

Molecular Toxicity of Lead

by

Laura Jacox

Submitted to the Department of Earth, Atmospheric and Planetary Sciences

in Partial Fulfillment of the Requirements for the Degree of

Bachelor of Science in Earth, Atmospheric and Planetary Sciences

at the Massachusetts Institute of Technology

May 12, 2008 [June 2008]

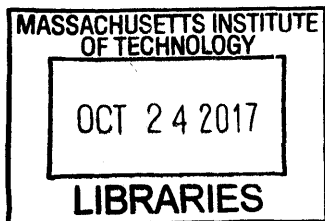
Copyright 2008 Laura Jacox. All rights reserved.

The author hereby grants to M.I.T. permission to reproduce and distribute publicly paper and electronic copies of this thesis and to grant others the right to do so.

Author Signature redacted
Department of Earth, Atmospheric and Planetary Sciences
May 12, 2008

Certified and accepted by Signature redacted
Samuel Bowring
Thesis Supervisor

Chair, Committee on Undergraduate Program



ARCHIVES

The author hereby grants to MIT permission to reproduce and to distribute publicly paper and electronic copies of this thesis document in whole or in part in any medium now known or hereafter created.

Introduction

Lead is a heavy metal that has been in use for over 8000 years (White, 2007). It was first smelted in 4000BC as a byproduct of silver processing. Since then, Pb has played a dynamic role in history, possibly contributing to the fall of the Roman Empire (Nraigu, 1983). Pb is a highly malleable and ductile Group IVa metal. It has been utilized in a variety of products including makeup, water pipes, cooking vessels, wine bottle seals, glass, batteries, solder, electronic components, paint, and antiknock fuel additives (White, 2007). Its prevalent, long-term use has distributed anthropogenic Pb across the planet in soil, air-borne dust, and water (White, 2007). As a result, human exposure can occur via inhaled air, dust, food, and drinking water. Pb has no known biological functions, yet it has numerous detrimental effects on the body, several of which have been recognized for millennia.

There are several routes of lead absorption into the body. For adults, the predominant site of absorption is the respiratory tract, which has an absorption efficiency of approximately 40 percent of total lead inhaled (Fischbein, 1998). Respiratory exposure is often due to airborne lead dust from the scraping, sanding, or burning of leaded paint surfaces as well as various smelting processes. Ingestion of lead followed by gastrointestinal absorption can also occur, but the absorption efficiency is only 10 to 15 percent in adults. In children, however, gastrointestinal absorption is the major route of lead poisoning, as their GI tract absorption efficiency is approximately 50 percent. Children tend to introduce leaded products into their body through ingestion. Because lead is so similar to biologically essential metals, intestinal transport proteins do not

exclude it. Additionally, organic, non-additive lead from gasoline can be absorbed through the skin of both adults and children (Fischbein, 1998).

Once inside the body, lead is transported by blood, with 99 percent bound to erythrocytes (Rabinowitz, 1991). The 1 percent of unbound lead is free in the plasma to exchange with soft and mineralized tissues. The kidney excretes erythrocyte-bound lead relatively quickly, with an average half-life of about 30 days, if renal function is normal (Rabinowitz, 1991). However, if a patient has long-term lead exposure, compromised kidneys, or large bone stores, the half-life can be longer (Hryhorczuk, 1985).

If exposure ceases, the half-life for lead removal from the human body is dependent on the type of tissue. Within soft tissues, such as the brain or kidney, lead has a half-life of about 40 days, whereas lead within mineralized tissue (i.e. bone) has a half-life of decades (Rabinowitz, 1991). Because bones can store large volumes of lead which substitutes for calcium atoms, bones contain about 95 percent of the body's lead burden. Thus, BLL is not always a good reflection of total body lead burden. During periods of bone turnover, such as pregnancy, breast-feeding, menopause, and hyperthyroidism, lead stored in bone can be released, increasing blood lead levels (BLL) (Riess, 2007; Gulson, 1998; Goldman, 1994). Elevated BLLs are of particular concern for pregnant women because lead readily crosses the placenta and can effect fetal development (Riess, 2007).

Lead storage in bones varies by age because fetuses and children are forming bone at much higher rates than adults. As a result, they will store a greater portion of their lead burden in mineralized tissue than adults. As a result, adults will ultimately retain about 1 percent of lead that enters their body, while children younger than two

years of age retain about one-third of all absorbed lead, primarily in their bones (Agency, 2008).

Lead has well-characterized effects on most major body systems, and it has no apparent threshold (Lidsky, 2003). In adults, most toxic effects are reversible if lead poisoning is identified early, but chronic exposure or high levels can cause irreversible damage to the central and peripheral nervous system and kidneys. Growing children often have irreversible nervous system impairment due to lead exposure.

Adults with elevated BLLs often present with abdominal pain, muscle and joint aches, headache, fatigue, difficulty concentrating, impaired short-term memory, anemia, nephropathy, peripheral neuropathy, hypertension, and/or basophilic stippling of blood cells. Men who have chronic occupational exposure may also suffer from decreased fertility and sperm count (Alexander, 1996). In women, lead has been linked with increased numbers of miscarriages and stillbirths, reduced birth weight, and cognitive impairment of the offspring (Fischbein, 1998). The presence and intensity of symptoms vary based on the individual, the patient's BLL, and the length of exposure; acute health effects are usually well correlated with BLL (Levin, 2000).

Children with mildly elevated BLLs are often asymptomatic. They are typically diagnosed due to a lead screening program where BLLs are obtained. Childhood BLLs greater than 10mcg/dL are associated with irreversible neurocognitive deficits and are considered toxic. Moderate to high BLLs are sometimes associated with abdominal pain, vomiting, mental retardation, behavioral disorders, pervasive developmental disorders, encephalopathy, seizure disorders, anemia, and iron deficiency. Children are more

susceptible to lead poisoning because they are still undergoing physical and cognitive development.

Despite a century of documentation on the symptoms of lead poisoning, the molecular effects of lead on the human body have only become a focus of research in recent years. By substituting for catalysts' divalent cations, primarily Zinc, Calcium, and Magnesium, lead has been shown to hinder an organism's ability to form erythrocytes, metabolize energy, transport metals, and signal through the nervous system. To delve into this in more detail, I will talk about lead's effects on numerous cation-containing proteins next. I will describe lead's effect on heme biosynthesis, sperm formation, calcium sensing, cell cycle regulation, nerve signaling, apoptosis, mitochondrial respiration, DNA replication, DNA repair systems, and transcription. Until recent research, it was unclear how lead induced the symptoms associated with lead poisoning. With our new knowledge of lead's molecular effects, the source of symptoms is becoming known.

