Hemopoietic, Hemostatic and Mutagenic Effects of Lead and Possible Prevention by Zinc and Vitamin C

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Abstract: Lead is used extensively in industrial and community settings and hence exposure to it is inevitable. The exposure occurs through respiratory or gastro-intestinal route or through skin due to contaminated air, food and water. Acute toxicity of lead is well known for its haematological, neurological and renal effects but of serious concern is chronic exposure to low-level lead exposure associated with cognitive dysfunction, neurobehavioral disorders, hypertension, cardiovascular abnormalities, chromosomal aberrations, somatic and germ cell mutations and genotoxicity. Chelation is the conventional recommendation in the case of blood lead levels associated with acute toxicity. Chronic toxicity can be prevented and even treated with alternate non-pharmacological methods such as dietary or herbal agents. In this article hemopoietic, hematotoxic, hemostatic and genotoxic effects of lead will be highlighted. In addition, possible preventive role of dietary components such as zinc and vitamin C will be discussed

**Key words**: Lead, heme synthesis, drug metabolism, blood coagulation, zinc, vitamin C

Lead, the 5th most abundant metal in the earth's crust, is known to man since the beginning of the civilization. Due to its versatile properties like ductility, high resistance to erosion and corrosion, lead has been used by man since centuries for making tools, pots, statues, water pipes and other hardware. In the modern civilization lead has found a variety of industrial applications by humankind. As a consequence, today it has become one of the most widely distributed pollutant in environment. Lead is not biodegradable and therefore persists in the soil, in the air, in drinking water, and in homes. It continues to accumulate where it is deposited and get biomagnified to toxic levels in animals and human beings. The main sources of human exposure to lead include leaded gasoline, industrial processes such as lead smelting and coal combustion, lead-based paints, lead-containing pipes or lead-based solder in water supply systems, battery recycling, making grids and bearings etc., Vehicular traffic was the single largest source of environmental lead pollution in the world including India until its use in gasoline was stopped. Introduction of unleaded petrol in the year 2000 has resulted in substantial lowering of mean blood lead levels in Indian population. Exposure to lead occurs mainly through the respiratory and gastrointestinal routes. Approximately 30-40 percent of inhaled lead is absorbed into the bloodstream [1]. Gastrointestinal absorption varies depending on nutritional status and age. Infants can absorb up to 50 percent of lead ingested from food, water, contaminated dust, or soil, while adults absorb only 10-15 percent [2]. Inorganic lead (food, water, paint, toys, vinyl products) is minimally absorbed through the skin, but tetraethyl or alkyl-lead (leaded gasoline), which is still legally allowed in aircraft, watercraft, and farm machinery is absorbed through the skin [3]. Once absorbed, 99 percent of circulating lead is bound to erythrocytes for approximately 30-35 days

(only one percent of absorbed lead is found in plasma and serum) and is dispersed into the soft tissues - liver, renal cortex, aorta, brain, lungs, spleen and following 4-6 weeks exposure in teeth and bones. Due to the short half-life (35 days) in the bloodstream, blood lead levels cannot be used to diagnose or rule out evidence of exposure that occurred more than six weeks prior to testing [4].In adults approximately 80-95 percent of retained lead is stored in the bone, while in children approximately 70 percent is stored in bone, resulting in more soft tissue lead in children compared to adults. Lead is stored in bone for extended periods of time, with half-life estimates of 20-30 years. Toxicity of Lead has been known for thousands of years. Greek physicians made the first clinical description of lead poisoning in the first century B.C. Hippocrates described lead colic in 370 B.C. Lead is a systemic poison and can adversely affect every body system to which it is distributed. neurologic, hematopoietic, gastrointestinal, renal and immunological systems are most often involved. Reproductive abnormalities, mutagenicity and carcinogenicity have also been reported in animals and industrial workers. Abnormalities and clinical signs may vary with species, and dose and duration of exposure. Signs and symptoms of lead toxicity in man – anorexia, abdominal pain, anaemia, hyperactivity and inattentiveness are quite non-specific and hence are often overlooked and hence remain undiagnosed. At high levels, lead poisoning causes coma, convulsions and death. At low levels - levels far below those that present obvious symptoms - lead poisoning in childhood causes reductions in IQ and attention span, reading and learning disabilities, hyperactivity, impaired growth, behavioral problems, and hearing loss. These effects are long-term and may be irreversible. Diagnosis of lead toxicity has traditionally been based on significantly elevated blood lead levels. However, data now implicates low-level exposures and blood lead levels previously considered normal as causative factors in cognitive dysfunction, neurobehavioral disorders, neurological damage, hypertension, renal impairment, chromosomal aberrations, somatic and germ cell mutations and genotoxicity. Chelation is the conventional recommendation in the case of blood levels associated with acute toxicity and encephalopathic damage. Chronic toxicity can be prevented and even treated with alternate nonpharmacological methods such as dietary or herbal agents.This paper will mainly discuss hemopoietic, hemostatic and genotoxic effects of lead and possible prevention by Zinc and Vitamin C.

