

LEAD CONTENT IN BREAST MILK

by

Susie Wilson Rockway

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SIGNED: Staci Wilson Rockney

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

Charles W Weber

CHARLES W. WEBER

Professor of Nutrition and
Food Science

December 14, 1981

Date

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ABSTRACT

Lead was measured in the milk of lactating women to determine whether quantities posed a toxicological hazard to the suckling infant. Blood and hair of these women were also analyzed to establish a correlation with the lead in the milk and with the environmental lead levels of Tucson, Arizona. Three-day dietary histories were evaluated for their food composition using a computer program, and proximate analysis was done on each milk sample. Mean levels of lead in milk, blood, and hair were found to be 23, 119, and 2,002 ng/ml (ppb), respectively. These levels are not considered high enough to pose a threat to the mother or her child. The mean percentages of fat, solid, moisture, protein, and ash in human milk were found to be 3.16, 12.15, 87.78, 1.52, and 1.54, respectively. Descriptive statistics, correlation coefficients, and bivariate (scatter) plots were obtained for all data.

INTRODUCTION

A variety of environmental toxicants are introduced into the air, soil, and water of industrially active nations. In urban areas, for example, lead is more densely concentrated (i.e., auto exhaust) and contributes greatly to environmental contamination. Man not only inhales lead but ingests it from contaminated plants and water. A potential health hazard exists for lead poisoning because exposure to lead is continuously occurring. Children and infants are particularly at risk since they may ingest large amounts of lead while playing in dirt. Also they absorb more and excrete less lead than adults.

Many animal studies have shown lead to be transferred from lactating rats to their suckling pups. Although the processes are unknown, maternal lead stores (i.e., bone) become available for transfer and excretion via milk. This route of lead exposure to the infant may be significant at high levels. In human milk, lead has been reported to occur at low concentrations. However, it is not known if milk lead levels are elevated in women living in urban areas. Therefore, more research is needed to determine if infants are being exposed to high lead levels via breast milk.

LITERATURE REVIEW

Environmental Lead

The concentration of lead in the environment has risen sharply since the advent of the industrial revolution (Hawkins, 1979). Lead use is an integral practice of industrialized nations even though it is known to be a health hazard. The major use of non-recoverable lead is in the manufacture and application of alkyl-lead fuel additives (World Health Organization (WHO), 1977). In the United States alone, 600,000 tons of lead are released into the environment each year (National Research Council (NRC), 1980; Ember, 1980). In urban areas, where sources of lead are densely concentrated, the effect of environmental contamination is most evident. Unfortunately, there are few areas in the world where lead is at its natural background level (NRC, 1980). The National Academy of Sciences (Ember, 1980) recently reported that every member of the general population of the U.S. is exposed to elevated levels of lead in air, drinking water, and foods. Urban children who might consume leaded paint chips or soil with lead-laden dust are especially at risk (Cavalleri et al., 1981). Also at risk are adults exposed to lead in the workplace as well as to the environment (Mahaffey, 1977; Ember, 1980; Singhal and Thomas, 1980; Keller and Doherty, 1980a; Gloag, 1981).

The concentration of lead in the air varies with traffic density. The highest concentration is near busy roadways where houses or agricultural crops may be located. Lead in air is widely dispersed

and varies from 2 to 4 $\mu\text{g}/\text{m}^3$ in large cities with dense automobile traffic to less than 0.2 $\mu\text{g}/\text{m}^3$ in most suburban areas (WHO, 1977; Billick, Curran, and Shier, 1980). Gloag (1980) reported values as high as 8 to 10 $\mu\text{g}/\text{m}^3$ in some metropolitan areas of high traffic, but stated that monthly averages were less.

The importance of environmental air lead as a potential health hazard to man can be further appreciated by noting its increased absorption rate in the lungs. Atmospheric lead is readily absorbed from all portions of the respiratory tract, including the nasal passages. Of inhaled lead, 30% to 50% is absorbed by this route, and the amount is even higher when the particle size is very small. In comparison, alimentary absorption of lead approximates only 5% to 10% of the total amount ingested (Goodman and Gilman, 1975; Underwood, 1977; Morrow et al., 1980). Children and infants are a more susceptible target group because they have a higher intestinal absorption rate and a reduced elimination rate of lead (NRC, 1980; Cavalleri et al., 1981). Recently The Australian Medical Journal (Anonymous, 1980) reported that the daily intake of 3 to 4 mg of lead would produce toxic symptoms in man in a period of months; whereas, an intake of 0.6 mg per d could be tolerated without toxic effects occurring. Unfortunately, it is not known whether low concentrations of lead over long-term exposure, as occurs from breathing lead-contaminated air, will cause chronic health effects in man.

The absorption and metabolism of lead is continually being studied because lead has no known physiological role in man (Cohen, 1980) and, therefore, any concentration of this metal in the tissues

can be considered hazardous. Its excretion rate is so low (Brown, Cherry, and Forbes, 1979) that only a slight excess over the average daily intake may result in a positive lead balance (Goodman and Gilman, 1975). However, the exact point at which accumulation begins is not known. The amount of lead in the body is based on the interactions of the offsetting processes--absorption, distribution, storage, mobilization, and excretion. The complex responses with internal and external factors are not yet understood (NRC, 1980). Additionally, a two-fold increase in lead absorption was observed in lactating rats receiving 2 mg Pb/l of drinking water. About one-half of the absorbed lead was transferred to the litters (Momčilovic, 1979).

After lead is absorbed into the blood, it is distributed to body tissues in accordance with tissue affinity (Green, Wise, and Callenbach, 1976). Organ sensitivity for lead varies among species (Singhal and Thomas, 1980). Lead is gradually incorporated into the long bones where a considerable pool size builds up, and whether or not it is toxic to the bone is not known. Singhal and Thomas (1980) suggested that the lead in bone may cause anemia by a defection in erythropoiesis. However, it is known that lead can be mobilized out of the bone during certain physiological states; i.e., pregnancy. The factors that regulate the mobilization of skeletally stored lead, or how physiological stresses like pregnancy and lactation affect the balance of lead between tissues, is not known (NRC, 1980).

Pathological Effects of Lead

Monitoring the effects of lead exposure in humans represents a major public health problem. The focus of attention has recently been on nonoccupational exposure to this environmental pollutant because of the large range of people who are affected. Children in particular are considered an "at risk" group since they are exposed to many environmental lead sources, i.e., lead in air, food, water, paint, and pica, and because they have increased intestinal absorption (Cavalleri et al., 1981). The majority of clinical manifestations of lead poisoning occur in children between the ages of 1-6 years and, furthermore, a number of cases of lead poisoning have occurred in adults as well (Mahaffey, 1977). The effects of lead poisoning upon human metabolism can range from slightly elevated levels of lead in the blood to encephalopathy.

One well-known effect of lead is the pathway of heme synthesis (Waldron, 1974; Mahaffey, 1977; Singhal and Thomas, 1980; Hsu, 1981). Lead inhibits three enzymes: delta-aminolevulinic acid dehydratase, coproporphyrinogen oxidase, and ferrochelatase. As a consequence of decreased heme production, delta-aminolevulinic acid (ALA) accumulates and is excreted in the urine. With lead intoxication, there is a direct increase in urinary ALA (Singhal and Thomas, 1980). Moreover, overt anemia may develop before increased concentrations of urinary ALA are detected. One study showed that when blood-lead concentration in a group of young children increased from 37- to 100 $\mu\text{g}/100\text{-ml}$ blood, the incidence of anemia rose sharply (Mahaffey, 1977). However, a recent study done by Hsu (1981) was not able to determine how lead inhibited ALA. The inhibition may be direct or mediated by metabolic

changes in glutathione reductase (GSH), an enzyme necessary for the activation of delta-aminolevulinic acid dehydratase.

Another known effect of lead intoxication occurring primarily in children involves the central nervous system (CNS). The CNS effects of lead poisoning are those principally responsible for the morbidity and mortality in humans. In acute cases neurological symptoms (encephalopathy), such as ataxia, clumsiness, weakness, stupor, coma, and convulsions, may be present (Mahaffey, 1977; Singhal and Thomas, 1980). One factor in some of the neurological manifestations of lead toxicity is thought to be the inhibition of adenylate cyclase by low levels of this metal (Ewers and Erbe, 1980). Unfortunately, the minimal blood-lead concentration necessary to cause encephalopathy is not clearly documented due to the wide variation found among those afflicted individuals.

The least understood effect of lead on metabolism concerns the kidneys. The renal system's relationship with nephropathy and lead toxicity is still being debated (Waldron, 1974; Mahaffey, 1977; Singhal and Thomas, 1980). Long-term studies suggest that exposure to lead in childhood may result in renal malfunction later in life (NRC, 1980). However, evidence is sparse.

A case study by Qazi, Madahar, and Yuceoglu (1980) described an infant exposed in utero to high levels of lead who was found to have chromosomal breakage. The frequency of chromosome breaks, however, decreased with age, and the neurological examinations given later were within the normal limits. Qazi et al. concluded that although the fetal cells were more susceptible than adult cells to the

toxic affect of lead, the abnormal cells would be replaced by normal cells with time.

Dietary Implications

Experimental studies indicate that the nutritional status of people may be an important modifier in lead absorption and its toxic effects (Cerklewski, 1980). For example, low concentrations of protein, calcium, iron, selenium, zinc, and vitamin E in the diet exaggerate the toxic effects of lead in rats and possibly in humans. In particular, lead absorption has been shown to increase in rats when dietary iron was decreased (Kostial and Kello, 1979), or when vitamin D was increased (Hawkins, 1979; Cerklewski, 1979; NRC, 1980; Hart and Smith, 1981). These responses are thought to occur by competition for receptor sites in the intestinal mucosa (Hawkins, 1979).

Investigations have shown that iron deficiency in rats enhances lead toxicity as evidenced by increased lead concentrations in tissues. Suzuki and Yoshida (1979) tested whether iron and ascorbic acid had protective effects against moderately long-term exposure to lead. They also investigated the curative effect of iron and ascorbic acid on lead toxicity in rats which had previously received high intakes of lead. Their results showed that dietary iron and ascorbic acid had preventive effects on lead toxicity as measured by growth retardation and anemia. However, these two dietary factors had negligible curative effects on rats previously exposed to lead. Apparently iron and ascorbic acid prevent lead toxicity by inhibiting its absorption; however, once the lead is absorbed, they have no effect on reducing accumulation of lead in tissues.