Despite headway in the field, much work needs to be done. Following my discussion of lead's effects on enzymes and the associated symptoms, I discuss several possible directions for future research. Then I describe a field of related investigation where scientists and epidemiologists are associating lead exposure with a range of systemic, chronic conditions including Alzheimer's disease, osteoporosis, and behavioral disorders. The molecular effects of lead and the resulting impact on the nervous and skeletal systems have been linked to these conditions and may provide causes for these diseases whose origins were previously unknown. I conclude with a brief summary of how research into lead's molecular toxicity holds promise for improving our

understanding of lead poisoning's symptoms, learning how to improve treatment of lead poisoning, and possibly offering insight into the prevention and treatment of disorders such as Alzheimer's disease, osteoporosis, and behavioral disorders

Molecular Sites for Lead

Proteins are capable of selectively attract cations to their cation binding sites. Zinc-coordinated proteins create a zinc affinity pocket by populating their zinc-binding sites with sulfur and nitrogen and having low coordination numbers (Frausto, 2001). Such sites are not favorable for calcium, which prefers an active site with a coordination number of 6 or 7 and a wide, evenly charged environment to allow rapid ion exchange (Frausto, 2001). By tailoring the active site to the preferences of a specific cation, a protein can determine which atom it will bind. However, such selectivity breaks down when elements are introduced that are not normally present in cells and possess partial ionic-molecular mimicry with cellular cations; proteins have not evolved to selectively expel such elements (Garza, 2006). One particularly toxic element is Pb. Pb can interact with oxygen and sulfur in a wide range of coordination numbers; oxygen and sulfur are common in many protein metal-binding sites (Godwin, 2001).

Calcium and magnesium are often coordinated with oxygen while zinc is coordinated with sulfur. Calcium, magnesium, and zinc are three of the most important cations in biological systems, and they are also the most readily replaced by lead (Kern, 2000; Ghering, 2005; Godwin, 2001). Lead has a larger ionic radius and is more electronegative than calcium, magnesium, or zinc. These features allow it to establish highly favorable interactions with oxygen and sulfur coordinating groups. As a result, Pb

highly favorable interactions with oxygen and sulfur coordinating groups. As a result, Pb coordinates with cation-binding sites with greater affinity than calcium, magnesium, and zinc, and will replace these cations in proteins (Kern, 2000; Ghering, 2005; Godwin, 2001).

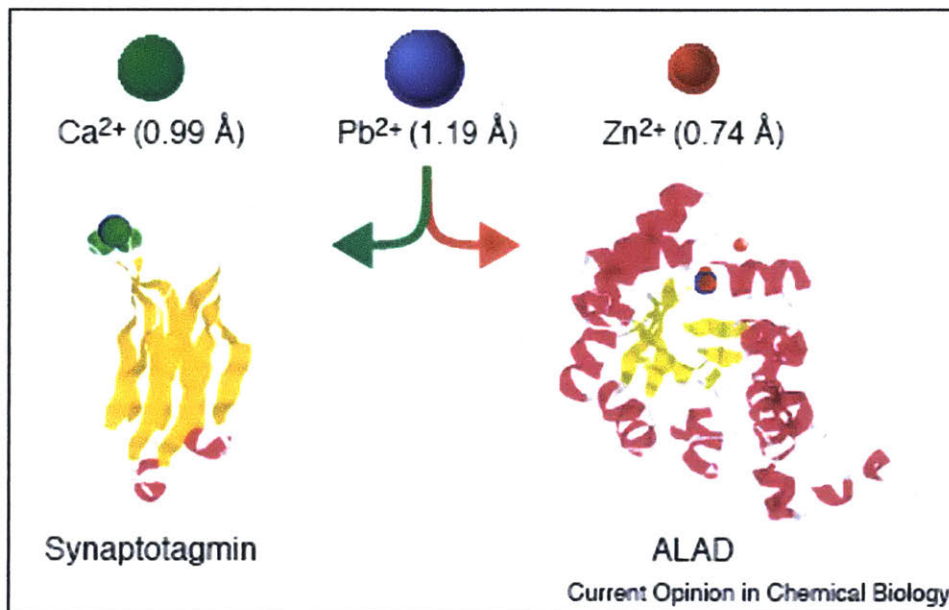


Figure 1: Cation-associated proteins Affected by Lead Substitution

Lead interferes with cations in cation-associated proteins including calcium and zinc. Two such cation-associated proteins are synaptotagmin, a calcium sensor in neurons, and ALAD, the second enzyme in the heme biosynthetic pathway. Crystallization assays demonstrated that lead can substitute for calcium in synaptotagmin and for zinc in ALAD despite size differences. (Taken from Godwin, 2001)

A lead substitution alters the normal structure and function of a protein because it has a different distribution of charge than zinc, magnesium, or calcium. Lead has a lone pair of electrons, while calcium, magnesium, and zinc have a spherical distribution of charge (Figure 1). As a result, the substitution of calcium, magnesium, or zinc with lead distorts the distribution of coordinating groups in a protein, altering its structure and function. This effect is particularly pronounced in zinc-bound proteins with few ion-coordinating groups.

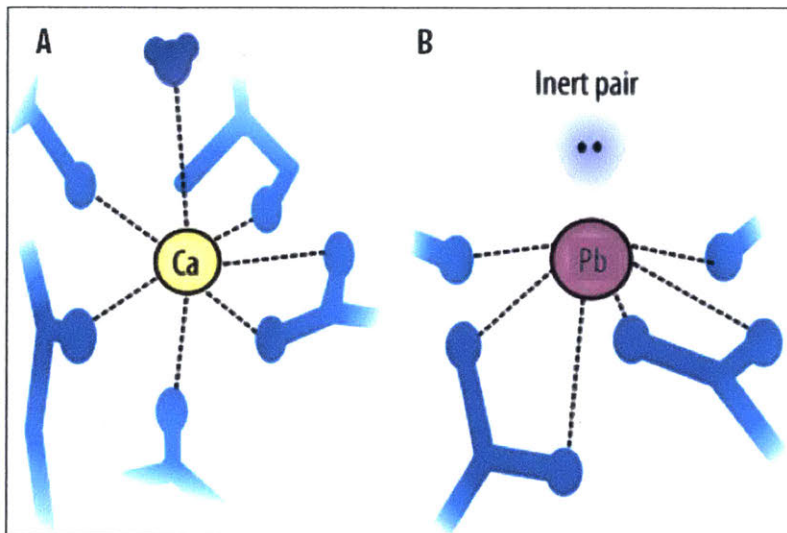


Figure 2: Schematic representation of coordinating groups for Ca and Pb.
 A) Holodirectional (pentagonal bipyramidal) coordination sphere of Ca. There is a regular distribution of coordinating oxygen groups. A water molecule, shown in black, participates in the coordination. B) Hemidirectional coordination sphere of lead. The irregular distribution of coordinating groups is due to the inert lone pair of electrons. (Figure taken from Garza, 2006)