Hemopoietic Effect: Anaemia as a serious haematological manifestation of lead toxicity was reported as early as 1831 by Laennec and role of lead in porphyrin biosynthesis was known since 1895. Lead can induce two types of anemia, often accompanied by basophilic stippling of the erythrocytes [5]. Acute high-level lead exposure has been associated with hemolytic anemia. Frank anaemia is manifestated only when the blood lead level is significantly elevated for prolonged periods. In chronic lead exposure, lead induces anemia by both interfering with heme biosynthesis and by diminishing red blood cell survival. Hemoglobin levels begin to decline at lead levels of 40-60 µg/dl and prevalence of anaemia increases with increase in blood lead level. However, the correlation between blood lead levels and haemoglobin levels are low (Values of -0.15 and -0.16 have been reported by Tola et

al., 1973 [6]. Frank anaemia, which is a result of reduced hemoglobin production and shortened life span of erythrocytes, is seen in adults at blood lead concentrations of 80µg/dL and in children at concentrations of 70 µg/dL. The anaemia in lead-exposed individuals is of the hypochromic and normocytic (also microcytic) type and is accompanied by reticulocytosis with basophilic stippling. The shortened life span of erythrocytes is due to increased fragility of the blood cell membrane and reduced hemoglobin production is due to decreased levels of enzymes involved in heme synthesis[7]. The key enzymes involved in the synthesis of heme are - aminolevulinic acid synthetase (ALAS), a mitochondrial enzyme that catalyzes the formation of - aminolevulinic acid (ALA) from glycine and succinyl CoA, and ALA dehydratase (ALAD), a cytosolic enzyme that catalyzes formation of porphobilinogen. Through a series of steps, coproporphyrin and protoporphyrin are formed from porphobilinogen, and, finally, the mitochondrial enzyme ferrochelatase catalyzes the insertion of iron into protoporphyrin to form heme. Lead inhibits the body's ability to make hemoglobin by interfering with several enzymatic steps in the heme pathway. Specifically, lead decreases heme biosynthesis by inhibiting ALAD and ferrochelatase activity. ALAD inhibition is first noted at blood lead levels of 10-20 µg/dl but heme biosynthesis is not decreased until the activity of ALAD is inhibited by 70-80% [6]. Ferrochelatase, the enzyme which catalyses transfer of iron from ferritin to protoporphyrin to form heme, is another enzyme in heme biosynthetic pathway that is inhibited by lead. Its inhibition causes increased excretion of coproporphyrin in urine and accumulation of protoporphrin in erythrocytes (EP). EP levels begin to increase at blood lead concentration of 15µg/dl [8].

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Figure I
Haem Synthesis Pathway