Furthermore, Kostial and Kello (1979) studied lead bioavailability in rats fed "human" diets by measuring the whole body retention of radioactive lead after a single oral dose. They fed the rats various types of foods normally consumed by adults or infants. Their results showed that the highest amount of lead was absorbed (17 to 20% of the initial dose) when the rats were fed cow's milk and fruit. The researchers speculated this high absorption was the cause of low iron levels in the diet. They also found that absorption rates of radioactive lead were 3 to 8% when other types of human diets (i.e., baby foods) were used. Kostial and Kello concluded that the absorption values found in their study closely approximate the known 10% absorption rate found in humans. This study further indicates that the bioavailability of lead is dependent upon dietary habits.

A study by Cerklewski (1980) found that undesirable quantities of lead could be transferred to the newborn rat via lactation as a result of maternal lead exposure. Milk-lead concentration was reduced 34% in rats receiving additional dietary iron (30 or 150 ppm) plus lead as compared to the controls consuming low iron plus lead. Also, the lead concentration in the maternal rats' blood and livers were markedly as shown in Figure 1. This study showed the protective effect of iron on reducing lead in liver by 44% and in blood by 54%. Therefore, the magnitude of the mother's lead exposure could be significantly influenced by her present iron status. This effect may have clinical significance for women who are occupationally or environmentally exposed to lead and simultaneously iron deficient (Cerklewski, 1980).

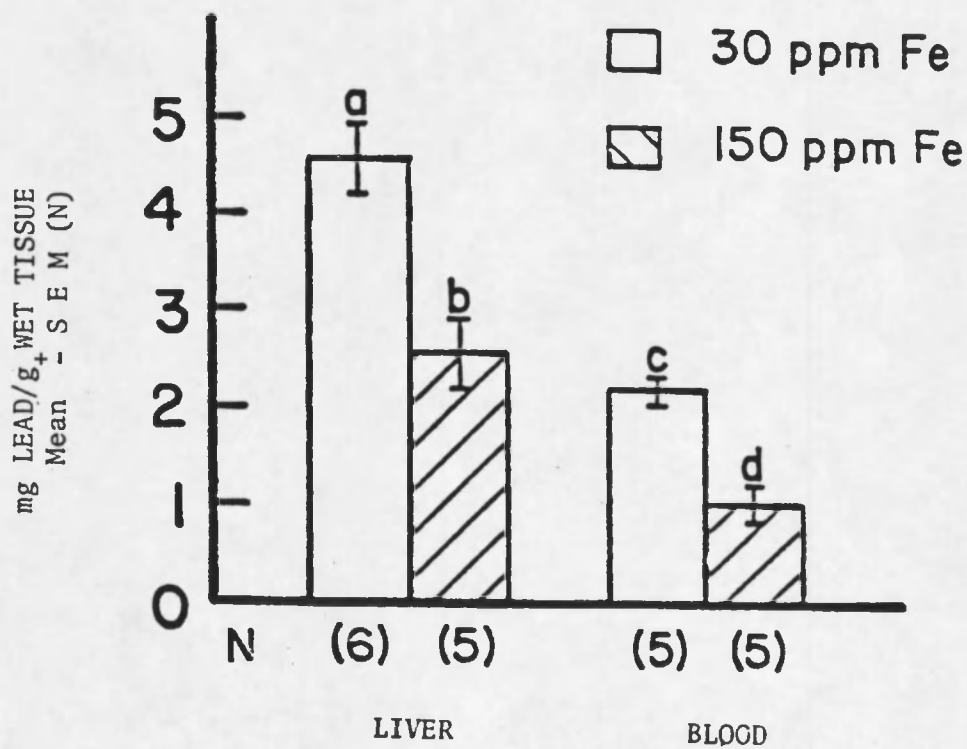


Figure 1. Maternal tissues from leded rats

On the other hand, a low dietary calcium intake in lactating mothers may increase lead mobilization from bone and cause an increase of lead secretion into the milk (Keller and Doherty, 1980b). A significant fraction (25%) of the initial maternal dose (absorbed dose) was transferred to the suckling pups (Keller and Doherty, 1980a), but the physiological processes and parameters are still unclear and need further investigation.

The relationship between lead in whole blood or plasma and the lead concentration in milk is not known. Keller and Doherty (1980) studied lead and calcium distributions in blood, plasma, and milk of the lactating mouse. They administered radiolabeled lead (both intravenously and orally) to lactating and nonlactating female mice and determined lead concentrations in whole blood, plasma, and milk during a 21-day period. A sizable difference in lead elimination was observed between the two groups. In the lactating group, 25% of the initial dose was transferred to the suckling pups. The concentration of lead in the milk-to-plasma ratio was constant over time. The rate of lead elimination in the lactating mice was increased compared to the rate of elimination in the nonlactating mice. The results indicated a physiological process which established a high milk-to-plasma ratio of lead concentration. Keller and Doherty (1980a) also noted a similar calcium concentration ratio. The researchers concluded that plasma lead concentration was a more accurate index for the estimation of lead in milk than whole blood. However, blood lead is still the preferred indicator for lead exposure. Keller and Doherty's work provides further evidence

that a common mechanism or pathway may be involved in the accumulation of lead and calcium in milk.

Other researchers have studied the effects of dietary zinc upon rats during pregnancy and lactation. Nutrition Reviews (Anonymous, 1980) published a study demonstrating that zinc has a protective effect against lead poisoning. The study investigated the effects of dietary lead and zinc in pregnant rats and found that zinc had a protective action against lead for the fetus, but not for the mother. Cerklewski (1979) also demonstrated a protective effect of dietary zinc on lead transfer from mother rats to their offspring. Milk lead concentration was reduced as reflected by a 15% reduction in pup tibia lead concentration, a 20% reduction in inhibition of pup liver delta-aminolevulinic acid dehydratase activity, and a 31% reduction in pup blood porphyrin. Overall, the results indicated that the nutritional zinc status of lactating rats can reduce lead toxicity in offspring.

Another aspect of dietary implications in lead poisoning deals with children who practice pica. Approximately 50% of all children between the ages of 1 and 3 practice pica, a perverted appetite for non-food items such as clay or paint chips (Johnson and Tenuta, 1979). This practice can result in lead poisoning from the high concentration of lead ingested in the dirt (Johnson and Tenuta, 1979; NRC, 1980; Singhal and Thomas, 1980). A recent theory proposed that a child deficient in calcium may discover that lead relieves some of the deficiency symptoms, therefore making the practice of pica a rewarding experience. This theory has been demonstrated in rats; however, one can only conjecture as to its relevance for humans (Johnson and Tenuta, 1979).

Johnson and Tenuta (1979) evaluated the possibility that calcium deficiency in children leads to pica. They evaluated the diets of 43 children and compared them with the children's blood levels of lead and to their practice of pica. They found that the children who consumed fewer servings of milk, and who also consumed the lowest calcium diets, were the children who had the highest blood levels of lead. In fact, pica was more prevalent among children with high levels of lead in their blood. Although results from this study suggested that low calcium intakes by children leads to pica, the dietary data were insufficient to conclude that there was an initial deficiency of calcium in these children (Johnson and Tenuta, 1979).

In conclusion, individuals whose diets are deficient in certain essential nutrients such as iron, calcium, and zinc are thought to either absorb more lead or to mobilize more lead from the bones (NRC, 1980). In either case, lead in milk may increase. The research in this area is very important because of the possible detrimental effects of lead exposure to fetuses and breast-fed babies (Singhal and Thomas, 1978; Kellman and Walter, 1980; Alexander and Delves, 1981). The toxicological significance of lead in milk is evidenced by rat pups who become encephalotrophic after being exposed to lead from lactation. Often there is no indication that the mothers have large amounts of circulating lead in their bodies since no clinical symptoms are observable (Keller and Doherty, 1980a, -b). The implications may be serious to humans for the following reasons:

1. The environmental air levels of lead in urban areas may pose high risk for long-term exposure.

2. Women who are pregnant or lactating may mobilize high levels of stored lead that can lead to toxicity in the neonate or in the infant while the women themselves are unaffected.
3. The combination of lead from milk, air, water, and food and/or pica may simply overwhelm the young child's system and cause lead poisoning.

Problems in the Assessment of Lead

The problems encountered in assessing lead are two-fold. A biological measurement must be made that is easily accessible and a good indicator of body stores. The other problem lies in the methodology used to analyze the lead content in the biological material.

Biological Measurement

Total body burden of lead in humans is determined by surrogate measures such as lead levels in blood, urine, feces, teeth, milk, and hair. Consequently, the measurement of lead concentration using these biological parameters accounts for only a small fraction of the body burden, due to the short half-life of lead in these tissues (Brown et al., 1979). On the other hand, skeletal lead concentration contains at least 90% of the body burden, has a half-life in terms of years, and reflects long-term exposure. Unfortunately, the only practical method at present for direct measurement of lead in the skeleton is by autopsy. This obviously cannot be used in monitoring large population samples for exposure to lead. Thus, indirect measures must be used to monitor lead exposure in spite of the known limitations stated.

Blood-lead measurements are regarded by many as the most reliable tests available for indicating recent lead exposure (Brown et al., 1979; Schlick, Kaman, and Friedberg, 1980; Piomelli et al., 1980; Pan, 1981). Sartor and Rhondia (1980) concluded from their study that blood-lead is a good indicator because it tends to be in equilibrium with environmental lead. WHO (1977) reported that the exchangeable body burden of lead (represented by the soft tissues) was the preferred measurement because it was the best indicator of available lead which could cause toxicological damage. Therefore, lead in blood may not be a reliable index of body burden, but it does represent a feasible means of estimating, at least qualitatively, whether recent exposure to lead has occurred.

Although debatable, some researchers feel that hair is a good parameter to determine the amount of absorbed lead. For example, Kello and Kostial (1978) compared the lead concentration in hair with total body lead level in rats and found it to be a good indicator. Husain et al. (1980) also stated that hair was a suitable index for lead because: (1) it was easily accessible and (2) during growth, hair was exposed to circulating blood, lymph, and extra-cellular fluids, and therefore kept a continuous record of the changes in trace element concentrations. However, it is not known how well hair reflects a body metal burden, because hair represents only 0.5% to 1.0% of the total body pool. Some feel that lead in hair does not reflect the body burden because it does not increase with age as does bone lead concentration (Hammer et al., 1971). Regardless of which parameter is used to monitor lead exposure, it should be kept in mind that the lead in

any one tissue is responding to many internal and external factors in ways that are as yet understood (NRC, 1980).