Zinc-Associated Proteins

Zinc assumes both structural and catalytic roles in the cell. Zinc often complexes with polycysteine coordination arrays, facilitating interaction between zinc and sulfur (Frausto, 2001). Zinc tends to distort the electron cloud of sulfur, giving the coordination bond a stable, covalent character. However, lead forms stable complexes with sulfur more readily than zinc, and will replace Zn, inhibiting the activity of proteins (Simmons, 1995). Sulfhydryl-dependent enzymes involved in heme synthesis are inhibited in this fashion; they include δ -aminolevulinic dehydratase (ALAD), δ -aminolevulinic synthetase (ALAS), and mitochondrial sulfhydryl enzyme (Hammond, 1977).

ALAD catalyzes the second reaction in the heme biosynthetic pathway. When ALAD was co-crystallized with lead, it was shown that lead binds to the enzyme's three-cysteine site (Erkshine, 1997). Lead binding non-competitively inhibits substrate

binding. As a result, the log of ALAD activity in erythrocytes decreases linearly with an individual's BLL (Millar, 1970). The body's inability to efficiently form heme molecules in the presence of lead causes anemia: a common symptom of lead poisoning with BLL over 40 mcg/dl.

Surprisingly, other zinc-based catalysts are seemingly unaffected by lead. It is postulated that lead only affects heme biosynthetic enzymes because of their three-cysteine residue site, while other zinc-based catalysts contain fewer than three cysteine residues (Godwin, 2001).

Lead's deleterious effects on normal cell function extend beyond protein catalysts, as it has been found to hinder the normal function of zinc-coordinated DNA-binding proteins (Godwin, 2001). Lead has been shown to replace zinc in human protamine 2 (HP2), a protein important in spermatogenesis (Quintanilla-Vega, 2000). HP2 replaces histone proteins in sperm by binding, organizing, and stabilizing DNA. Lead-bound HP2 is unable to function normally. This may account for the increased infertility rates in men occupationally exposed to lead. The effect of lead on HP2 suggests that lead would alter the DNA-binding activities of zinc-coordinated transcription factors (Quintanilla-Vega, 2000).

Several crucial transcription factors have been found to contain four cysteine residues or three cysteine residues plus a histidine residue, reminiscent of zinc-bound, heme synthetic catalysts (Godwin, 2001). These transcription factors modulate gene expression needed for proper development. They include steroid receptors and retroviral nucleocapsid proteins. Lead coordinates with the protein's zinc-binding sites, causing the transcription factors to fold improperly (Payne, 1999). The change in structure alters

their function, which in turn may contribute to many of the developmental delays associated with lead poisoning in children (Godwin, 2001).

Calcium-Associated Proteins

Calcium-binding sites are based on oxygen-containing groups such as carboxylates. Oxygen-containing groups have a lower affinity for Pb than thiol groups by several orders of magnitude (Garza, 2001, Godwin, 2006). As a result, one might conclude that the effects of lead on zinc proteins would be more deleterious to a cell than those of lead on calcium proteins. However, calcium is so crucial to key cellular reactions that Pb's interference with Ca-regulated proteins is one of the main physiopathogenic mechanisms of Pb toxicity.

There are two types of calcium-binding structures in proteins: the "EF-hand" domain and the C2 domain (Bouton, 2001). Both domains have a high affinity to lead, and are found in proteins most effected by elevated lead concentrations including calmodulin, protein kinase C (PKC), and synaptotagmin I. Calmodulin is a protein with four EF motifs and is one of the cell's main calcium sensors (Kuboniwa, 1995; Chattopadhyaya, 1992). Calmodulin changes conformation and activity according to the number of EF domains bound by calcium. Lead and calcium can cooperatively activate calmodulin, as both can bind the protein's EF domains (Kern, 2000). Calmodulin can then be activated at a lower calcium concentration than that required for calcium alone. Active calmodulin initiates numerous signal cascades to modulate gene expression and regulates ion channels such as calcium-activated potassium channels, NMDA receptors, calcium channels, and intracellular inositol triphosphate receptors (Garza, 2006).

Besides modulating gene expression and ion channels via calmodulin, lead was shown to alter expression of early-response genes in a PKC-dependent fashion; the induced genes include *c-fos*, *c-jun*, and *erg-1* (Kim, 2000). It is not currently clear if *in vivo*, lead interacts with a transcription factor upstream of PKC or if it directly activates PKC, a protein with a calcium-binding C2 domain. It was shown *in vitro* that lead is a more potent activator of PKC than calcium, PKC's physiologic activator (Markovac, 1988). PKC also becomes active at lower lead concentrations than those required to activate calmodulin (Markovac, 1988).

Active PKC stimulates the expression of *c-jun* and *c-fos* via phosphorylation of other transcription factors (Kim, 2000). *C-fos* acts with *c-jun* to induce protein expression necessary for cells to progress through the cell cycle (Lodish, 2004). *C-fos* and *c-jun* are proto-oncogenes that cause excessive cellular proliferation, en route to cancer, when inappropriately regulated. Lead's ability to interfere with genes crucial to cell cycle regulation may result in the higher incidence of cancer observed in rodents exposed to lead (Buzard, 2000; Koller, 1985). Recent epidemiological studies have indicated an association between lead exposure and various types of cancer including bladder, kidney, lung and stomach tumors (Fu, 1995, Lustberg, 2002).

PKC family proteins regulate learning and memory on an organismal level (Newton, 1995). PKC and the signal transduction pathways it controls are essential for the complex cellular orchestration required for learning to occur. Lead's interference with calcium-regulated PKC may further contribute to neurological problems associated with lead poisoning (Godwin, 2001).

