AN ABSTRACT OF THE DISSERTATION OF

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Title: Lead Toxicity at Various Dosages in Naeemi Lambs in Kuwait

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Environmental contamination along roadways with lead from processed petroleum and automotive residues has been reported. Toxicity to the herbivores grazing these areas has not been well studied. Comparison of lead concentrations adjacent to roads in Kuwait and in Oregon, USA was studied. Soil samples were taken from three sites at three different distances from the highway (0, 3 and 10 meters) adjacent to King Fahad Highway in Kuwait and Interstate 5 (I-5), Highway 34 (H-34) and Highway 20 (H-20) in Oregon. Soil was analyzed for lead concentrations. The mean lead concentrations in soil samples along King Fahad Highway were significantly higher ($p < 0.05$) than those along I-5, H-34 and H-20 in Oregon [4943.6 ppm (mg/kg) vs 129 ppm, 94.9 and 81.67 ppm respectively].

In a field trial animal toxicity studies were conducted on sheep grazing near roadway in Kuwait and also in a controlled barn studies. Fifty lambs ranging in age from 4 to 9 months and grazing on Kuwait pasture adjacent to the King Fahad Highway were tested for blood lead. Levels were determined by Inductively Coupled Plasma-Atomic

Emission Spectrometry (ICP-AES). Blood lead levels of these lambs ranged from 0.05-1.00 ppm. Only 12% of the tested population exceeded the blood lead above 0.1 ppm (the high normal value). None demonstrated any clinical signs of lead toxicosis.

In addition, a controlled feeding trial was conducted with sheep ingesting similar concentrations of lead as were found along the roadways. These sheep were observed for clinical, gross and histopathological changes. Using the intensive lamb production system common in Kuwait, twenty five lambs ranging in age from 2-10 months were orally fed 0, 2, 4, 8 and 16 mg lead acetate/kg body weight/day in a controlled study. Blood lead levels were tested in these lambs at time zero, week two, and then at monthly intervals until the 14th week. All lambs were slaughtered and necropsied with select tissues analyzed for lead concentrations.

Levels of lead in the blood were directly related to the daily administrated lead acetate ($P<0.05$). Neither gender, age nor breed of the sire had any affect on blood lead levels except for the 14th week where blood lead levels of the young lambs significantly exceeded ($P<0.05$) those in the older lambs with mean values of 0.54 and 0.34 ppm respectively. In general, lead levels in all the tested tissues were directly related to the amount of the daily oral administration of lead acetate. Differences between the tissue levels of lead in the experimental and control lambs ($N= 25$) were statistically significant ($P<0.05$) in liver, bone and kidney but were not significant in trachea, testis, brain, diaphragm, ovary, lung, muscle, rumen, aorta, spleen, tongue, eye, intestine, heart and esophagus. Lead accumulation was the highest in bone at the lower ingested lead concentrations, but was the highest in the kidney at higher lead dosages. Lead values were significantly greater ($P< 0.05$) in the livers of female lambs compared to those of the

male. Bone, liver and kidney of the young lambs had significantly higher ($P< 0.05$) levels of lead than older lambs with means of 19.24, 7.31 and 54.54 compared to 6.34, 3.59 and 21.31 ppm respectively. Gross lesions were not found in any of the 25 necropsied lambs. Histopathological changes of intranuclear inclusion bodies were found in 100% of the kidneys in lambs administered 8 mg/Kg/day and above and in 50% of the livers of the lambs administered the same dosages. Thirty three per cent of lambs administered 2 and 4 mg/kg/day had intranuclear inclusion bodies in their kidney but not in the liver. The controls had no inclusion bodies in any of these matching tissues. No clinical signs of lead toxicosis were observed in any lambs during the 14 weeks of the experiment.

The same lamb population was used to compare blood lead levels and the growth performance of lambs (feed intake, weight gain and feed conversion) in relation to different dosages of lead acetate. Although there was a tendency for lambs ingesting the two higher lead doses to eat less feed, gain less weight; and have a lower feed conversion ratio, these differences were not statistically significant ($P> 0.05$).

The conclusion of these studies reveal some concern. Levels of lead as found near the highways of Kuwait were high enough to cause elevated tissue lead concentrations, particularly in liver and kidney, of lambs grazing adjacent to these highways. These levels cause tissue abnormalities in lambs and could be hazardous to human health eating the internal organs of these lambs.

Lead Toxicity at Various Dosages in Naeemi Lambs in Kuwait

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Tariq A.Y.AL-Sabbagh

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CHAPTER I
GENERAL INTRODUCTON

Scientists and long term planners are spending more effort to develop and improve agriculture to diminish hunger. The danger of hunger and poverty are climbing anywhere in the globe, particularly in third world countries with a corresponding overusing of energy resources. Nutrition surveys show the rate of severe malnutrition among children has increased up to 78% in some locations because of rising acute food shortages. More than 1.2 million people in Sudan alone are facing famine conditions FAO, 1998).

World population is expected to grow from 5.5 billion to about 8 billion in the year 2020 with up to 44% of the world population expected to reside in urban areas, this is up from 30% in 1980 (IFPRI, 1995). This increased population may raise the current demand for meat from 206 million tons to 310 million tons in the year 2020 (FAO, 1998). Therefore, the increased production of animals for meat and dairy along with their parallel industries are important agriculture means to maintain. Parallel to this concern is the desire for food safety and the development of animal health and production technologies.

Animal disease causes major economic loss through mortalities, reduced productivity, lower fertility, condemned products and restricted access to potential markets. Lead poisoning is a continual threat for animal disease. Lead toxicosis in animal also has a potential to reach and affect human health particularly children. In

spite of new and advanced technologies for screening, prevention and treatment we still lose human and animal lives due to the prevalence of lead exposure that is ubiquitous since the advert of industrialization.

Lead poisoning causes multiple physiological dysfunctions that pose a major threat to the lives of animals. Lead is considered one of the major environmental pollutants causing accidental poisoning in domestic animals (N.A.S., 1972). Concerns about the acute effects of lead ingestion on animal health have been identified (Allcroft, 1950; Hatch and Funnell, 1969; Osweiler et al., 1973). Animals grazing near smelters or heavily traveled highways have elevated lead tissue content (Hussain et al., 1996; Ward and Savage, 1994). However, little attention has been given to the subclinical effects of low lead levels on livestock (Dinius et al., 1973; Kelliher et al., 1973).

The goal of this study was to determine if sheep grazing grass adjacent to contaminated highway in Kuwait would have clinicopathological changes and tissue levels potentially dangerous to human health. The levels of lead deposition in soil adjacent to highways affected by varying densities of motor vehicle traffic in Kuwait and the United States of America was assessed. Two animal studies were also conducted. A survey of blood lead levels was done in a group of sheep grazing grass near contaminated highway in Kuwait. In a second controlled barn study the

clinicopathological effects and the growth performance of Naeemi lambs (males and females) ingesting different dietary levels of lead acetate for 3.5 months under Kuwaiti environmental conditions was conducted.

CHAPTER II
LITERATURE REVIEW

Introduction

Lead is the most ubiquitous toxic metal and is detectable in practically all phases of the inert environment and in all biological systems where there is no demonstrated biological need for it (Hamir, 1984). Lead is a soft, dull gray, heavy naturally occurring metallic element of principal toxicologic concern for major health effects in both human beings and animals (Hammond, 1973). It does not degrade or gradually lose its toxicity once released to the environment. It has no characteristic taste or smell. Metallic lead does not dissolve in water and does not burn. Because of its presence and properties, it is a health hazard of global properties.

Greek physicians made the first clinical description of lead poisoning in the first century BC (before Christ) when it was produced as a byproduct of silver. At that time it was used as a cosmetic to whiten the skin and darken the hair. Only during the last three decades of the twentieth century has an interest been focused on the presence and significance of environmental lead pollution (Maresky and Grobler, 1993).

Lead has been found in all tissues of human beings and animals (Pearl et al., 1983). It is an accumulative poison in human beings and animals that leads to microscopic as well as gross lesions (Ahmed and Shalaby, 1991; Papaioannou et al., 1998). Lead poisoning has affected livestock, especially sheep and cattle throughout

agricultural history and has been considered one of the most important causes of poisoning in farm animals (Ahmed and Shalaby, 1991). Recent work has indicated that body concentrations which were previously regarded as safe may have a detrimental effect on the mental development of children in spite of the absence of the classical symptoms of lead poisoning (Waldron and Stofen, 1974; Bryce-Smith and Stephens, 1978). Lead is reported as the most common cause of poisoning in dogs (Zook et al., 1969), cattle (Buck, 1969), and children as an accidental poisoning in Chicago area (Perlstein and Attala, 1966). During recent decades these incidences have increased because of the greater use of the element and its compounds in industrial products allowing more exposure to higher concentrations as well as exposure to years of residue buildup in the environment (Takla et al., 1989).

Oral consumption is the major route of exposure for animals. Grazing pastures near highways or lead industries results in ingestion of grass with high lead content (Hammond and Anronson, 1964; Lagerwerff and Specht, 1970; Motto et al., 1970; Ward et al., 1977; Ward et al., 1978; Tjell et al., 1979; Collins, 1984; Ndiokwere et al., 1984; Deroanne et al., 1987; Ward 1990a; Ogonsola et al 1994; Ward and Savage, 1994; Koh and Judson, 1995; Hussain et al., 1996; Nriagu et al., 1996). It is generally agreed that the principal source of lead in the air in the urban environment is the result of the combustion of leaded gasoline (Ferguson, 1986; Saleh et al., 1996). Interest in investigation of the lead pollution of roadside dust and vegetation has grown because of

lead's hazardous effect on health (Cannon and Bowels, 1962; Deloph et al., 1970; Dinies et al., 1970; Page and Gannje, 1970; Fell, 1984; Boechx, 1986; Ferrier, 1986; Majdi and Person, 1989; Ward, 1990). Motto et al. (1970) found levels of lead in grass as high as 255 mg/kg adjacent to highways with a decline in contamination with increasing distance from the highway. Deaths and costs of prevention and treatment are of major economic loss to the livestock industry. (Jensen et al., 1988). Concern over the effects on animal health and production losses have resulted in several studies that have offered explanations and unveiled some information about the challenges facing animal production. It is known that grazing animals absorb metal particulate derived from surface contamination of pasture and soil (Ward and Savage, 1994). However, only a limited amount of information is available about the uptake of lead by the grazing of animals around highways and its effects (Ward, 1991).

Lead toxicosis in animals

Lead poisoning in domestic animals has been well documented due to water and other environmental contamination (Blaxter, 1950; N.A.S., 1972; Dinius et al., 1973; Hapke, 1973;Gelder et al., 1973; Van Neathery and Miller, 1975; Fick et al., 1976; Ammerman et al., 1977; Pearl et al., 1983). Large numbers of farm animals die annually due to lead toxicity. Among farm animals, lead toxicity affects sheep and cattle to the highest degrees; they are followed by horses, poultry and swine (Blood and Hederson,

1968). Horses and sheep are commonly chronically poisoned while grazing contaminated pastures or inhaling fumes in a contaminated area (Blaxter, 1950). Lead poisoning occurs in all ages (sheep below the age of two years are more susceptible than older ones), sexes and breeds of sheep;(Kimberling, 1988). Calves (Oliver et al., 1987) and puppies (Chrisman, 1991) often chew and lick woodwork that is rich in lead from old paint. Such activity also causes toxic biochemical changes in liver and kidney functions in dogs (White, 1977) and in cattle (Milhaud et al., 1979).

Lead has also been used in treatment of various health conditions. Lead poisoning was reported in lambs receiving treatment for rickets in North Derbyshire in England (Sharma, 1976). Lead arsenate has been widely used as an anticestodal compound in sheep (Allen and Jongeling, 1948; Foster and Haberman, 1948; Morgan et al., 1950). This practice was a factor in the lead poisonings reported by Bennett and Schwartz (1971).

Soil and pastures adjacent to lead and other smelters have been proven to be polluted with metal residues (Allcroft, 1951; Tiller et al., 1975; Cartwright et al., 1977). As early as 1924, Morgan observed chronic lead poisoning in sheep in lead mining districts. Lead toxicosis is generally chronic in sheep when they graze in areas polluted from lead mining activities (Summers et al., 1995).

Lead toxicity has increased in sheep with the increased use of the element and its compounds in industrial products allowing greater exposure of animals to the metal residues in the environment. There is evidence that mixed metals are found in contaminated roadside surface soil and vegetative species (Ward et al., 1977; Ward, 1988 and Ward, 1990b) due to transportation activities including fuel combustion (Deroanne et al., 1987; Ward and Savage, 1994). Exhaust emissions from vehicles have been shown to contaminate roadsides (Ward et al., 1978; Mahmood et al., 1985; Reina et al., 1990; Hewitt and Rashed, 1991; Gratani et al., 1992; El-Gamel et al., 1993; Swarup et al., 1993). Automotive lead emissions is a source of lead toxicosis to grazing animals when they are allowed to eat grass adjacent to highways (Motto et al., 1970; Bloom et al., 1976; Ward et al., 1977; Collins, 1984; Takla et al., 1989; Ward and Savage, 1994). Since grazing animals consume small amounts of surface soil, grazing roadside pastures causes metal poisoning since soil is a giant reservoir of tiny particles of lead (Howard Mielke, 1999). Elemental contamination of these animals can result when roadsides become pastures to animals (Healy, 1967). Lead levels in soil along highways have been reported at extremely high levels; Nriagu (1992) reported finding roadside lead contamination to be more than 5000 ppm in Egypt (Nriagu 1992) and Ogunsola et al. (1995) found levels as high as 7000 ppm in Nigeria .

Lead toxicity in sheep results in digestive, nervous, blood alterations as well as disturbances to several other systems (Sharma, 1971; Carson et al., 1973; Neathery and Miller, 1975; Harrison, 1977; Koh and Judson, 1986; Kimberling, 1988). Interference with the reproductive system has been attributed to lead toxicity (Baumann, 1933; Kehoe et al., 1933; Flurry et al., 1934; Coffigny et al., 1994). Lead toxicosis in male Barki sheep was reported by Ahmed and Shalaby (1991) to be the most frequent cause of reduced fertility in males when they were exposed to as low as 1 mg of lead acetate per kilogram body weight three times a week for 12 months. They attributed lead toxicosis, under Egyptian environmental conditions, to depriving both production and reproduction. Early literature reported that low levels of lead in the body reduces the resistance of animals to infectious diseases (Cook et al., 1975; Underwood, 1977; Cook and Karns, 1978; Koller, 1980; Sakaguchi et al., 1982; Lawrence, 1985; Undeger et al., 1996) although Hoffman et al. (1980) found that lead as high as 16 mg per kilogram of body weight fed orally did not impose a state of immunological deficiency in sheep.

In brief, lead toxicity is of great concern for grazing animals. Concerns are especially important when poor quality pastures drive animals to graze in unusual places where the possibility of having lead-laden material is very high. Lead toxicosis has been observed in animals grazing near trash dumps or on sides of major highways where rain fall accumulates and soil and forage are contaminated from vehicle emissions (Motto et al., 1970; Bloom et al., 1976; Ward et al., 1977; Hussain et al., 1996). Such lead

poisoning is very common and has been reported in many countries from New Zealand and Africa, to the United States as well as in many other countries (Sharma, 1976). Concern for lead toxicosis in sheep is important as it occurs in all countries where sheep production has been commercialized (Kimberling, 1988), therefore it has potential to affect not only the health of the livestock but eventually the health of the human populating consuming it.

Sources of lead

Lead is a naturally occurring element that has been used almost since the beginning of civilization (Ferguson, 1986). Lead is known to be toxic for human beings and has been of interest to many biologists (Ammerman et al., 1973; Merck, 1973; Neathery and Miller, 1975). Because of the many industrial activities that have brought about its wide distribution, lead is ubiquitous in the environment today (Chao and Wang, 1994; Gromov and Emelina, 1994). As a primary result of exposure to manmade products containing lead, all humans have lead in their bodies, (N.A.S., 1972). Today, the major environmental sources of metallic lead and its salts are paint (Zook, 1973; Kowalczyk, 1976; Mushak and Crocetti, 1989), auto exhaust (Cannon and Bowels, 1962; Deoph et al., 1970; Dinies et al., 1970; Lagerwerff and Specht, 1970; Page and Ganje, 1970; Ward, 1977; Tjell et al., 1979; Fergusson et al., 1980; NRC, 1980; Collins, 1984; Nasralla, 1984; Ndiokwere, 1984; Deroanne-Bauvin et al., 1987; Majdi and

Pearson, 1989; Takla et al., 1989; Ward, 1990a; Madany et al., 1990; Gromov and Emelina, 1994; Ward and Savage, 1994; Saleh et al., 1996), food (Underwood, 1977; Sherloock, 1987; Alegria et al., 1990), water (Underwood, 1977) and medications in the form of folk remedies and herbal medications (EPA, 1998). Lead also comes from battery manufacture, lead refineries, pigment manufacture, printing, shipbuilding, smelting plants, welding, contaminated alcohol, firing ranges, lead ammunition manufacture, pottery glaze, burning of solid waste, windblown dust, volcanoes, exhaust from workroom air, burning or weathering of lead painted surfaces and even cigarette smoke (Agresti et al., 1958; Bryson, 1989; Dreisbach, 1983).

Lead based paint remains the most common source of environmental lead exposure. Although the Consumer Product Safety Commission banned the manufacturing of interior and exterior paint exceeding 0.06% lead by weight in 1978, lead based paint is still available for industrial, military and marine use. Since the lead content of paint was not regulated until the late 1970's, many older structures, residential and commercial, have old leaded paint that is now peeling, flaking, and chipping. The most dangerous source of lead exposure for children is the estimated 3.8 million residential units in which they live that have decaying and deteriorating lead paint. The unique characteristics of children's developmental behaviors and their physiological differences make them a vulnerable population. For children, the most important pathways are ingestion of chips from lead-painted surfaces (Mushak and Crocetti 1989).

Children's hand to mouth activity and the efficiency of the gastrointestinal absorption of contaminated "pica" (eating non-food substances) place children at greater risk for lead poisoning than adults (CDC, 1991). Children can ingest loose paint as a result of pica and through mouthing of items contaminated with lead from paint, dust, and soil. High levels of lead in soil and house dust have been associated with increased blood lead levels in children. The Center for Disease Control and Prevention (CDC) in Atlanta describes lead as one of the most common pediatric health problems in the United States (Mielke, 1999).

Automobile emissions have been an important source of lead exposure for urban residents (Ferguson, 1986), particularly in areas with congested traffic (Ogunsola et al., 1994). Although inhalation of lead from gasoline is no longer considered a public health problem, the lead from dust in automobile emissions has been deposited in the soil because of the many years of previous use (Ward and Savage, 1994). Children playing near roads and freeways may come in contact with contaminated soil. Not only automobile emissions, but also evaporation losses incurred during transport and handling leaded petrol are considered a major source of lead.

Another prime exposure to lead is in food. Food and beverages may become contaminated through the deposition of lead-containing dust from atmosphere on crops or from uptake of lead by plants from the soil. Agricultural vehicles are not required to use unleaded gasoline, consequently, lead can be deposited on and retained by crops,

particularly leafy vegetables. Grain, cereal products and vegetables are important sources of lead for adults while milk is for young children. Food containers used in processing and storage also contain lead which may be leached into food. Acidic foods have been found to leach lead from lead solder used in cans and lead glazes used in making pottery and ceramicware (U.S. Food and Drug Administration 1993 [FDA consumer August 1993; Update Feb. 1997], (Mielke, 1999). Because the point of origin of many lead-glazed pottery items is unknown, there is a potential for confusion and therefore a hazardous finish may be overlooked. Even "safe" ceramicware can become harmful; dishwashing may chip or wear off the protective glazes and expose lead-containing pigments. Water from leaded pipes, faucets, soldered plumbing or water coolers can be other potential sources of lead exposure (Bond and Kubin, 1949; Dreisbach, 1983).

Lead releases from industries into the air consequently contaminate water and soil (Chao and Wang, 1994 ;Gromov and Emelina, 1994, Casarett et al., 1996) lead acid battery manufacturing, brass foundries and manufacturing of tetraethyl and tetramethyl lead. In addition, the disposal of lead in municipal and hazardous waste dump sites also adds lead to soil. Stationary or point sources of lead include mines and smelters.

Lead acetate, sugar of lead, is one of the most bioavailable forms of lead that is an ingredient of cosmetic products. The Food and Drug Administration allows inclusion of as high as 6000 ppm of lead in some cosmetic products (Mielke 1999).

Cigarettes contain lead in addition to other substances having adverse effects on human health. Although cigarette smoking is decreasing in Western civilizations as a result of education and awareness of its health hazards, this habit is still increasing among the Middle Eastern communities endangering smokers and second hand smokers as well.

Several folk remedies used in this country have been shown to contain large amounts of lead. Two Mexican folk remedies are azarcon and greta, which are used to treat "empacho," a colic-like illness. Azarcon and greta are also known as liga, Maria Luisa, alarcon, coral and rueda. Lead-containing remedies and cosmetics used by some Asian communities are chuifong tokuwan, pay-looah, ghasard, bali goli, and kandu. Middle Eastern remedies and cosmetics include alkohl, kohl, surma, saoott, and cebagin (EPA, 1998).