Analytical Methodology

Accurate measurements of lead in environmental and clinical samples are an important component of evaluating lead status. Contamination of lead either during collection or during preparation is a major concern to scientists (Lagesson and Andrasko, 1979). The possibility of contamination of the sample can occur from the laboratory air, furnishings, apparatus, containers, reagents, and analyst himself (NRC, 1980). Lead concentration in tissues, i.e., blood, is low and often the sample size is small. The major problems in analysis occur when the lead concentrations are low; the problems of accuracy increase as concentration of lead in the tissue decreases. In an attempt to overcome this problem, samples are often concentrated (i.e., ashing) for more accurate detection; however, this process can lead to further contamination (Snee, 1981). Also, loss of the metal through volatilization in the ashing procedure is a problem many investigators neglect to consider (Pleban, Kerkay, and Pearson, 1981). The National Research Council (1980) reported that the actual magnitude of the inaccuracy problem is difficult to group and impossible to estimate. Because there is much variation among laboratories' analyses of similar samples, standardization of analytical methods used in lead analysis is needed so that accurate comparisons of results can be made (WHO, 1977).

Lead Concentration in Human Milk

Experiments described previously suggest that lead storage in bones poses a threat to suckling infants by transfer of lead through milk. Since milk is the major source of nutrients for breast-fed infants and, since the risk of human milk having excessive lead levels becomes greater as more nations become industrialized, there is special concern to quantify the amount of lead in human milk (Jelliffe and Jelliffe, 1979).

Murthy and Rhea (1971) completed a study comparing the lead content of evaporated milk and infant formulas with human milk. They found the average lead concentration in evaporated milks and formulas to vary from 0.012 to 0.329 $\mu\text{g/ml}$ (ppm) while concentrations in human milk were significantly lower. Lamm et al. (1973) did similar studies and found their results to be in agreement with those of Murthy and Rhea (1971). Considering the lead values found in these two studies, and based on an average daily consumption of 615 ml of milk, an infant would ingest approximately 0.007 mg of lead from human milk and 0.203 mg from evaporated milk. The intake of 0.4 to 0.5 mg per d for a year is cited as the amount needed for accumulation to occur (Murthy and Rhea, 1971). However, other researchers feel that this level is too high, and a value of .300 mg/per d is more likely to cause accumulation of lead in the body (Dillon, Wilson, and Schaffner, 1974).

Dillon et al. (1974) analyzed 29 human samples for lead. The range of concentrations was 0.006 to 0.058 $\mu\text{g/ml}$, with a mean lead concentration of .026 $\mu\text{g/ml}$. After comparing their results with previous

results, Dillon et al. concluded there was no increase of lead in human milk during the past 40 years.

In addition, a study conducted by Ryu, Ziegler, and Fomon (1978) on four different human milk samples from women not exposed to lead were analyzed and found to agree with those levels of lead reported by Dillon et al. (1974). However, one woman in Ryu et al.'s study worked in the manufacture of electrical storage batteries during her pregnancy and was exposed to high levels of lead dust. At 3 w of lactation, her milk lead levels were 19 to 62 $\mu\text{g/l}$ (.019-.062 $\mu\text{g/ml}$). The following month a range of 36 to 63 $\mu\text{g/l}$ was measured. Thereafter the concentration of lead gradually decreased to a mean value of 24 $\mu\text{g/l}$ (0.024 $\mu\text{g/ml}$). Furthermore, Chatranon et al. (1978) analyzed 164 breast milk samples for lead and obtained a mean lead concentration of 0.085 $\mu\text{g/ml}$, a slightly higher value than reported in the literature. The findings of Walker's (1980) work with lead levels in milks are in agreement with those stated previously at 0.024 $\mu\text{g/ml}$. Their data indicated that the concentration of lead in infant formula, evaporated milk, and non-fat dry milk exceeds that in fresh cow's milk and human milk.

The most recent study, done by Larson et al. (1980), found lead levels in human milk to be 0.0002 $\mu\text{g/ml}$; a value one-tenth of the literature values. These low levels may indicate either loss of lead through volatilization or simply very low levels.

Further investigations of milk lead concentrations in women living in urban areas are still needed. These studies are necessary to thoroughly evaluate the potential hazard of lead exposure to

infants through breast feeding. Therefore, it was the purpose of this research to evaluate lactating women for lead concentration in their milk, hair, and blood. Correlations between the three stated variables and atmospheric lead levels were to be determined.

METHODOLOGY

Introduction

Forty breast-feeding mothers of Tucson, Arizona, ranging from ages 22 to 47, volunteered to be subjects for this study. Each subject signed a human consent form agreeing to donate at least one breast-milk sample and to give a blood and a hair sample once during their participation in the study. Each subject was interviewed to determine whether exposure to lead may have occurred in her residential area (i.e., high traffic density) or work place. Information on the women's age, number of previously breast-fed children, and treatment of hair was also gathered during the interview. Subjects were asked to submit a diet history in which they were to write down everything consumed over a 3-day period. The diets were then analyzed for necessary nutrients as given in the USDA Agricultural Handbook 8 and were then run on a computer program.

Monthly, each subject hand-expressed milk into an 8-oz acid-washed jar--the amount could be collected over a period of days. If the subject so desired, consecutive milk samples were also obtained.

Once during the study the subjects had 20 ml of blood drawn at the Tucson Medical Laboratories. Ten ml of the blood was used for Sequential Multiple Analysis Computerized (SMAC) analysis, and the remaining 10 ml was used for lead analysis at The University of Arizona. Blood was drawn into blue-topped heparinized tubes. The blood for lead analysis was stored at 10°C until all samples were ready to be run. The day the blood was drawn hair samples were taken.

from the women and stored in acid-washed glass vials until time for analysis.

Lead Analysis

Lyophilized milk samples were oven-dried, weighed to the fourth decimal place, and ashed overnight in a muffle furnace at 450°C. The white ash was dissolved in 1% nitric acid and quantitatively transferred into a 10-ml volumetric flask and read by a flameless Polarized Zeeman Hitachi Atomic Absorption Spectrophotometer. The heating program was as follows:

<u>Stage</u>	<u>Temperature (°C)</u>		<u>Time (sec)</u>
Dry	80	120	30.0
Dry	120	120	60.0
Ash	400	400	30.0
Atom	2,000	2,000	7.0
Clean	2,400	2,400	3.0

Values were expressed as parts per billion of the dried milk sample.

Whole blood and hair samples were collected once from each individual during the time of the study. Ten ml of blood was used in a SMAC analysis and the remaining 10 ml of whole blood was used for the lead analysis. The whole blood was diluted with deionized water--a range from 10- to 50-fold dilution (i.e., 200 µl of blood to 4.0 ml of deionized water). The samples were read using atomic absorption as described above. The conditions used for the graphite furnace were as follows:

<u>Stage</u>	<u>Temperature (°C)</u>		<u>Time (sec)</u>
Dry	80	120	90.0
Dry	120	120	30.0
Ash	400	400	40.0
Atom	2,000	2,000	8.0
Clean	2,400	2,400	3.0

At least two absorbance readings of less than 10% difference were taken for each sample so an average could be obtained. Standard curves were made each time samples were run.

The hair samples were washed and digested in nitric acid according to Reinhold's (Reinhold, Kroury, and Arslanian, 1968) method. After the acid was fumed off, the samples were quantitatively transferred to a 5-ml volumetric flask and brought up to mark with deionized water. Samples were read on AAS using the same conditions as described in the milk sample section.

Proximate Analysis

To determine the moisture content of human milk samples, the frozen milk was weighed before and after lyophilization. The weight difference was used to calculate the percentage of moisture. The standard AOAC method was used to determine the percentage of ash. Crude fat was obtained by placing a 1-g sample of oven-dried lyophilized milk into an alundum thimble and extracted with hexane in a Goldfisch Extraction Apparatus for 5 h. The extracted oil was weighed to the fourth decimal place and the percentage of oil calculated (Association of Official Analytical Chemists (AOAC), 1970). The protein content of the milk was determined by the AOAC micro-Kjeldahl digestion method with Nessler's reagent modification (Reid, personal

communication, 1980). The digested samples were cooled and transferred to 100-ml volumetric flasks and brought up to mark. Forty μ l of this digest was transferred to a 10-ml volumetric test tube and brought up to mark. Two ml of Nessler's reagent were added to each tube, and after 10 min the samples were read on a Bausch and Lomb spectrophotometer at 500 nm. Standards were prepared with each series of samples.

RESULTS AND DISCUSSION

Lead in Milk

Currently it is not known whether biological effects of lead are strictly proportional to dose or whether protective mechanisms or tolerances exist that produce a threshold of toxicity for lead in humans (NRC, 1980). To set up a definite study to provide a final answer is virtually impossible (Christophers, 1980). Therefore, I chose to focus more on obtaining levels of lead in milk, blood, and hair of lactating women to see if the levels posed a threat to either the women or their breast-fed babies. I also chose to compare the lead values obtained with the type of area (traffic density) in which these women reside. Of course, this route of investigation has its limitations. For instance, I grouped together women's lead values for each variable for those who lived in a "busy" section of town without consideration of the distance of each house from the street. The level of lead exposure would vary directly with distance from street. Neither did I consider the amount of hours the women spent in their car per day (where the highest exposure to lead exists). This last factor may have more importance for the women who live in the rural areas and who have to use the bus system or cars more often to get around. The only accurate way to get an evaluation of lead exposure through air would be to take air samples specifically for each individual in the study. I chose a more general method of grouping the women together in accordance with location (rural vs. urban)

and assumed that the exposure level of lead was the same for all the women in the same type location because of the cost and the time involved doing individual air analysis.

The lead levels in milk in lactating women are presented in Table 1, where 63 human milk samples from 40 donors were analyzed. The months of lactation ranged from 1 to 22, with the median at 6 months. The mean level of lead found was 0.023 ppm which agreed with most of the values cited in the literature. For example, one study (Dillon et al., 1974) found the mean lead concentration to be 0.026 from 29 human milk samples. The range of values for this study was from 0.006 to 0.058 ppm. Lamm et al. (1973) obtained a mean lead value of 0.02 ppm from 14 human milk samples, while Walker's (1980) study of 40 human milk samples had a mean lead value of 0.02 ppm, with a range of 0.00 to 0.05 ppm. On the other hand, Chatranon et al.'s (1978) work with 164 milk samples had a mean lead value of 0.085 ppm, with a range of 0.014 to 0.222 ppm. Their samples of milk ranged monthly from 1 d postpartum to 9+ months' postpartum. They found no specific pattern in the differences of lead levels in breast milk at the various stages of lactation. Our mean lead value (0.020 ppm) was much lower than Chatranon et al.'s (0.085 ppm); however, both levels of lead were higher than the levels published in two other studies--Murthy and Rhea (1971) and Larson et al. (1981). Murthy and Rhea obtained lead values from 13 human milk samples averaging 0.012 ppm. Larson et al. reported low lead levels and obtained results from 41 milk samples with an average lead value of 0.002 ppm and a range of 0.0005 to 0.009 ppm. This lead value seems particularly low and the accuracy

Table 1. Lead values (ppb) of milk, blood, and hair from lactating women

	Mean (ppb)	SD	Smallest		Largest	
			Value	z Score*	Value	z Score
Milk	23	± 13.4	7	- 1.2	82	4.4
Blood	119	93.9	32	- .9	530	4.4
Hair	2,002	1,815.5	193	- 1.0	8,128	3.4

$$*z \text{ Score} = \frac{X - \bar{X}}{SD}$$

at such low ranges is suspect. A more realistic range for lead analysis would be between 5 to 50 ppb (ng/ml) using an extremely sensitive graphite furnace atomic absorption spectrophotometer. In spite of the possible inaccuracies in the results of the last study, the main point was that the lead levels were quite low and would therefore not pose a toxicological hazard to the suckling infant.