The third calcium-binding protein affected by lead is synaptogamin (syt). Syt possesses a calcium-binding C2 domain like PKC, and is essential to nerve signaling (Godwin, 2001; Garza, 2006). In normal nerves, an action potential will propagate down the membrane of a presynaptic terminal (Campbell, 2002). The depolarization of the membrane triggers voltage-gated calcium channels to open, allowing an influx of calcium into the presynaptic cell. Syt binds calcium at its C2 domain, which causes a conformational change; the structural change allows syt to associate with syntaxin, its protein partner. The protein pair then triggers synaptic vesicles containing neurotransmitter to fuse with the presynaptic membrane and release neurotransmitter molecules via exocytosis into the synaptic cleft. The neurotransmitter molecules will diffuse across the synaptic cleft and bind receptors in the postsynaptic neuron to stimulate the opening of sodium channels. The opening of sodium channels causes a sodium influx that depolarizes the postsynaptic membrane. The resulting action potential will propagate down the neuron and eventually stimulate calcium channels to open. By this mechanism, signals will be passed from neuron to neuron. Such signaling is crucial to normal nervous system function (Campbell, 2002).

In organisms with elevated BLLs, lead passes through voltage-gated calcium channels during presynaptic depolarization. In fact, voltage-gated calcium channels have a higher affinity for transporting lead than calcium (Cloues, 2000). Once inside the neuron, lead is capable of substituting for calcium in syt's C2 domain (Bouton, 2001). Lead-bound syt is unable to bind syntaxin. Without the syt-syntaxin complex, neurotransmitter release cannot occur, and neuronal signaling is impaired. It has been demonstrated that lead interferes with the ability of calcium to trigger exocytosis of

neurotransmitters in neurons (Manalis, 1973). The above mechanism can account for this observation and may contribute to the pervasive neurological problems in adults and children exposed to lead (Godwin, 2006).

Another proposed source of lead's effect on nervous system function stems from its ability to stimulate apoptosis. Apoptosis is programmed cell death. Low to moderate lead exposure was found to stimulate apoptosis in primary cultural neuronal cells (Oberto, 1996; Scortegagna, 1997) and in rod and bipolar cells of developing and adult rats (Fox, 1988; Fox, 1997). Lead is transported into mitochondria, and tends to be concentrated in the organelle (Lidsky, 2003). High intracellular lead can act like high intracellular calcium by stimulating the opening of the mitochondrial permeability transition pore (PTP). PTP has an internal binding site for metals with an oxidation state of +2. Normally, high calcium levels caused by cell death signals or cellular stress trigger the pore to open. The pore is located in the inner mitochondrial membrane, and its opening allows protons to flow down their concentration gradient from the intermembrane space to the mitochondrial membrane (He, 2000; Campbell, 2002). This decouples the electron transport chain from ATP synthase, and thus ATP is not generated. The depolarization event initiates the cytochrome c-caspase cascade, which ultimately results in apoptosis (He, 2000).

High intracellular lead can stimulate the opening of PTP by binding in the protein's metal site in place of calcium (He, 2000). During apoptosis, high intracellular lead concentrations also increase intracellular calcium levels *in vitro* and *in vivo* (Fox, 1992; Medrano, 1994). Rises in lead and calcium concentrations increase the fraction of PTP bound to metals. Metal-bound PTP will open, leading to mitochondrial

depolarization and activation of the c-caspase apoptotic cascade. Additionally, lead favors the generation of free radicals and reactive oxygen species (ROS) (Lidsky 2003). ROS promote cell death and can mutagenize DNA. The combined action of ROS generation, calcium overload, and PTP opening causes apoptosis to occur independently of a natural impetus for cell death. Inappropriate apoptosis was shown to occur in rat retinal and rod cells exposed to lead (He, 2000). Apoptosis in retinal and rod cells is functionally significant, as humans, rats, and monkeys have long-term visual system deficits associated with low to moderate developmental lead exposure (Fox, 1997)

Lead not only stimulates apoptotic pathways and blocks heme synthesis within mitochondria, it also inhibits the most basic function of the organelle: energy generation. Mitochondrial energy generation requires an electron transport chain to establish a proton concentration gradient. The chain is made up of iron-associated cytochromes that alternate between a +2 and +3 oxidation state. The chain is also associated with magnesium and zinc atoms. Exactly how lead interferes with the electron transport chain is still unclear, but interaction with or substitution for the metal atoms is a likely mechanism (Lidsky, 2003).

The effect of lead on mitochondrial function and associated metabolism was observed in several *in vivo* assays. Suckling rat pups were fed lead-tainted milk over several weeks and their weight was tracked (Holtzman, 1976; Holtzman, 1978). Pups were sacrificed at various time points. Their brains were weighed, and their neural mitochondria were isolated. The lead-poisoned rat pups had decreased weight compared with controls within 2 days of switching to a lead enriched diet; normal metabolism was impeded by the lead. Pups also had a drop in cerebellum weight within a week of

adopting the lead tainted diet; neural development was hindered. Finally, their neural mitochondria were found to have lower levels of oxygen consumption when compared with mitochondria isolated from control rats. Oxygen consumption is a proxy for energy generation because oxygen is reduced to water by the electron transport chain (Holtzman, 1976; Holtzman, 1978). Similarly, when adult rats were exposed to chronic administration of low lead doses at levels similar to environmental exposure in people, energy metabolism in adult brain nerve endings was altered (Rafalowska, 1996, Lidsky, 2003).

Lead's effect on neural development and metabolism in rats is phenotypically similar to its influence on humans, suggesting that lead alters human and rat neural systems via a common mechanism (Holtzman, 1976; Holtzman, 1978). Humans exposed to lead lose brain volume and have an increased incidence of white brain lesions (Stewart, 2006). When individuals with elevated BLLs are analyzed by magnetic resonance spectroscopy, they have a reduction in N-acetylaspartate/creatine and phosphocreatine ratios in their frontal gray matter (Trope, 20001; Trope, 1998). Phosphocreatine and creatine ratios are a reflection of brain metabolism, and a decrease in these parameters indicates a drop in neural function. Declines in metabolism and brain mass could contribute to many of the neurological symptoms associated with lead exposure in children and adults including cognitive and behavioral developmental delay, impaired short-term memory, difficulty concentrating, and fatigue (Goldman, 2008).

Magnesium-Associated Proteins

Apart from inhibiting normal brain function, high doses of lead can cause patients to develop a rare form of anemia, called hemolytic anemia. Red blood cells will sickle in a manner similar to erythrocytes from patients who have sickle cell anemia. Lead causes this condition by disrupting magnesium coordination in eukaryotic pyrimidine 5'-nucleotidase type 1 (P5N-1). P5N-1 catalyzes the dephosphorylation of pyrimidine 5'-mononucleotides, which is needed for erythrocyte structural integrity and function (Rees, 2003; Bitto, 2006). When lead is introduced into blood cells, it binds within the active site of P5N-1 such that it impedes normal function of magnesium's cationic cavity (Bitto, 2006). The cationic cavity is crucial for the recognition and binding of nucleotides' phosphate groups. If P5N-1 is unable to recognize or act upon its substrate due to lead, it is essentially inactivated (Bitto, 2006). The functional deficiency of P5N-1 causes the accumulation of phosphorylated pyrimidine 5'-mononucleotides, resulting in basophilic stippling of red blood cells (Rees, 2003; Bitto, 2006). Patients with severe lead poisoning (BLL greater than 80mcg/dL) often present with basophilic stippling on a blood smear and have hemolytic anemia (Goldman, 2008).