Many occupations, hobbies, and other activities result in potential exposures to high levels of lead and can put the entire family at risk of lead poisoning. More than one million workers in over 100 different occupations may be exposing their families to lead when showers and changes of clothing are not provided at the end of a work day (Agency for Toxic Substances and Disease Registry, ATSDR, 1992). Lead toxicity in pregnant women results in transplacental lead exposure, which places the viability of a growing fetus at risk. Adult lead exposure is subsequently becomes a pediatric concern.

Lead is ubiquitous in the environment since the advent of industrialization. The variety of sources and practices involving lead directly or indirectly result in wide-scale dispersion of lead into the environment exposing human beings and animals to the danger of lead toxicity.

Absorption of lead

Millions of tons of lead are produced every year making the human and animal exposure more likely. Lead may enter the body through ingestion, inhalation, penetration or pass through the skin. Species, age, body composition, sex, genetic factors, presence of pathology and nutritional factors are all possible influential parameters affecting lead absorption.

Oral absorption of lead is slow and incomplete (Osweiler, 1996) but this is considered the most significant pathway for lead uptake (Rabinowitz et al., 1977). Lead absorption through the gastrointestinal tract is small compared to via the respiratory tract (Saryan and Zenz, 1994). However, the more time that lead spends in the gastrointestinal tract increases the absorption (Oehme, 1972). The temperature of the environment is another factor that can affect lead absorption by the gastrointestinal tract (Oehme, 1972; Jones and Hunt, 1983).

Lead is relatively insoluble. Soluble forms form insoluble compounds (metal and metalloids) in the gastrointestinal system; however, they dissolve readily in gastric secretions and are absorbed in the small intestine (Blaxter, 1950). Although, Dieter et al. (1993) found that lead concentrations in the kidney were ten times greater in rats fed more soluble compared to the less soluble lead compounds. Absorption depends in part on the surface area of the lead particles; finely divided lead is more toxic.

Up to 50% of ingested lead is absorbed in the digestive tract, depending on the species type, physiological status, dietary influence and even the season of exposure (Smith, 1996). Different species absorb lead from the gastrointestinal tract differently. Also, different species may have differing lead absorption rates throughout the gastrointestinal tract (Kostial et al., 1974; Levander et al., 1977; Hart and Smith, 1981; Mykkanen and Wasserman, 1981). The amount of lead absorbed is about 1- 2% in sheep and rabbits while it is 5-10% in human beings and small lab animals (Blaxter, 1950).

In the mature animals studied by Kimberling (1988) and Summers (1996) only 2% of the ingested lead was absorbed. Lead absorption through the alimentary canal in human infants and rat pups is more efficient than in adults of both species (Forbe and Reina, 1972; Alexander et al., 1973). This is a general trend; adults absorb about 10 to 15% of the ingested lead in food and water (Kumar et al., 1997; Department of Health Service (dhs), 1997; The Centers for Disease Control, 1998) as opposed to about 50% in children and pregnant women (Oehme, 1972; Ibels and Pollock, 1986; Fox, 1987;

Kumar et al., 1997). Adults retain less than 5% of the absorbed ingested lead whereas children absorb between 41.5% with a 31.8% net retention, making age an important factor for absorption and retention of lead (Casarett et al., 1996). The same trend was observed in rats; Forbes and Reina (1972) found that lead absorption was up to 89% in younger suckling rats while it dropped to 15% at about the time of weaning. Blaxter (1950) estimated the lead absorption in sheep to be around 1.3% when lead was given as lead acetate or nitrate, or in the form of naturally contaminated hay. In general, there is a higher tendency for lead poisoning among younger members of a species which is due to in part to higher lead absorption from the diet (Underwood, 1977). This higher rate of absorption in young animals also facilitated the penetration of lead across the blood brain barrier and accounts for the higher incidence of neurologic signs among young animals (Bratton and Kowalczyk, 1989).

Acidic diets may increase lead absorption (Bratton and Kowalczyk, 1989). Fasting and nutritional status (dhs, 1997) also affect gastrointestinal absorption. Fasting conditions are suggested to increase the absorption quantity significantly (Bratton and Kowalczyk, 1989) as well as increase iron or calcium deficiency (Fox, 1987; Rabinowitz et al., 1980). Pearl et al. (1983) found that dietary calcium reduced the concentration of lead deposited in liver. In 1987, Spivey Fox reported low calcium and iron intakes increased lead absorption.

Dietary minerals (Ca, Fe, Zn and Cu) also influence lead absorption (Six and Goyer, 1970; Cerklewski and Forbes, 1976, 1977; Suzuki and Yoshida, 1979; Mahaffey and Rader, 1980; Petering, 1980; F. Fox, 1987; Osweiler, 1996). A diet rich in calcium and phosphorus was found to decrease the absorption of lead and; hence, lead was found to accumulate less in bone and kidney tissues (Sobel et al., 1938; Six and Goyer, 1970). Low dietary calcium and other minerals such as potassium, phosphate and magnesium, provide an increase in the absorption of lead in rats (Six and Goyer, 1970; Osweiler, 1996). On the other hand, Pearl et al. (1983) did not find any difference in lead concentration in blood, bone and soft tissues in sheep fed 0.25 or 0.5% dietary calcium in 1000 mg Pb/kg with the exception in the liver which had more lead concentration with lower calcium intake. Diets deficient in zinc may enhance lead absorption as well (Osweiler, 1996).

Low iron diets have shown enhanced lead uptake in rats (Six and Goyer, 1972). However, Khoo (1975) did not find any effect on lead absorption in rats offered low iron diets.

Vitamin D has also been implicated in lead absorption. Smith (1996) found a direct correlation between lead absorption and a high lead poisoning during the summer.

A low dietary protein level generally increases lead absorption as well as its retention in the organs (Barltrop and Khoo, 1975; Rowland, 1991; Osweiler, 1996).

Milev et al. (1970) as well as Barltrop and Khoo (1975) found that any deviation from the normal levels (higher or lower) of dietary protein would increase lead uptake in laboratory animals.

The quantity and type of dietary fat has been found to affect lead absorption (Rowland, 1991). Barltop and Khoo (1975 and 1976) found that a diet high in fat could lead to an increase in lead absorption. Stowe and Vandevelde (1979) made similar statements regarding a high fat low calcium diet increasing lead absorption in a dog's gastrointestinal tract.

During periods of physiological stress, such as pregnancy, lactation, or aging, lead can be mobilized back into the blood. This increases the blood lead level affecting the body itself or the embryo, in case of pregnancy (FDA, 1997).

Generally, metallic lead and the sulfide form are less well absorbed than the acetate, phosphate, carbonate, oxide and hydroxide salts (Casarett et al., 1996). The poorly absorbed forms of lead produce toxicity only when it is entrapped in the stomach for a prolonged time. It is the ionic species that are absorbed no matter what is the particular form of lead that is incorporated.

Therefore, lead absorption is affected by many factors including type of compound, animal species, age, stage of lactation or pregnancy. It is also affected by the fullness of the gastrointestinal tract, the availability of minerals in diet, and the acidity of the rumen or stomach (Allcroft, 1950; Allcroft and Blaxter, 1950; Blaxter, 1950a).

Inhaled lead deposited in the lower respiratory tract is completely absorbed (Centers for Disease Control and Prevention Guidelines, 1998; Cal/OSHA, 1997). Absorption from the respiratory tract is rapid, entering directly into general circulation without passing through the liver like ingested type (Bryson, 1989). Casarrett and Doull (1975) mentioned that 37% of the lead that is inhaled is retained in the lungs. Lead absorption through the lungs is affected by many factors such as volume of air respired per day, size of particles, form of lead (whether particle or vapor). Most of inorganic lead emissions are less than 10 microns in diameter (about 1/5 the size of a human hair), a particle size that can easily be inhaled and deposited into lung tissue (Casarett et al., 1996).

Oral intake and respiration are the two major routes through which lead compounds enter the body (Oehme, 1972 ; Saryan and Zenz, 1994; dhs, 1997). Inorganic lead enters the body primarily through inhalation and ingestion (Oehme, 1972) and does not undergo biologic transformation. In contrast, organic lead, found primarily in gasoline as tetraethyl lead, enters the body through inhalation and skin contact and is metabolized in the liver. The epidermis usually works as a barrier against inorganic lead compounds, but when the skin is injured it is considered to be another route for lead to enter the body. While inorganic lead cannot penetrate the skin except through wounds

(Bryson, 1989), organic lead compounds like tetraethyl and tetramethyl lead (metals and metalloids) can enter the body through the skin. Subcutaneous and intramuscular sites can also be a way for lead to be absorbed (Goodman and Gillman, 1966).

Storage and distribution

Lead and lead compounds can be highly toxic when eaten or inhaled. Although lead is absorbed very slowly into the body, its rate of excretion is even slower. The lead that is not immediately excreted is stored in soft tissues and bone. Thus, with constant exposure, lead accumulates gradually in the body. It is absorbed via calcium channels as a divalent ion. It is carried mainly by the red blood cells as diphosphate compounds (Freeman, 1970 b) and circulated through the body where it becomes concentrated in soft tissues, especially the liver and kidneys (Freeman, 1970a). Once lead absorbed, it is found in all tissues; however, 60 to above 90 % of the body's lead burden is accumulated in bone (Kimberling, 1988), where it has a half life of years to decades (dhs, 1997) as a relatively insoluble lead triphosphate (Freeman, 1970b). It does not remain in the bone permanently but it is slowly released into the blood stream. The half-life of lead in the blood is about 30- 35 days (Lauwerys, 1983). Reports from analysis of organs has indicated 25% and 4% of absorbed lead resides in the liver and kidneys respectively (Kimberling, 1988). The half-life of lead in soft tissues is reported to be around 40 days (Lauwerys, 1983).

Inhaled lead enters the blood more rapidly than ingested lead. It goes to the soft tissues like liver, kidneys, spleen, brain and others (CDC, 1998).

Lead excretion

Lead is excreted via the kidneys and the feces and to a lesser amount in sweat, hair and milk (Caserett and Doull 1975). However, Bratton and Kowalczyk (1989) indicated the major route of lead excretion varies among species. Tsuchiya (1977) stated that most lead leaves the body in the urine while some is eliminated in gastrointestinal secretions, hair, sweat and milk. Other studies have shown lead to be excreted via feces five to ten times greater than in the urine. Osweiler (1996) stated blood with low levels of lead are excreted into the bile by active transport while high blood lead levels are excreted in the urine. Five percent of intravenously administrated lead is excreted in the urine during the first 24 hours (Casarett and Doull 1975).

Arai et al. (1998) found about 4% of the administered amount of lead was excreted into the urine during the 7 days after the injection of single dose of 5.4 mg Pb/kg body weight to rabbit while about 68% was excreted in the feces; fecal excretion of total lead being about 17 times as great as the urinary excretion. Of the urinary excretion of total lead, 85% was composed of diethyllead (Et2Pb2); 92% of the fecal excretion of total lead consisted of inorganic lead (Pb2). The major chemical species of lead excreted in the urine was found to be Et2Pb2, while the major species excreted in

the feces was Pb2. These results were similar to the findings reported on the administration of tetraethyllead (Et4Pb) to rabbits (Arai et al., 1998). However, an opposite effect was found in the case of ingested or inhaled lead; here, lead was excreted primarily through the urine with smaller amounts in feces, sweat, hair and nails (dhs, 1997).

Measurement of lead in the urine is most commonly used to follow lead excretion of patients while on chelation therapy for lead poisoning. This monitoring of chelation therapy begins 24-hours prior to chelation. Measurement of Lead in the urine lead is also used to indicate exposure to lead. Only 5% of the blood lead concentration is reported to be excreted in milk (Osweiler 1996) which is an important source of lead for infants and toddlers (Fox, 1987). Sharma et al. (1982) also found an increase in Pb concentration in milk when sheep were fed a 31.45 mg Pb/kg diet but not in sheep with lower lead concentrations in the diet.

Mechanism and metabolic effects of lead

Lead is not an essential (necessary) element and serves no useful purpose in the body (Casarett et al., 1996). As new information emerges about the toxicity of lead, low exposures, that in the past were thought safe, are now considered hazardous.

Lead enters the body primarily by breathing in or swallowing lead dust, fumes or mist and is absorbed into the blood stream and distributed throughout the body. Thus,

many parts of the body can be affected by lead. An additional concern for pregnant women occurs because lead easily crosses the placenta and can harm the developing child. Lead poisoning can occur with few or no obvious effects; therefore, it is important to realize that lead can cause damage to the body without knowledge and the damage can be permanent.

Lead may enter the body through several routes. One to two percent of orally administered inorganic lead is absorbed by the gastrointestinal tract (Osweiler, 1996). Lead is initially retained in the soft tissue and later in bone. Inorganic lead can not cross the epidermis except through open wounds. On the other hand, organic lead can penetrate the skin rapidly. Both types of lead can enter the body through the respiratory system (Goodman and Gilman, 1966). Although blood lead levels usually rise fast within the first half day to 3-or-even 4 ppm, while subsiding to 1.5 ppm may take two to three days; this rate of decline emphasizes the slow excretion of the cumulative lead toxin.

The pathophysiology of lead toxicity is not fully understood (Osweiler, 1996). Lead compounds are not specific and tend to affect all major organs. In general, it enhances peroxidative damage to membranes and, therefore, it interferes with cellular functions (Sandhir and Gill, 1995). Lead combines with erythrocyte proteins causing them to be fragile (Chrisman, 1991; Smith, 1996).

Additionally, lead can interfere with hemoglobin production causing anemia (Green Peace Report, 1996; Hilliard et al., 1973; Kumar et al., 1997). It depresses the

bone marrow, thereby decreasing red cell production (Alexander et al., 1996). Lead, at the subcellular level, interferes with enzymes with free-sulfydryl groups (Clarke, 1973; Osweiler, 1996) including the heme synthesis enzyme (Fox, 1987). It blocks the metabolism of aminolevulinic acid as well as blocking the incorporation of iron into the heme molecule (Hammond, 1973; Smith, 1996; Kumar et al., 1997). The resulting inhibition of protoprphyrin formation (Klein, 1962) by slowing coproporphynogin oxidase (Bryson, 1989) or its conversion to heme by acting on ferrochelatase (Bryson, 1989) causes an increase in free portoporphyrin in the erythrocytes (Freeman, 1970b). Lead also inhibits aminolevulonic acid dehydretase that catalyzes aminolevulinic acid into porphobilinogin (Bryson, 1989). Serum (Feldman et al., 1969) and urinary accumulation of amenolevulinic acid will depress heme production. In a word, impairment of heme biosynthesis and the acceleration of red blood cell destruction or impairment of RBC survival cause lead-induced anemia (Landrigan, 1994).

The central nervous system is affected by lead via several ways. Lead can damage blood capillaries (Osweiler 1996) leading to edema or a collapse of the small arteries that supply the brain. It can physically damage the blood brain barrier and lead to edema, an increase of intracranial pressure or even ischemia. Lead can lead to the dysfunction of the astrocytes signals in the blood brain barrier. Lead interferes with calcium that is needed to activate protein kinase C that is important in brain differentiation, proliferation (Markovac and Goldstein, 1988) and may retard brain

growth (Krigman and Hogan, 1974; Underwood, 1977). It is also believed that lead interferes with neurotransmitters like gamma amino butyric acid (GABA) and dopamine uptake by the synaptosomes (Osweiler, 1996). Studies show that lambs born from dams exposed to subclinical levels of lead during gestation require twice the time and number of trials to learn a two-choice visual discrimination operant task than lambs born form unexposed dams (Carson et al., 1973). Lead toxicity can also lead to the reduction of the DNA content of the cerebellum of suckling rats (Michaelson and Sauehoff, 1974).

One of the insulting actions of lead on the peripheral nervous system maybe segmental demylination and possible axonal degeneration interfering with nerve conduction (Landrigan, 1994; Casarett and Doull, 1996; Kumar et al., 1997). This action may cause the paralysis of the masseter muscle in dogs and the recurrent paralysis of the laryngeal nerve that causes roaring in horses (Osweiler, 1996). Levels as low as 40 μg/dl of lead in blood can have an affect on motor nerves and can lead to their dysfunctioning (EPA, 1986).

Lead can cause the rupture of lysosomes and the release of acid phospatase that is required for energy production and protein synthesis. Lead interferes with synaptic mechanisms of transmitter release by substituting for calcium or zinc in ion dependent events (Casarett and Doull, 1996).

Lead causes insult to several organs in the body. It causes degeneration and necrosis of renal tubule cells and can lead to irreversible damage to nephrons and a

gradual reduction in the efficiency of uric acid excretion. Lead may cause hepatic cells degeneration and interefere with its biologic functions. Lead affects the metabolically active growth centers of long bones and may lead to some bone deformaty (Greenpeace report, 1996).

In females, lead crosses the placenta (Allcroft, 1951; Abadin et al., 1997). Thus the effects of lead are present to affect the develping embryo very early.

Some studies show that lead can reduce the resistance to bacterial infection by inhibiting antibody production. Hemphill et al. (1971) report such activity in mice.

Clinical signs of lead toxicosis

Symptoms and possible signs of lead poisoning in adults could be summarized as mild to moderate toxicity and include symptoms ofincluding: headaches, forgetfulness, memory loss, nausea, persistent fatigue, difficulty sleeping, weight loss, weakness, irritability, nervousness, anemia, clumsiness, lack of coordination, muscle or joint aches, high blood pressure, tremors of the hands or head, metallic taste in mouth, dizziness, loss of appetite, decreased sex drive, difficulty in concentrating, stomach aches, infertility, depression, constipation, and pregnancy problems; many of these symptoms may be confused with those of other health conditions (Underwood, 1977; Caserett et al. 1996; Osweiler, 1996). Occasionally lead tosicosis can lead to permanent brain damage or even death (Panariti and Berxholi, 1998). Children ingesting chips of old

lead-contaminated paint or who are exposed to dust from the deterioration of such paint may exhibit several symptoms but the most common are neurological.

Some individuals may experience toxic symptoms when their blood lead levels reach 25 μg/dl (Kosnett, 1994); others may have much higher lead levels and yet have few or no symptoms. An exact threshold level for the effects of lead has been established; signs have been observed at levels as low as 10-15 μg/dl and less (ATSDR, 1988). Generally, the severity of symptoms worsen with increasing blood lead levels (dhs, 1997).

Lead is called a race poison indicating its effects are passed on to the progeny of the exposed individuals (Hamilton, 1925) and it has an adverse effects on both males and females. Lead toxicity is associated with infertility and neonatal deaths (Casarett and Doull, 1996) and it is known to impede the male reproductive function (ASTDR, 1993) with an unclear mechanism (Thoreux-Manlay et al., 1995). Assnato and his colleagues (1986) found a reduction in sperm counts and abnormal sperm motility and morphology in lead exposed males (Landrigan, 1994) with blood lead levels of 40 μg/dl (Alexander et al., 1996). A decrease in testicular endocrine function was found to be related to duration of lead exposure of adult men with a mean blood lead level of 60 μg/dl (Rodamilans et al., 1988). Consistent with these findings, Thoreux-Manlay and his coworkers (1995) found a 50% drop in testosterone production with leydig cells from lead-exposed rats. Sexual drive could be decrease as a result of lead poisoning (Bryson,

1989). In females, lead could be present in breast milk (Abadin et al., 1997) endangering the young dependant it for nurishment during the early period of their lives. Lead exposure can decrease fertility in females (Bryson, 1989; Landrigan, 1994) and decrease gestation time (EPA, 1998) in addition to causing congenital malformations in children (CDC, 1991). It is documented that lead is transferred from the pregnant heifer to the embryo (O'Hara et al., 1995). They found that 71.7%- 84.3% of the lead concentration was transferred compared to the lead concentration in the blood and liver respectively. Spontaneous abortion in women (ASTDR, 1993) with lead toxicity were reported early by Flury and Blei (1934) and continue to be (Lewis, 1997). In ancient times, women ingested lead to induce abortions (EPA, 1998). In the last century, female lead workers had high rates of miscarriages and stillbirths (EPA, 1998). Alcroft and Blaxter (1950) as well as Howard (1981) stated that abortions are also common among animals exposed to high and even low levels of lead during pregnancy. Pregnant sheep can be aborted when a transitory infertility is associalted with chronically ingested metallic lead (Sharma and Buck, 1976; Radostits et al., 1983). Needleman and Bellinger (1990) agree that adverse effects on pregnancy are a result of transplacental lead toxicity and have effects such as: increased miscarriages, stillbirths, and minor malformations in neonates such as skin tags and hydrocele. Reduced birth weight and premature birth can be a consequence of prenatal exposure to low levels of lead (CDC, 1992; EPA 1998). After an episode of lead poisoning in Delhi, milk yield in lactating Buffalo was reported to be reduced by almost

80% (Dey et al., 1996). When exposure is excessive during early childhood, a latent development may occur due to brain and nervous system damage (EPA, 1998). On the other hand, Coffigny et al. (1994) found that rats inhaling lead oxide inhalation (5 mg/cubic m) did not disturb reproductive function in their male offspring. However, a comprehensive review of lead toxicity done by Bryson (1989) documented an increase in death rates among the newborn especially during their first year.