The fluctuation of lead values between milk samples is indicated by the large standard deviation in Table I. This wide range (7 to 82 ppb) is explained by a combination of reasons. Most important is individual variation. Each woman would be expected to have different exposure levels, different absorption rates, and different excretion rates. The total accumulation from life-long exposure and deposits in the bones would vary greatly. In theory, a woman who has breast fed more than once would possibly have lower milk levels of lead than a woman who was breast feeding for the first time, simply because the former had more time to mobilize lead from her bones and decrease her body pool of lead. This total decrease in body lead, however, can easily be replaced by current exposure. Animal studies have shown this relationship of increased lead elimination from lactation (Keller and Doherty, 1980; Kelman and Walter, 1980). The problem of various lead sources from either air, food, and water combine to complicate matters and increase variation among subjects. The length of lactation period would also have an effect on the body loss of lead.

The variation of milk lead on a month-to-month basis is shown in Figure 2. The number of samples in each month varied from 1 to 8 and, therefore, any extreme value obtained per given monthly collection

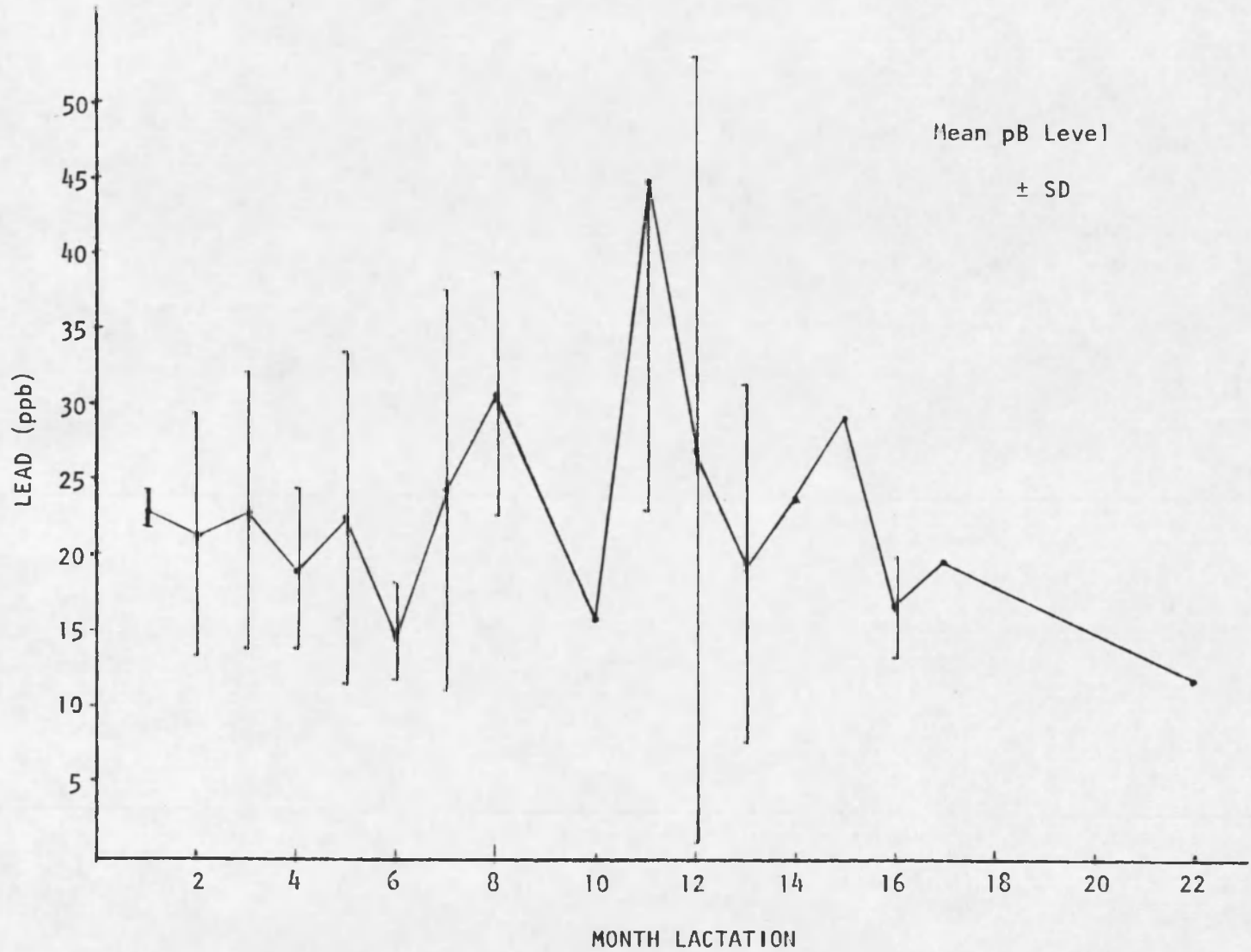


Figure 2. Variation of milk lead on a month-to-month basis

would influence the average value for that particular month. The histograms in Figure 3 show the average lead values by grouping together several months of lactation. Both Figure 2 and Figure 3 show an increased lead value between the 7th and the 11th month (N = 19 milk samples). If this increase is not due to sampling error, it may indicate a time of increased lead mobilization from the bones. The calcium levels in the blood of these women may have become depleted at this late stage of lactation; if so, then the parathyroid hormone would be stimulated and increase lead (along with Ca) mobilization from bones which, in turn, causes an increase of lead elimination via milk. This hormone was not measured in the lactating women so this idea is only speculative.

The concentration of lead in milk did not correlate significantly with blood or hair levels, or with the location of residence of the women. The number of women who lived in rural areas was quite small and because of this a mean lead value of their pooled milk levels would not be a good estimation of the population. Additionally, one of the women living in a rural area had the highest milk level of lead found in this study. This single value caused the distribution to become skewed and shifted the mean to a higher value. Had the sample size for both groups been larger, I may have been able to show a relationship between them. Also, the blood and hair were not taken on the same day as the milk sample (the milk was collected over a period of days which may not have included the day blood and hair were taken), and this could cause enough fluctuation to negate any correlation that may exist.

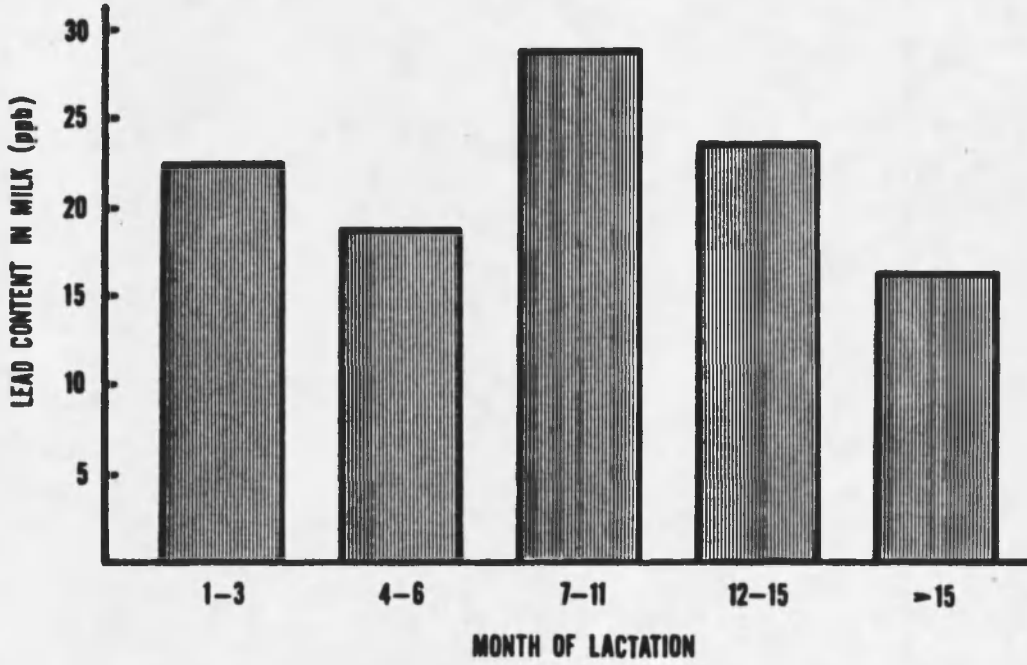


Figure 3. Histograms of lead values by grouping several months of lactation

Although not significant, I saw a slight decrease in the concentration of lead in blood as the lead in the milk increased. Visually this makes sense. As the blood carries lead throughout the body, some mechanism favors the transport of lead to the mammary gland. As more lead is excreted into the milk, the pool size in the blood decreases. This would eventually lead to decreased amounts in the milk unless the bone output of lead was further increased. However, despite failing to establish any direct relationships among the variables, my results do indicate that the amount of lead in human milk (overall) is, fortunately, quite low. For example, if the average intake of milk per day is 615 ml, then 0.014 mg lead/day would be ingested by the suckling infant. If the highest value obtained in this study was used to calculate the amount ingested (instead of the mean value of 0.023 ppm), then the maximum intake by milk would be 0.05 mg/day--a value still considered safe by many investigators (Murthy and Rhea, 1971; Dillon et al., 1974; Mahaffey, 1977). Legislation has yet to update the maximal permissible daily intake of 0.30 mg/d for infants and children. At some time in the near future this value will probably be decreased for infants since they are more susceptible to the hazards presented by lead. Reasons why this is a necessary move were stated by Clair C. Patterson (NRC, 1980):

1. Babies place objects and their hands in their mouths.
2. Much time is spent on or near surfaces which contain high concentrations of lead residues and, therefore, objects and hands become highly contaminated with lead deposits.