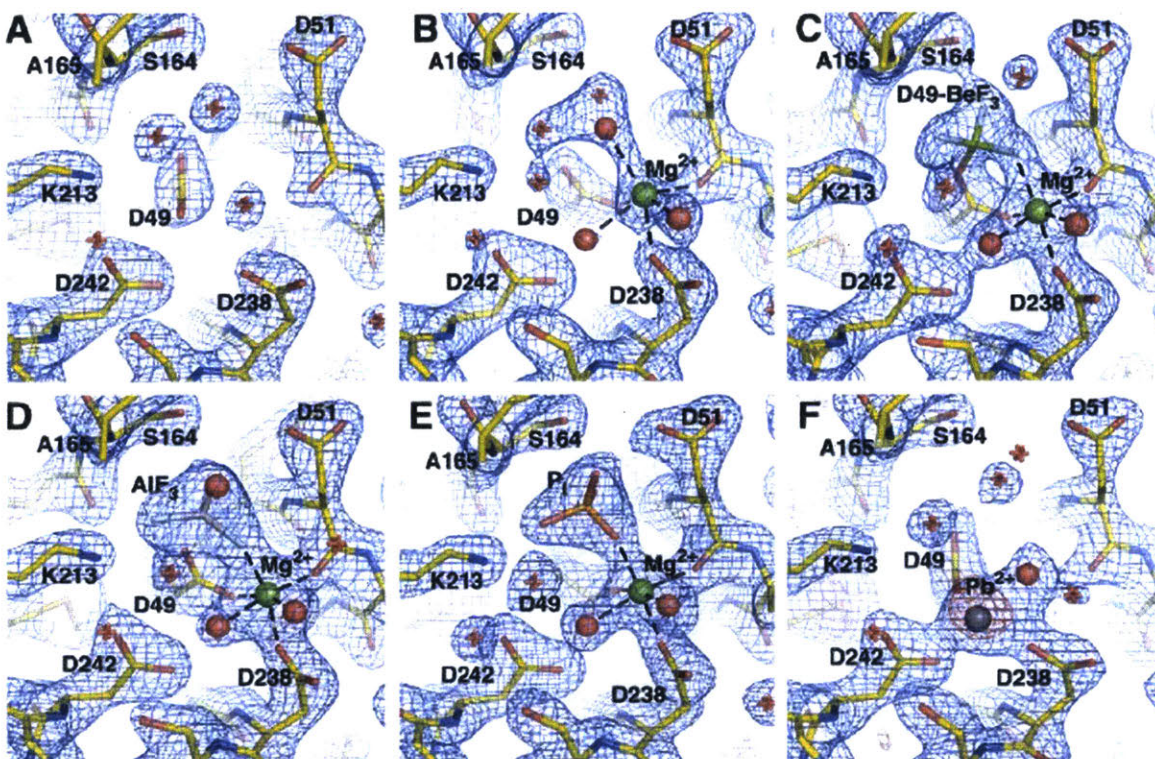


Figure 3: Structural snapshots of the P5N-1 Reaction cycle. The blue netting represents the electron density of the P5N-1 complex, while the sticks beneath the netting represent amino acid backbones and R groups. The *yellow* stick sections are composed of carbon. *Red* and *blue* spheres and stick sections represent polar oxygen and nitrogen, respectively. Mg(II) is represented by a *green* sphere. Coordinated waters are represented by *red* spheres. Other water molecules are represented by *red* three-dimensional crosses, and coordination bonds are represented by *black* dashed lines. A *grey* sphere represents lead. Comparing the distribution of atoms and electrons during the normal P5N-1 cycle with the lead bearing cleft shows marked differences as one or two oxygen atoms are absent; the arrangement of amino acids' R groups is markedly different. It is thus not surprising that the cationic activity of the cleft is greatly hindered by lead, which interacts with the surrounding atoms quite differently. (Figure image taken from Bitto, 2006)

DNA and RNA

Lead has been shown to increase the rate of mutations in DNA and RNA by effecting DNA and RNA polymerases. Using *in vitro* replication and transcription systems, it was shown that adding lead to the incubation mixture decreased the fidelity of DNA and RNA polymerases (Sirover, 1976; Hoffman, 1977). In the replication assay,

lead significantly increased the rate of mutations and was subsequently classified as a mutagen (Sirover, 1976; Silbergeld, 2000; Silbergeld, 2003).

To safeguard against replication errors and random mutations, cells possess DNA repair systems. However, lead impairs these systems such that mutations will never be corrected (McNeill, 2004). The nuclease Ape1 is part of a DNA repair system; lead binds to the nuclease, inhibits its operation, and thus allows the accumulation of mutagenic damages. Lead first causes replication infidelity, increasing the rate of mutations, and then prevents the correction of errors, compounding the risk of deleterious genetic alterations. Mutations in DNA are particularly dangerous because all daughter cells will have altered DNA, including somatic and germline cells. Somatic mutations can lead to cancer, while germline mutations can cause parental infertility or birth defects in offspring. Such mutations may explain why men chronically exposed to lead have decreased sperm concentrations and total sperm count (Alexander, 1996; Robins, 1997). Children of lead poisoned parents also have higher rates of learning disabilities even though their parents had normal BLLs when procreating (Hu, 1991).

Besides altering DNA polymerase function, lead was shown to increase RNA polymerase infidelity, upsetting transcription. Inaccurate transcription will lead to the production of mutant mRNA and subsequently mutant proteins (Hoffman, 1977). This can disrupt normal cell cycle regulation and metabolism, eventually leading to apoptosis. Lead has been shown to stimulate cell death, particularly in the central nervous system (Fox, 1997, Oberto, 1996).

