates, smaller litter sizes, or higher incidence of resorption events. It should be noted though, that ovulation rates and litter sizes in *C. gapperi*, but not *P. maniculatus*, appeared lower in females with elevated molybdenum concentrations, but these differences were not statistically significant.

This study illustrates two critical points for conducting field toxicological studies. First, there was a poor relationship between the pattern of molybdenum in the livers of herbivorous small mammals and the pattern of molybdenum measured in soils. Small

mammals may not show these general patterns of contaminant loadings because they may be ranging over larger areas than assumed for the design of this study. It was also postulated in Chapter 2 that the specific diet of small mammals may not contain high enough levels of molybdenum due to either varying levels of uptake of molybdenum in the plants (Kubota 1975) making up the diet of small mammals, or due to the avoidance of vegetation containing toxic concentrations of molybdenum as demonstrated by lab trials with rats (Monty and Click 1961). The analysis of stomach contents in Chapter 3, however, demonstrated that the molybdenum concentration in the diet of small mammals is elevated and that the levels are within the range that is toxic to rats (eg. Fungwe *et al.* 1986, 1989, Gray and Daniel 1954, Van Reen 1954).

Perhaps more interesting to this study is the number of individuals captured in areas outside of the mine's influence that had elevated molybdenum concentrations in their livers. Again, this may relate to the size of their home ranges, i.e., they are travelling into areas of mining activity. However, it is more likely due to the influence of small areas within control sites that contain high levels of molybdenum in the soils, and presumably, the vegetation. The mine is located in an area of molybdenum mineralization and localized patches of surficial molybdenum deposits occur outside of the mine's influence (Mathieu 1995).

This section of the study illustrates the importance of determining contaminant loading in individuals rather than composite sampling. Composite sampling may not be sensitive enough to determine the pattern of contaminant loading in wildlife and may, therefore, obscure patterns. This section also illustrates the importance of avoiding the assumption when designing field toxicity studies that contaminant loading of individual animals will follow the overall pattern observed in the environment (see Green 1979). Had this study been dependent on composite samples, differences between molybdenum concentration of small mammals from treatment and controls would not have been detected. Furthermore, composite sampling would have made it impossible to re-design this study to determine specific effects of molybdenum toxicity.

The second critical point relating to field toxicological studies that was illustrated

by this study involves the apparent lack of observable effects despite elevated concentrations of molybdenum in the livers of small mammals. These elevated levels are similar to those in rats which exhibited toxic effects in controlled experiments. (eg., Fungwe *et al.* 1989, Miller *et al.* 1956, Nederbragt 1980). Toxic effects may not have been obvious due to small sample sizes and consequent reduction in the power of the investigation. It was difficult to achieve sufficient sample size for some aspects of the study when species, gender, area, and season, were taken into account. However, in most cases trends were not evident to even suggest toxic effects, except in one case mentioned above.

The lack of toxic effects may relate to the fact that species, and populations within species, react differently to the same exposure levels to a contaminant. First, there may be differences between the species of rodents used in the experiments and those captured in the field (i.e., Chapman 1995). Thus, the elevated concentrations of molybdenum measured in the livers of *P. maniculatus*, *M. pennsylvanicus* and *C. gapperi* may not be sufficient to cause toxic symptoms in contrast to the various species of rats used in the laboratory studies. Second, the populations of small mammals found around the mine area may have developed a resistance to the toxic levels of molybdenum over evolutionary time. Environmental contaminants are known to be powerful selective forces (Moriarty 1983) and the development of resistance to toxic substances by populations is common (Ford 1975, Moriarty 1983). This hypothesis seems likely in regards to the results presented in Chapter 2 that showed elevated molybdenum concentrations in both soil samples and the livers of small mammals from control areas. Naturally occurring areas of molybdenum mineralization may have led to the evolution of resistant populations of small mammals in the area of the mine.