3. Babies breathe air that contains higher than ordinary concentrations of lead dusts because of their proximity to sources of these dusts.
4. Their high requirements for calcium elevate their absorption of lead from intestines.
5. Their immature developing cells are more susceptible to damage from lead than mature cells.

Human milk was the only indicator available to me for the estimate of lead exposure in infants. Infant blood and hair would have provided a more direct estimation of the total circulating lead levels in their bodies, however, I could not obtain these samples.

To summarize, my null hypothesis states that there would be no difference between the mean concentration of lead in milk in women living in urban areas (high traffic density) and those living in rural areas at a significant level of 5%.

$$H_0: \mu_{\text{(rural)}} - \mu_{\text{(urban)}} = 0$$

$$H_1: \mu_{\text{(rural)}} - \mu_{\text{(urban)}} \neq 0$$

The obtained t value for this study was below the critical t value needed to reject the H_0 . Therefore, it was necessary to accept my null hypothesis that there was no difference between levels of lead in these two groups of lactating women.

Lead in Blood

The mean level of lead found in whole blood from lactating women is listed in Table 1. This value of 0.120 $\mu\text{g/ml}$ is below

0.170 $\mu\text{g}/\text{ml}$, the value Underwood (1977) cited as "normal" in his study. Underwood's value came from combining blood-lead concentrations from 16 countries with a range of 0.15 to 0.40 $\mu\text{g}/\text{ml}$. He stated that the upper limit of "normal" is often given as 0.50 μg of lead per ml, but that clinical poisoning will not occur below 0.80 $\mu\text{g}/\text{ml}$ in adults. Christophers (1980) also stated that blood-lead levels must rise to 0.80 $\mu\text{g}/\text{ml}$ or more in children to see evidence of encephalopathy; it is not known to occur at levels lower than that number. The resulting concentrations of lead in blood in the present study ranged from 0.032 to 0.530 $\mu\text{g}/\text{ml}$, but only one subject had a blood level of lead exceeding 0.33 $\mu\text{g}/\text{ml}$.

I did not find a significant correlation between blood-lead levels and site of location for the women. This may be due to the small sample size (39 blood samples). The t value obtained was below the critical t value needed for significance at the 0.05 level (degrees of freedom equaled 38). However, if I could extrapolate to 65 degrees of freedom, then the obtained t value would have been significant. In conclusion, had my sample size exceeded 65, I would have found a direct relationship between blood values and location (relative to traffic density) as have other researchers if the same distribution occurred. Alexander and Delves (1981) showed that women living in an urban area had significantly higher blood-lead levels than did those women in a rural area. Snee (1981) evaluated major studies on the effects of airborne lead on blood-lead levels of adults and children and found, on the average, a slope of 1.0 for adults and a slope of 2.0 for children when blood-lead was plotted on the ordinate axis

and the air-lead plotted on the abscissa. The studies considered by Snee had sample size ranges from 61 to 1,935 subjects. This further indicates the necessity of having large sample sizes.

From other studies involving environmental lead and blood-lead concentrations, it has been found that blood concentrations of lead increase 0.01 to 0.02 $\mu\text{g}/\text{m}^3$ for each 1 $\mu\text{g}/\text{m}^3$ of lead in air (WHO, 1977). The average lead concentration in Tucson, Arizona, is 0.66 $\mu\text{g}/\text{m}^3$ (a range from 0.38 to 1.18 $\mu\text{g}/\text{m}^3$). If approximately 20 m^3 of air are inhaled per day, then the average person would inhale 13.20 μg of lead. This level is below the national average of 15 $\mu\text{g}/\text{day}$ (NRC, 1980). The levels of lead in Tucson are probably not high enough to cause an elevation of lead in blood.

Lead in Hair

The concentration of lead in hair was found to be highly variable among subjects. Table 1 shows the range of 193 to 8,128 ng/g (ppb) in the hair of lactating women with a mean value of 2,002 ng/g (or 2 $\mu\text{g}/\text{ml}$). Waldron and Stofen (1974) reported that the normal contemporary value of lead in hair was 16.23 $\mu\text{g}/\text{g}$, an actual decrease from a century ago. Some researchers feel that women have higher hair lead levels than do males (Waldron and Stofen, 1974), while others report that males have the higher amount (WHO, 1977).

Two infant (male) hair samples were obtained and were found to average 7.1 μg lead/g hair. The hair of normal children has been reported to range from 2 to 95 $\mu\text{g}/\text{g}$. All hair samples analyzed in the present study had low concentrations of lead when compared to the

normal range and to patients suffering from chronic plumbism (lead poisoning) with hair lead levels of 282 $\mu\text{g/g}$ (Underwood, 1977).

There were no correlations between hair lead levels and any other variables. However, a slight increase of lead in hair was shown to occur as the lead in the blood decreased. This possible negative relationship could be explained in terms of one pool (blood) size decreasing as the pool of another (hair) was increased. Unfortunately, correlations are difficult to determine when sample sizes are less than 100, unless the difference between the two variables happens to be very large.

Proximate Analyses of Milk

The results of the proximate analyses on human milk samples ($N = 63$) are depicted in Table 2. The values obtained, except for one, agreed with those reported by Jelliffe and Jelliffe (1978). Any individual differences seen may have been due to sampling error as the women hand-expressed their milk into the jars; i.e., the let-down reflex may not have occurred if the subject was not completely relaxed. The protein content was the only value that differed from Jelliffe and Jelliffe's findings. My value of 1.54% was higher than the cited value of 1.10%. This may be due to the procedure used since I determined all nitrogen present in the milk rather than just protein nitrogen. There was a significant relationship between month of lactation and percentage of moisture (Figure 4); therefore, a relationship between month of lactation and percentage of solids (Figure 5). The correlation coefficients are shown in Table 3. The results show

Table 2. Proximate analysis of human milk

	Mean (% Wet Weight)	SD	Smallest		Largest	
			Value	z Score*	Value	z Score
Moisture	87.78	± 2.4	80.14	- 3.18	93.69	2.46
Solid	12.15	2.3	6.31	- 2.55	19.86	3.37
Fat	3.16	1.2	0.61	- 2.07	6.80	2.95
Protein	1.52	0.53	0.67	- 1.61	4.20	5.11
Ash	1.54	0.56	0.09	- 2.59	3.02	2.76

$$* z \text{ Score} = \frac{X - \bar{X}}{SD}$$

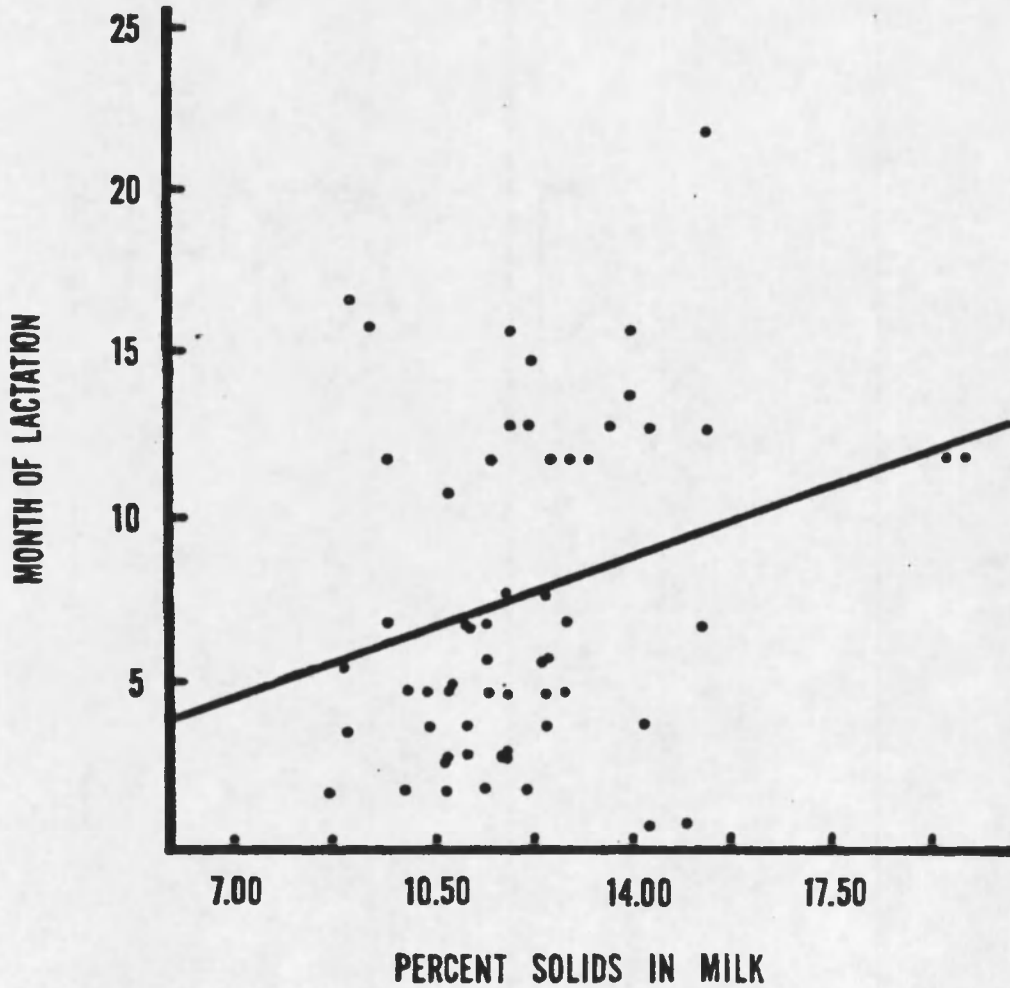


Figure 5. Linear regression of month of lactation vs. percentage of solids in human milk

Table 3. Correlation coefficients between different variables obtained from lactating women

	Month Lactation	Lead in Hair	Lead in Milk	Lead in Blood
Month Lactation	1.00	0.16	0.03	0.18
% Moisture	-0.25*	+0.04	0.16	0.05
% Solid	-0.27*	-0.04	-0.17	0.05
% Fat	-0.04	-0.03	-0.23*	0.09
% Protein	0.21	-0.11	-0.17	0.11
% Ash	-0.36**	-0.15	0.14	-0.05
Hair Lead	0.16	1.00	0.20	0.07
Milk Lead	0.03	0.20	1.00	-0.16
Blood Lead	0.18	-0.07	-0.16	1.00
Calcium	-0.10	-0.29	-0.23	-0.17
Location of women	0.04	-0.07	0.16	-0.26