Future Research

Despite investigation into lead's molecular toxicity being a recent focus of research, there has been significant headway in the field. The data are elucidating the methods by which lead disrupts normal function of essential proteins. Lead compromises the ability of proteins needed for nervous function, blood formation, and maintaining intact DNA. Not surprisingly, many of the symptoms associated with lead poisoning arise in systems dependent on these proteins. However, many of the assays are *in vitro*, and have yet to be confirmed in animal models or human *in vivo* experiments. Instead of just exposing purified proteins to lead in a test tube, it would be insightful to expose animals to lead, purify a protein of interest, and demonstrate compromised function. Such assays would show that lead was able to reach the protein and interact with it under physiologic conditions. *In vivo* assays would also allow researchers to directly associate compromised protein function with cellular and organismal phenotypes. Further use of animal models and study of human cadavers will help to close this research gap.

Another area of potential work would include studying the effect of lead on global gene function using microarray techniques. Previous studies were aimed at specific genes that one would expect to be hindered by lead because of their signal transduction pathway, the gene product's association with cations, or the gene's role in the body. However, there may be genes whose expression is greatly altered by lead that one would not expect initially. A global look at protein production would allow us to identify such genes and help us to understand the diverse ways lead can alter cell cycle regulation, metabolism, and protein cascades.

Insights into Lead-Associated Conditions

Continued research in the field holds promise in providing a cause for diseases such as Alzheimer's, osteoporosis, and criminal behavioral disorders. Lead exposure has been investigated as having a possible environmental link to Alzheimer's disease (AD) pathogenesis, particularly because AD appears sporadically in populations. Genetically linked diseases tend to have heritable patterns, but conditions induced by environmental factors lack such predictability. AD is one such disease that appears sporadically in populations without a clear genetic linkage, and lead's effect on the central nervous system pinpointed it as a possible initiator of AD.

Epidemiologists, however, did not hold the same hypothesis; they did not consider the possibility that childhood lead exposure could impact disease processes whose symptoms do not manifest until old age (Basha, 2005). As a result, there have been few epidemiological studies to ascertain whether there is an association between lead and AD. Other epidemiological studies have established associations between lead poisoning and other central nervous system disorders such as Parkinson's disease and amyotrophic lateral sclerosis (Gorell, 1999; Kamel, 2002).

Scientists have, however, studied the association between lead exposure and AD using *in vitro* and *in vivo* analyses. AD is associated with deficiencies of chaperone proteins needed to assist in protein folding in the endoplasmic reticulum. In lead exposed astrocytes, cells were found to have a marked deficiency of chaperone proteins (White, 2007). Without chaperones, functional, correctly folded proteins form at a lower rate, leading to a myriad of cellular problems such as disrupted regulation, proliferation, and cellular organization.

In AD affected brains, tissue is riddled with amyloid plaques and associated proteins (White, 2007; Wu, 2008). The plaques are primarily made up of β -amyloid ($A\beta$), a short peptide cleaved from the $A\beta$ precursor protein (APP). To assay lead's effect on APP expression, young rats were transiently exposed to lead (Basha, 2005). This stimulated a temporary rise in APP expression, but it shortly returned to normal. However, there was a marked increase in APP expression, as the animals approached old age, leading to increased production of the $A\beta$ cleavage product and age-dependent development of AD (Basha, 2005). When rats were exposed to lead only in adulthood or old age, there was no increase in APP expression, indicating that time of lead exposure may be determining factor in AD development.

In rats and monkeys, developmental exposure was associated with the formation of amyloid plaques and other pathological features of AD in aged animals (Wu, 2008). Primates have a similar central nervous system to humans, and exhibit AD-like pathology in old age (Price and Sisodia, 1994). Thus, the higher incidence of AD pathology in lead-exposed primates suggests that childhood lead poisoning could be a contributing factor to the development of human AD in old age (Wu, 2008).

Because of lead's pervasive effect on nervous system function, the tie between neurological dysfunction and lead exposure is not surprising. Similarly, lead's storage in bone suggests that it may contribute to bone-related disease such as osteoporosis. The increased rate of bone turnover associated with osteoporosis can also release bone lead burdens into circulation, leading to recurrent lead poisoning, and potentially accelerating osteoporosis further (Shannon, 1988). Substantial epidemiologic, *in vitro*, and *in vivo* studies have been undertaken to explore the tie between osteoporosis and lead poisoning.

It was found that lead indirectly alters bone cell differentiation and function by altering plasma levels of calciotropic hormones (Goyer, 1994). Because lead can disrupt calcium-mediated signal cascades, lead-exposed bone cells may also be unable to respond normally to hormones that control proliferation and activity. In accordance with this, rats with lead exposure were found to have defective bone remodeling, altered growth plate thickness due to a loss of proliferating cells, and disorganization of growth plate architecture (Hamilton and O'Flaherty, 1994). Lead exposure has also been shown to delay fracture healing in mice and induce fibrous disunions (Carmouche, 2005). More importantly, environmental lead exposure is associated with a decrease in bone mineral density in both rats and humans (Campbell, 2007; Campbell, 2004). The loss of bone density and alteration of structural integrity of bone is associated with osteoporosis and thus, lead may serve as an important determinant in osteoporosis' pathogenesis.

Finally, the effects of lead on cognitive function and behavior lead researchers to look at ties between aggressive behavioral disorders and childhood lead poisoning. For years, mothers had observed that their children's behavior permanently worsened after lead exposure (Wakefield, 2002). It was not until recently that their observations were taken seriously, as lead intoxication has been reported as the variable with the highest predictive value for the development of criminal behavior (Dietrich, 2002; Stretesky, 2001; Stretesky and Lynch, 2004). When researchers looked at BLL and the number of reported delinquent acts from the lowest levels of exposure to the highest, there was a linear relationship (Wakefield, 2002). However, children raised with high lead exposure also tend to have compromised social and economic circumstances, which expose them to risks that may add or multiply the risks posed by lead or toxic substance exposure

(Wakefield, 2002). As a result, it is difficult to establish a clear cause and effect relationship, and the nature of the relationship between lead and crime rate is still being debated (Garza, 2006).

Concluding Thoughts

An expanded knowledge of lead's molecular toxicity not only holds promise for understanding the origin of symptoms and systemic disease, but also may be useful for clinical treatment of lead poisoning. Currently, the only proven, recommended treatment for lead poisoning is to reduce exposure (Goldman, 2008). Doctors will also prescribe cation-chelating agents for patients with exceptionally elevated BLLs. Otherwise, patients must prevent further exposure and wait for their BLL to drop to normal through urinary excretion of lead. Once we know the most basic mechanisms of lead's toxicity, more potent, sophisticated treatments can be developed to combat the neurologic and oncogenic effects and also limit storage and release of lead. Such medications would be particularly pertinent for pregnant women with large bone lead stores who have no means of preventing lead release during pregnancy and breast feeding. Additionally, discovering the role of lead exposure in the development of AD, osteoporosis, and behavioral disorders may lead to prevention and improved treatment methods. Research into lead's molecular toxicity is still at its beginning and has the potential to offer fascinating insights into a wide array of associated conditions and potential treatments.