This aspect could be studied by conducting controlled feeding experiments. Small mammals from the mine site which are presumably resistant, and small mammals which have not been exposed to elevated levels of molybdenum, could be fed varying concentrations of molybdenum in their diet. The toxicological investigation could then test for differences in response.

LITERATURE CITED

Anke, M., Groppel, B. and Grün, M. 1985. Essentiality, toxicity, requirement and supply of molybdenum in human and animals. *In*: Trace Elements in Man and Animals 5. C.F. Mills, I. Bremner and J.K. Chesters (eds.). Commonwealth Agric. Bureau, UK. pp 154-157.

Antonovics, J., Bradshaw, A.D. and Turner, R.G. 1971. Heavy metal tolerance in plants. Adv. Ecol. Res. 7:1-85.

Arrington, L.R. and Davis, G.K. 1953. Molybdenum toxicity in the rabbit. J. Nutr. 51:295-304.

Arthur, D., Motzok, I. and Brannion, H.D. 1958. Interaction of dietary copper and molybdenum in rations fed to poultry. Poultr. Sci. 37:1181-1189.

Bachmann, R.W. and Goldman, C.R. 1964. The determination of microgram quantities of molybdenum in natural waters. Limnol. Ocean. 9:143-146.

Barshad, I. 1951. Factors affecting the molybdenum content of pasture plants: I. Nature of soil molybdenum, growth of plants, and soil pH. Soil Sci. 71:297-313.

Bremner, I. Humphries, W.R., Phillippo, M., Walker, M.J. and Morrice, P.C. 1987. Iron-induced copper deficiency in calves: dose-response relationships and interactions with molybdenum and sulphur. Anim. Prod. 45:403-414.

Buck, W.B. 1978. Copper/molybdenum toxicity in animals. *In*: Toxicity of Heavy Metals in the Environment. Part 1. F.W. Oeheme (ed.). Marcel Dekker Inc. New York. pp 491-515.

Chapman, P.M. 1995. Exotoxicology and pollution - key issues. Marine Poll. Bull. 31:167-177.

Chappell, W.R. 1976. The molybdenum project: An interdisciplinary program. *In*: Molybdenum in the Environment. W.R. Chappell and K.K. Peterson (eds.). Marcel Dekker Inc. New York. pp 1-7.

Chesnin, L. 1972. The Fertilizer Handbook. W.C. White and D.N. Collins (eds.). Fertilizer Institute. Washington, DC.

Comar, C.L., Singer, L. and Davis, G.K. 1949. Molybdenum metabolism and interrelationship with copper and phosphorus. J. Biol. Chem. 12:37-43.

Compere, R., Burny, A., Riga, A., Francois, E. and Vanuytrecht, S. 1965. Copper in the treatment of molybdenosis in the rat: Determination of the dose of the antidote. J. Nutr. 87:412-418.

Cunningham, I.J. 1950. Copper and molybdenum in relation to disease of cattle and sheep in New Zealand. *In*: Copper Metabolism: A Symposium on Animal, Plant and Soil Relationships. W. D. McElroy and B. Glass (eds.). John Hopkins Press, Baltimore. pp 246-273.

Cunningham, I.J. and Hogan, K.G. 1959. High molybdenum intake and the thrift of young shiip. New Zealand J. Agric. Res. 2:134-137.

Davies, R.E., Reid, B.L., Kurnick, A.A. and Couch, J.R. 1960. The effect of sulfate on molybdenum toxicity in the chick. J. Nutr. 70:193-198.

Day, F.H. 1963. The Chemical Elements in Nature. Reinhold. New York.

Dick, A.T. and Bull, L.B. 1945. Some preliminary observations on the effect of molybdenum on copper metabolism in herbivorous animals. Austr.Vet. J. 21:70-72.

Dick, A.T., Dewey, D.W. and Gawthorne, J.M. 1975. Thiomolybdates and the copper-molybdenum-sulphur interaction in ruminant nutrition. J. Agric. Sci. Camb. 85:567-568.