* = Level of significance of 0.05 for two-tailed test

** = Level of significance of 0.01 for two-tailed test

that the percentage of moisture decreased as the month of lactation increased. This relationship is shown in Figure 4. There are few data in the literature concerning late-stage lactation with which to compare our results. However, this process of decreasing moisture (and thereby increasing solids) as month of lactation increases, makes sense because it would provide the infant with a higher concentration of nutrients as it continues growing at a rapid rate. On the other hand, perhaps the increase in solids was actually due to dehydration in some women in late-stage lactation since many of the samples were taken during the summer months; however, the mean percentage of solids for this group was definitely within the normal range of 12-13% and, therefore, could not be explained as being caused by dehydration. Furthermore, the results show that the mineral content (percent ash) in the milk significantly decreased with time of lactation (Fig. 6). This agrees with the results found by Vaughan, Weber, and Kemberling (1979). A possible reason for a decrease of mineral content with time may be due to the lactating women's diet. It is often observed that lactating women fail to consume the high calories needed to produce milk while maintaining their weight. These women believe that dieting along with breast feeding is a good time to shed the pounds that were gained during pregnancy and, therefore, they cut down drastically in their intake of calories. Some women in my study reported doing this. As time progresses, the mineral content of the milk would decrease if each woman was not consuming enough food to provide the necessary amounts of minerals needed for her maintenance and the baby's growth. This may not be a problem with the babies who are not solely breast fed

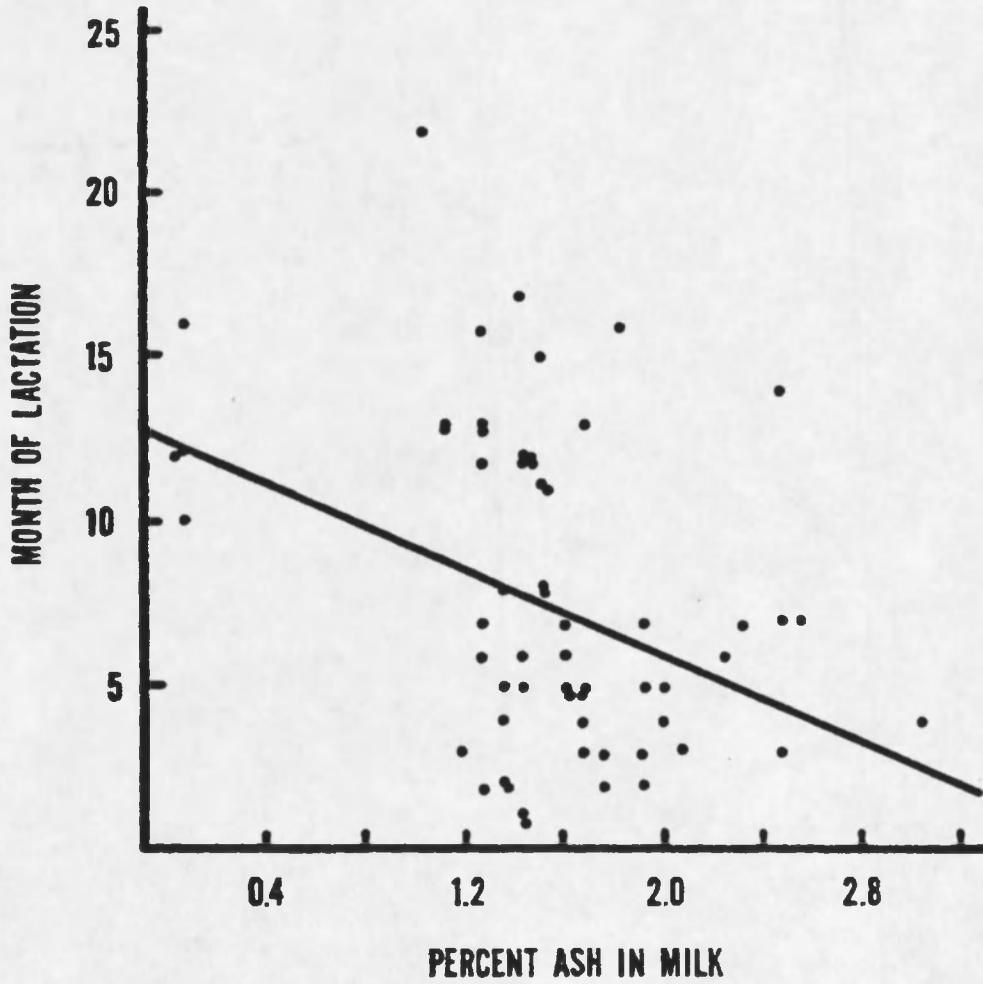


Figure 6. Linear regression of month of lactation vs. percentage ash in human milk

since they would presumably be getting extra nutrients elsewhere. A somewhat surprising significant relationship was found, although not a practical one, between fat content in milk and lead content in milk. As the lead content increased in milk, the percentage of fat decreased (Figure 7). There is really no explanation for this relationship.

Dietary Evaluation

The results from a 3-day diet history taken during the subjects' participation in this study are presented in Table 4. The mean percentage of calories are below the recommended dietary allowances (RDA) of 2,500 calories. Many of the women were far below the RDA for some of the other nutrients, too. The ranges (extreme values) given a better indication of the wide variation in the dietary habits of these women. For example, calcium intake has a mean value of over 100% of the RDA, but the low value is only 30% of the RDA and the high value is nearly 500%! This extreme value skews the distribution and shifts the mean to a higher value for this group of women. A mode would have given a better indication of the average dietary intakes for these women. This wide range of intakes is seen in the other nutrients, especially for ascorbic acid and vitamin A. Therefore, it should be kept in mind that the mean values given do not necessarily indicate the true averages because the intakes of these nutrients were not normally distributed. Looking at the dietary eating habits of these women individually is evidence that not all lactating women are meeting their nutritional needs. Mineral and vitamin supplements are not the answer if the overall calories are not being consumed in sufficient amounts

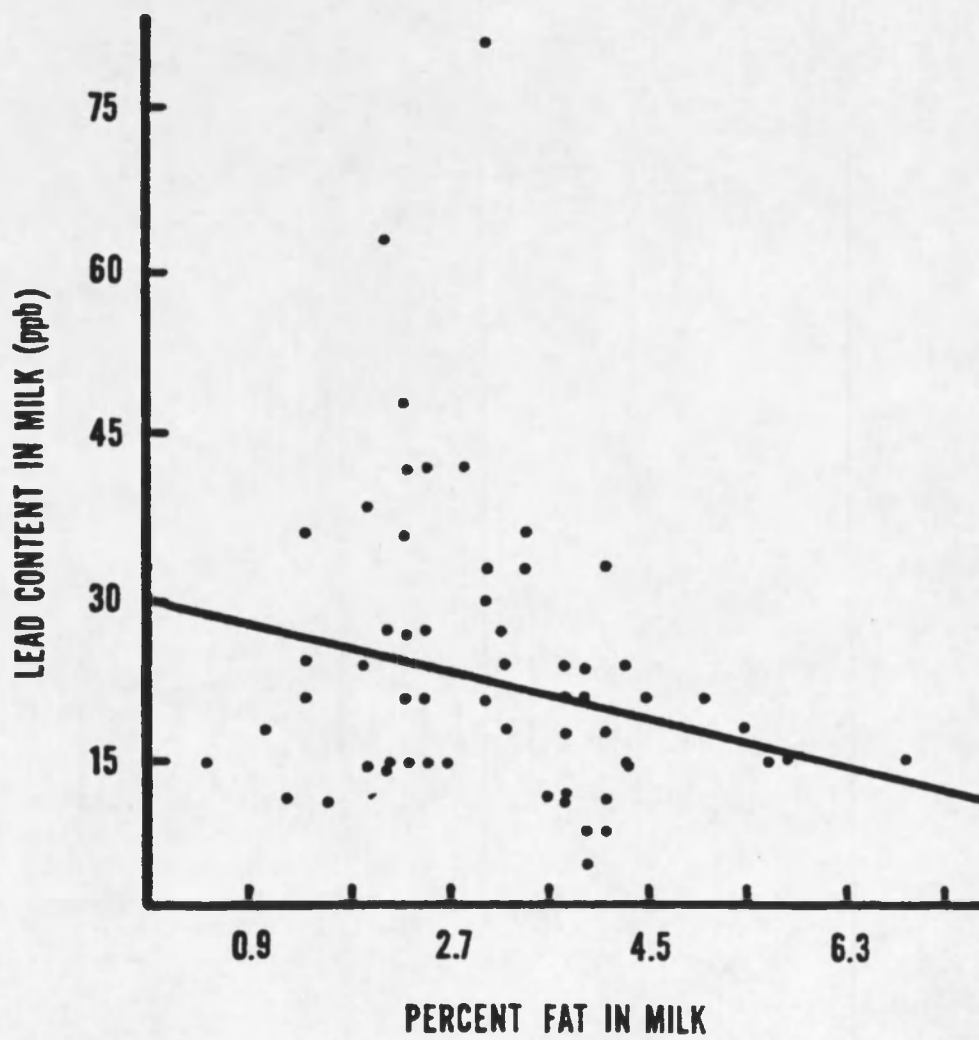


Figure 7. Linear regression of lead content in milk vs. percentage of fat in human milk

Table 4. Average intake from diet during 3 days in lactating women*

	Mean (% RDA)**	± SD	% RDA			
			Smallest		Largest	
			Value	z Score	Value	z Score
Calories	85.39	16.86	47.60	- 2.24	120.10	2.06
Iron	80.51	32.17	38.00	- 1.32	174.10	2.91
Vitamin A	126.36	94.47	27.80	- 1.04	423.70	3.15
Thiamin	112.90	63.95	41.40	- 1.12	311.20	3.10
Riboflavin	120.21	60.75	44.00	- 1.25	353.50	3.84
Niacin	119.68	44.57	78.40	- 0.93	261.20	3.18
Ascorbic Acid	243.83	141.56	29.80	- 1.51	520.00	1.95
Calcium	104.19	80.36	29.80	- 0.93	466.40	4.51

*Supplements of vitamins and minerals not included

**Food and Nutrition Board, Recommended Daily Dietary Allowance, 1974

by breast feeding women--either their own health or that of their infants can suffer from the lack of essential nutrients.

Blood Profiles

Blood values were obtained by SMAC analysis to see if there was any evidence of anemia or other disorder in these lactating women. Table 5 summarizes some of the components analyzed. Overall, the values fell into the normal range. One woman had a low-serum iron count (32 $\mu\text{g}/\text{dl}$), but this did not seem to affect her lead levels in milk, blood, and hair (no high lead levels in these parameters). All values that were suspected of being too low or too high were reviewed by a physician for further comment and the women were notified.