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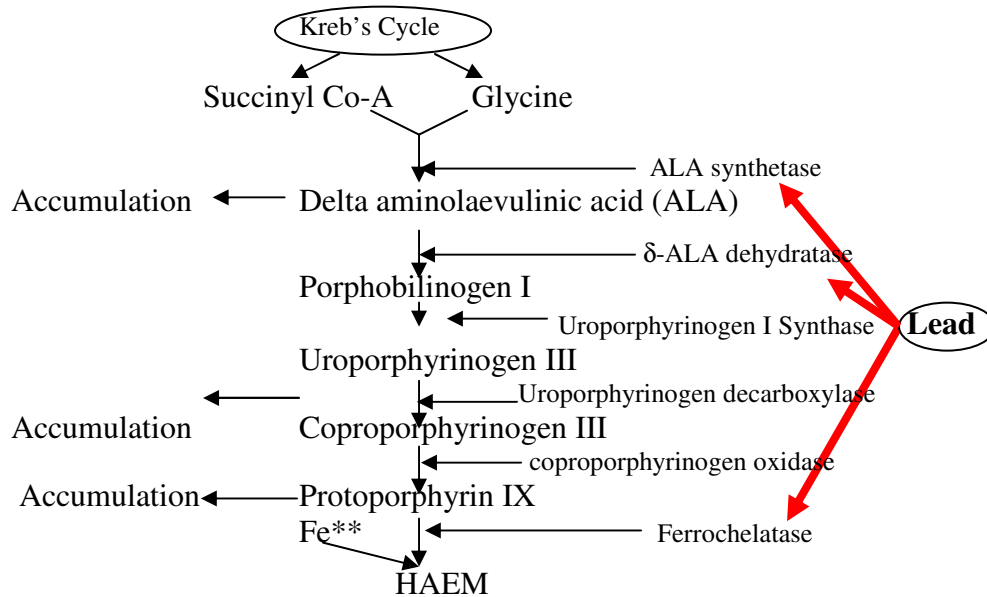
ALA synthetase, ALA dehydratase and ferrochelatase are the three enzymes inhibited to the greatest degree by lead. Other intermediary enzymes such as uroporphyrinogen decarboxylase and coproporphyrinogen oxidase may also be affected.As erythrocyte ALAD activity is inhibited, ALAS activity is stimulated; therefore, ALA levels in blood are increased, leading to elevated levels of ALA in urine. The threshold for detecting elevated ALAS activity and blood and urinary ALA levels is 40 µg/dl in both adults and children, but evidence indicates that the threshold may be as low as 15-20 µg/dl. Erythrocyte ALAD is one of the most sensitive indicators of exposure to lead. The threshold blood lead level for ALAD activity is less than 10 µg/dl. Coproporphyrin levels are elevated in individuals with blood lead concentrations of 40 µg/dl. Although erythrocyte ALAD activity may be the most sensitive measure of lead exposure, detection of erythrocyte zinc protoporphyrin is probably the most reliable indicator of lead exposure, because it is a measure of exposure due to the mobilizable fraction (toxicologically active fraction) of bone lead. In lead-exposed individuals, zinc is inserted into the porphyrin moiety instead of iron. The threshold for detecting elevated zinc protoporphyrin levels is 25-30 µg Pb/dl of blood in adults and 16 µg Pb/dl in children. Patil et al., 2006 reported decreased hemoglobin and increased erythrocyte- zinc protoporphyrin levels in blood and increased urinary ALA and porphobilinogen (PBG-U) levels in workers employed in battery manufacturing unit for over 15 years[9]. However, there was no significant change in activated ALAD activity despite high levels of lead in blood (in the range of 25.8 – 78.0 μg/dl (mean ± SD, 53.63 ± 16.98) in exposed group (n=28). In unexposed control group (n = 35) the blood lead levels were in the range of 2.8 – 22.0 µg/dl (mean ± SD, 12.52 ± 4.08). Susceptibility to lead may depend upon the allelic form of ALAD, an individual inherits as $ALAD_2$ binds more strongly to lead as compared to $ALAD_1$.In-vivo studies in animals have shown that lead like many other metals such as cobalt, nickel, tin and cadmium induces heme oxygenase, an enzyme involved in catabolism of heme which could exacerbate deficiency of heme. However, whether occupational exposure to lead can also induce heme oxygenase is not certain. nhibition of heme synthesis by lead has implications on many other processes in the body as heme is an integral part of myoglobin, catalase, mitochondrial and microsomal cytochromes, tyrosine hydroxylase, nitric oxide synthase etc., Hence heme related functions such as mitochondrial respiration, microsomal drug metabolism and neurotransmitter synthesis may also get compromised due to lead toxicity.