Bibliography

- Agency for Toxic Substances and Disease Registry. Case Studies in Environmental Medicine (CSEM): Lead Toxicity Cover Page.
www.atsdr.cdc.gov/csem/lead/pbcover_page2.html (Accessed on May 2, 2008).
- Alexander, B., et al. (1996), Semen quality of men employed at a lead smelter. *Occup Environ Med*, 53, 411.
- Basha, M. et al. (2005), The fetal basis of amyloidogenesis: exposure to lead and latent overexpression of amyloid precursor protein and beta-amyloid in the aging brain, *J Neurosci*, 25, 823-829.
- Bitto, E. et al. (2006), Structure of Pysrimidine 5'-Nucleotidase Type 1: insight into mechanism of action and inhibition during lead poisoning, *J of Bio Chem*, 281:29, 20521-20529.
- Bouton, C. et al. (2001), Synaptotagmin is a molecular target for lead, *J Neurochem*, 76, 1724-1735.
- Buzard, G. and K. Kasprzak (2000), Possible roles of nitric oxide and redox cell signaling in metal-induced toxicity and carcinogenesis: a review. *J Environ Pathol Toxicol Oncol*, 19, 179.
- Campbell, N. and J. Reese (2002), *Biology*, 6th ed, San Francisco: Benjamin Cummings. p.1033-1034
- Campbell, J. and P. Auinger (2007), The association between blood lead levels and osteoporosis among adults- Results from the third national health and nutritional examination survey (NHANES III), *Environ Health Perspect*, 115:7, 1018-1022.
- Campbell, J. et al. (2004), The association between environmental lead exposure and bone density in children, *Environ Health Perspect*, 112:11, 1200-1203.
- Carmouche, J. et al. (2005), Lead exposure inhibits fracture healing and is associated with increased chondrogenesis, delay in cartilage mineralization, and a decrease in osteoprogenitor frequency, *Environ Health Perspect*, 113:6, 749-755.
- Chattopadhyaya, R. et al. (1992), Calmodulin structure refined at 1.7 Å resolution, *J Mole Biol*, 228, 1177-1192.
- Cloues, R., Cibulsky, R., and W. Sather (2000), Ion interaction in the high-affinity binding locus of a voltage-gated Ca²⁺ channel, *J Gen Physiol*, 116, 569-586.
- Dietrich, K. et al. (2001), Early exposure to lead and juvenile delinquency, *Neurotoxicol Teratol*, 23, 5110518.

- Erkshine, P. et al. (1997), X-ray structure of 5-aminolaevulinic dehydrase, a hybrid aldolase, *Nat Struct Biol*, 4, 1025-1031.
- Fischbein, A. (1998), Occupational and environmental exposure to lead. In: Environmental and Occupational Medicine, Rom, WN (Ed), Philadelphia, Lippincott-Raven Publishers, p.973.
- Fox, DA, Campbell, ML, and Y. Blocker, (1997), Functional alterations and apoptotic cell death in the retina following developmental or adult lead exposure, *Neurotoxicol*, 18, 645-664.
- Fox, D., and L. Chu, (1988), Rods are selectively altered by lead: II. Ultrastructure and quantitative histology, *Exp. Eye Res*, 46, 613-625.
- Fox, D., and M. Katz (1992), Developmental lead exposure selectively alters the scotopic ERG component of dark and light adaptation and increases rod calcium content, *Vision Res*, 32, 249-255.
- Frausto da Silva J. and R. Williams (2001), The Biological Chemistry of the Elements: the Inorganic Chemistry of Life. 2nd. New York: Oxford University press.
- Fu, H. and P. Boffetta (1995), Cancer and occupational exposure to inorganic lead compounds: a meta-analysis of published data, *Occup Environ Med*, 52: 2, 73-81.
- Garza, A., Vega, R., and E. Soto (2006), Cellular mechanisms of lead neurotoxicity, *Med Sci Monit*, 12:3, RA57-65.
- Ghering, A. et al. (2005), Spectroscopic and functional determination of the interaction of Pb²⁺ with GATA proteins, *J Am Chem Soc*, 127, 3751-3759.
- Godwin, H. (2001), The biological chemistry of lead, *Bio-inorganic Chem*, 5, 223-227.
- Goldman, R. et al. (1994), Lead poisoning from mobilization of bone stores during thyrotoxicosis. *Am J Ind Med*, 25, 417.
- Goldman, R. et al. (2008), Adult lead poisoning, *Up-to-date*, 1-9.
- Gorell, L. et al. (1999), Occupational exposure to manganese, copper lead, iron, mercury, and zinc and the risk or Parkinson's disease. *Neurotoxicol*, 20, 239-247.
- Goyer, R. et al. (1994), Environmental risk factors for osteoporosis, *Environ Health Perspect*, 102: 4, 390-394.

- Gulson, B. et al. (1998), Mobilization of lead from the skeleton during the postnatal period is larger than during pregnancy, *J Lab Clin Med*, 131, 324.
- Hamilton, J. and O'Flaherty (1994), Influence of lead on mineralization during bone growth, *Toxicol Sci*, 26:2, 265-271.
- He, L. et al. (2000), Lead and calcium produce rod photoreceptor cell apoptosis by opening the mitochondrial permeability transition pore, *J Bio Chem*, 275:16, 12175-12184.
- Hoffman, D. and S. Niyogi (1977), Metal mutagens and carcinogens affect RNA synthesis rates in a distinct manner. *Science*, 198, 513.
- Holtzman, D. and J. Hsu (1976), Early effects of inorganic lead on immature rat brain mitochondrial respiration, *Pediatr Res*, 10, 70.
- Holtzman, D., Shen, J., and P. Mortell (1978), In vitro effects of inorganic lead on isolated rat brain mitochondrial respiration, *Neurochem Res*, 3:2, 195-206.
- Hammond, P. (1977), Exposure of humans to lead. *Annu Rev Pharmacol Toxicol*, 17, 197.
- Hryhorczuk, D. et al. (1985), Elimination kinetics of blood lead in workers with chronic lead intoxication. *Am J Ind Med*, 8, 33.
- Hu, H. (1991), Knowledge of diagnosis and reproductive history among survivors of childhood plumbism. *Am J Public Health*, 81, 1070.
- Kamel, F. et al. (2002), Lead exposure and amyotrophic lateral sclerosis, *Epidemiology*, 13, 311-319.
- Kim, K. et al. (2000), Immediate Early Gene Expression in PC12 Cells Exposed to Lead: Requirement for Protein Kinase C, *J Neurochem*, 74:3, 1140-1146.
- Koller, L., Kerkvliet, N., and J. Exon (1985), Neoplasia induced in male rats fed lead acetate, ethyl urea, and sodium nitrite, *Toxicol Pathol*, 13,50.
- Kern, M. et al. (2000), Inorganic lead and calcium interact positively in activation of calmodulin, *Neurotoxicology*, 21, 353-363.
- Kuboniwa, J. et al. (1995), Solution structure of calcium-free calmodulin. *Nat Struct Biol*, 2, 768-776.
- Levin, SM, and M. Goldberg (2000), Clinical evaluation and management of lead-exposed construction workers. *Am J Ind Med*, 37, 23.