Summary

The results of this study indicate that the three biological parameters under investigation did not correlate significantly with each other with respect to their lead content. When the levels of lead in milk, blood, and hair were compared to the location of these women (environmental air-lead) there was again no correlation. Other researchers have found blood lead to correlate with environmental lead; however, my sample size was probably too small to detect any such relationship. More importantly, this study has shown that the levels of lead present in the milk of these women are at levels considered safe for infants. Furthermore, the concentration of lead in blood and hair were found to be below values considered "normal". Therefore, it does not appear that these women have high body burdens

Table 5. Biochemical profile on blood of lactating women

	Mean	SD	Normal Range*	Smallest		Largest	
				Value	z Score	Value	z Score
Hb (g)	14.44	± 0.76	12- 16	12.80	- 2.16	15.80	1.79
Hc (% total volume)	41.26	2.18	37- 47	36.80	- 2.04	45.70	2.03
Fe (µg/dl)	101.38	37.33	50-160	32.00	- 1.86	186.00	2.27
Ca (µg/dl)	9.42	0.41	8.5- 11	8.20	- 2.98	10.30	2.16
BUN (mg/dl)	17.60	7.37	10- 26	2.00	- 2.12	36.00	2.50
Bilirubin (mg/dl)	.53	0.31	0.2-1.5	0.20	- 1.06	1.90	4.36
Cholesterol (mg/dl)	170.14	25.08	140-260	133.00	- 1.48	221.00	2.03

*Normal range as indicated by Tucson Medical Lab.

of lead (from any source) as indicated by their milk, blood, and hair levels.

APPENDIX A

LEAD IN BREAST MILK

<u>Month of Lactation</u>	<u>N</u>	<u>Mean (ppb)</u>	<u>± SD</u>
1	2	22.94	1.05
2	5	21.38	8.53
3	6	23.64	9.11
4	5	19.31	4.74
5	8	22.58	11.42
6	5	14.80	3.14
7	6	24.14	16.19
8	3	30.60	8.35
9	0	---	---
10	1	15.66	---
11	2	44.73	24.49
12	7	26.57	25.58
13	5	19.72	10.07
14	1	23.88	---
15	1	29.32	---
16	3	16.86	2.93
17	1	19.92	---
18	0		
19	0		
20	0		
21	0		
22	1	11.78	---
Total	62	23.4	± 13.4

APPENDIX B

DEFINITION OF TERMS

Definitions used by the Center for Disease Control for classification of children examined in blood lead screening programs are as follows:

1. Child lead poisoning:
 - a. Two successive blood lead levels ≥ 70 $\mu\text{g/ml}$ with or without symptoms.
 - b. Erythrocyte protoporphyrin level ≥ 250 $\mu\text{g/ml}$ whole blood and a confirmed elevated blood lead ≥ 50 gm/dl with or without symptoms.
 - c. Erythrocyte protoporphyrin level > 109 $\mu\text{g/dl}$ with blood-lead level ≥ 30 gm/dl with compatible symptoms.
 - d. Blood-lead $> 49\mu\text{g/dl}$ (ppm).
2. Lead toxicity--Biochemical or functional derangements caused by lead.
3. Elevated blood lead level--Confirmed blood lead 30 $\mu\text{g/dl}$ or greater.
4. Disease process can be divided into arbitrary states:
 - a. No observed biological Δ 's.
 - b. Biochemical or physiological Δ 's of uncertain significance.
 - c. Physiological Δ 's of a pathological but compensable nature unaccompanied by overt symptoms of impaired health.
 - d. Overt illness.

There are no sharp boundaries between the stages of the process, and a given level of lead in the body can produce different degrees of change in different individuals. Thus, the definition of non-detrimental degree of biological change often involves an attempt to define the boundary between stages.

APPENDIX C

HUMAN SUBJECTS COMMITTEE LETTER



THE UNIVERSITY OF ARIZONA

TUCSON, ARIZONA 85724

HUMAN SUBJECTS COMMITTEE
ARIZONA HEALTH SCIENCES CENTER 2305

TELEPHONE: 626-6721 OR 626-7575

February 6, 1980

Charles W. Weber, Ph.D.
Department of Nutrition and Food
Science
309 Agricultural Sciences Building
University of Arizona
MAIN CAMPUS

Dear Professor Weber:

We are in receipt of your letter outlining procedural and consent form revisions for your project entitled, "Trace Mineral Content of Human Milk". Since these minor changes involve no further risk to the subjects and have been approved by your Departmental Review Committee, reapproval is granted effective 6 February 1980.

The following modifications are noted:

1. Eight ounces of milk will be collected from the subject monthly.
2. A single blood sample of 20 ml will be collected from the subject.
3. A request is included for a small hair sample from the subject's infant. This request is optional.

Reapproval is granted with the condition that the consent form will bear the title of the project. It is also understood that no further changes will be made in the procedures followed or the consent form used (copies of which we have on file) without the knowledge and approval of the Human Subjects Committee and the Departmental Review Committee. Any physical or psychological harm to any subject must also be reported to each committee.

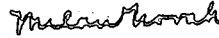
A university-wide policy requires that all signed consent forms be kept in a permanent file in the Departmental Office to assure their accessibility in the event that university officials require the information

Professor Charles W. Weber
Re: Trace Mineral Content of Human Milk
6 February 1980

Page 2

and the principal investigator is no longer on the staff or unavailable for some other reason.

Sincerely yours,



Milan Novak, M.D., Ph.D.
Chairman
Human Subjects Committee

MN/jm

cc: Bobby L. Reid, Ph.D.
Departmental Review Committee

APPENDIX D

LA LECHE LEAGUE INTERNATIONAL LETTER



La Leche League International

9616 Minneapolis Avenue Franklin Park, IL 60131, U. S. A.
Telephone: 312-455-7730

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- Sheila D. Williams, D.M.

May 12, 1980

Dr. Charles W. Weber
Ms. Susie Rockway
Nutrition and Food Science
University of Arizona
Tucson, AZ 85721

Dear Dr. Weber and Ms. Rockway:

I assume that Carolyn Vemulapalli informed you that your proposed study of lead levels in human milk was being resubmitted to our Research Review Committee before being approved for League participation.

The committee has read your proposal and approved it. You can now go ahead with collecting milk samples. Please note that any changes in the procedure should be submitted to the LLLI offices.

Also, we'd appreciate receiving copies of the results of your study, especially since studies of hazardous substances in mother's milk can cause great and unnecessary alarm among nursing women. We'd like to see studies such as yours stimulate efforts to clean up the environment--and not suggest to mothers that they should quit breastfeeding.

Good luck with your project!

Sincerely,

Gwen Gotach
Coordinator of LLLI
Participation in Research

Encl.

cc: Carolyn Vemulapalli
Maryann Thurston, AC for AZ

*Good Mothering
Through Breastfeeding*



The World Over

La Leche League International

9616 Minneapolis Avenue Franklin Park, Illinois 60131

LLLI Policy with Regard to Participation in Research Projects

All projects must be cleared by LLLI's Research Review Committee. The following requirements govern LLL participation in such projects:

1. Participation is Voluntary. The mothers will be approached first by the La Leche League liaison person who will explain the project and exactly how they can participate. The names of the volunteers may then be given to the researcher to contact personally, or the LLL liaison person may handle this.
2. Babies are not to be deprived, even for a very short period, of either their mother's milk or of mother herself.
3. Babies are under no circumstances to be given food, formula or anything other than mother's milk for the purpose of the study. If during the course of the research project a baby shows he/she is ready for solids, the mother should put her baby's need ahead of the project. If babies are older and already on solids, they should not be given foods other than those to which they've already been introduced.
4. LLL groups and/or individuals who volunteer should not be put to any expense for the study. The LLL liaison person's only responsibility is to inform the women about the project and give them the opportunity to volunteer if they would like to do so. On her own, the liaison person may agree to take on additional responsibilities.

A League group may decide to undertake to act as a collection point for milk samples if they wish. However, collections and transportation are the responsibility of the researcher and there is no reason for League mothers to feel obligated to take this on, or to be burdened in any way.

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APPENDIX E

PATIENT HANDOUT

Susan Rockway
Department of Nutrition & Food Science
Home Economics Bldg., Room 324
University of Arizona
Tucson, AZ 85721
Phone (602)626-4050

Dear Participating Mothers,

Thank you for volunteering in this breast milk project. What we hope to do with the milk (hair & blood) you donate is analyze it for lead and then compare these values with the known environmental lead values. We want to see if there is any correlation between the lead that's in the air and your milk. Because lead can be toxic when inhaled or ingested and it is a cumulative poison, we want to see if your breast milk is transferring this metal to your infant.

We are also interested in knowing if you've worked in any capacity where you may have been exposed to lead. For example, working in a copper smelter or using paints with a lead base.

The following will give you more details for the actual milk collection.

- 1) Hand express the milk into the sterilized jar given to you. You will not be able to fill it at one feeding, so perhaps fill it in a few days. If possible, try to vary the times you donate the milk since morning milk varies from evening milk. Also, the milk's composition changes during the feeding - it becomes higher in fat content, for example, as the baby suckles. Take a felt pen and just mark a line on the tape to the level you filled the jar, then date it. You may want to start hand expressing one breast for a while then feed your baby on the other breast. Use any combination that works for you and your baby. After you have finished hand expressing the milk into the jar, close the lid tightly and just place it in your freezer section until you are ready to add more. Please write on the jar what month of lactation you are in. We hope you will stay with this project longer than one month, however, if you cannot we understand perfectly.
- 2) At some point during the time you are donating milk, we'd like you to go to the Associates in Laboratory Medicine, Inc. where a licensed medical technologist will draw some blood. Some of the blood will be analyzed for about 38 different items at the lab. The rest will be sent to us so we can analyze for lead.
- 3) Once during this study, we'd like to collect some hair from the nape of your neck, about half the size of a thumb (400 mg). This is so we can also analyze it for lead. Lead levels found in the hair may indicate any recent exposure your body has had to lead.

- 4) We'd like you to fill out the dietary information sheets once (for a three day period) during your participation in this project. We will analyze your intake of protein, calories and fat for these 3 days.

Sincerely,

Susan Rockway

Susan Rockway

DIRECTIONS FOR RECORDING DIET RECORD

1. Record at the time eaten, the exact amount of all foods you eat and the beverages you drink except water.
2. The amount of milk taken should be recorded in ounces (cups) or by carton size. The kind of milk should also be recorded as whole milk, evaporated milk (diluted or undiluted), 2% nonfat milk, buttermilk, chocolate milk, filled milk, imitation milk, skim milk, dry non-fat milk (regular or instant). Give brands.
3. Amount of fruit juice taken should be recorded in ounces (cups) or by glass size. List the kind of juice as orange, grape, etc. List whether sweetened or un-sweetened. List brand.