Effect of Lead on Drug Metabolism: Interference in heme synthesis by lead may affect availability of heme, the prosthetic group of hemoprotein- cytochrome P450, the terminal oxidase in mixed function oxidase system involved in catabolism of drugs. The ability of lead to affect hepatic cytochrome P450 levels and mixed function oxidase enzyme activities microsomal is well documented, particularly following acute injections of the metal [10 -13].In these studies, it was found that intraperitoneal lead acetate increased hexabarbital sleeping time, decreased cytochrome P450 and b5 levels and aminopyrine demethylase and aniline hydroxylase activities. The observed decrease in P450 levels in metal treated animals

is mostly due to decrease in ALAS activity and increase in microsomal heme oxygenase activity, a primary enzyme in heme degradation [14]. Data suggesting that microsomal enzyme activities are affected in humans with known exposures to metals are limited; however, in studies of chronic lead toxicity children showed decreased ability to metabolize drugs [15-16]. Also, in acute lead intoxication in adults, Meredith and co-workers (1977) and Fischbein et al., (1977) have reported increases in in-vivo drug metabolism rates following chelation therapy[17-18]. Cytochrome oxidase activity was reported to be depressed in muscle biopsies obtained from patients with lead exposure with acute porphyria [12].Another aspect of heme deficiency relates to its requirement for tryptophan pyrrolase activity an enzyme needed for tryptophan degradation [19]. Any decrease in tryptophan pyrrolase activity may be associated with increase in tryptophan levels and 5-hydroxy tryptamine giving rise to serotonin syndrome.

The activity of another erythrocyte enzyme, pyrimidine-5-nucleotidase (Py-5-N), is significantly reduced in lead-exposed individuals at blood levels of 5 to 30 µg/dl. The consequence of reduced Py-5-N activity is thought to be accumulation of cellular nucleotides, reduced erythrocyte stability and survival, and reduced mRNA and protein synthesis related to production of the globulin chain. Furthermore, inhibition of erythrocyte Py-5-N activity may be indicative of a widespread impact on pyrimidine metabolism in other tissues besides blood. The possibility of generalized effects on pyrimidine metabolism and heme biosynthesis has serious implications regarding the health hazards of very low levels of lead, especially in children.

Effects of Lead on Hemostasis: Blood coagulation abnormalities in lead exposed individuals have not been studied much. In 1955, Saita and co-workers reported a decrease in prothrombin time and Factor VII in workers exposed to lead, which normalized after a single injection of vitamin K[20]. A similar impairment in blood coagulation was reported in workers in storage battery plant. Experimental studies in rats and rabbits have also been reported. Few studies on cardiovascular effects of lead have shown that chronic lead exposure causes hypertension and cardiovascular disease by promoting oxidative stress, limiting nitric oxide availability, impairing nitric oxide signalling, augmenting adrenergic activity, increasing endothelin production, altering the renin-angiotensin system, raising vasoconstrictor prostaglandins, lowering vasodilator prostaglandins, promoting inflammation, disturbing vascular smooth muscle Ca^{2+} signalling, diminishing endothelium-dependent vasorelaxation and modifying the vascular response to vasoactive agonists. Moreover, lead has been shown to cause endothelial injury, impede endothelial repair, inhibit angiogenesis, reduce endothelial cell growth, suppress proteoglycan production, stimulate vascular smooth muscle cell proliferation and phenotypic transformation, reduce tissue plasminogen activator, and raise plasminogen activator inhibitor-1 production. Several studies have focused on the interaction of lead with cellular Ca^{2+} and Ca^{2+}-dependent signalling pathways [21-24]. These investigations revealed that lead can potentially compete with Ca^{2+} for the transport systems, such as channels and pumps involved in physiological movements of ions, particularly Ca^{2+}, into and out of the cell [25] Shin et al., (2007) suggested that lead exposure can provoke procoagulant activity in erythrocytes by

Phosphatidylserin exposure, contributing to enhanced clot formation [26]. Exposure of the cellular constituents of the sub-endothelial tissue to lead appears to shift the balance of fibrinolytic and anti-fibrinolytic forces in favor of the latter, thereby raising the risk of thrombosis

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Table- 1

Effects of Lead on Coagulation
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- Endothelial injury….. Facilitation of coagulation via contact with subendothelial tissue
- Reduced nitric oxide availability…. Increased platelet adhesion and Aggregation
- Reduced tissue plasminogen activator….. Inhibition of fibrinolysis
- Increased Plasminogen activator inhibitor-1   production…. Inhibition of fibrinolysis