Dutt, B. and Mills, C.F. 1960. Reproductive failure in rats due to copper deficiency. J. Comp. Path. 70:120-125.

Ferguson, W., Lewis, A.H. and Watson, S.J. 1938. Action of molybdenum in nutrition of milking cows. Nature (London) 141:535-547.

Ford, E.B. 1975. Ecological Genetics. 4th ed. Chapman & Hall. London.

Fungwe, T.V., Buddingh, F., Yang, M.T. and Yang, S.P. 1986. Effect of molybdenum (Mo) supplementation on reproduction in the female sprague-dawley rat. J. Nutr. 116(6):xxxvi [abs. 95].

Fungwe, T.V., Buddingh, F., Yang, M.T. and Yang, S.P. 1989. Hepatic, placental, and fetal trace elements following molybdenum supplementation during gestation. Biol.

Trace Elem. Res. 22:189-199.

Fungwe, T.V., Buddingh, F., Demick, D.S., Lox, C.D., Yang, M.T. and Yang, S.P. 1990. The role of dietary molybdenum on estrous activity, fertility, reproduction and molybdenum and copper enzyme activities of female rats. Nutr. Res. 10:515-524.

Gliwica, J. 1983. Age indicator in the spiny rat *Proechimys semispinosus*. Tropical Ecol. 24:299-304.

Gray, L.F. and Daniel, L.J. 1954. Some effects of excess molybdenum on the nutrition of the rat. J. Nutr. 53:43-51.

Green, R.H. 1979. Sampling Design and Statistical Methods for Environmental Biologists. John Wiley & Sons. New York.

Gupta, U.C. and Lipsett, J. 1981. Molybdenum in soils, plants, and animals. Adv. Agron. 34:73-115.

Hall, G.A. and Howell, J. McC. 1969a. Histological observations on foetal resorption in copper-deficient rats. Br. J. Nutr. 23:47-52.

Hall, G.A. and Howell, J. McC. 1969b. The effect of copper deficiency on reproduction in the female rat. Br. J. Nutr. 23:41-45.

Hall, G.A. and Howell, J. McC. 1973. Lesions produced by copper deficiency in neonate and older rats. Br. J. Nutr. 29:95-105.

Halverson, A.W., Phifer, J.H. and Monty, K.J. 1960. A mechanism for the copper-molybdenum interrelationship. J. Nutr. 71:95-100.

Higgins, E.S., Richert, D.A. and Westerfield, W.W. 1956. Molybdenum deficiency and tungstate inhibition studies. J. of Nutr. 56:539-559.

Hornick, S.B. and Baker, D.E. 1976. Crop production and animal health problems associated with high soil molybdenum. *In*: Molybdenum in the Environment. W.R. Chappell and K.K. Peterson (eds.). Marcel Dekker, Inc. New York. pp 665-683.

Jackson, D.R., Lindsay, W.L. and Heil, R.D. 1975. The impact of molybdenum-enriched irrigation water on agriculture soils near Brighton, Colorado. J. Environ. Qual. 4:223-229.

Jarrell, W.M., Page, A.L. and Elseewi, A.A. 1980. Molybdenum in the environment. Residue Rev. 74:1-43.

Jeter, M.A. and Davis, G.K. 1954. The effect of dietary molybdenum upon growth, hemoglobin, reproduction and lactation of rats. J. Nutr. 54:215-220.

Johnson, H.L. and Miller, R.F. 1961. The interrelationship between dietary molybdenum, copper, sulfate, femur alkaline phosphatase activity and growth of the rat. J. Nutr. 75:459-464.

Johnson, R.H., Little, J.W. and Bickley, H.C. 1969. Some effects of molybdenum on connective tissues. J. Dental Res. 48:1290-1295.

Kubota, J. 1975. Areas of molybdenum toxicity to grazing animals in the western states. J. Range Manage. 28:252-256.

Kulwich, R., Hansard, S.L., Comar, C.L. and Davis, G.K. 1953. Copper, molybdenum and zinc interrelationships in rats and swine. Proc. Soc. Exp. Biol. Med. 84:487-491.