Impaired cognitive development has been observed in children with prenatal lead exposure (Bellinger et al., 1987) since lead can readily cross the placental barrier (Abadin et al., 1997). Slowed mental growth has been observed in infants born to mothers who have been exposed to lead levels considered safe for children (Bellinger et al., 1987). Impaired academic performance and deficits in motor skills have been reported even after blood lead levels return back to normal (Needleman, 1990). Reduction of I.Q. and attention span, reading and learning disabilities, impaired growth and hearing loss were reported in children exposed to low levels of lead (CDC, 1991;ATSDR, 1993; EDF, 1994). Van Gelder et al. (1973) stated that encephalopathy, hyperirritability and aggressive behavior can result from lead toxicity in children. Severe lead exposure in children can cause coma, convulsions and even death (CDC, 1991).

In adults, lead can affect memory and decrease reaction time (ASTDR, 1993). Landriga (1994) found that blood lead levels as low as 40-70 μg/dl increased mood

depression, fatigue, impaired concentration and contributed to subtle behavioral changes. In severe cases, toxicity can result in lead encephalopathy causing seizures and even coma (Chrisman, 1991; Cal/OSHA, 1997).

Levels of environmental lead considered non-toxic may also be involved in increased hypertension, although Korrick et al., (1999) found no association between hypertension and blood or tibia lead concentration. However, the U.S. Center for Disease Control has been downgrading the acceptable levels of environmental lead that had normally been considered safe.

Neurologic signs in animals are due to cerebral edema. These signs include circling, restlessness and pushing against objects (Kimberling, 1988; Smith and Sherman, 1994). In animals displaying excitement and convulsive seizures, the prognosis is poor (Neathery and Miller, 1975; Osweiler et al., 1985). However, findings are inconsistant as Radostits et al. (1983)observed no excitement, tetany or convulsions in sheep with lead poisoning. Blindness (Neathery and Miller, 1975; Osweiller et al., 1985; Oliver et al., 1987; Bratton and Kowalcyzk, 1989; Chrisman, 1991; Smith and Sherman, 1994), muscle twitching and hyperirritability may occur also in some cases of lead poisoned animals (Buck, 1975; Osweiler and Rruhr, 1978; Osweiller et al., 1985; Smith, 1996).

The carcinogenicity of lead in humans is inadequate understood (ASTDR, 1993). Dinghall-Fordyce and Lane (1963) did not find any association between lead exposure

and cancer mortality. On the other hand, a study performed by Cooper et al., (1985) showed an excesses for total cancer mortality, stomach cancer and lung cancer among battery plant workers and lead smelter workers. In an observational reviewed study by Steenland et al. (1992) on patterns of death for smelter workers between 1940 and 1987, findings showed an excess mortality from chronic renal disease that was directly related to duration of employment. The same results were obtained by Cooper et al. (1985) from a study conducted on battery plant workers with blood lead level around 80μg/dl. All of the available studies lack quantitative exposure information, including information on smoking status. Also, all studies included exposures to other metals such as arsenic, cadmium, and zinc but made no adjustment for their presence. The cancer excesses observed in the lung and the stomach were relatively small ($n<200$). No site consistency among the various studies, and non of the studies showed any dose-response relationship. Thus, the available human evidence is inadequate to refute or demonstrate any potential carcinogenicity for humans from lead exposure.

Animal carcinogenicity is better documented. The carcinogenic potential of lead salts (primarily phosphates and acetates) administered via the oral route or by injection has been demonstrated in rats and mice by many investigators (Casarett and Doull, 1996). The most characteristic cancer response is bilateral renal carcinoma. Rats given lead acetate or subacetate orally have developed gliomas; lead acetate also has produced lung adenomas in mice after intraperitoneal administration. Most animal studies have

found a carcinogenic response only at the highest doses. Almost all lead compounds tested in animals have been soluble salts. Metallic lead, lead oxide and lead tetralkyls have not been adequately tested. Studies of inhalation exposure could not be found in the literature.

Lead poisoning may cause aminoacidurea, glycosuria and hyperphosphateuria due to its direct affect on mitochondrial respiration and phophorelation and hence cause impaired reabsorption of amino acids, glucose, phosphate and citric acid (CDC, 1992; ATSDR, 1992; Casarett and Doull, 1996). A hypochromic microcytic anemia causing an increase in the numbers of reticulocytes and basophilic stippling can be present in the blood as a result of lead poisoning especially in children (Paglia et al., 1975). This type of anemia is a chronic lead poisoning anemia that is not seen in the early phases of lead poisoning; it differs from the acute form that produces hemolytic anemia (ATSDR, 1992).

In adults, there is evidence for an association between high blood pressure and an elevated body burden of lead (EPA, 1989b; Lockitch, 1993; ASTDR, 1993). These findings are supported by the Health and Nutrition Examination survey for the U.S population between 1976 and 1980 in their strong association of hypertension and blood lead levels in 40 to 59 aged males. On the other hand, Korrick et al. (1999) found no association between high blood pressure (systolic > 140, diastolic > 90mm Hg) and blood lead concentration in women nurses. The discrepencey can possibly be explained

by the sex difference as Tyroler and colleagues (1988) found a systolic increase of 1.5-3.0 mm Hg for every doubling of blood lead concentration in adult males where as the increase was found to be only 1-2 mm Hg in females.

Lead exposure can lead to a decrease in serum vitamin D (Casarett and Doull, 1996). The effect of lead toxicity on the immune system has been studied by many.

Koller (1979) found lead and cadmium to directly suppress the B cell, accounting, in part, for findings reporting suppression of humeral and cell-mediated immune responses. Haneef and his colleagues (1995) support Koller's findings in a study on goats. They reportedcell-mediated immune response as monitored by coetaneous hypersensitivity reaction (CHR) [measured by average increase in skin thickness] to dinitrochlorobenzene, was suppressed. Reihold (1992) states that there are problems with the interpretation of the results because lead is usually injected with infectious microorganisms which may enhance the microbe rather than interfering with the immune response.

Kehoe et al. (1933) described lead colic in man as being characterized by a sharp onset and recurrent spasms in which the patient writhes in pain, retracts his legs spasmodically to his abdomen, groans, clenches his hands, grits his teeth, and has beads of sweat on his brow. A transient constipation may occur early followed by diarrhea (metals and metalloids) and severe constipation can be manifested with lead levels exceeding 100 μg/dl in blood (Saryan and Zenz, 1994).

Anorexia has developed in animals poisoned by lead (Alcroft and Blaxter, 1950; Daeschner, 1983; Osweiller et al., 1985;Bratton and Kowalcyzk, 1989; Bryson, 1989; Chrisman, 1991) which is also believed to be a consequent insult to the nervous system (Osweiller, 1996). Lead poisoned animals tend to be solitary and be depressed (Chritian and Tryphonas, 1971; Osweiller et al., 1985). Alcroft and Blaxter (1950) noticed decreased water consumption in sheep with lead poisoning. Animals may show excessive salivation, grinding teeth and tucked abdomen (Neathery and Miller, 1975; Howard, 1981; Osweiller et al., 1985; Bratton and Kowalcyzk, 1989; Smith and Sherman, 1994; Smith, 1996), frequent colicky abdominal pain (Bratton and Kowalcyzk, 1989). In cattle, rumen motility is decreased or even abolished in some cases (Osweiler, 1996).

A bluish line on the gum, the Burtonian lead line, that is formed by precipitation of lead sulfide is a well known feature of prolonged lead exposure (CDC, 1992; Saryan and Zenz, 1994).

Lead lines on radiographs can be seen in lead exposed species. These lines are due to increase bone density because of the interference of lead with normal remodeling of calcified cartilage and primary bone trabeculae in the epiphyses (Kumar et al., 1997).

Lambs have reportedly developed rickets when they were kept in areas surrounding the lead mines of North Derbyshire in England (Gardner, 1924). Clegg and

Rylands (1966) observed osteoporosis in young grazing lambs in lead mining areas. However, signs only occured in lambs 3-12 weeks of age and never in adults (Radostits et al., 1983).

Lead was recognized to be nephropathic very early in the twentieth century (Lockitch, 1993). Only with blood levels greater than 0.6 ppm will lead produce its nephropathic effect (Casarett and Doull, 1996). Several researchers (Bryson, 1989; Osweiler, 1996) reported elevated levels of aminolevulinic acid, coproporphyrinogenin serum and urine. There is a positive relationship between chronic lead exposure and gouty nephropathy (CDC, 1992) due to the reduction of uric acid excretion excerpted causing hyper uricenia leading to the development of saturnine gout (Saryan and Zenz, 1994; Casarett and Doull, 1996).

Wrist or ankle drop is a classic clinical picture of lead poisoning (CDC, 1991; ASTDR, 1993; Mielke, 1999); as well as mild slowing of nerve conduction velocity especially in the ulnar nerve at blood lead levels of 30-40 μg/dl (Landrigan, 1994). Young and adult guinea pigs showed a reduction in the motor nerve conduction velocity when they were orally dosed 0.5-1 g/kg of lead acetate for about 5.5 months (Fullerton, 1966). Stiffness of gait , lamness and posterior paralysis are common signs in sheep with lead poisoning (Radostits et al., 1983). The posterior paralysis is due to lameness of the vertebrae, resulting in the compression of the spinal cord (Radostits et al., 1983).

Laryngeal and pharyngeal nerve paralysis may occur especially in horses and lead anal sphincter paresis (Oliver et al., 1987), dysphonia, roaring and aspiration pneumonia with a remarkable weight loss (Knight and Burau, 1973).

The mortality rate among those diagnosed with lead poisoning and untreated is much higher (65%) than among treated patients (25%) (Chan, 1998). Treatment for lead poisoning initially involves removing the animal from the source of lead. Second, lead compounds need to be removed from the gastrointestinal tract (G.I.T) in order to decrease further absorption; This can be accomplished by emetics or by surgery if the lead object is a solid. Magnesium or sodium sulphate can be used to pericipitate lead and prevent further absorption (Bratton and Kowalczyk, 1989). Thirdly, lead can be removed from the blood and tissues by using chelation therapy; nontoxic water soluble complexes bind with lead to facilitate its excretion. The highest volumes are eliminated in urine on day two of chelation therapy as the lead is removed from the blood stream and then drop slowly to level off on day three to five. Blood lead level should not be taken for at least 2 weeks after chelation to allow equilibration of the lead between the soft tissues and the bloodstream to occur. A blood lead done immediately after chelation will give a falsely low value since most of the lead in the blood has been removed.

Calcium chelate [calcium disodium ethylene diamine tetraacetate] (CaNa2EDTA) is one such compound that is used to removed lead from the body and it also helps to prevent hypochalcemia. It is given at a rate of 100 mg/Kg body weight

daily for two to five days. The daily dose is diveded into four subcutaneous diluted dosages (10 mg chelate/ml of 5% dextrose solution (Chrisman, 1991). Olkowski et al. (1991) found that an addition of thiamine to the EDTA will improve biliary and urinary excretion up to 842% in sheep. Lidocaine can be mixed with the solution to prevent pain at the injection site. Treatment should not continue more than five days to lessen the chance of any renal damage that could be caused by the CaNa2EDTA. Such a treatment can be repeated, if neccessary, after a resting period of five days. Since CaNa2EDTA has a negative effect on the renal system, an adequate intake of fluids with the chelation therapy should be part of the treatment regime. In rare cases, CaNa2EDTA has caused proteinuria, microscopic hematuria, proximal tubule damage, hypercalcemia and fever. These side effects require its use be closely monitored to avoid any complication. CaNa2EDTA should carefully be administered to animals that are going to be used as a source of food since administration of CaNa2EDTA causes translocation of lead from bone into muscle (Hammond, 1973).

The dithiol groups are not the only binding sites for lead (Oehme, 1972) which explains the high affinity of lead to bone and the ineffectiveness of Dimercaprol® in treating lead poisoning. Another widely used drug is the D-Peicillamine which can be used in animals that does not have penicillin allergies. It is the only oral chelating agent (8 mg/Kg, four times daily) used a lone or following parental calcium EDTA therapy (Osweiler, 1996).

CHAPTER III
GENERAL MATERIALS AND METHODS

Lamb Management

Twenty-five of local Kuwaiti breed of lambs were allocated to this fourteen weeks experiment. All lambs were between 2, 4, 6 or >6 months of age and not older than 10 months of age. Both male and female lambs were used and equally distributed between the five groups. Each group received a single dose of lead acetate daily as follows: Group 1 was the control group and received no lead in the diet. Group 2 received 2 mg/kg/d lead acetate. Group 3 received 4 mg/kg/d lead acetate. Groups 4 and 5 received 8 and 16 mg/kg/d lead acetate respectively. Lead acetate was given as an oral solution using graded syringe. It was given early in the morning before their first meal. Each lamb was kept in an individual pen (1.7 m X 1.7 m) with straw bedding that was cleaned regularly. All lambs were fed feed ad libitum of feed containing 70% concentrate and 30% of alfalfa with feed divided into two meals one in the morning and the other one in the afternoon. All groups had a free access for water.

All lambs were examined and found to be healthy before the experiment started. All lambs were vaccinated to protect them against common diseases. They received vaccines to protect them against Clostridial diseases including enterotoxemia (Vaxual 8, manufactured by Websters Australia). Pastocidin manufactured by Hoechest was used to

protect them against pasteurellosis. Regular examination were made to detect any abnormalities among the experimental lambs. Before the experiment started all lambs had blood withdrawn to measure lead for a time zero concentration.

Feed intake and weight gains

At the end of each week, feed intake and body weights were measured for each lamb. Two meals of approximately 2 kg of feed were given in the morning and in the afternoon. Before the next morning meal, the residual feed was taken from the troughs and placed in a bag , with the lamb number recorded. The residuals accumulated for one week and then weighed. Then the amount of the residuals was subtracted from the total weekly feed weight to calculate the daily feed intake.

Lambs were weighed individually using a balance with a two-door cage to facilitate the entrance and exit of each lamb. To reduce errors, the one person consistently read the scales for both measurements. Weight gain or loss was calculated accordingly. The weight of the lambs every week was used to calculate the lead acetate dosage given daily to the lambs.

Blood withdrawal

blood was drawn at time o and at 2 weeks, then at monthly intervals. The lambs' necks were sheared over the jugular vein to facilitate blood withdrawal and

avoid contact with the wool that might have lead in it from the feed. Using blood collection tubes with heparin, (vacutainers). One needle per lamb was used to prevent contamination. Samples of blood were refrigerated at 4 C right away and then sent directly to the center of analytic laboratories (CAL) at Kuwait institute for scientific research for the subsequent analysis of the blood lead concentration totaling up to 200 samples at the end of the experiment. Using mechanical rollers, samples were all mixed thoroughly before performing the tests.

Blood samples preparation

About 10 g of blood sample was taken from the original sample. The subsamples were digested with Aristar grade (BDH,UK) nitric and perchloric acids mixture in a beaker at 100-180EC. After a complete digestion the final volume is made to 25-ml. Lead concentration were determined by inductively coupled plasma -Atomic emission spectrometric (ICP-AES) method using HORIZON Model, from Fison, UK. It is im nitrogen purged cserny-Turner spectrometer with 27.12MHz RF generator. The instrument is calibrated with lab made standards prepared from individual metal standards solutions obtained from E-Merck, Germany. Following were the optimal operating conditions were power is 650W, plasma gas is 0.8 liter/hr, cool gas is 7.5 liter/hr and carrier gas is 0.8 liter/hr using Reinhard type for Nebulose with peristaltic pump.

The analysis were controlled using certified reference standards such as bovine liver SRM1577b and oyster tissue SRM1566a obtained from National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA. The measurable detection limits for Pb were 0.01 mg/L in the solution by Icp-AES. The precision of ICP-AES method for the metal analyzed were SD of 5% for range 1 - 100 mg/Kg and 10% SD for concentration range of 0.1 - 1 mg/kg in the actual samples.

Clinical signs and behavioral changes

Lambs were watched closely every day for any clinical or behavioral changes that might arise from lead intake. A continuous observation throughout the day by different people was there to insure observing any changes on the animals.
A form to be filled by the observer for the sign observed, the time of observation and the identification number of the symptomatic lamb. That form was filled three times, once at each mealtime and once in between, daily to ensure that we do not miss any sign.

Necropsy

At the end of the experiment all lambs were necropsized. That was done on a 5 days window that is 5 animals (one from each group) each day. Each necropsized animal was watched closely for any gross changes that could be observed in any of the organs. Samples (n=850) from different parts (Testis, ovary lung, kidney, heart, liver, small

intestine, bone, masseter muscle, rumen, diaphragm, eye, spleen, tongue, esophagus, brain, trachea and aorta) were taken and divided into two parts. One part was frozen and sent to Central Analytical Lab for subsequent analysis of lead content using ICP as a method for analysis. The other half were preserved in 10% Formalin for subsequent check for the histopathological changes.

Organs (head, lung, heart, liver, spleen kidney, ovary, testes, tail and wool and leg shafts) were weighed as well as the carcasses for comparisons and correlation, if any, within the groups.

Data analysis

Data were analyzed using General Mixed Linear Model of the Statistical Analysis System (SAS, 1988). The Student-Newman-Keul test was used to determine significant differences between means (Steel and Torrie 1960). All main effects were included in the analysis. The main effects examined included the dependent variable (lead concentration), highway, site within the highway and distance from the highway. The sites within highways efeects were considered random. General linear model was used with repeated measures for analysis like lead content in blood, as well as feed intake and weight gain for the lambs over the experimental period. Evaluation of organ

weights, lead content in tissues were analyzed using GLM with means and least square means using student Numan Keul to separate the means. Correlation analysis was used for most of the analysis.

CHAPTER IV
EXPERIMENT I
ASSESSMENT OF LEAD STATUS IN SOIL AROUND MAJOR HIGHWAYS IN THE STATE OF OREGON AND IN THE STATE OF KUWAIT AND IN LAMBS GRAZING NEAR A MAJOR HIGHWAY IN KUWAIT

Introduction

Lead poisoning has been recognized as the number one environmental threat to the health of both human and animals (Hammond, 1973). In Rhode Island, almost half of the children reported blood lead levels in excess of what is considered harmless by the Centers for Disease Control. Farm livestock poisoned with lead through a variety of sources of contamination has been well documented (Blaxter, 1950; Allcroft, 1951; Aronson, 1972; Clarcke, 1973; Dinius et al., 1973; Hammond, 1973; Froslie et al., 1985; Koh and Judson, 1986; Takla, et al., 1989; Ahmed and Shalaby, 1991; Dey et al., 1996). Air, water, food, dust and soil have all been implicated as pathways of exposure to lead. Dust and soil, however, have emerged as the primary culprits in recent years.

Lead is by far the most common contaminant and permanent resident residue in soils. Soils are a basic natural resource of any country upon which many other natural resources depend. Animal production in general and sheep production, in particular, are industries that depends on this soil resource. It can indirectly affect livestock by grazing them on pastures that are located beside major highways. Sheep could be exposed to lead by eating contaminated plants, eating the soil itself, or breathing soil dust (Maine Soil Testing Service 1998). Human activities such as using leaded gasoline in motor vehicle

tends, over time, to elevate the concentration of lead in soils to levels which may pose toxicity risk (Ferguson, 1986; Koh and Judson, 1986; Saleh et al., 1996). Exhaust emissions by motor cars are the main sources of lead in the atmosphere; it lead to an increase in the levels of lead in roadside soil and vegetation (Tjell et al., 1979; Ferguson, 1986; Saleh et al., 1996). Rodrgues and Castellon (1982) estimated that 70-80% of the gasoline lead content is emitted to the environment in automobile exhaust. Livestock grazing on contaminated soils are at risk by breathing dust (dust is very common in areas such as Kuwait at certain times of the year) or eating the soil itself (Ward and Savage, 1994) or indirectly at risk by eating contaminated pastures growing near highways (Mahmood et al., 1985). Animals attend to graze beside highways can accumulate lead in their internal organs and tissues (Ward et al., 1978, Froslie et al., 1985; Husain et al., 1996).

In sheep, studies have shown that toxicity from Pb poisoning can cause anorexia, abdominal pain and diarrhea (Neathery and Miller, 1975). Such results from lead toxicity could add to the difficulties that Kuwait is facing in razing sheep industry. Additionally, the internal organs of sheep are edible by both man and animals and therefore contribute a health risk to both (Hankin et al., 1975). Husain et al (1996) found an elevated mean lead concentration in kidney and liver samples of goats (427 and 130 μg/Kg) and sheep (145 and 125 μg/Kg) in Kuwait and attributed the heavy lead concentration to grazing of these animals near highways.

Lead toxicity becomes particularly important where urbanization places an exceptional stress on natural resources endangering livestock and consequently man

himself. In spite of these concerns, limited studies on lead in soil are available (EPA, 1997). A study by Olszowy and co workers (1995) found that 40% of soil samples taken near a busy road in Sydney and Brisbane exceeded 300 ppm, the threshold standard for health and environmental investigation of

contaminated land set by the National Health and Medical Research Council (NHMRC) and the Australian and New Zealand Environment and Conservation Council (ANZECC).