Mutagenic effects of lead:

Lead has been suspected to be mutagenic and carcinogenic in human beings but cytogenic studies performed on peripheral blood lymphocytes present a confusing picture. In-vitro studies with Chinese hamster V79 cells showed that lead is a weak mutagen [27]. Studies using human cells dosed with lead *in vitro* have suggested that lead ions decrease the fidelity of DNA synthesis or repair (Skreb et al., 1975*)* and inhibit the activity of DNA polymerase β and ligase [28]. It cross-links proteins and DNA, binds to the phosphate groups of DNA and changes its conformation [29]. Montaldi (1985) asserted that the covalent binding of lead ions and DNA, chromosomal protein, DNA polymerase, or substrate nucleotide precursors might jeopardize DNA replication and repair or expression of genes [30]. DNA impairment and genotoxic effects may be magnified when repair is inhibited. In an *in vitro* study, Loeb and Mildvan, 1981 added lead chloride to the cell suspension [31]. They found that lead oxide disrupted DNA synthesis by increasing the frequency of DNA synthesis error, thus undermining the accuracy of RNA synthesis. Hartwig et al. (1994) suggested that lead may inhibit DNA repair; they postulated that the mechanism might involve the interaction of lead and repair enzymes (*e.g.*, polymerase or ligase) or calcium-regulated interference and that these effects were related to DNA synthesis and repair [32]. Regarding the ability of lead to produce chromosomal aberrations in the somatic cells of people occupationally or accidentally exposed to lead some positive reports have been obtained in industrial workers, people living in contaminated areas and bus drivers. The investigations carried out in the peripheral lymphocytes of industrial workers [33], painters exposed to lead-containing pigments [34] and battery plant workers [35] showed a significant increase in the frequency of chromosomal aberrations in the workers when compared to the controls. Significant increase in frequency of chromosomal aberrations with increasing years of lead exposure (1%, 2.2% and 4.8%) suggested lead as the causal factor for genotoxicity. There was significant increase in mean chromosomal aberrations (lead exposed 2.2% Vs control 0.8%), micronuclei (lead exposed

10.48/1000 cells Vs control 1.82/1000 cells) and sister chromatid exchanges (10.21/cell Vs control 4.2/cell) in lead exposed population. Anwar and Kamal (1988) reported significantly higher percentage of chromosomal aberrations as well as the mean sister-chromatid exchange among the traffic policemen than in the control group [36]. Kapka et al., 2007 reported significantly increased frequency of micronuclei in peripheral lymphocytes of the children at blood lead levels of 5.29 ± 2.09 µg/dl as compared to children with blood lead levels of 3.45 ± 1.20 µg/dl, although the levels are much below the value of the biological exposure limit of 10 µg/dl [37]. Chromosomal aberrations in somatic cells may lead to carcinogenesis and those in germ cells to genotoxic consequences

Reproductive and Genotoxicity of Lead:
Effect of lead exposure on male and female reproductive system is analysed by clinical, experimental and epidemiological surveys related to fertility, morphological abnormalities of spermatogenesis, functional integrity of hypothalamus-hypophysis-gonadal axis and chromosomal abnormalities. Due to complexity of such an approach and number of variables involved the results are often contradictory and inconclusive. At very high blood lead levels, lead is a powerful abortifacient. At lower levels, it has been associated with miscarriages and low birth weights of infants [38]. Females employed at lead smelter were more likely to experience miscarriages in later pregnancies if their husbands were also employed at a smelter, suggesting that long-term exposure of the males was required to affect reproductive outcome [38]. Women with blood lead level 5-9 µg/dl were two to three times more likely to have spontaneous abortion than women with blood lead level lesser than 5 µg/dl [39].Studies have shown that pregnancy outcome may be affected by paternal exposure to lead indicating reproductive toxicity of lead on male lead workers, but the results have been inconsistent [40–42]. Lancranjan et al. (1975) observed increases in asthenospermia, hypospermia, and teratospermia in two groups of occupationally exposed workers whose mean blood lead levels were 74.5 and 52.8 µg/dL compared with groups that experienced lower exposures (41 and 23 µg/dl) [43]. Apostoli (1998) remarked that significant effects on reproductive capacity are not seen below a blood lead level of 50µg/dl, but blood lead concentrations of >40µg/dl may affect sperm morphology and function [44]. A high percentage of males exposed to lead have been reported to have childless marriages. The rate of miscarriages and stillbirths was increased, and the male: female ratio of offspring was increased [45-46].Recent studies have focused primarily on the effect of low level paternal exposure to lead on male reproductive parameters and reproductive outcome. Epidemiological survey in battery workers [47] revealed decrease in various reproductive end points like fertility, live births and increase in abortions, still births, congenital malformation, neonatal deaths, etc., among families of lead exposed workers with blood lead levels between 18-28 µg/100 ml. In view of this, reproductive and genotoxicity of lead needs to be re ascertained.