Lidsky, T. and J. Schneider. (2003), Lead neurotoxicity in children: basic mechanisms and clinical correlates, *Arch Intern Med*, 126:1, 5-19.

Lodish, H. et al. (2004), *Molecular Cell Biology*, 5th Ed., New York: W.H. Freeman & Co.

Lustberg, M. and E. Silbergeld (2002), Blood lead levels and mortality, *Arch Intern Med*, 162: 21, 2443–2449.

Manalis, R. and G. Cooper (1973), Presynaptic and postsynaptic effects of lead at the frog neuromuscular junction, *Nature*, 243, 354-356.

Markovac J. and G. Goldstein (1988), Picomolar concentrations of lead stimulate brain protein kinase C, *Nature*, 334, 71-73.

McNeill, D. et al. (2004), Inhibition of Ape1 nuclease activity by lead, iron, and cadmium, *Environ Health Perspect*, 112, 799-804.

Medrano, C., and Fox, D. (1994), Substrate-Dependent Effects of Calcium on Rat Retinal Mitochondrial Respiration: Physiological and Toxicological Studies, *Toxicol. Appl. Pharmacol*, 125, 309–321.

Miller, J. et al. (1970), Lead and delta-aminolevulinic acid dehydrase levels in mentally retarded children and in lead-poisoned suckling rats, *The lancet*, 2, 695-698.

Newton, A. (1995), Protein kinase C: structure, function, and regulation, *J Biol Chem*, 270, 28495-28498.

Nriagu, JO, (1983), Saturnine gout among Roman aristocrats. Did lead poisoning contribute to the fall of the Empire?. *N Engl J Med*, 308, 660.

Oberto, A., Marks, N., Evans, H. L., and A. Guidotti (1996), Lead (Pb⁺²) promotes apoptosis in newborn rat cerebellar neurons: pathological implications, *J Pharmacol Exp Ther*. 279:1, 435–442.

Payne, J., ter Horst, M., and H. Godwin. Lead fingers: Pb(II) binding to structural zinc-binding domains determined directly by monitoring lead-thiolate charge-transfer bands, *J Am Chem Soc*, 121, 6850-6855.

Price, D. and S. Sisodia (1994), Cellular and molecular biology of Alzheimer's disease and animal models, *Annu Rev Med*, 45, 435-446.

Quintanilla-Vega, B. et al. (2000), Lead interaction with human protamine (HP2) as a mechanism of male reproductive toxicity, *Chem Res Toxicol*, 13, 594-600.

- Rees, D., Duley, J., and A. Marinaki (2003), Pyrimidine 5'nucleotidase deficiency, *Br J Haematol*, 120, 375.
- Riess, ML and J. Halm (2007), Lead poisoning in an adult: lead mobilization by pregnancy?, *J Gen Intern Med*, 22, 1212.
- Robins, T. et al. (1997), Semen quality and fertility of men employed in a South African lead acid battery plant. *Am J Ind Med*, 32, 369.
- Rabinowitz, MB. (1991), Toxicokinetics of bone lead, *Environ Health Perspect*, 91, 33.
- Scortegagna, M., and Hanbauer, I. (1997), The effect of lead exposure and serum deprivation on mesencephalic primary cultures, *Neurotoxicology* 18, 331–339.
- Shannon, M. et al. (1988), Recurrent lead poisoning in a child with immobilization osteoporosis, *Vet Hum Toxicol*. 30:6, 586-588.
- Silbergeld, E. (2003), Facilitative mechanisms of lead as a carcinogen, *Mutation Res*, 533, 121-133.
- Silbergeld, E., Waalkes, M., and J. Rice (2000), Lead as a carcinogen: Experimental evidence and mechanisms of action, *Amer J of Industrial Med*, 38:3, 316-323.
- Simmons, T. (1995), The affinity of human erythrocyte porphobilinogen synthase for Zn²⁺ and Pb²⁺, *Eur J Biochem*, 234, 178-183.
- Sirover, M. and L. Loeb (1976), Infidelity of DNA synthesis in vitro: screening for potential metal mutagens or carcinogens. *Science*, 194, 1434.
- Scortegagna, M., and Hanbauer, I. (1997), The effect of lead exposure and serum deprivation on mesencephalic primary cultures, *Neurotoxicology* 18, 331–339.
- Stretesky, P., and M. Lynch (2004), The relationship between lead and crime, *Ann Rev of Med*, 55, 209-222.
- Stretesky, P. and M. Lynch (2001), The relationship between lead exposure and homicide, *Arch Pediatr Adolesc Med*, 155, 579-582.
- Trope, I. et al. (2001), Exposure to lead appears to selectively alter metabolism of cortical gray matter. *Pediatrics*, 107, 1437.
- Trope, I., Lopez-Villegas, D., and R. Lenkinski (1998), Magnetic resonance imaging and spectroscopy of regional brain structure in a 10-year-old boy with elevated blood lead levels. *Pediatrics*, 101, E7.
- Wakefield, J. (2002), The lead effect?, *Environ Health Perspect*, 110, A574-A580.

White, L. et al. (2007), New and evolving concepts in the neurotoxicology of lead, *Tox Applied Pharm*, 225, 1-27.

Wu, J. et al. (2008), Alzheimer's disease (AD)-like pathology in aged monkeys after infantile exposure to environmental metal lead (Pb): evidence for a developmental origin and environmental link for AD, *J Neurosci*, 28:1, 3-9.

Acknowledgments

I would like to thank Professor Bowring for all of the geology and life lessons he has taught me. I would also like to thank the EAPS department for making my time at MIT amazing; I am so glad I chose to major in course12! Finally, I would like to thank my Mom for all of her support and love.