Lalich, J.J., Groupner, K. and Jolin, J. 1965. The influence of copper and molybdate salts on the production of bony deformities in rats. Lab. Invest. 14:1482-1493.

Lord, RD. 1959. The lens as an indicator of age in cottontail rabbits. J. Wild. Manage. 23:358-360.

Mason, J. 1978. The relationship between copper, molybdenum and sulphur in ruminant and non-ruminant animals: A preview. Vet. Sci. Comm. 2:85-94.

Mason, J. 1981. Molybdenum-copper antagonism in ruminants: a review of the biochemical basis. Irish Vet. J. 35:221-229.

Massey, H.F. and Bailey, H.H. 1967. Relation of extractable molybdenum to soil series and parent rock in Kentucky. Soil Sci. Soc. Amer. 31:200-204.

Mathieu, S.F. 1995. Molybdenum : A summary of environmental research conducted at Placer Dome Canada Ltd., Endako Mines Division from 1989 to 1994. Unpublished Report. 82 pp.

Miller, R.F. and Engel, R.W. 1960. Interrelations of copper, molybdenum and sulfate sulfur in nutrition. Federation of American Societies for Experimental Biology. Federation Proceedings 19:666-677.

Miller, R.F., Price, N.D. and Engel, R.W. 1956. Added dietary inorganic sulfate and its effect upon rats fed molybdenum. J. Nutr. 60:539-547.

Mills, C.F. 1960. Comparative studies of copper, molybdenum and sulphur metabolism in the ruminant and the rat. Proc. Nutr. Soc. 19:162-168.

Mills, C.F. and Davis, G.K. 1987. Molybdenum. *In*: Trace Elements in Human and Animal Nutrition. W. Mertz (ed.). pp 429-463.

Mills, C.F. and Mitchell, R.L. 1971. Copper and molybdenum in subcellular fractions of rat liver. Br. J. Nutr. 26:117-121.

Mills, C.F., EI-Gallad, T.T. and Bremner, I. 1981. Effects of molybdate, sulfide, and tetrathiomolybdate on copper metabolism in rats. J. Inorg. Bioch. 14:189-207.

Monty, K.J. and Click, E.M. 1961. A mechanism for the copper-molybdenum interrelationship. III Rejection by the rat of molybdate containing diets. J. Nutr. 75:303-308.

Moriarty, F. 1983. Ecotoxicology. 2nd ed. Academic Press Ltd. London. pp 71-102.

Nagy, J.G., Chappell, W. and Ward, G.M. 1975. Effects of high molybdenum intake in mule deer. J. Anim. Sci. 4:412.

Nederbragt, H. 1980. The influence of molybdenum on the copper metabolism of the rat at different Cu levels of the diet. Br. J. Nutr. 43:329-338.

Neilands, J.B., Strong, F.M. and Elvehjem, C.A. 1948. Molybdenum in the nutrition of the rat. J. Biol. Chem. 172: 431-439.

Norecol Environmental Consultants Ltd. 1990. Environmental assessment of molybdenum releases from Endako Mine on the waters, soils and biota surrounding the mine. Prepared for Placer Dome Inc. Endako Mines Division, Endako, BC. 60 pp.

Osman, N.H.I. and Sykes, A.R. 1989. Comparative effects of dietary molybdenum concentration on distribution of copper in plasma in sheep and red deer (*Cervus elaphus*). Proc. New Zealand Soc. Anim. Prod. 49:15-19.

Ostrom, C.A., Van Reen, R. and Miller, C.W. 1961. Changes in the connective tissue of the rats fed toxic diets containing molybdenum salts. J. Dental Res. 40:520-528.

Phillippo, M., Humphries, W.R., Bremner, I., Atkinson, T. and Henderson, G.1985. Molybdenum-induced infertility in cattle. *In*: Trace Element Metabolism in Animals 5. C.F. Mills, I. Bremner and J.K. Chesters (eds.). Commonwealth Agricultural Bureau, UK. pp 176-180.