Since lead is used as petrol additive in Kuwait from the time they used automotives in the fourties till the present time, it is necessary to document the magnitude of lead contamination of roadside and evaluate its environmental implications. The present experiment measured the levels of lead around a major highway in Kuwait and compare it to several highways in Oregon. Additionally, the study was designed to assess the effect of distance and the density of the traffic on the amount of lead accumulation on the sides of a highway in Kuwait and Oregon. Thirdly, it measured the toxicity effect on lambs grazing near a major highway in Kuwait using their blood lead level as an indicator for that effect since it is believed to provide a reliable indication of the exposure of sheep to lead (Rolton et al., 1978). Additional data using blood lead levels as an indicator for lead toxicity is presented in another paper.

Materials and Methods

The first part of the study was conducted in November 1997 in Kuwait around King Fahad Highway that links Kuwait with Saudi Arabia. Soil samples were collected from areas that is about 20 kilometers to the south of kuwait city (figure IV. 1). King

Fahad Highway is one of the major highways in Kuwait and more that 70000 cars is passing through it each day. Soil was Sampled at 0, 3 and 10 meters from roadway within each site of the three sites that were in rural areas and away from exits or intersections that might be influential on the results of the study.

The second part of the study was conducted in late summer of 1998 in Oregon. Soil samples were collected from three areas associated with highways of different traffic volume, Interstate 5 (122,000 cars per day), Highway 34 (11,800 cars per day) and highway 20 (11,400 cars per day) in Oregon State (Transportation Systems Monitoring Unit 1997). Soil was Sampled at 0, 3 and 10 meters from roadway within each site of the three sites sampled at each highway. Samples were collected from interstate 5 were collected in the part that connect Albany to Salem; from Highway 34 at the region that connect Corvallis to Lebanon in the area that lead to Interstate 5 exit 228; from Highway 20 at the region that connect Corvallis to Albany (FigureIV.1) avoiding any factors that could interfere with the study such as exits and intersections.

In general sites within any highway were approximately 2 kilometers a part.

Dust from the top of the soil was taken at the edge of the highways, three meters and ten meters away from the edge. Using a small brush dust particles were swept into a small polythene bag. Each bag was documented with location, site number and distance from the highway margin.

Soil prepation

Soil samples were dried at 50°C for 7 days. Subsequently 2 g of each sample was digested with a high purity nitric acid and perchloric acid mixture that was heated over a hot plate. The final solution , 25 ml volume, is made with 5% nitric acid. Samples were filtered and then analyzed for lead content by inductively coupled plasma-Atomic emission spectrometer (ICP-AES). The calibration is made with standard lead solutions prepared from 1000 ppm stock solutions obtained from BDH, UK.

Blood collection and analysis

Blood samples were taken from 50 grazing lambs around King Fahad high way in the south region of Kuwait. Using heparinated tubes, blood samples were withdrawn from the jugular veins on the sides of the lamb's neck. Samples were shaken in the tubes, kept in a freezer chest and sent to the Central Analytical Laboratory (CAL) of Kuwait Institute for Scientific Research for subsequent analysis of blood lead content. Lead concentration was determined by (ICP-AES) using a HORIZON Model from Fison, UK as described earlier in chapter III.

Data were analyzed using General Mixed Linear Model of the Statistical Analysis System (SAS, 1988). The Student-Newman-Keul test was used to determine significant differences between means (Steel and Torrie 1960). All main effects were included in the

analysis. The main effects examined included the dependent variable (lead concentration), highway, site within the highway and distance from the highway. The sites within highways efeects were considered random.

Results and Discussion

Table IV. 1 shows the levels of lead in the soil at 0, 3 and 10 meters from Interstate 5 (122,000 cars per day), Highway 34 (11,800 cars per day) and highway 20 (11,400 cars per day) in Oregon State (Transportation Systems Monitoring Unit 1997) and King Fahad Highway in Kuwait (>70,000 cars per day). The shorter distances from the highway correlated with the higher concentrations of lead in the soil ($p=0.005$). Results shows 1379.8 ± 27.03, 1317.8 ± 27.03 and 1239.3 ± 27.03 μg lead/g dry weight soil at distances of 0 meter, 3 meter and 10 meters from the highway respectively. In support of this, Dierkes et al (1998) conclude from a study on pollution retention capabilities of roadside soils, that Pb concentration is decreased with increasing depth in the soil and distance from the street. Analogous to that, Howard and Sova (1993) conducted an experiment in Michigan showed that Pb concentration increases with the increase proximity to the highway (although they worked with distances further from the road (10, 30, 60 and 100 meter) than what we did). Also in agreement are Collins (1984) findings indicating lead concentration in soil adjacent to a busy highway (23000 vehicle per day) in New Zealand decreased with distance from the road and that concentrations of lead ranges from 8 -23 ppm at a distance of 300 meters from the road. Although, this present

study did not include this distance, theoretically as well as determined by this study's regression equation for each highway the lead concentration would be null at this distance; the regression equation of Collin's data will be similar to the equation used in this study if it was calculated using a distance of 10 meter from the highway. Collins found concentrations ranging from 177- 262 ppm at a distance of 4.2 meters from the road which is close to the higher concentration found at Interstate 5 at a similar distance (180 μg/g at 3 meters), although unleaded gasoline has been used in United states for almost two decades which shows how hard for the environment to bioremediate itself from such a toxic metal. This was also shown in 1996 study done in Tampa, Florida Hafen and Brinkmann to assess the amount and distribution of lead in soils adjacent to a major highway. They found that In spite of the weak correlation between lead soil content and distance from highway, a negative relationship was present at distances of 0.81, 2.43 and 7.29 meters from the edge of roadside. More than one third of their samples exceeded the concentration of 500 ppm while non in the present study reached that high a level. This finding could be due to differences in atmospheric turbulence and other microclimatic factors between the two states as well as attributable to the difference in automotive density. In general, lead in soils near highways is related inversely with distance from the road (Cannon and Bowles, 1962; Lagerwerff and Specht, 1970).

Concentrations of lead in the soil around King Fahad Highway in Kuwait (4943.6 ± 31.22) was significantly higher ($p=0.0001$) than those in soils of the tested highways in Oregon, i.e Interstate 5 (129 ± 31.22), Highway 34 (94.9 ± 31.22) and

Highway 20 (81.7±31.22). This reference is likely a result to leaded gasoline the only type of fuel that was used in Kuwait at the time of the experiment. Another reason contributing to differences in lead is that the heavy vegetation found on the sides of Oregon highways could act as a physical barrier against the travel of lead particles.There are no data from Kuwait allowing direct comparison with lead concentration in soil. However, Jeddah , a city on the red sea in Saudi Arabia (south and west of Kuwait), was tested for the concentration of lead in the dust of its streets. Lead levels were found to be close to a 1000 ppm (Nasralla 1984). These findings proceeded this study by 14 years allowing a longer time for lead to accumulate. Additionally, fires that were started by the Iraqi regime before leaving Kuwait created the largest environmental disaster in the century and had a disastrous affect on the accumulation of pollutants in the soil. Another study reporting high roadside lead levels is Ward (1986). The lead content in the soil surface was measured 5 meters away from London Orbital Motorway (M5) and found it to be on an average of 5244 ppm and 8400 ppm in areas of South Mimms and Watford and respectively. Levels of lead either similar (Watford) or exceeding (South Mimms) to the present study. The similarity could be due to the use of leaded gasoline in United Kingdom at that time while the excess could be due to the higher traffic density in Watford area (102 000 vehicle/day).

Concentrations of lead around several Oregon highways (Interstate 5, Highway 34 and Highway 20) were statistically similar; although there was a tendency for an increase of lead content in the soil of the highway with the higher traffic density. That is in agreement with Ogunsola et al (1994) who found a positive correlation of the

concentration of Pb as vehicular emissions were compared with the traffic density. They found lead concentrations were higher in soils by highways than in other tested areas such as residential areas, industrial areas, marine areas and near bus stops. They concluded that automotive emissions were the main contributing to Pb concentrations in the environment and particularly roadside dust that is settles on the surface of the soil. Supporting this finding, a study done by Mareskey and Grobler (1993) that used step-wise reduction to elucidate a progressive decline in the blood lead levels among South Africans and petrol lead additives used in automotive. Similarly, a study was done by Mielke (1999) that found that soil- lead accumulation to follow traffic volumes. Although lead is presently removed from most gas used in many places such as United States it is still important to test soil lead levels. The clay and organic matter in soil weakly binds lead and it remains in the soil for long time (Mielke 1999). That is proven by the study of Hafen and Brinkmann in the mid nineties which showed hazardous levels exceeding 500μg of lead /g (ppm) of dry soil In spite of the use of the unleaded gas for more than two decades. These levels could be a real threat to livestock grazing near that areas since grazing animals consume components of surface soil (Healy, 1967).

Krishnayya and Bedi (1986) reported on testing the lead concentration in plants at various distances from the Baroda highway in India and found an inverse relationship between them supporting the trend found also in soil lead values found by several studies. A study in Pakistan by Mahmood and co workers (1985) also found that crops growing near a highway accumulated lead with an inverse relationship to the distance from that highway. Similarly, a Hungarian team lead by Poti (1997) found a 30- 60% decrease in

the concentration of lead in the grass at a distance of 15 meters compared to 1 meter away from a road. These findings agree with the soil assessment in the present study in Oregon which shows a decrease ranging from 36% at Highway 34 to 49.5% at Interstate 5 when the lead soil content at 0 meters was compared with that at 10 meters away from the highway see. However, the decrease in lead levels at these distances measured in Kuwait were in the average of 7.5% See Figure IV.2. Sand storms hit Kuwait and many other countries of that region which may help in homogenizing the content of lead at the surface levels of the soil regardless of the distance from the highway. The 1991 oil fires started by the Iraqi regime burned all over Kuwait leaving residues that deposited everywhere, could be also a valid explanation of the small decrease.

In general, the many studies dealing with concentration of lead in soil, plants, blood of animals grazing adjacent to roadways (Ward and Savage 1994) or in water and milk (Abdullah et al 1993) all agree that lead concentration in these different tested parameters increase with the close approximity to motorways. This provides an indication that to date the major contributor to toxic metal (lead) in areas near highways due to motor vehicles.

In the present study fifty lambs that were grazed near the highway in Kuwait were tested for the lead concentration in their blood. Table IV. 2 shows the blood lead content of these lambs. The overall average of lead in the blood was 0.08 ppm ranging from 0.05 to 1 ppm. Only 12 per cent of the sheep population were exceeded the normal blood lead levels while the majority (88%) were within the normal lead blood levels (< 0.1). This finding due to natural tendency of sheep to avoid contaminated pastures. Secondly, it

might be a result to the nature of the scattered thin grasses and their low ability to hold lead particles. Third, unlike the previous typical practices, shepherds at the time of this study grazed their lambs at distances further the highways in order to comply with security orders as explosion accidents (due to mines left by the Iraqi regime) were possible at certain areas close to the highway even several years after the liberation. Additionally, increased human activities in the region as efforts to clear the area from mines has made vegetation scares.

Lambs at the sites in Kuwait pastures were clinically normal showing no signs of lead poisoning. These observations were made by the shepherd (1993) who did not find any signs of lead poisoning in the animals (cattle and buffaloes) that were grazing near a Delhi urban area. The same animals had blood lead levels of 0.38 ppm after grazing hay with levels of 140 ppm. Lower levels of lead shown in lambs in the present study may be due to several reasons. These reasons affecting ability to lead accumulation and uptake include species differences and the difference in pasture type. Sheep were also reported clinically normal in a study in South Australian conducted by Koh and Judson (1986) where they studied sheep that were grazing in a contaminated area next to a lead smelter. With blood lead values up to 0.5 ppm, sheep kept next to London Orbital motorway (78000 -100 000 vehicle/day) showed no clinical signs (Ward and Savage 1994). Sheep blood values range from 0.15-0.51 ppm in sheep grazing near the motorway (M5) with a traffic density of more than 100 000 vehicle /day (Ward and Savage 1994). Ward and his colleagues in (1978) found higher ranges of blood lead levels in sheep in New Zealand when they were grazing near a highway with a lower density (5000 vehicle/day). That

shows that although density on the highway plays a role in soil contamination with lead, lead blood content in the animals does not depend directly on it but is affected by other factors such as the actual intake by the animals through breathing, eating top soil or contaminated pasture which is affected by its ability to hold toxic particles within its leaves.

In experiment II in this study lambs with blood lead levels up to 1 ppm that was found in a lamb in the present experiment, will have liver and kidney lead levels with 10 and 138 ppm respectively. These levels were exceeding the maximum legal provisional Pb limit of 2 ppm for human (Avigdor 1987) and show how grazing animals at such contaminated area could be a hazard practice that is needed to be avoided.

Table IV.1 Mean levels of soil lead concentration μg/g dry weight at different distances 0 meter, 3 meter and 10 meter away from interstate 5, Highway 34, Highway 20 in Oregon and King Fahad Highway in Kuwait.

Highway	Traffic density vehicle/ day	Location	Means of Soil Lead Content μg/g dry weight at Different Distances from the Highway		
			0 meter	3 meter	10 meter
King Fahad highway	70000	Kuwait	5136**ax** (n=3) (4600-5605)	4950**ax** (n=3) (4250-5580)	4744.7**ax** (n=3) (4250-5104)
Interstate 5	122000	Oregon	165.7**bx** n=3 (148-175)	137.3**bx** n=3 (89-180)	84**bo** n=3 (61-120)
Highway 34	11800	Oregon	114**bx** n=3 (100-125)	97.7**bx** n=3 (92-101)	73**bo** n=3 (65-84)
Highway 20	11400	Oregon	103.3**bx** n=3 (98-110)	86.3**bx** n=3 (75-95)	55.3**bo** n=3 (45-66)

a,b Means in column with different superscripts differ ($p < 0.0001$).

x,o Means in a row with different superscripts differ ($p < 0.0001$).

n = Number of sample collected.

() Numbers in brackets represent the range values.

Table IV.2 Blood lead concentrations [mg/kg (ppm)] in lambs grazing near a major Highway in Kuwait

Lamb no.	Blood Lead Content (ppm)	Lamb no.	Blood Lead Content (ppm)	Lamb no.	Blood Lead Content (ppm)	Lamb no.	Blood Lead Content (ppm)
1	0.05	15	1	29	0.09	43	0.06
2	0.06	16	0.05	30	0.1	44	0.09
3	0.05	17	0.05	31	0.05	45	0.1
4	0.05	18	0.05	32	0.05	46	0.1
5	0.08	19	0.05	33	0.06	47	0.12
6	0.05	20	0.05	34	0.07	48	0.08
7	0.05	21	0.05	35	0.05	49	0.05
8	0.05	22	0.05	36	0.05	50	0.05
9	0.05	23	0.05	37	0.06		
10	0.05	24	0.05	38	0.08		
11	0.05	25	0.05	39	0.05		
12	0.05	26	0.07	40	0.05		
13	0.05	27	0.05	41	0.12		
14	0.05	28	0.05	42	0.06		

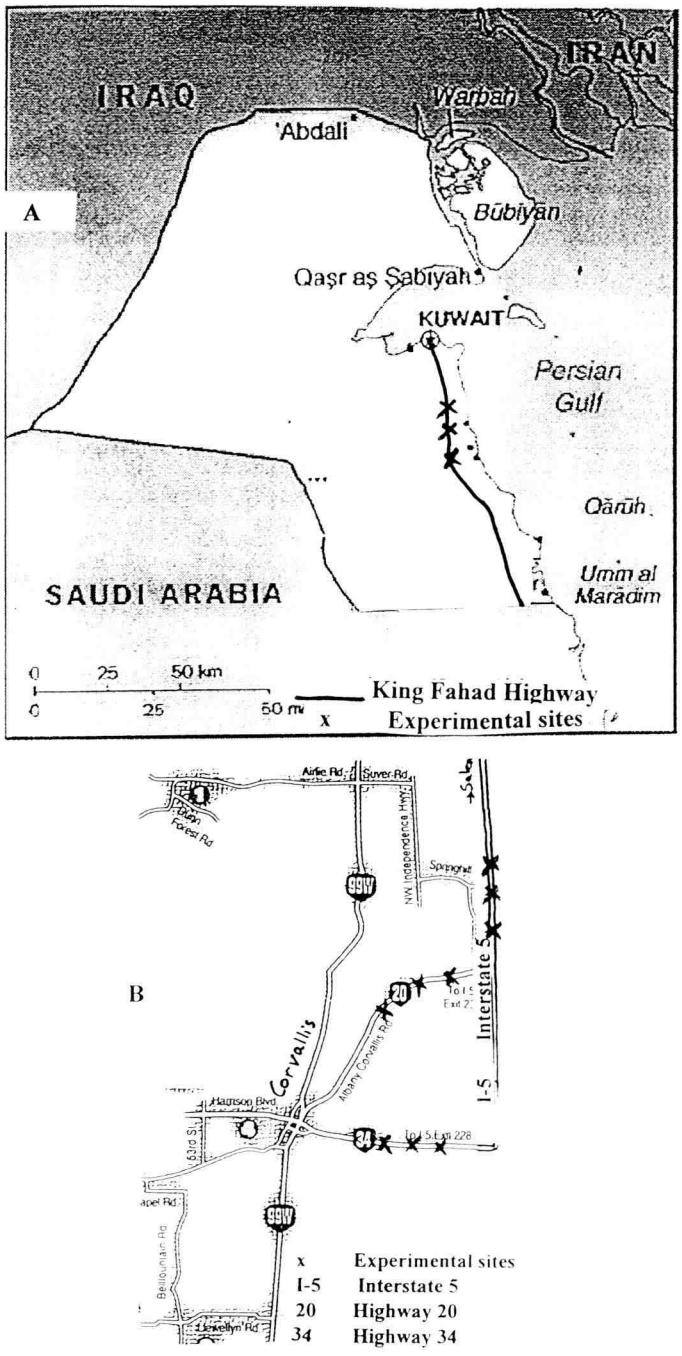
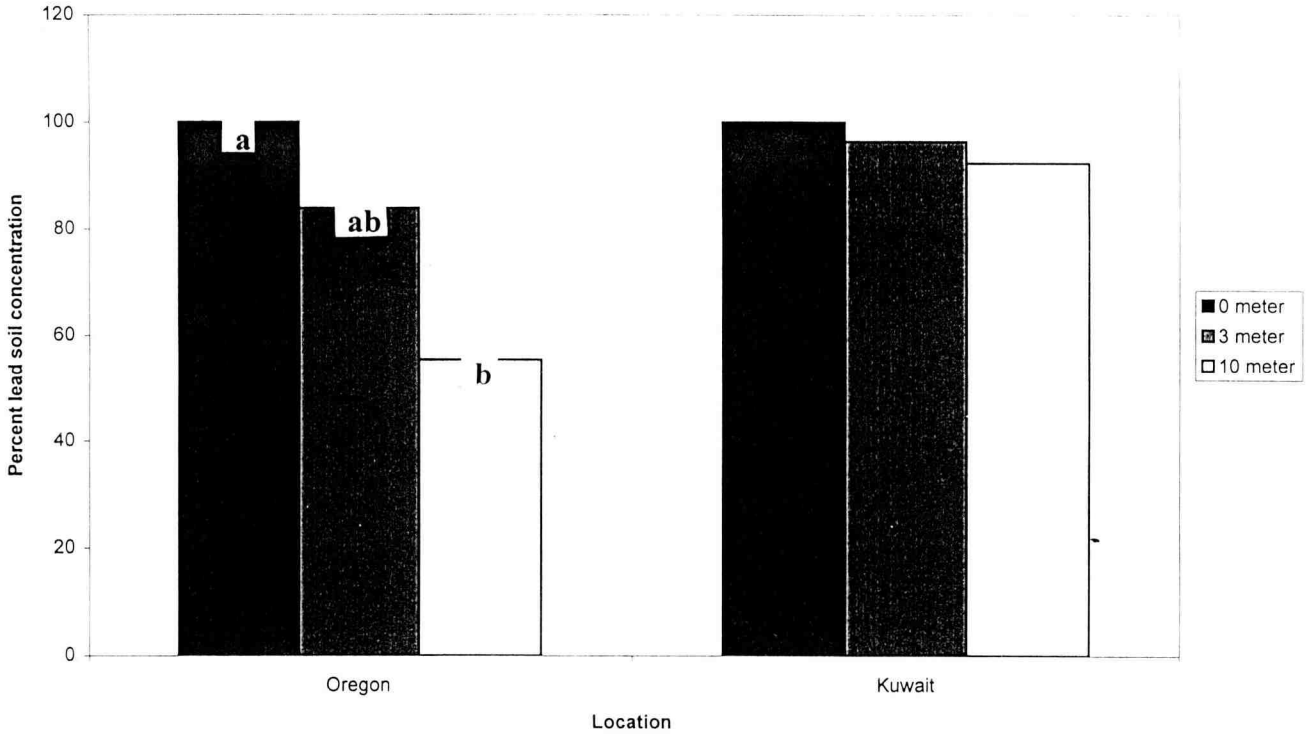


Figure IV. 1 A- Kuwait map showing King Fahad Highway.
B- Interstate 5 and Highways 34 and 20 at Oregon.

Figure IV.2 Percent of soil lead concentration from the edge of the highway at 0, 3 and 10 meters from the highway in Oregon and Kuwait.