Canned Fruits--record by name and amount in cups. List brand. List whether heavy or light syrup and amount if syrup is used. Note if home-canned.

Whole Raw Fruits--should be recorded by name, number, and size, as small, medium or large.

Frozen Fruits--should be recorded by name and amount in cups. List brand. List whether heavy or light syrup and amount if syrup is used.

4. Vegetables--list name and how prepared. Specify "baked in skin," etc.

Cooked Vegetables--record number and size of pieces--such as two carrot sticks four inches long or by cup portions.

Frozen Vegetables--record by name and amount in cups. List brand, or home-frozen.

Canned Vegetables--record by name and amount in cups. Record vegetable and juice separately if juice is used. List brand. Specify if fresh, as: mushrooms, asparagus, etc.

5. Cereals--list specific name as Cheerios, or oatmeal.

Cooked--record tablespoon or cup portions, level measurements, after cooking. List brand. List type of rice, pasta, etc., and specify if enriched or not. List "rolled oats" if measured raw; "oatmeal" if cooked.

Dry--record level cup portions or tablespoons.

Biscuit cereals--record as number of biscuits eaten.

6. Breads--record as wheat, white, rye, tortilla; list whether corn or wheat flour, enriched or restored. List brand. Give recipe for French toast or home-made bread. Specify if bakery goods are enriched or not.

7. Meats, Poultry, Fish--list kind and how prepared as baked, broiled, boiled or fried. Give grade plus other description, as "lean" hamburger, cut of beef or pork, or piece of chicken. Give approximate portion size as 1 1/4 x 1/2 x 2". Specify type of wieners from label, as: "all-meat," "dry milk added," etc.

Cheeses--list kind and amount in cups or size of piece in inches. Include yogurt, cottage cheese. Cottage cheese--specify dry curd, small curd, creamed, etc. Cheese--specify type and brand from label, as "Longhorn," "Swiss," "Processed."
8. Fats--record in level teaspoons or tablespoons--include those used in cooking. If butter is used, list as butter; if margarine, list brand; if oil, list kind, as corn oil or olive oil. Include cream, sour cream, and imitation sour cream. Include non-dairy cream substitutes (liquid or powder).
9. Desserts--describe size of portion in cup portions and list descriptive name. Give recipe or attached sheet.
10. Candies should be listed with exact amount and number, such as 5 large gumdrops.
11. Nuts and peanut butter--nuts in cup portions and peanut butter in tablespoon portions. Record brand and type of peanut butter.
12. List amount of ingredients in mixed dishes, such as stews, soups, home-made desserts, tacos, casseroles, pizza. Use attached sheet.
13. List sugar added to cereals, tea, or used on other foods.
14. Beverages--coffee, tea, alcoholic, herb teas, hot chocolate. List as cups. Include sugar and cream, cream substitutes, lemon, in teaspoons. List method of mixing of dry beverages, e.g. Tang, Quick, if different from directions on package. List in ounces the kind and amount of carbonated beverages taken.
15. Snacks--size of bag or number of pieces. Give brand name. Give type and description of snack crackers from label.
16. Legumes (dried beans, peas, lentils)--list kind and amount in cups after cooking. If used with other foods, list ingredients on recipe page. Peanut butter--specify if added fat or added sweetener (from label); give brand and type ("chunky").
17. Eggs--give size (small, medium, or large). If omelet or scrambled eggs, give recipe.
18. Gravies and sauces--give recipe and amount eaten.
19. Miscellaneous--syrups, toppings, jams, jellies, pickles, condiments and seasonings, yeast, e.g. chili sauce, peppers, tomato sauce, soy sauce, ketchup, mustard. Specify c, tb, tsp. Include type of vinegar. Lemon juice--specify fresh or bottled (canned). Specify type of yeast (dry, compressed, Brewers').
20. Include number and brand name of vitamin, mineral, yeast pills, bone meal, alfalfa meal, wheat germ, in teaspoons or tablespoons.

APPENDIX II

Diet History

Subject's Code: _____

Date: _____

How many times do you usually eat these foods? As I name a food, please tell me how many times a day, week, or month you eat it.

<u>Food</u>	<u>Times</u>	<u>Interval</u>
	1	1 - never
	2	2 - daily
	3	3 - weekly
	4	4 - monthly
	etc.	5 - seasonly

Milk (including milk used on cereals, fruits, and puddings)

Meat Alternative

	<u>Times</u>	<u>Interval</u>
Fresh, whole milk		
Fresh skim milk		
Fresh 2% milk		
Evaporated		
Non-fat dry milk		
Chocolate milk		
Yoghurt		

	<u>Times</u>	<u>Interval</u>
Eggs		
Cottage cheese		
Hard cheese		
Pinto beans		
Lima beans		
Peanut butter		
Soy beans		
Other _____		

Meats

Fruits

	<u>Times</u>	<u>Interval</u>
Ground beef		
Beef steaks		
Beef roasts		
Luncheon meats & franks		
Organ meats		
Corned beef		
Prok chops, roasts		
Ham		
Bacon, sausage		
Chicken		
Turkey		
Duck		
Deer meat		
Lamb		
Other _____		

	<u>Times</u>	<u>Interval</u>
Citrus fruit		
Citrus juice		
Banana		
Apple		
Peaches		
Pear		
Apricot		
Pineapple		
Other _____		

Fish

Vegetables

	<u>Times</u>	<u>Interval</u>
Tuna fish		
Salmon		
Fin fish		
Shrimp, crab, lobster		
Oysters, scallops		
Other _____		

	<u>Times</u>	<u>Interval</u>
Peas		
Green beans		
Spinach		
Broccoli		
Kale		
Greens		
Squash		
Carrots		
Asparagus		
Potato		
Tomato		
Turnip		
Beets		

Times Interval

Corn
Cabbage
Cauliflower
Brussel sprouts
Rutabagas
Onion
Lettuce
Celery
Cucumbers
Other _____

Cereals and breads

Enriched breads
Pancakes, waffles
Rice
Pastas
Dry, unsweetened cereal
Dry, presweetened cereal
Cooked cereals
Homemade breads
Cornbread
French Toast
Other _____

Fats

Butter
Margarine
Vegetable oil
Shortening
Mayonnaise
Salad dressing
Non-dairy creamer
Other _____

Soups

Clear type
Cream type
Vegetable
Other _____

Desserts

Cakes
Donuts, rolls
Cookies
Puddings
Ice Cream
Other _____

Times IntervalSweets

Jams, jellies
Syrups
Honey
Hard candy
Other candies

Snack Foods

Nuts
Jello
Chips
Popcorn
Pretzels
Pickles
Other

Beverages

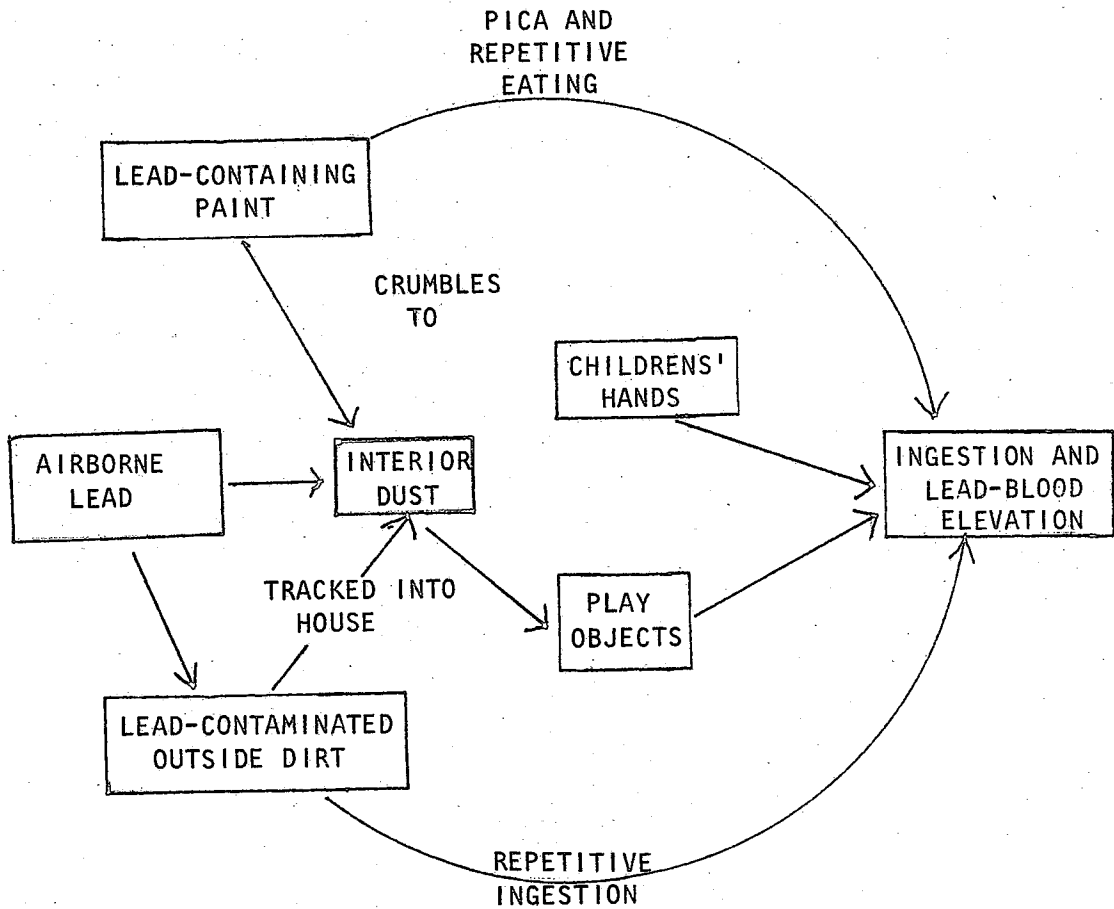
Soft drinks
Artificially sweetened drinks
Coffee, tea
Alcoholic drinks
Beer
Wine
Other

Vitamin or Mineral Supplements

Brand name _____
Amount Taken Daily _____
Time of Day Taken _____

APPENDIX F

INCREASED LEAD ABSORPTION IN INNER CITY CHILDREN:
WHERE DOES THE LEAD COME FROM?



Source: Charney, Sayre, and Coulter (1980), p. 230.

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