Phillippo, M., Humphries, W.R., Atkinson, T., Henderson, G.D. and Garthwaite, P.H. 1987. The effect of dietary molybdenum and iron on copper status, puberty, fertility and oestrous cycles in cattle. J. Agric. Sci. Camb. 109:321-336.

Pojar, J. and Nuszdorfer, F.C. 1988. Biogeoclimatic and Ecoregion Units of the Prince Rupert Forest Region. 1:500,000 Map. British Columbia Ministry of Forests. Smithers, BC.

Rajagopalan, K.V. 1987. Molybdenum - An essential trace element. Nutr. Rev. 45:321-327.

Runnells, D.D., Kaback, D.S. and Thurman, E.M. 1977. Geochemistry and sampling of molybdenum in sediments, soils and plants in Colorado. *In*: Molybdenum in the Environment. W.R. Chappell and K.K. Peterson (eds.). Marcel Dekker Inc. New York. pp 387-423.

Sandell, E.B. 1946. Abundance of tungsten in igneous rocks. Amer. J. Sci. 244:643-657.

Solomons, C.C. and Bekemans, K. 1976. Biological effects of molybdenum. *In*: Molybdenum in the Environment. W.R. Chappell and K.K. Peterson (eds.). Marcel Dekker Inc. New York. pp 229-241.

Spence, J.T. 1976. Reactions of molybdenum coordination compounds: Models for biological systems. *In*: Metal Ions in Biological Systems. H. Sigel (ed.). Dekker Inc. New York. pp 279-324.

Spence, J.A., Suttle, N.F., Wenham, T., El-Gallad, T. and Bremner, I. 1980. A sequential study of the skeletal abnormalities which develop in rats given a small dietary supplement of ammonium tetrathiomolybdate. J. Comp. Path. 90:139-153.

Suttle, N.F. 1980. The role of thiomolybdates in the nutritional interactions of copper, molybdenum and sulphur: Fact or fantasy? Annals N.Y. Acad. Sci. 355:195-207.

Suttle, N.F. 1974. Recent studies of the copper-molybdenum antagonism. Proc. Nutr. Soc. 33:299-305.

Suttle, N.F. 1991. The interactions between copper, molybdenum and sulphur in ruminant nutrition. Annu. Rev. Nutr. 11:121-140.

Swain, L.G. 1986. Water quality criteria for molybdenum. British Columbia Ministry of Environment and Parks, Resource Quality Section, Water Management Branch. Victoria, BC. 38 pp.

TetraTech, 1986. Recommended protocols for measuring metals in Puget Sound water, sediment and tissue. Publ. #TC-3090-04.

U.S. EPA. 1986. Test methods for evaluation solid waste, physical/chemical methods. Publ. #SW-846, 3rd ed., Washington, DC 20460.

Underwood, E.J. 1977. Trace Elements in Human and Animal Nutrition. 4th ed. Academic Press. New York.

Van Reen, R. 1959. The specificity of the molybdate-sulfate interrelationship in rats. J. Nutr. 68:243-250.

Van Reen, R. 1954. The influence of excessive dietary molybdenum on rat liver enzymes. Arch. Bioch. Biophys. 53:77-84.

Vlek, P.L.G. and Lindsay, W.L. 1977. Molybdenum contamination in Colorado pasture soils. *In*: Molybdenum in the Environment. W.R. Chappel and K.K. Peterson (eds.). Marcel Dekker Inc. New York. pp 619-650.

Voegeli, P.T. Sr., and King, R.U. 1969. Occurrence and distribution of molybdenum in the surface water of Colorado. Water Supply Paper, United States Geology Survey. 1535-N.

Wetzel, RG. 1975. Limnology. W.B. Saunders Co., Toronto.

Winston, P.W. 1981. Disorders of Molybdenum Metabolism. Academeic Press Inc. New York.