CHAPTER V
EXPERIMENT II
HISTOPATHOLOGICAL AND CLINICOPATHOLOGICAL CHANGES IN FORMALIN- FIXED TISSUES AND LEAD DISTRIBUTION IN FROZEN TISSUES OF NAEEMI LAMBS ADMINISTERED ORAL LEAD ACETATE

Introduction

Lead is considered to be one of the most common environmental pollutants as well as an important cause of farm animal poisoning (N.A.S,. 1972; Ahmed and Shalaby, 1991). Lead exposures are continual problems for veterinary medicine. Every year, large numbers of animal deaths are attributed to lead (Carson et al., 1973). In addition, lead causes overall poor health and a reduction in efficiency (Blaxter, 1950). Despite its common presence and noted detrimental effects, little data are available from the literature regarding clinicopathological and histopathological changes in farm animals.

Lead accumulates in the body with constant exposure. However, it is absorbed into the body very slowly and there are differences in the amount of lead distributed to and stored at different sites such as in the various soft tissues of organs and bone. Its rate of excretion is even slower. The presence of lead in the liver, kidneys, nervous system and bone marrow is the major sign of lead toxicity (Bratton and Kowalczyk, 1989). Lead is carried primarily by red blood cells (Freeman, 1970b) and thereby it is circulated through the entire body where it subsequently becomes concentrated in soft tissues,

especially the liver and kidneys (Freeman, 1970a). Ninety percent or more of the lead burden in the body is accumulated in the bone; there it has a half-life of years to decades (dhs, 1997; Freeman, 1970b). Lead does not permanently remain in bone but is slowly released into the blood stream. The half-life of lead in blood is about 30-35 days (Lauwerys, 1983). Analyses of lead accumulation reveal that 60% of lead accumulates in bones, whereas 25 % accumulates in the liver and 4% in the kidneys (Kimberling, 1988). Lead has a half-life of approximately 40 days in these soft tissues (Lauwerys, 1983).

Histopathological and clinicopathological changes in tissues due to lead poisoning vary widely depending on dose, route of intake, lead absorption and factors that influence it. Several scientists have described lead poisoning in various species and reported the clinical symptoms associated with it. Hammond and Aronson (1964) describe several effects associated with lead poisoning in cattle and horses while Hemphill and his colleagues (1971) describe the suppression of the immune response as an effect of lead poisoning in mice. Gross lesions are rarely reported in the literature and, when presented, are mild and nonspecific (Osweiler, 1996). Microscopically, intranuclear and intracytoplasmic presence of lead inclusion bodies is the most typically observed pathognomonic lesion, especially in the proximal convoluted tubules in kidney (Beaver et al., 1961; Clegg and Ryland, 1966; Choie and Richter, 1972; Cramer et al., 1974; Fowler et al., 1980; Murakami et al., 1983; Kanakoudis et al., 1988). The cytoplasm of osteoblast is another site where lesions associated with lead have been observed (Hamir et al., 1985), however, this finding is rare in the liver (Wilson and Lewis, 1963; Zook ,1972; Davis and Libke, 1976). Kaldrymidou et al. (1994) report advanced changes in

kupffer and hepatic cells of lambs orally dosed 1-3 mg of lead acetate daily for four months while finding little evidence of any degenerative changes in the kidneys . Additional studies report spongiosis, gliosis and hyperplasia of vessels in the cerebral cortex in several lead poisoned animal species (Chritian and Tryphonas, 1971; Zook, 1972; Hamir et al., 1984).

The objectives of the present study were, first, to highlight some of the clinicopathological and histopathological effects, if any, of orally administrated lead acetate, given daily for 98 days, in varying doses (from 0 to 16 mg/kg body weight) to Naeemi sheep under environmental conditions in Kuwait. Secondly, the study was undertaken to detect tissue lead levels and determine the distribution of the absorbed lead at various organ and tissue sites in the body. Lastly, the study correlated the amount of lead acetate administered orally to Naeemi lambs (independent variable) with the histopathological changes, clinicopathological changes and amount of lead distributed in the various tissues and organs of lambs (dependent variables). This subchronic study will establish the no-observed-adverse-effect-level of lead (NOAEL) and further obtain the lowest-observed-adverse effect-level of lead (LOAEL) for Naeemi lambs. The study was designed to demonstrate and characterize the toxic effects that lead can produce in these species. Lead tissue distribution will provide background information on the level of lead intake that is hazardous to these lambs and at which organs or tissues the lead is stored. This finding will be important since these organs and tissues are used for human consumption and, presently, there are no specific data available regarding these lead parameters in lambs. The study objectives were undertaken in order to add information

to literature that is important for the sheep industry itself as well as contribute important information regarding the intake of lead levels that are acceptable for human consumption.

Materials and Methods

The 25 lambs used in the experiment were from the Naeemi breed (fat tail lambs); they were either pure (n = 6) or crossed with Australian dams (n = 19). The lambs were 2, 4, 6 or >6 months of age and not older than 10 months of age. Both males and female lambs were used and well distributed between the five groups (n = 5). Each group received a single dose of a different level of lead acetate daily; group 1 served as the control group and received 0 mg of lead acetate per kg body weight per day (kg/d), group 2 received 2 mg lead acetate/kg/d, group 3 received
4 mg/kg/d lead acetate, and groups 4 and 5 received 8 and 16 mg lead acetate /kg/d respectively. Lead acetate was given as an oral solution using a graded syringe. The lead solution was given early in the morning before the lambs had access to their first meal.

Lambs were closely observed every day over the duration of the study for any clinical signs that might rise or any behavioral changes that might appear. A form was filled out by the observer indicating the signs observed, the time of observation and the identification number of the symptomatic lamb. This form was filled out three times a day, once at each of the two feeding times and once in between, to ensure that no signs were missed.

At the end of the experiment the lambs were necropsized. Fresh samples were cut

from trachea, bone, testes, brain, diaphragm, ovary, lung, muscle, rumen, aorta, liver, spleen, tounge, intestine, eye, heart, kidney and esophagus and directly frozen at -20°C for tissue lead content determination. Ten grams of the fresh frozen tissue samples were digested with Aristar grade (BDH,UK) nitric and perchloric acid mixture in a beaker at 100 heated to 180°C. After complete digestion, the final volume was made to 25 ml. Lead levels was determined by inductively coupled plasma-Atomic emission spectrometry (ICP-AES). The instrument used was a HORIZON, from Fison, UK. It was a 1 m nitrogen purged czerny-Turner spectrometer with a 27.12MHz RF generator. The instrument was calibrated with laboratory made standards prepared from individual metal standard solutions obtained from E-Merck, Germany. Optimal operating conditions were as follows: carrier gas 0.8L/H, plasma gas 0.8L/H, cool gas 7.5L/H. A Meinhard type was used as a nebuliser with peristaltic pump. Analyzed certified standard materials such as bovine liver SRM1577b and oyster tissue SRM1566a were used as control references; these were obtained from National Institute of Standards and Technology (NIST), Gaithersburg, MD, USA. The measurable detection limits for lead were 0.01 mg/l in the solution by ICP-AES. In the actual samples, the precision of ICPfor the lead analysis was SD= 0.05 for a range 1-100 mg/kg and 0.10 for concentrations ranging from 0.1- 1 mg/kg. Data obtained for lead the distribution in the various organs were analyzed using the Student-Newman-Keul test. The general linear model (GLM) of the statistical analysis system (SAS) was used.

Results and Discussion

All tested tissues except bone liver and kidney showed not to be lead dosage dependent. The mean lead concentrations found in bone, brain, liver and kidney samples after the five levels of oral lead administrations are shown in Table V.1. The distribution of lead among the organs and tissues was found to be dependent on the dosage given to the lambs. In general, feeding lead to Naeemi lambs for 98 days increased lead concentration in bone, kidney and liver ($P < 0.05$) and only slightly in brain and spleen ($P = 0.05$). The lead concentrations were shown to be significant only at the highest levels, 8 and 16 mg of lead acetate /kg body weight. Accumulation of lead was highest in the kidney, followed by bone and, then, liver. In spite of the absence of significance, at the lower levels, namely 0, 2 and 4 mg of lead acetate/kg body weight, lead was accumulated in the highest amount in bone, then kidney and followed by liver. Highest accumulations of lead have also been noted in bone in studies on man, laboratory and farm animals (Underwood, 1977). Present findings agree with findings reported by Fick et al. (1976) of levels of accumulated lead in the bone exceeding that in the kidney at lower levels of lead intake (0, 10,100, 500 mg of lead acetate/kg feed) in lambs, whereas the lead levels in kidney were found to exceed that of bone at higher dosages (1000 mg of lead acetate/kg feed). Neathery and Miller (1975) explain such a finding; they indicate most lead is deposited in the bone until it reaches a possible threshold and additional lead intake is then deposited in other tissues, mostly the kidney using blood as an intermediary transport medium. Although at higher intake levels, kidney was the organ showing the highest lead concentration levels, however, bones have the highest in total contribution to

body burden since they represent more percentage of body mass than do kidneys. Kimberling (1988) states the distribution of lead is about 60% in bones, 25% in liver and a concentrated 4% in kidneys. Whereas, a study by Hamir et al. (1981) reports lead accumulated most in bone regardless of the amoun of lead fed to dogs. The difference in findings is probably due to species differences. However, consistent with present findings that study reported lead levels in bone and kidney exceeded levels in other tissues. Willoughby et al. (1972), Fick et al. (1976), Suzuki and Yoshida (1979) and Pearl et al. (1983) also found the same trend.

Figures V.1,V.2,V.3 and V.4 shows the acumulation of lead in bone, brain, liver and kidney tissues respectively based on daily oral administration of various dosages of lead acetate (mg/kg body weight/day) to Naeemi lambs. Lead levels in the bone, kidney and liver samples from all the experimental groups in this study exceeded the average Provisional Tolerable Weekly Intake (PTWI) of 25 μg/kg body weight for humans (Galal-Gorchev, 1993). Such organs are hazardous for human consumption at these levels of lead. Levels of lead in liver and kidney samples of the experimental groups exceeded 2000μg/kg which agree with Cantarow and Trumper (1944) indicating normal liver and kidney is unlikely to exceed that level in the human.

It was anticipated that muscle, lung and heart would be sites where lead accumulation would be the least. Lead accumulation in the lambs did not exceed an average of 0.09 ppm except in the group of animals that were administered 16 ppm daily where the levels then jumped to almost 0.4 ppm in muscle, 0.14ppm in heart and 0.24

ppm in lung tissues. This finding is consistent with Allcroft (1950) who found that levels of lead accumulated in spleen, lung, heart and brain were detectable but at much smaller amounts than in kidney and liver.

High doses of orally administrated lead significantly affected the accumulation of lead in bone (P= 0.016), kidney (P= 0.017) and liver (P = 0.0001) indicating higher concentrations of orally administered lead resulted in higher accumulation of lead at these tissue sites. Pearl et al. (1983) also found an increase of lead accumulation in all tissues of sheep orally administered 1000ppm of lead acetate. In addition, Hamir et al. (1981) observed higher levels of lead in tissues of dogs fed lead compounds than in tissues of the control group.

The level of lead accumulated in the bone, liver and kidney samples was also affected by age (Figure V.5), younger lambs accumulated more lead in their bone, liver and kidney than older ones ($P < 0.05$). Casarett and Doull (1996) indicate the young are the most susceptible population to lead poisoning; lead accumulation is noted to exert most effect in young organs. High accumulation at this age is due to, in part, to higher lead absorption from the diet (Underwood, 1977).

The sex of lambs was a significant factor only in the accumulation of lead in liver samples. Female lambs tended to accumulate higher levels of lead in liver than males (P= 0.02).

Being a Naeemi or Naeemi cross lamb was not a factor that affected lead accumulation in the various tissue sites. This finding was uniform in all experimental groups.

Pearson correlation coefficient analysis revealed the expected results; findings showed concentrations of lead in bone, brain, liver and kidneys to be highly correlated with the rumen lead content ($P < 0.005$), which in turn correlated with the amount of lead orally administered to the lambs ($r = 0.57$, $P = 0.007$).

Bone lead concentrations were highly correlated with the lead content in ovary, rumen, aorta, liver, spleen, tongue, intestine, eye and esophagus samples. Lead content in bone negatively correlated with age ($r = 0.40$, $P = 0.066$) showing the tendency of

younger lambs to have more lead in their bone than older ones. The higher the oral dosage administered to the lambs resulted in higher lead concentrations in their bones ($r=0.73$, $P = 0.0001$).

Lead concentration in the brain highly correlated with lead content in spleen ($r=0.53$, $P = 0.014$), and kidney ($r= 0.69$, $P = 0.0003$). Brain lead concentrations also highly correlated with the oral dose of lead administered to the lambs ($r = 0.67$, $P=0.0006$).

Liver lead concentration was highly correlated to the lead content in bone, ovary, rumen, spleen, tongue, intestine, eye, heart kidney and esophagus. The dose of lead administered to the lambs was again highly correlated to liver lead content ($r = 0.75$, $p=0.0001$).

Finally, kidney lead concentration was correlated highly to brain, lung, rumen, liver, spleen and heart. Its lead content correlated with the dose amount of orally administered lead to the lambs ($r =0.70$, $p = 0.0002$) as did the other tissues.

Microscopic investigations of lambs were made using haematoxilin and eosin (HE) for all tissues and Ziehl-Neelsen (ZN) for bone, liver and kidney sections to detect acid fast inclusions which are positive for inclusion bodies (Ahmed and Shalaby, 1991). Results revealed that all tested lambs (100%) in the first and second groups (16 and 8 mg lead acetate/kg body wight) had intranuclear inclusion bodies in the kidney (Figure V.6A) while it is only 50% in liver (Figure V.6B). Investigation on inclusion bodies revealed that they tend to follow the concentration of lead in tissue rather than the administrated amount (Table V. 2). Hamir and co workers (1983) in experiments 2 and 3 found 37 % and 68 % of dogs given lead had acid fast inclusion bodies in the liver and kidney. They reported that inclusion bodies in the tissues of dogs were correlated to the amount of lead administrated and retained in the tissues. In the present study inclusion bodies were not found in liver of lambs administrated 4mg/kg/day or lesser; this is in agreement with Hamir et al (1983) who found in experiment 3 that none of the dogs fed 5mg lead/kg/day had any inclusions in the liver. None of the tested bone tissues gave evidance of any histological changes even in tissues that retained the highest lead level. This is in agreement with Zook (1972) who found inclusions in liver and kidney in 59% and 84% respectively of his dogs while none of the sections of the tested bones showing any. Consistent with these findings, Hamir and his colleagues (1983) his fourth experiment found no inclusions in the bone in spite of the high skeletal lead content. Supporting that Hamir et al (1999) also did not find inclusions in bones of racoons fed lead up to 4mg/kg/day. However, in experiment 3 1983 Hamir et al found 90% of the treated dogs

(5 mg lead/kg/day) had inclusion bodies in the bone although inclusions were absent in the liver and kidney of the same aimals. These differences could be due to duration of lead administration and animal species differences. Ahmed and Shalaby (1991) found no inclusions in liver but in kidney in lambs fed with 1 mg lead/kg three times a week for one year. This is similar to what was seen in the present study with the lambs fed 2 mg lead/kg/day. In general the present study showes that inclusion bodies start earlier in the kidney with a dosage of 2 mg/kg body weight /day followed by the liver at 8mg/kg body weight /day. This agrees with a study done by Hamir et al (1999) on raccoons although inclusions manifested at different dosages. That was expected since species from the two experiments were different. In brief inclusion bodies in liver and kidney correlated to the lead level that is retained in the tissue rather than the amount administrated.

The daily observations of the experimental lambs failed to detect any evidence of clinical or behavioral changes. Lambs continued to behave normally. All lambs, including the highest dosed group, survived to the end of the experiment with no obvious ill-effects. Carson et al. (1973) also did not observe any clinical signs of lead poisoning in his sheep fed up to 4.5 mg lead /kg body weight for a six month period. Papaioannou et al, (1998) observed neurological signs in dogs each given ten intraperitoneal shots with 12 mg lead acetate over on a 20 day period. Tremer, ataxia and hypersensitivity were observed among these dogs. These differences in findings could be due to such things as route of administration and species difference. However, in further support of the present findings, Allcroft and Blaxter (1950) state the highest dose in their experimental sheep (8 mg lead acetate /kg body weight) was tolerated for many months and that it could be

years before any changes would be observed with doses of 6 mg lead acetate/kg body weight. Unfortunately, this was not the case with the pregnant ewes in a study by Sharma and Buck (1976). They reported 50% of pregnant ewes died following administration of 1 mg lead acetate /kg body weight before lambing and 25 % of them aborted lambs. The rate of lambing dropped from 100 % to 18 % in sheep exposed to metallic lead compounds during gestation.

Gross lesions were not detected in any of the internal organs in any of the lambs in the experimental groups. Even in the Papaioannou experiment on dogs (1998) they were unable to find any diagnostic lesion except for brain and cerebellum congestion. An experiment conducted by Hamir and Sullivan (1983) reported gastric lesions; several large deep ulcers were found in a male dog administered 60 mg/kg body weight daily for 21 days. Differences in species and the amount of lead administered are probable contributors to the differences in these findings.

Table V. 1. Mean level of tissue lead concentration (mg/kg) of different categories for Naeemi lambs administered oral lead acetae daily for 98 days

Category	Total (n) of Samples	Days of Treatment	Means of Tissue lead concentration (mg/kg)				Acid-fast iclusion in			
			Bone	Brain	Liver	Kidney	Bone*	Brain*	liver*	Kidney
Dosage mg/kg body weight										
0	5	98	1.71a (4)	0.05 (4)	0.06a(5)	0.07a(4)	0%	0%	0%	0%
2	5	98	4.38a (4)	0.07 (5)	2.20a(4)	3.33a(5)	NT	NT	0%	33%
4	5	98	3.84a(5)	0.10 (5)	1.30a(4)	2.83a(4)	NT	NT	0%	33%
8	5	98	14.8b(5)	0.32 (4)	11.64b(5)	26.90b(5)	NT	NT	50%	100%
16	5	98	34.50c(4)	1.97(5)	10.00b(4)	138.6c(5)	0%	0%	50%	100%
Sex										
Males	7	98	11.07(6)	0.34 (5)	2.68a(5)	42.99 (5)				
Females	18	98	11.82(16)	0.61 (17)	5.83b(17)	35.60 (18)				
Breed										
Pure	6	98	7.58(6)	0.15 (6)	3.97(6)	8.63(6)				
Cross	19	98	13.13 (16)	0.70 (16)	5.54 (16)	47.29(17)				
Age										
Young	12	98	19.24a(9)	0.54 (10)	7.31a (9)	54.54a (11)				
Old	13	98	6.34b (13)	0.56 (12)	3.59b (13)	21.31b(12)				

Numbers Between Brackets Resembles Number of Samples Tested for each Tissue.
a,b Means in column within a category with different superscripts differ ($p < 0.05$).
*Percentages represents 100 X(lambs with inclusion bodies/tested lambs); NT= Not tested.

Table V.2 Tissue lead concentration and acid fast inclusions in lead treated lambs.

		Lead Concentration		Acid-fast inclusions	
Dosage mg/kg/day	Lamb No.	Liver	Kidney	Liver	Kidney
16	2	15	30	+	NT
16	3	14	187	+	+
16	4	6	13	-	+
16	5	5	159	-	+
8	7	10.8	24.5	+	+
8	8	13	24	+	+
8	9	13.6	34	-	+
8	10	10.8	30	-	+
4	12	1.43	2.5	-	-
4	13	1.36	2.5	-	NT
4	14	0.8	1.3	-	-
4	15	1.6	5	-	+
2	17	1.3	3	-	-
2	18	2.4	3.28	-	-
2	19	2.6	3.8	-	NT
2	20	2.38	3.76	-	+
0	22	0.1	0.1	-	-
0	23	0.05	0.05	-	-
0	24	0.05	0.05	-	-
0	25	0.05	0.08	-	-

NT, non tested tissues

Figure V.1 Mean lead concentration in bone of Naeemi lambs administrated various dosages of oral lead acetate (0, 2, 4, 8 and 16 mg/kg/day) daily for 98 days.