Preventive action of Zinc and Vitamin C on toxicity of lead: Preventive action of any agent could be either due to faster removal of lead from the body for example chelating action or by reversal of inhibitory action of lead on the enzymes such as

ALAS, ALAD, Ferrochelatase and may be other SH dependent enzymes or by compensating for the nutritional deficiencies such as zinc, calcium and vitamin C created by it.Earlier studies have shown that zinc can replace lead from the binding site of ALAD and bring about a conformational change in the enzyme structure so as to restore its activity. Lead has also been shown to reduce tissue levels of zinc and vitamin C. Studies carried out in our laboratory and those in others have shown that zinc and vitamin C can protect against toxic effects of lead by reverting lead induced inhibition of ALAD and UPS, maintaining normal levels of heme and hence microsomal cytochrome P450 required to carry out metabolism of drugs [13,48].Many of the factors of coagulation pathway including factor XIII and fibrinogen contain sulfhydryl groups at the active site. Lead is known to bind sulfhydryl groups and is likely to interfere with its activation. Zinc plays an important role in normal hemostasis. It is needed for normal platelet function particularly arachidonic acid metabolism leading to thromboxane formation. Chronic toxicity to lead causes deficiency of zinc and therefore lead toxicity is likely to impair blood coagulation. Role of vitamin C in blood coagulation was cited as early as 1961 by Dayton and Wenier [49]. It was found to influence platelet function, thromboplastin activity and formation of prothrombin complex. Further lead induced oxidative stress increases the need for vitamin C to scavenge free radicals, regenerate oxidized glutathione and tocopherol and may cause ascorbic acid deficiency. L-ascorbic acid potentiates nitric oxide synthesis in endothelial cells, which is not only a vasodilator but also an inhibitor of platelet aggregation [50]. Studies carried out at DIPAS have shown that lead induced hemorrhagic tendencies in rats administered lead acetate (20 mg/kg bw, ip for 3 consecutive days) as both intrinsic and extrinsic coagulation pathway were inhibited by lead. Supplementation of zinc and vitamin C were able to restore clotting potential of rats. Rudrama et al., 2008 reported that lead is mutagenic and genotoxic in mice and Amla (phylantus) fruit extract which is rich in vitamin C could prevent genotoxic effects in mice somatic cells [35, 51]. Genotoxicity could occur due to lead directly attacking DNA and causing strand breaks [52 -54] or indirectly by generating free radicals which then cause genotoxicity [55].Vitamin C can function in both the cases. It can chelate out lead and thus reduce its effect [56](Goyer and Cherian, 1979). It can also neutralize lead induced free radicals thereby reducing the genotoxicity caused by these radical species[57-58]. Chronic exposure of rats to high lead levels leads to the accumulation of lead in the brain in association with high levels of lipid oxidation products and changes in components of the oxidant defense system [59]). Lucila and Oteiza (2006) demonstrated that Zn deficiency can increase the susceptibility of neurons to lead induced oxidative stress [60]. Hence zinc supplementation would prevent lead induced oxidative stress and its obvious consequences on cellular components including DNA. Reports on benefits accrued by dietary components like zinc and vitamin C supplementation to lead exposed individuals are quite encouraging. In addition there are some other nutritional deficiencies that emerge or get exacerbated during lead toxicity such as that of iron, calcium, glutathione and thiamine. The need of the hour is to carry out longitudinal studies to evaluate potential effectiveness of nutritional interventions to attenuate or reduce lead toxicity.

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