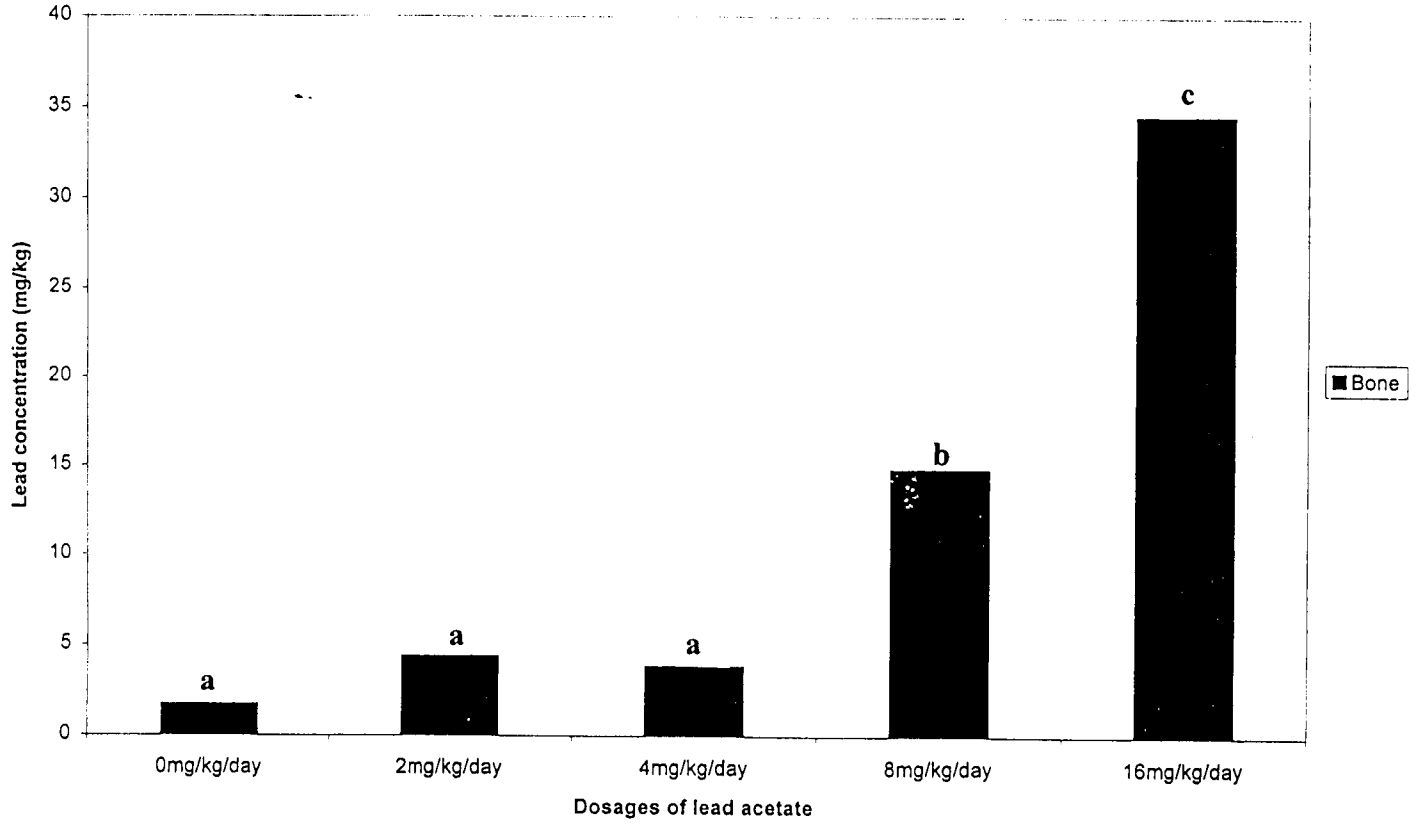


Figure V.2 Mean lead concentration in brains of Naeemi lambs administrated various dosages of oral lead acetate (0, 2, 4, 8 and 16 mg/kg/day) daily for 98 days.

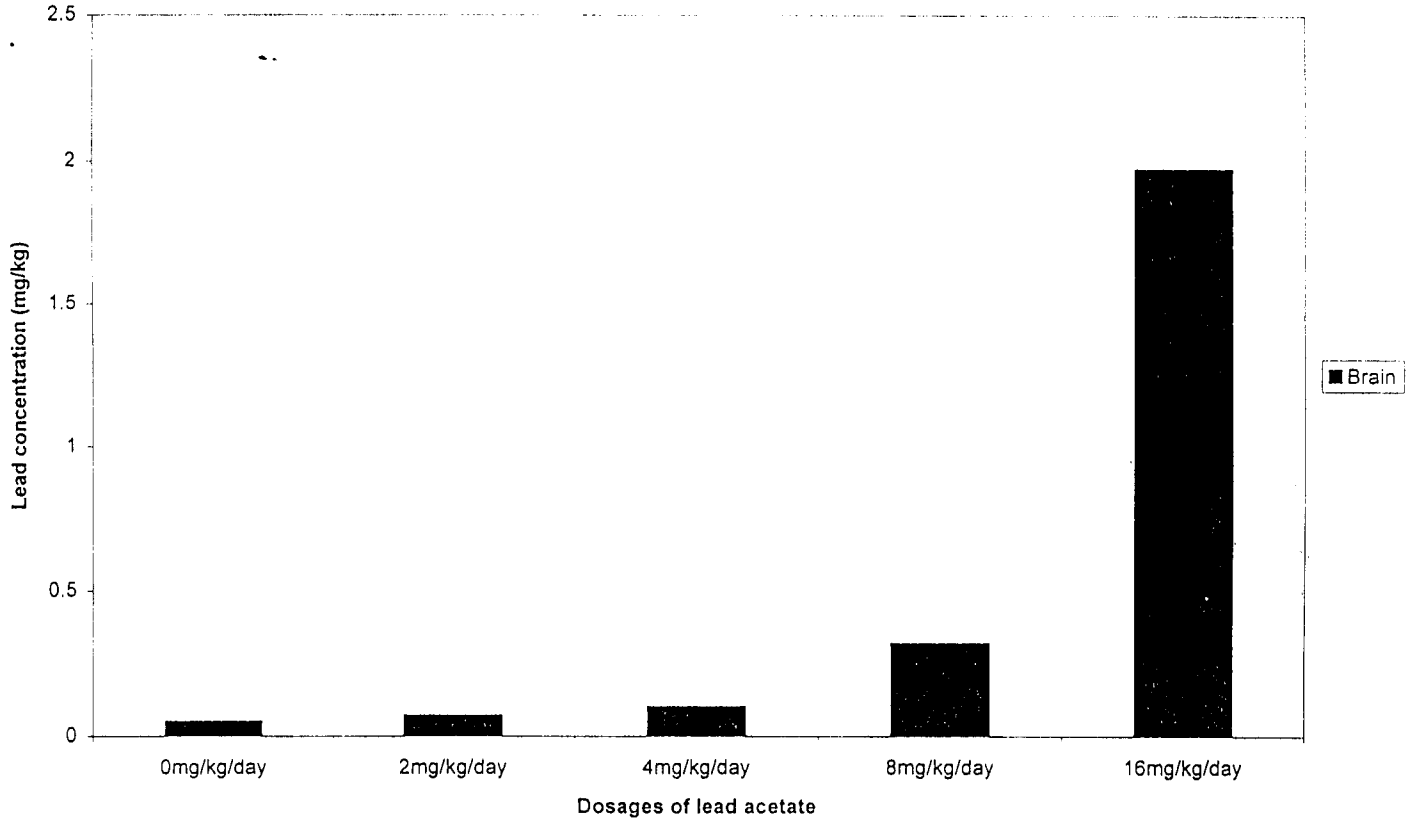


Figure V.3 Mean lead concentration in liver of Naeemi lambs administrated various dosages of oral lead acetate (0, 2, 4, 8 and 16 mg/kg/day) daily for 98 days

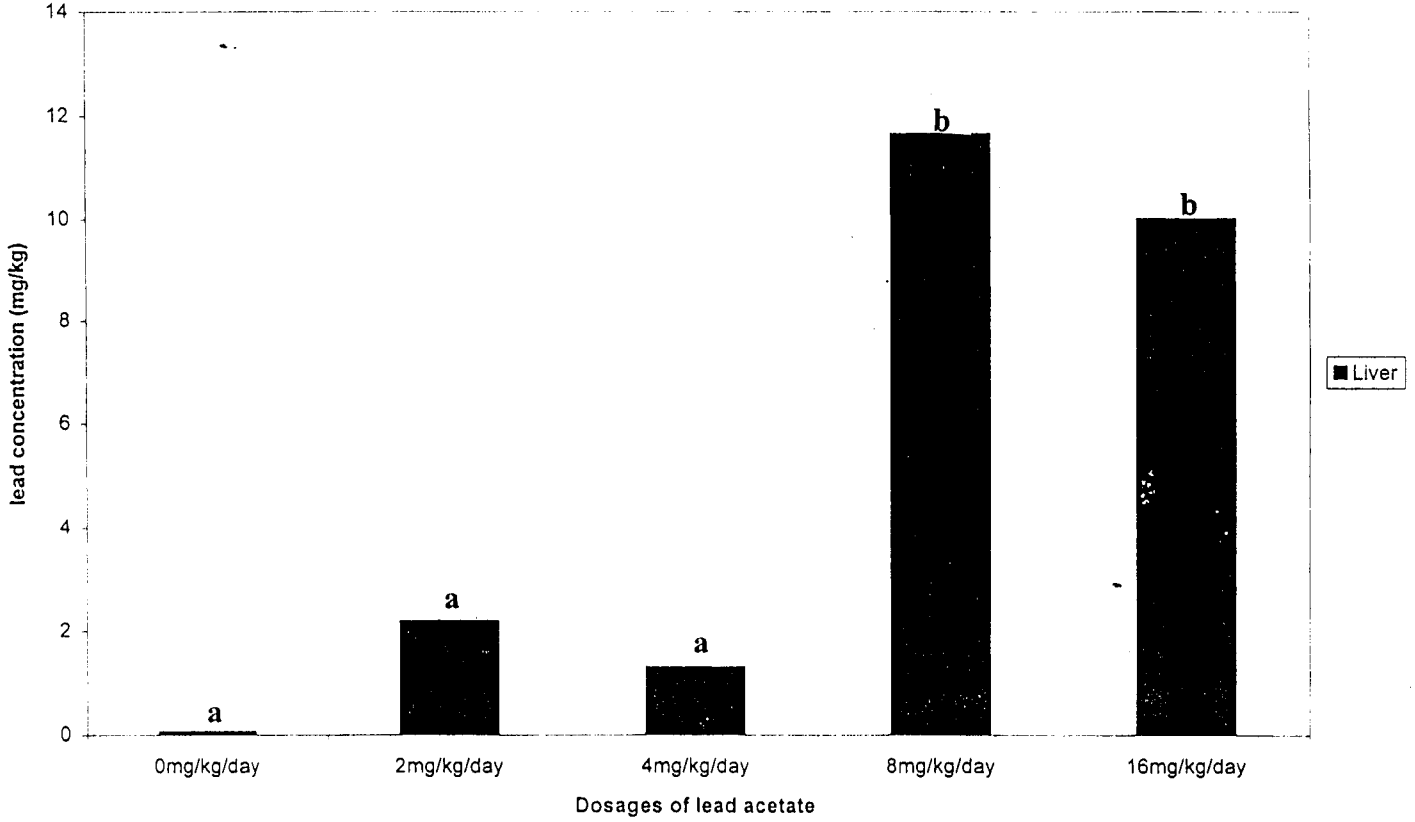


Figure V.4 Mean lead concentration in kidney of Naeemi lambs administrated various dosages of oral lead acetate (0, 2, 4, 8 and 16 mg/kg/day) daily for 98 days

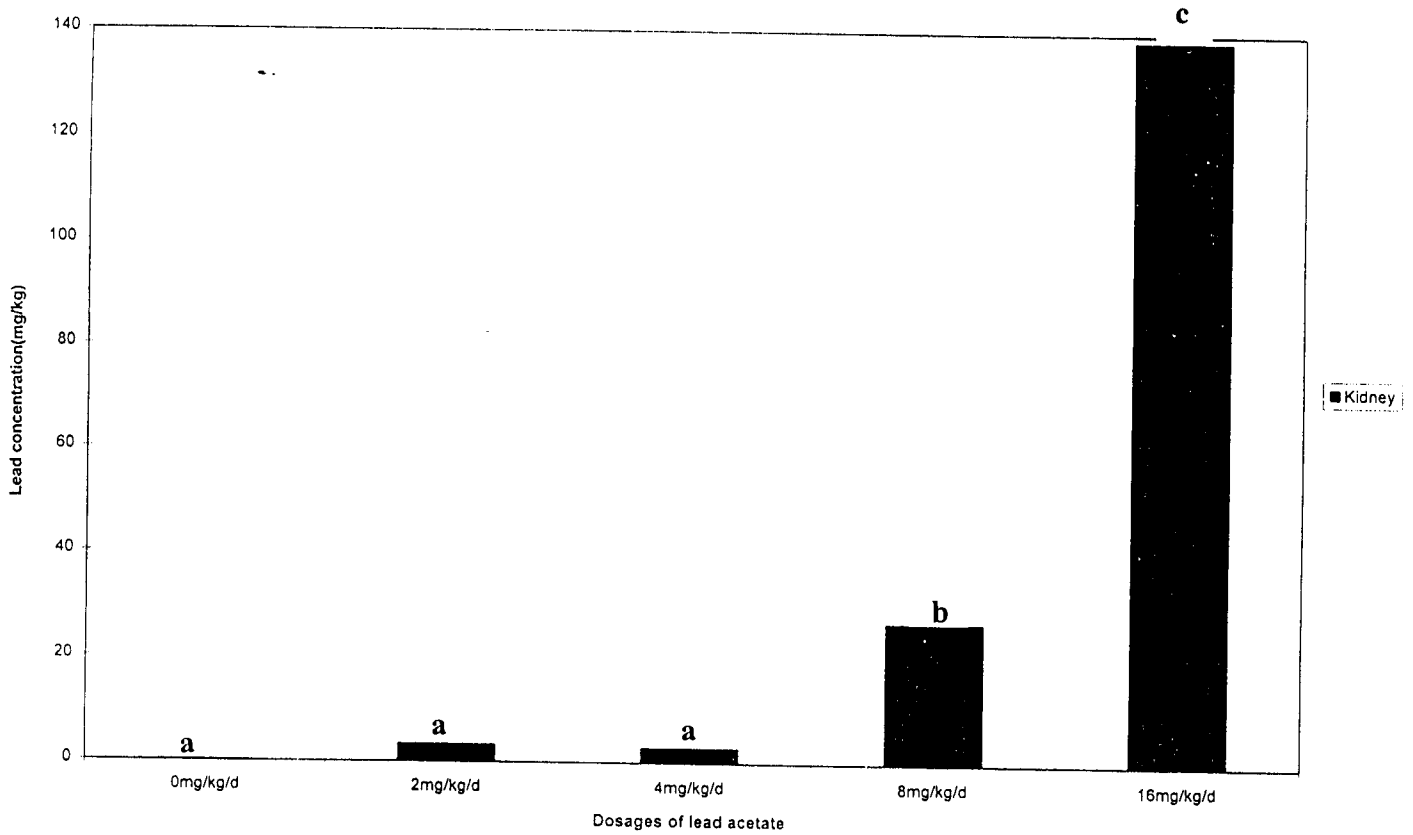
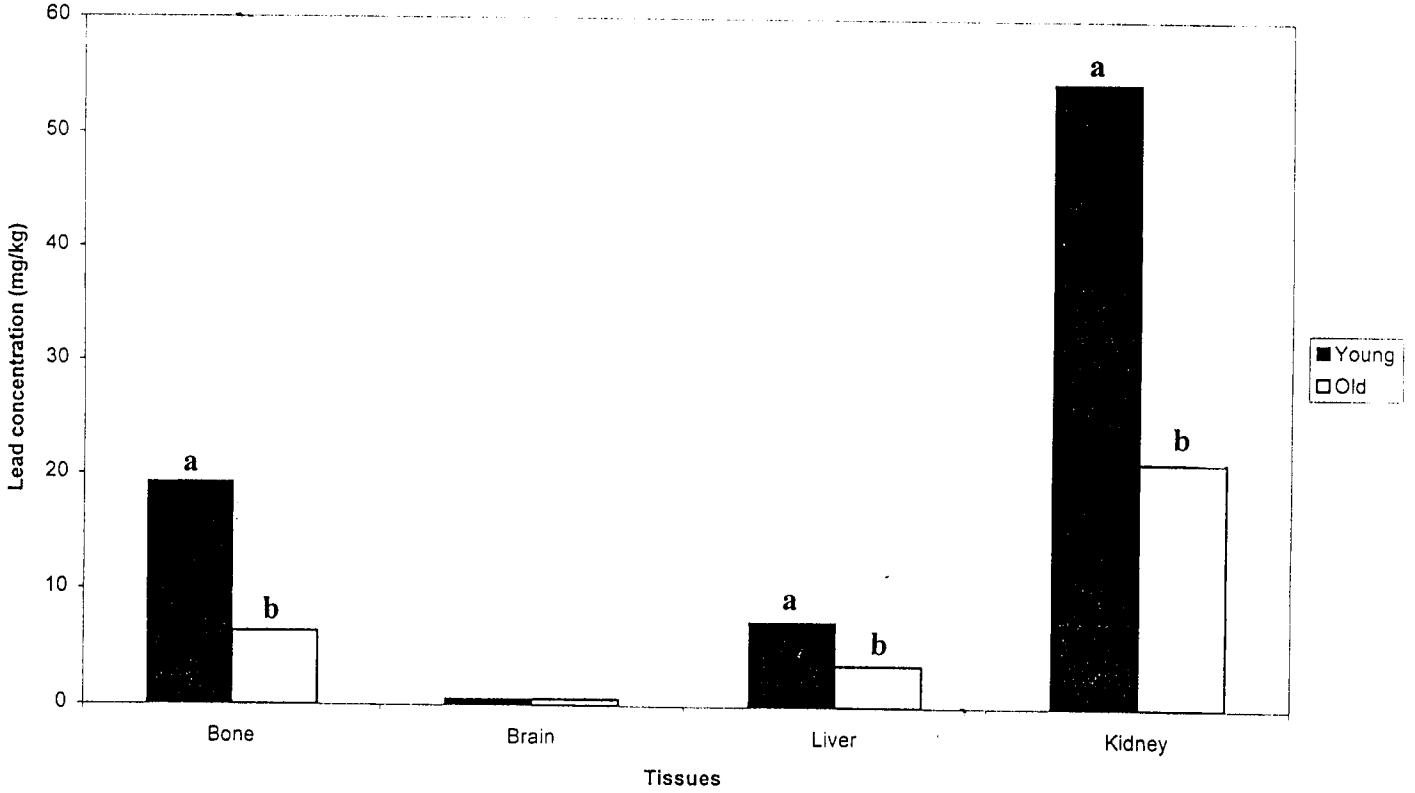


Figure V.5 Tissue lead concentration in young and old lambs administrated oral lead acetate for 98 days



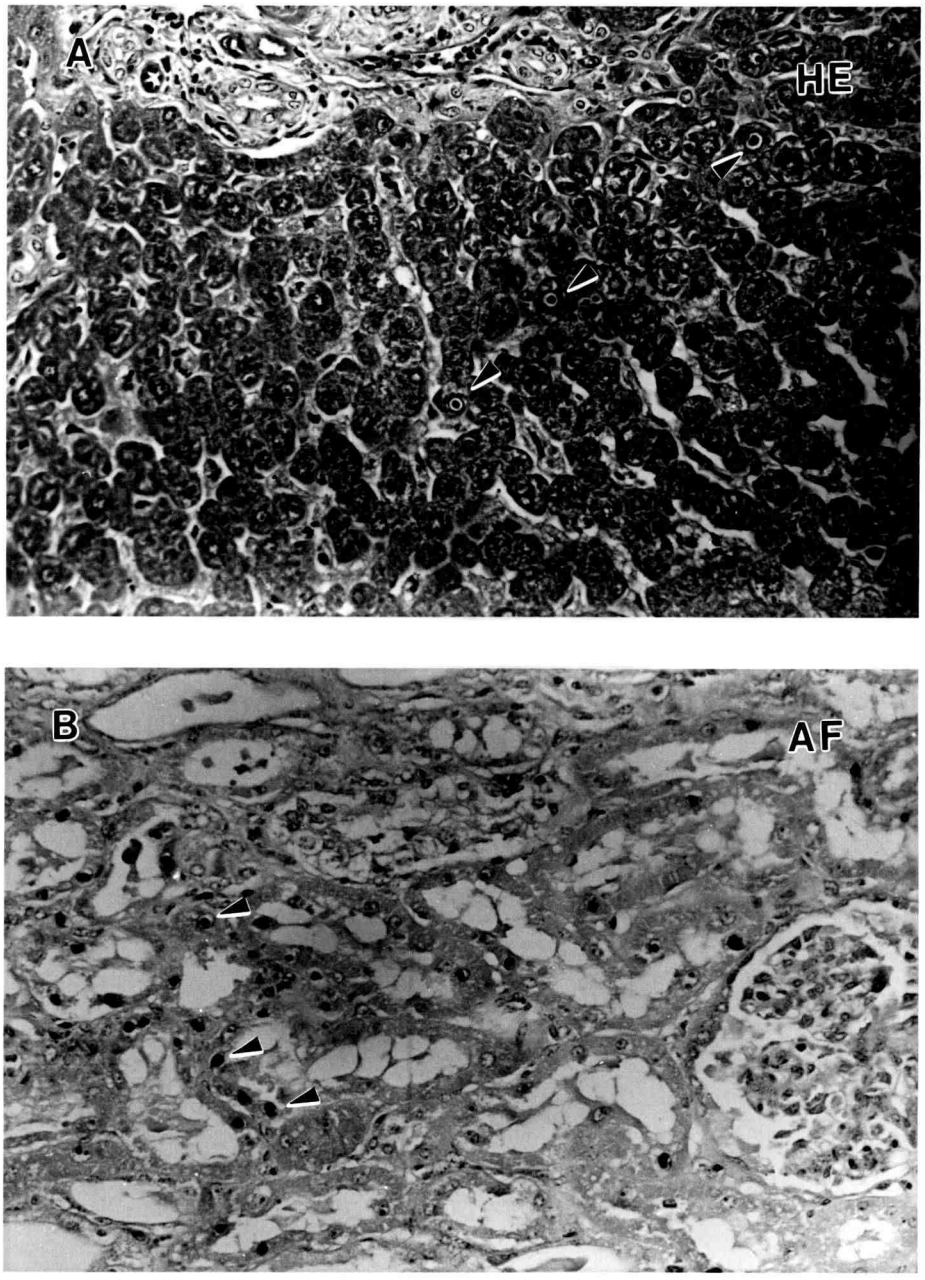


Figure V.6 A- Liver of lamb treated orally with lead acetate, showing intranuclear lead inclusions using (HE) Heamatoxylin and Eosin stain.
B- Kidney of lamb treated orally with lead acetate, showing (AF) acid-fast intranuclear inclusios.

CHAPTER VI
EXPERIMENT III
THE EFFECT OF LEAD ACETATE ADMINISTERED ORALLY AT DIFFERENT LEVELS ON THE PERFORMANCE OF NAEEMI LAMBS IN KUWAIT

Introduction

Lead poisoning is very well known as a threat to the health of man and animals. You cannot see or smell lead, and often, you cannot taste it. These characteristics make the toxic metal difficult to avoid. Lead may be found anywhere; it is present in ceramic dishes and crystals, brass and enameled metalware, paint on walls and fences, in food, and in the environment in water and soil. While lead is very toxic to people and animals, it has properties that make it ideal as a base for glazes, paints, solder, bullets, sinkers and plumbing fixtures. Lead production continues to increase worldwide (U.S. Bureau of Mines, 1992) in spite of the extensive efforts by many industrialized nations to reduce lead emissions. The released lead in the environment can not be economically recovered (Nariagu 1990a) and therefore it remains as a potential hazard to health.

Lead damages the red blood cells, nervous system, kidneys, brain and other organs. Young humans and animals, particularly those pregnant, are at the greatest risk of lead toxicity even when exposed to small amounts of lead.

Blood lead level is believed to be a reliable measure of the extent of recent (Rabinowitz et al 1977, 1980) exposure and indication of the hazardous level of lead toxicity (N.R.C., 1993). On the other hand, measurements of lead can be done on urine,

hair and nails but these have proved less useful because of within subject variability, susceptibility to contamination and the inadequate relationships between the exposure and the sample concentration (Ware, 1995).

Environments that are contaminated with lead as a result of industrialization have resulted in comparable increases in lead burden in human and animal populations. This burden is measured by deviations from the estimates of the natural level of lead in blood.

The lamb production systems in Kuwait are either identified as a range flock system, a semi-intensive flock system or an intensive flock system (Malik et al., 1998). However, the most common practice in Kuwait is the semi-intensive system where lambs are allowed to graze during times when natural grasses are available and offered rations (concentrate and roughage) during periods when pastures are unavailable.

Lamb performance is used as the major judge of lamb production. Feed intake, average daily gain and weight of carcass are affected by several pathalogical factors and hence have an affect on the economic feasibility of lamb production. The optimum combination of feed intake by the lambs and their average daily gain within a relatively short time is the optimum target of any lamb production industry. This has the producer cutting costs by reducing time in feeding, labor and selling the product earlier with the targeted carcass weight. Lead poisoning has affected livestock, especially sheep and cattle, throughout agricultural history and is considered one of the most important causes of poisoning in farm animals (Ahmed and Shalaby, 1991) and, therefore, has an economic effect on all livestock production, including the sheep industry. Lead toxicity in sheep has been proven to cause digestive, nervous, blood and other disturbances to several other

systems (Carson et al., 1973; Koh and Judson, 1986; Neathery and Miller, 1975; Kimberling, 1988; Harrison,1977; Sharma, 1971). In Kuwait, sheep mortality in range flocks has been noted as high as 38.8% in ewes and 32.1% in lambs in 1992 (Malik et al., 1998). Costs of prevention and treatment as well as deaths are of major economic loss in a production industry (Jensen et al., 1988). This high mortality rate added to the costs encumbered by preventing and treatment of lead poisoning add to economic difficulties of producing sheep in the region. Malik and his colleagues indicated disease associated problems as well as poor nutrition management are two other factors that have to be included in economics of the sheep industry in Kuwait.

This experiment was conducted to demonstrate how blood lead level in lambs is affected by differing doses of orally administered lead acetate to different groups of lambs for fourteen weeks. Second, the study was done to evaluate the effect on the performance of growing lambs (feed intake, weight gain, feed conversion, carcass and slaughter weight) at the various oral doses of lead acetate.

Materials and Methods

The same lamb population that was used in Experiment II was also used here. They were kept under the same environmental conditions in Kuwait. Lambs were 2, 4, 6 or >6 months of age and not older than 10 months of age. Both males and female lambs were used and distributed well between the five groups with n=5 in each group. A single dose of lead acetate was given daily. Group 1 was the control group and received 0 mg lead acetate per kg weight per day. Group 2 received 2 mg lead acetate/kg/d. Group 3

received 4 mg lead acetate/kg/d. Groups 4 and 5 received 8 and 16 mg lead acetate/kg/d respectively. Lead acetate was given as an oral solution using a graded syringe. It was given early in the morning before the lambs had access to their first meal.

(a) Animal management

All 25 lambs were kept in one housing shed. Each lamb was housed separately in a single pen of 1.7X1.7 meter. Free access to water was provided. Feed was offered adlibitum and was given to them twice a day at a known weight. Lambs were vaccinated for small pox and pasturollosis. They were checked by a veterinarian and found healthy. All were weaned before the start of the experiment.

The left over feed from each lamb's daily feeding was kept in a separate bag; this remaining feed was weighed at the end of each week in order to calculate the daily feed intake of each lamb. All lambs were weighed at the end of each week and calculation of daily weight gain was made accordingly.

(B) Blood samples

Blood samples were withdrawn before the beginning of the experiment, at two weeks and then at monthly intervals until the end of the experiment. Blood was drawn into heparinized vacutainors; a total of 200 samples were taken during the experiment. Blood was taken from the jugular veins on the sides of the lamb's neck. Lambs' necks were sheared when necessary to facilitate blood withdraws and eliminate any contact with

wool that might have lead on it (Ward et al, 1978). To avoid any contamination, only one needle per lamb was used for the blood withdrawal. Samples of blood were refrigerated at 4° C immediately upon drawing and sent directly to the Central Analytical Laboratory (CAL) at Kuwait Institute for Scientific Research for the subsequent analysis of the blood lead concentrations. Using mechanical rollers, all samples were thoroughly mixed before performing the tests.

(C) Internal organs

After the fourteen week ' s duration of the experiment, all lambs were weighed (slaughter weight) and then slaughtered. Internal organs (lungs, heart, liver, spleen, kidneys, ovaries and testes) as well as head, tail, legs and carcass were weighed.

(D) Statistical analysis

Data was analyzed using the general linear model (GLM) of the statistical analytical system (SAS) with a Student Newman Keul test to separate between the means. Repeated measures analyzed the daily levels of lead in blood, feed intake and daily weight gain. Person correlation analysis included age of the lambs, dosage of lead acetate administered orally, blood lead levels from the starting day of the experiment, 2nd week, monthly intervals until the end of the experiment, feed intake at 2, 4, 6, 8, 10, 12 and 14th week of the experiment, slaughter weight, carcass weight and weights of the internal organs .

Results and Discussion

Table VI.1. Shows the means of blood lead levels in control and experimental groups from the beginning of the experiment to the 14th week when it was terminated. Blood lead levels ranged from 0.05 up to 1.00 mg/liter. The highest blood lead level at the end of the experiment (14th week) was recorded for lambs administered the highest dosage (16 mg/kg/day). Doses of lead acetate were orally administered to the experimental lambs had a significant effect on their blood lead levels ($P < 0.0005$). This finding is supported by Pearson correlation coefficient analysis showing a positive correlation of lead administered to lambs with their lead blood levels at the second week, sixth week, 10th week and the 14th week of the experiment (83%, 77%, 86%, 92% respectively ($P < 0.01$). Table VI.4. Repeated measure analysis showed a significant effect of time alone ($P < 0.05$); in addition, there was an interaction with the dosage parameter ($P = 0.0001$) reflecting the strong effect of dosage on the blood lead level with time. Lambs in the control group, as well as those administered the lowest dosage (2 mg/kg/day), had blood lead levels significantly lower than those lambs that were administered the higher dosages (4, 8 and 16 mg/kg/day); the exception was during the 6th week where the blood lead level of lambs administered 2mg/kg/day was similar to those administered 4 mg/kg/day. All blood lead levels in the experimental groups peaked sharply at the 6th week and then leveled off with time (FigureVI.1). Non of the other tested categories (sex, breed of sire or age) significantly effected the levels of lead in lamb's blood as did the oral administration of lead acetate (Table V.1). In spite of the

absence of significance, females tended to have higher blood lead concentrations than males throughout the experiment. There was a tendency for younger lambs to accumulate higher levels of lead in their blood than older ones. There was an increase in the gap between the two concentrations, although, statistically they were alike up to the tenth week of the experiment. At week 14 of the experiment this difference become pronounced and at this time reached significance ($P < 0.0005$). The similarity could be attributed to the small age difference between the tested groups; 52% of the ages of the population were within 2-4 months of each other at the beginning of the experiment.

Daily feed intake of the lambs tended to be lower in lambs exposed to higher levels of lead acetate, however, this was not statistically significant (Table VI.2) FigureVI.2. This finding was supported by correlation analysis (Table VI.4) indicating a negative but non-significant correlation between them. Person correlation analysis showed daily feed intake by the lambs was negatively correlated with blood lead levels; the highest correlation occurred at the tenth week (40%, $P < 0.05$). Similarly, Fox (1987) found that lead toxicity caused a decrease in feed intake and growth in domestic animals.

Male lambs had a higher daily feed intake than females ($P < 0.05$). Although not significant, Malik and co workers (1998) found a tendancy for male Naeemi lambs to be superior in daily feed intake than females. Pure Naeemi lambs tended to eat less feed daily than the cross breed. Similar but significant findings were found by Malik et al. (1998). The data form the present study are consistent with Malik et al. (1998) but differ in the statistical significance. This difference could be attributed to the larger sample

number that was used in the Malik study. As expected, a positive correlation was found between daily feed intake and the weights of the lambs at the conclusion of the experiment ($P < 0.05$) (Table V.4).

Sire breed and the age of the lambs were not affected by daily feed intake, although the while male lambs ate more feed each day than did the females (Table V.3). Daily weight gain followed the same trend as feed intake except for breed of sire; in this case the superiority of the cross breed become significant ($P < 0.05$). Findings similar to Malik and his colleagues (1998) were found in this study found, male lambs gained more grams of body weight daily than females ($P < 0.01$).

Calculation of the daily feed conversion (daily Feed intake/daily feed weight) reveals that lambs exposed to higher doses of lead acetate need more grams of feed for conversion into body weight Figure VI.3. Female lambs also require more feed than males in order to gain similar body weight; a finding which is consistent with Malik et al. (1998). Young lambs, as well as cross- breed lambs, are superior in feed conversion compared to older and pure Naeemi lambs. Also consistent with Malik et al. the present study found the Naeemi X Border LeicesterMerino breed was superior in feed conversion ability than the pure Naeemi breed.

Least square means analysis showed that weight of lambs at the end of the experiment, slaughter weight (Figure VI.4), was affected by oral intake of lead acetate, sex of the lambs but not affected by age of the lamb or breed of sire Table VI.3. Starting weights of the lambs was used in the analysis as a covariate to avoid differences in weights at the beginning of the experiment. The analysis at that level showed that lambs

in the control group were heavier than those of the highest dosage (16 mg/kg/day) (p=0.048). Overall, there was a trend that lambs with higher dosage of lead acetate tend to be lighter in weight at slaughtering time than lambs with lower dosage groups FigureVI.4. These results were consistent with our previous findings were a trend of lower feed intake and weight gain by lambs exposed to higher dosages of lead acetate than lower ones Table VI.2. Since male lambs were eating more feed than females and gained more weight Table VI.2, it was expected that males will have heavier weights at slaughtering time than females (p=0.0017). Lambs administered higher dosage of lead acetate tend to gain lower body weight daily than those administrated lower levels Figure VI.5. Non of the internal organ's weight was affected by lead acetate dosage although internal organs of the control group tended to be the heaviest except for kidneys where it tended to be the lightest. Male lambs have heavier heart, liver and kidneys than females (p=0.04, 0.02, 0.005). That could be due to the more daily feed intake and weight gain by males than females in this experiment. The same reason could be refereed to the superiority of the internal organ's weight in the cross lambs. Lighter heart and liver weights (p=0.017, 0.013) as well as a tendency of having lighter kidneys and spleen in pure Naeemi was found. Age of lambs did no show any difference in
Liver, kidney and spleen weight although there was a tendency that older lambs had heavier organs than younger ones. Although it was a suggestive difference heavier heart weight in older lambs was expected since organs are growing in size with age.
Carcass weight is an important parameter and it is crucial to test it since lambs are not

sold in Kuwait by live weight but by carcass weight. Only half of the carcass (left side) was used in the analysis to give us an indication about that weight and how it was affected by different factors in the model. Least square means analysis showed that dosage of lead acetate administered orally to lambs did not affect on the carcass weight. while significance was not shown, lambs with lower intake of lead acetate tend to have higher weights for their carcasses than those fed higher amounts of lead acetate. That was supported by the correlation analysis that showed a negative but non-significant correlation for carcass weight with blood lead levels TableV.4. Age of lambs was not a factor that affected on carcass weight when we take all the other factors into account and corrected all of them to the starting weight. Carcass weight was affected significantly by the breed of sire where the pure Naeemi lambs had lower carcass weights than those of the cross bred ones ($p<0.05$). Identical results were found also by Al-Sabbagh et al in 1996. Analogous to ours, Malik et al in 1998 found that although breed did not affect slaughter weight; carcass weight was significantly higher in cross bred lambs than in pure Naeemi lambs. Sex of the lambs was not an effective factor on carcass weight although a slight superiority was accounted for male lambs that could be a result of feed intake and weight gain superiority in males Table V.3. Homologous findings were revealed by Al-sabbagh et al in 1996. This finding was significant in the 1998 experiment of Malik and his colleagues.

Correlation coefficient analysis revealed that a highly positive correlation was found for carcass weight with daily feed intake early, middle and at the end of the experiment ($p<0.05$) Table V.4.

Table VI. 1. Mean levels of blood lead levels (ppm) at begining of the experiment, second, sixth, tenth and the fourteenth week of different categories for Naeemi lambs administered oral lead acetate daily For 98 days

Category	Total (n) of Samples	Days of Treatment	Starting	2nd Week	6th Week	10th Week	14th Week
Dosage Mg/kg body weight							
0	5	98	0.05	0.05a	0.05a	0.05a	0.05a
2	5	98	0.05	0.10a	0.13ab	0.05a	0.05a
4	5	98	0.05	0.35b	0.42abc	0.45c	0.35b
8	5	98	0.05	0.46bc	0.67cd	0.70d	0.74c
16	5	98	0.05	0.58c	0.79d	0.90d	1.00d
Sex							
Males	7	98	0.05	0.24	0.39	0.31	0.36
Females	18	98	0.05	0.34	0.43	0.48	0.47
Breed							
Pure	6	98	0.05	0.32	0.52	0.48	0.39
Cross	19	98	0.05	0.31	0.38	0.42	0.45
Age							
Young	12	98	0.05	0.33	0.44	0.48	0.54a
Old	13	98	0.05	0.29	0.39	0.39	0.34b

a , b, c, d Means in column within a category with different superscripts differ ($p < 0.05$).

Table VI. 2. Mean level of daily feed intake (kg) and weight gain (g) and feed Conversion (kg of feed/ g of weight gain) of different categories for Naeemi lambs administered oral lead acetae daily for 98 days

Category	Total (n) of Samples	Days of Treatment	Daily Feed Intake kg	Daily weight gain g	Daily feed conversion Feed/weight Gain
Dosage Mg/kg body weight					
0	5	98	1.5	0.27	5.56
2	5	98	1.5	0.27	5.56
4	5	98	1.5	0.24	6.25
8	5	98	1.4	0.19	7.37
16	5	98	1.3	0.16	8.13
Sex					
Males	7	98	1.6a	0.2a	5.93
Females	18	98	1.3b	0.18b	7.22
Breed					
Pure	6	98	1.4	0.2a	7
Cross	19	98	1.5	0.25b	6
Age					
Young	12	98	1.4	0.26	5.39
Old	13	98	1.5	0.2	7.5

a , b, c Means in column within a category with different superscripts differ ($p < 0.05$).

Table VI. 3. Least square means of weights at slaughter (kg) heart weight (g), liver weight (g),kidney weight (g) and spleen weight (g) of different categories for Naeemi lambs administered oral lead acetate daily for 98 days

Category	Total (n) of Samples	Days of Treatment	Slaughter	Heart	Liver	Kidney	Spleen
Dosage Mg/kg body weight							
0	5	98	61.5a	201	773	126	80
2	5	98	60.5ab	190	648	131	79
4	5	98	58.1ab	175	676	138	73
8	5	98	55.9ab	164	641	136	65
16	5	98	54.7b	197	644	139	69
Sex							
Males	7	98	62.4a	202a	748a	149a	77
Females	18	98	53.9b	169b	605b	119b	70
Breed							
Pure	6	98	55.8	166a	596a	132	68
Cross	19	98	60.4	206b	757b	136	78
Age							
Young	12	98	57.6	164a	660	133	72
Old	13	98	58.7	206b	693	135	74

a , b Means in column within a category with different superscripts differ ($p < 0.05$)

Table V.4 Correlation coeffecients of some tested parameters in Naeemi lambs in Kuwait.

	Age	Blood2	Blood3	Blood4	Blood5	Feed 2	Feed 4	Feed 6	Feed 8	Feed 10	Feed 12	Feed 14	Heart	Liver	Kidney	Spleen	Carcass weight	Slaughter
Dosage	-.17	.84**	.77**	.86**	.92**	-.15	-.22	-.32	-.27	-.36	-.31	-.22	-.13	-.29	.05	-.40	-.07	-.22
Age		-.07	-.08	-.12	-.25	.31	.19	.18	.23	.17	.32	.22	.64**	-.06	.27	.20	.68**	.71**
Blood2			.77**	.82**	.83**	-.28	-.28	-.31	-.21	-.26	-.30	-.27	-.12	-.39	.04	-.51*	-.06	-.22
Blood3				.90**	.79**	-.16	-.19	-.26	-.13	-.33	-.24	-.39	-.17	-.41*	.08	-.38	-.05	-.18
Blood4					.91**	-.25	-.28	-.38	-.28	-.40*	-.36	-.39	-.25	-.38	.04	-.49*	-.14	-.29
Blood5						-.17	-.22	-.31	-.24	-.33	-.26	-.21	-.21	-.18	.05	-.44*	-.16	-.31
Feed2							.91**	.89**	.83**	.75**	.81**	.81**	.63**	.44*	.77**	.47*	.52**	.73**
Feed4								.92**	.82**	.77**	.69**	.71**	.46*	.38	.70**	.30	.36	.61**
Feed6									.92**	.88**	.77**	.80**	.51**	.41*	.74**	.46*	.38	.63**
Feed8										.92**	.85**	.76**	.55**	.42*	.77**	.51*	.41*	.63**
Feed10											.85**	.82**	.59**	.61**	.72**	.54**	.37	.58**
Feed12												.87**	.65**	.66**	.70**	.63**	.43*	.64**
Feed14													.64**	.69**	.70**	.54**	.40*	.61**
Heart														.48*	.67**	.55**	.73**	.85**
Liver															.48*	.63**	.21	.31
Kidney																.48**	.50*	.70**
Spleen																	.41*	.48*
Carcass weight																		.87**

** P<0.01; * P< 0.05

Age= Age of lambs; Blood2=Blood lead concentration at 2nd week; Blood3=Blood lead concentration at 6th week; Blood4=Blood lead concentration at 10th week; Blood5=Blood lead concentration at 14th week;Feed2= Feed intake by lambs at the 2nd week;Feed4= Feed intake by lambs at the 4th week;Feed6= Feed intake by lambs at the 6th week;Feed8= Feed intake by lambs at the 8th week;
Feed10= Feed intake by lambs at the 10th week;Feed12= Feed intake by lambs at the 12th week;Feed14= Feed intake by lambs at the 14th week;
Heart=Heart weight; Liver=Liver weight; Kidney=Kidney weight; Spleen=Spleen weight; Carcass weight= Weight of the left half of the lamb after slaughtering;
Slaughterweight=weight of the lambs alive at slaughtering.

Figure VI. 1 Means of blood lead levels (PPM) in Naeemi lambs administrated orally different dosages of lead acetate (0,2,4,8 and 16 mg/Kg/day) for 14 weeks

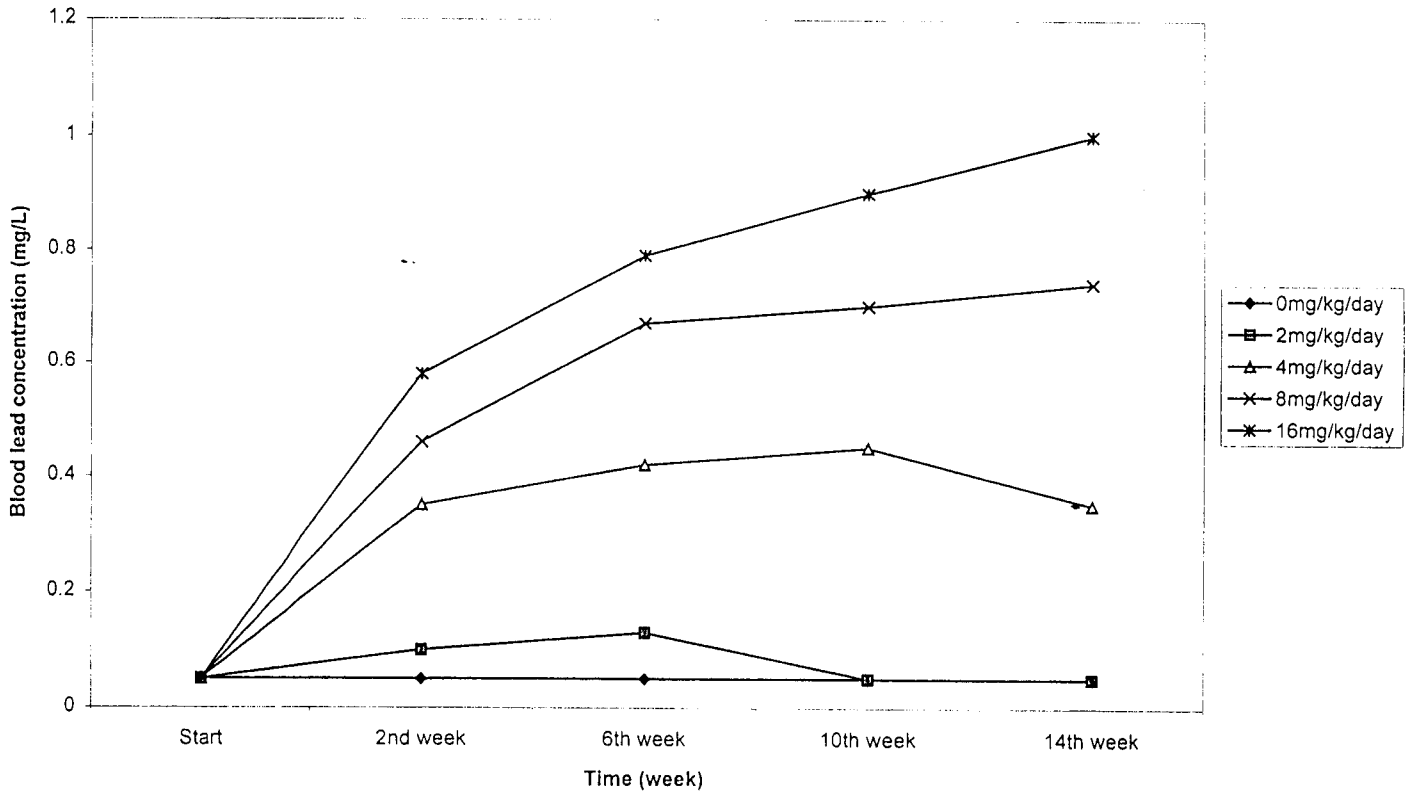


Figure VI. 2 Daily feed intake (kg) for Naeemi lambs administered various dosages oral lead acetate (0,2,4,8 and 16 mg/kg body weight/ day) for 98 days.

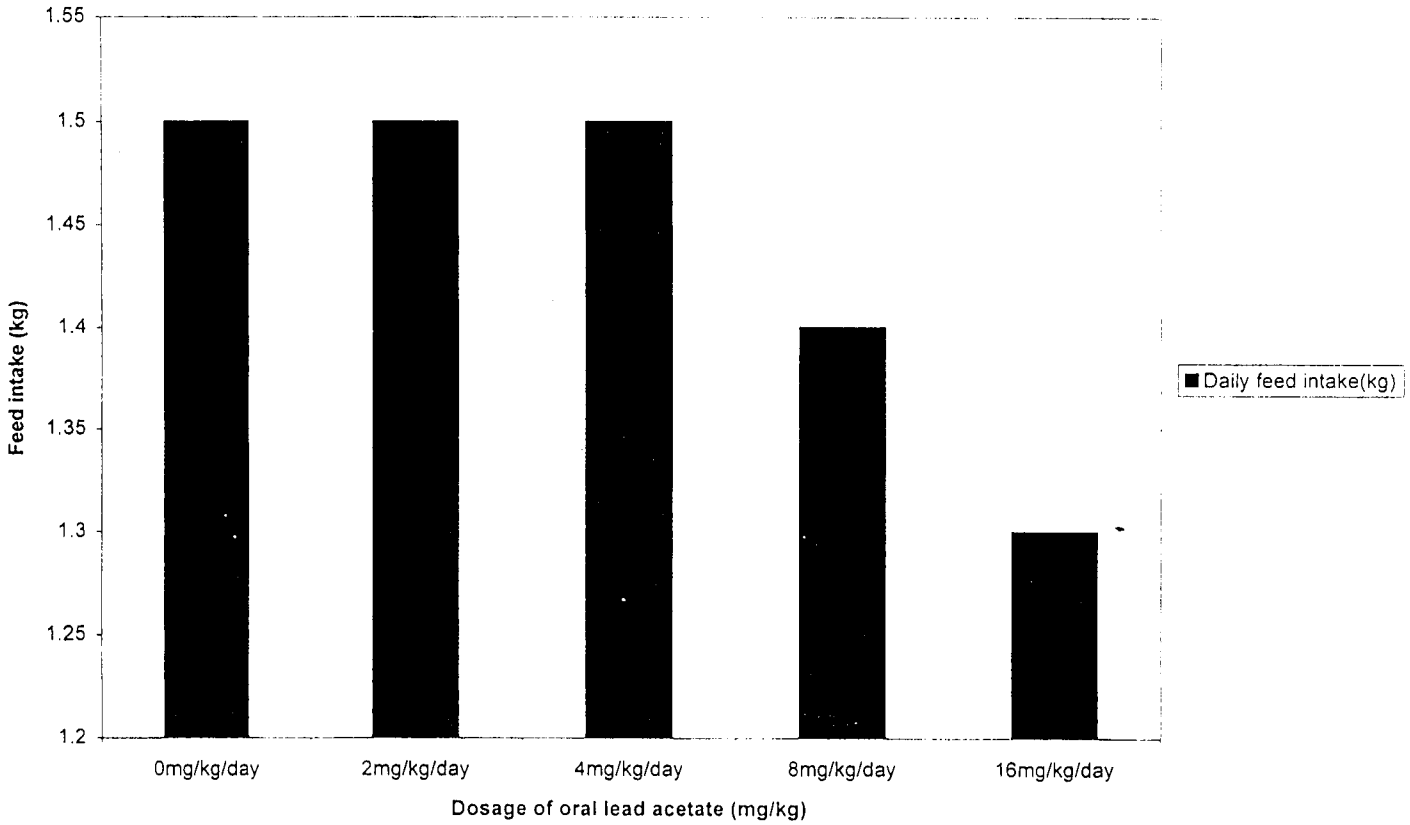


Figure VI. 3 Daily feed conversion (Feed/weight gain) for Naeemi lambs administrated various dosages oral lead acetate(0,2,4,8 and 16 mg/kg body weight/day) for 98 days.

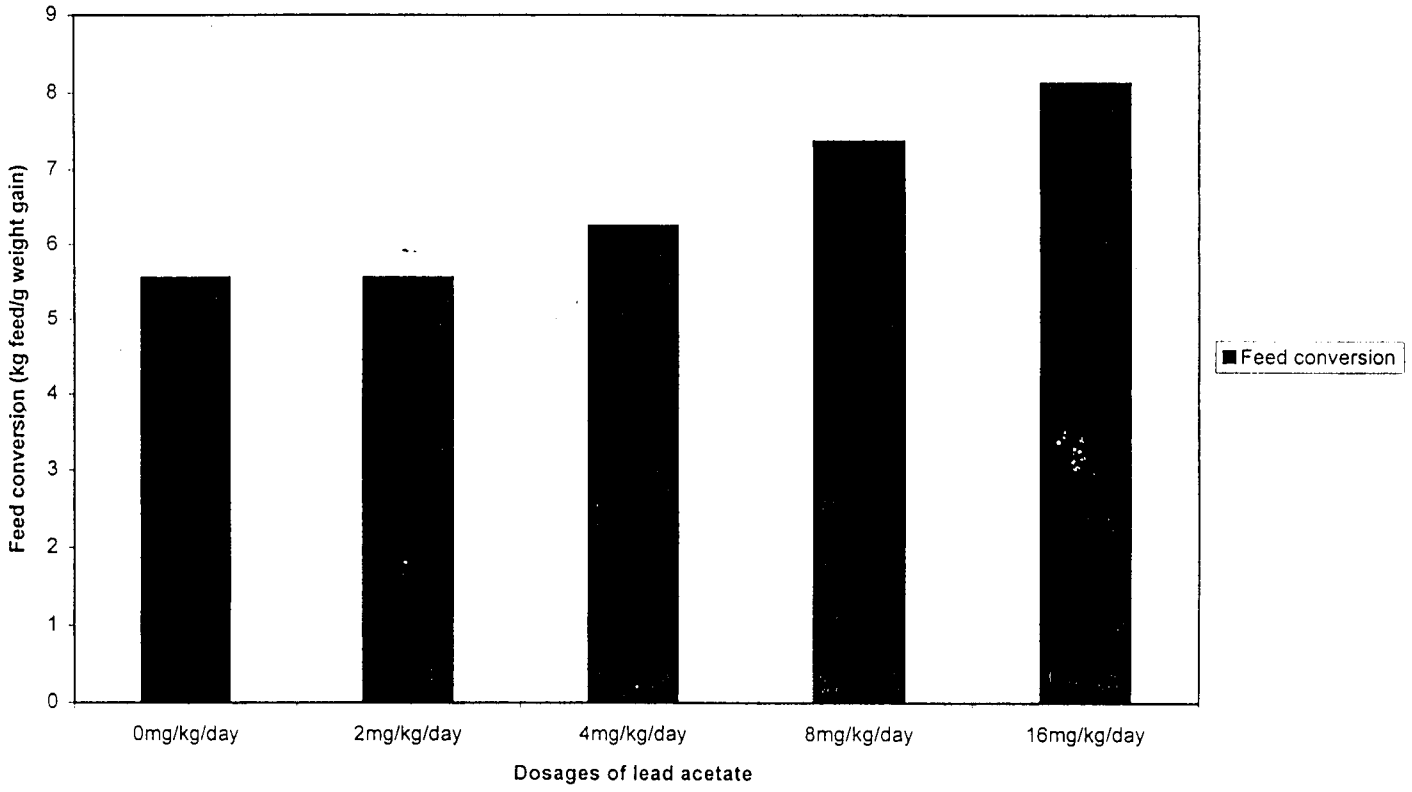


Figure VI. 4 Slaughter weight (kg) for Naeemi lambs administered various dosages oral lead acetate (0,2,4,8 and 16 mg/kg body weight/day) for 98 days.

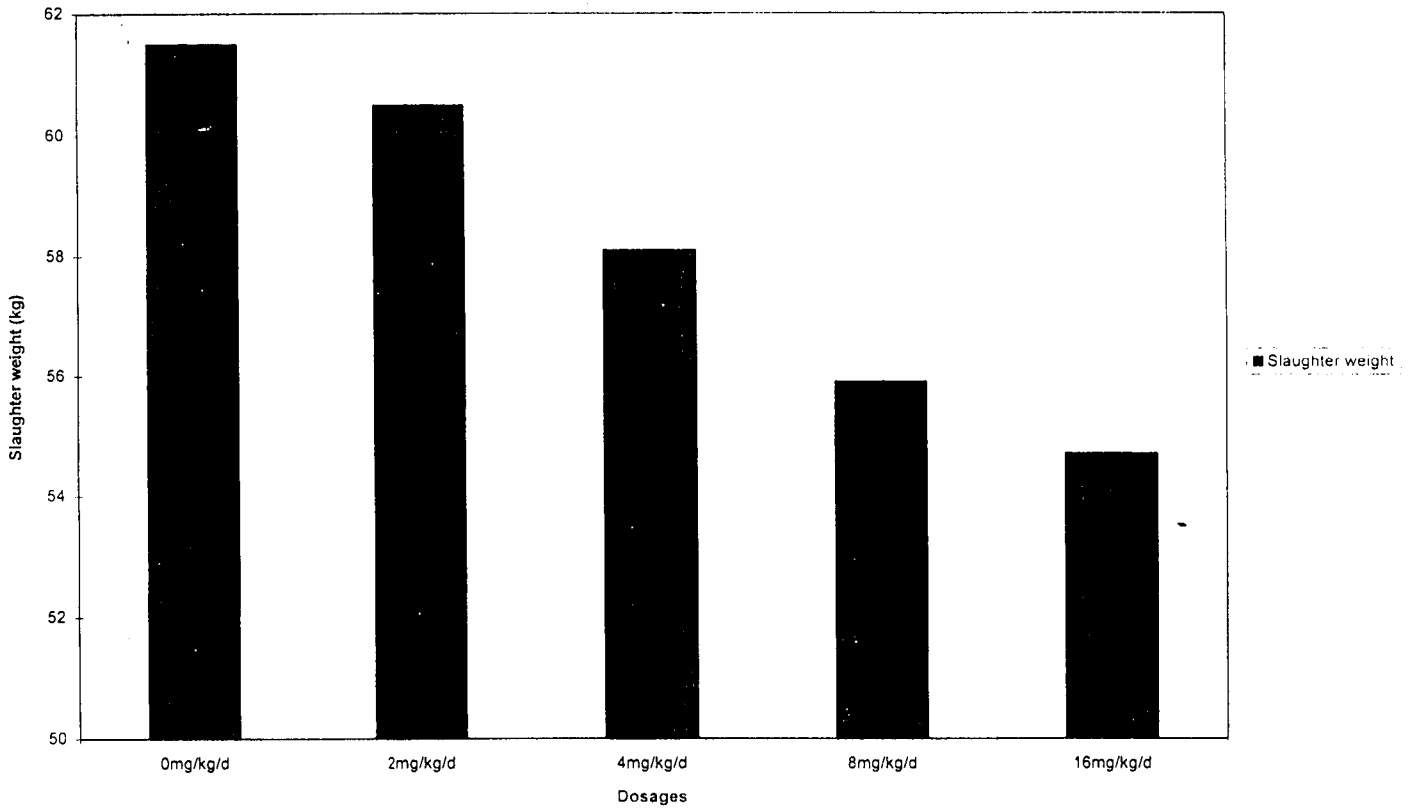
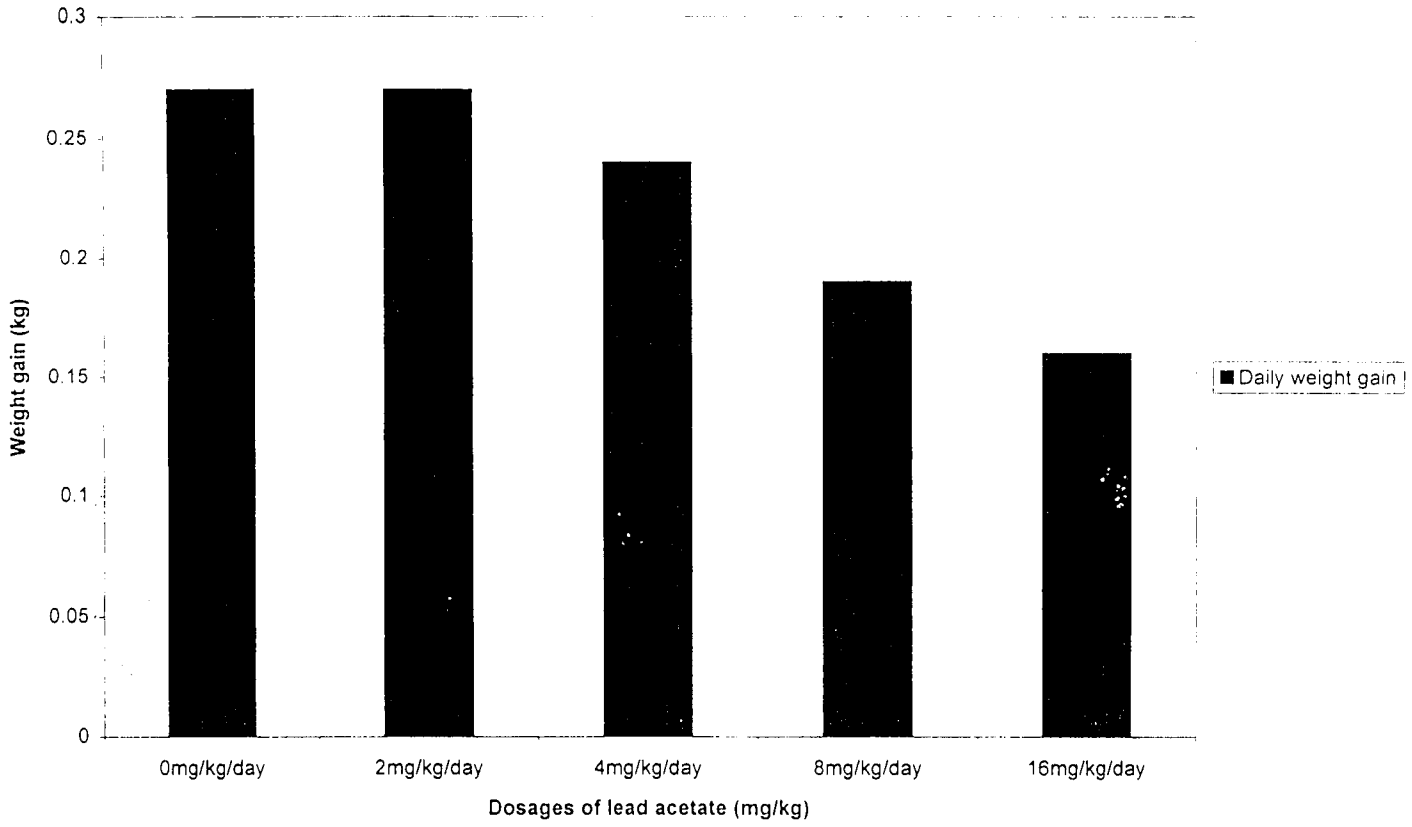


Figure VI. 5 Daily weight gain (g) for Naeemi lambs administered various dosages oral lead acetate (0,2,4,8 and 16 mg/kg body weight/day) for 98 days.



CHAPTER VII
CONCLUSION

Under the conditions of the study in the first experiment, it can be concluded that practicing lamb production as range flock or semi-intensive systems will be hazardous for lambs grazing up to ten meters from the King Fahad Highway in Kuwait. In this area lead concentration in the soil reached more than 5000 ppm. Lead concentration in the soil in the studied regions around Interstate 5, Highway 34 and Highway 20 in Oregon were much less than those found in Kuwait. Despite this, lead levels in Oregon climbed to levels more than 103.3 mg/kg at Highway 20 and 113 mg/kg at Highway 34 and165.7 mg/kg at Interstate 5; these lead levels exceed the standards mentioned by Shelleshear et al. (1975) and Chaney et al. (1986 and 1989). Lead soil concentration at these areas negatively correlated with distance from the highway at up to ten meters. The increase in traffic density tended to increase the concentration of lead in soil around the highway, although, not enough data were gathered to show the significance.

In the second experiment, oral lead acetate concentrations affected lead levels accumulated in bone, liver and kidney tissues. Clinical signs were absent in all animals even those receiving the highest lead dose (16 mg/Kg/day). Inclusion bodies were found in the kidney and liver were but not in the other tested tissues of lambs exposed to as low as 2 and 4mg/Kg/day respectively; lower doses produced neither clinical signs nor any histopathological changes. Inclusion bodies tend to correlate directly to the retained lead levels in tissues rather than the administered amount.

In the third experiment, oral doses of lead acetate affected the blood lead levels in lambs. Lambs administered higher oral doses of lead acetate accumulated higher levels of lead in their blood. Lambs exposed to higher oral doses of lead acetate needed more feed to convert into grams of body weight than those exposed to the lower doses. Oral doses of lead acetate also affected the slaughter weights; lambs exposed to higher oral doses of lead acetate had lower slaughter weights but similar carcass, heart, liver, kidney and spleen weights.

In a combined correlation analysis of the data from experiment II and III, findings revealed lead acetate dosing did not significantly ($p > 0.05$) directly affect feed intake. A high negative correlation was found between the concentration of lead in the brain and the amount of daily feed intake from day 30 in the experiment through day 60, at which point the significance disappeared. From this it can be inferred that higher levels of lead in the brain, which highly correlated to dose (67%, $P = <0.001$), may affect lambs' appetite and decrease feed intake. Ghosh et al. (1992) also found changes in brain tissue attributed to lead ingestion; these changes involved a decrease in RNase and DNase activity as well as lowered serotonin levels while elevated values of norepinephrine and dopamine were found.

Future studies are recommend to be conducted on lead levels present in the environment and concentrations found in tissues of exposed animals. More variables should be included in a similar experiment done on lead accumulation alongside of highways. Additionally, further distances from the highways should be included in order

to determine how much and where the drops in lead concentration occur. Further, a separate experiment on the lead dose effect on lead concentrations in lamb brain tissue and the affect on performance is recommended.

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