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# Lead Levels in Human Milk and Children's Health Risk: A Systematic Review

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# Abstract

Lead is a naturally-occurring element and industrially-produced metal that is highly toxic to children, causing intellectual and behavioral deficits, hyperactivity, fine motor function deficits, decreased intelligence quotient, hand-eye coordination, and problems in reaction time. Children's exposure to lead occurs mainly through ingestion of contaminated food, water and soil. Few discussions have been held on the magnitude and potential risk associated with exposure from the consumption of breast milk. Hence, this paper was designed to systematically review the scientific literature on published epidemiologic studies, with an emphasis on the study design and analytical procedures used for Pb assessment in breast milk. From a total of 112 selected articles published since the 1980s, 11 met the inclusion criteria. A review of the data indicated that Pb levels varied from 0.15 to 6.1  $\mu$ g L<sup>-1</sup> in mature milk samples, from 0.48 to 14.6  $\mu$ g L<sup>-1</sup> in colostrums samples, and were non-detectable in some samples. The milk/blood ratio, which estimates the mean efficiency transfer of lead from blood to milk, varied between 0.01 and 0.48. The heterogeneity of methods revealed by our assessment of the published studies emphasizes the need for harmonization of study designs and sample collection and analysis protocols to reflect specific exposure scenarios. Human milk seems to be one of the feasible biological matrices for use as a biomarker for assessing children's health risk to lead poisoning.

### Keywords

lead; human milk; children; exposure biomarker; health risk assessment

# INTRODUCTION

Lead, a known toxic element, is a public health problem due to its adverse effects, mainly those affecting the central nervous system in the most vulnerable populations, such as pregnant and lactating women and children. In the absence of a safe exposure limit of children to lead and because of its ability to accumulate in the body for a long time, a great interest in the evaluation of the adverse effects of this metal in low concentrations has emerged  $/1^{-7}/$ . In general, children are the most vulnerable to lead exposure because they

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have greater gastrointestinal absorption and less effective renal excretion. Lead is extremely damaging to the developing brain in utero. Experimental studies provide evidence that when compared with a more mature brain, the fetal brain presents greater sensitivity to the toxic effects of lead. The investigations also suggest that the encephalic barrier of the fetus is immature and does not promote protection against the metal /1,  $7^{-8}$ /.

Lead is readily transferred to the fetus through the placenta /9/. In the absence of placental barrier efficacy, the fetus would be exposed to lead in a concentration very close to that of the mother. Fetus vulnerability can occur even if the mother's exposure had ceased many years earlier  $/10^{-11}$ /. The interference of lead can be observed on early embryonic development and during the last months of pregnancy /1,12/.

In epidemiologic studies of lead poisoning, biological indicators of lead exposure must be considered whenever possible. Many such studies have used human milk when assessing risk to children's health. Measuring lead concentrations in this biological matrix would be the first step in establishing guidelines for safe breastfeeding.

The primary sources of lead in breast milk are maternal diet and bone lead. During pregnancy and lactation, up to 5% of bone mass is mobilized as a source of calcium /13<sup>-16/.</sup> Concomitantly, lead accumulated in bone from past exposures can be released into the blood and excreted into breast milk /4/; also/. Distinguishing how much of the blood lead derives from ongoing environmental exposure as opposed to the mobilization of bone lead is impossible. Although this phenomenon is of special concern in countries where environmental lead exposure is high, detectable levels of lead in breast milk have been documented in population studies of women with no current environmental or occupational exposures /17/.

A multinational study conducted by the World Health Organization /18/ in six countries comprising four continents, representing different degrees of industrial development, estimated lead levels in breast milk ranging from 2.0–17.8  $\mu$ g L<sup>-1</sup>. The study concluded that lead concentrations in breast milk samples varying from 2.0–5.0  $\mu$ g  $\mu$ g L<sup>-1</sup> might be considered within reference values. A review by Abadin et al /17/ also concluded that in a population not occupationally exposed to lead, concentrations of this metal in milk would be within the same range.

The aim of this paper was to review the scientific literature on published epidemiologic studies, with an emphasis on the study design and analytical procedures used for lead assessment in breast milk. We also intended to apply a set of selection criteria to obtain a broader picture of lead assessment in human milk.

# METHODS

A systematic review of the literature was conducted to retrieve studies published and indexed in MEDLINE (PubMed) and in Latin American and Caribbean Literature in Health Science (LILACS) databases using initially the key words *lead*, *human milk*, and *blood lead*. The inclusion criteria were studies published between 2000 and the first semester of 2010 (regardless of the sample collection period); those using biomarkers of lead levels in breast milk; human biomonitoring studies, and studies published in English or Portuguese. Exclusion criteria were studies that were not original, studies on animals; studies lacking data on lead exposure; studies lacking information for understanding the data (for example, units used), and studies with non-representative samples.

The flowchart presented in Figure 1 summarizes the search strategy adopted to identify and include studies. A total of 112 articles were selected as being relevant, based on the

information provided in the titles and abstracts. According to the pre-established inclusion and exclusion criteria, a total of 11 articles were selected for in-depth evaluation in this review.

During the evaluation process, the selected articles were classified in chronological order, milk time (colostrum or mature milk, for instance), analytical technique applied, detection limits, and epidemiologic study design. The indexes compiled from different studies considered in this research were estimated based on the mean concentrations of lead in tissues of interest. For consistency, both milk and blood lead levels were converted to microgram per liter.

# RESULTS

#### Scenarios of Lead Levels in Human Milk

Two review studies are worth mentioning. Dorea /18/ reviewed more than 30 studies reporting lead concentrations in mature milk and/or colostrum ranging from 0.11–791  $\mu$ g L<sup>-1</sup>. Gulson et al /5/ analyzed studies conducted for over 15 years, with concentrations varying from 0.7–209  $\mu$ g L<sup>-1</sup>.

In the present review, the mean concentrations of lead in breast milk varied, ranging over three levels of magnitude:

- below 1.0 µg <sup>L-l</sup>: Leotsinidis et al /20/
- between 1.0 and 10.0 μg <sup>L-l</sup>: Koyashiki et al, Ettinger et al, Chien et al, Kïrel et al, Ursinyova and Masanova, Anastacio et al, Hanning et al, Sowers et al, Gundacker et al /21<sup>,13,22-28/</sup>
- between 10.0 and 100.0 μg <sup>L-l</sup>: Turan et al /29/.

In Brazil, only two studies have analyzed lead levels in breast milk. Koyashiki et al /21/, in a cross-sectional study, observed concentrations of this metal ranging from  $1.0-8.0 \text{ L}^{-1}$  in a city in the South of the country. Anastacio et al /25/ also conducted a cross-sectional study in the city of Rio de Janeiro, and reported lead concentrations between < 0.1 and 11.9 L<sup>-1</sup>. In South America, a longitudinal analysis in Mexico City conducted by Ettinger et al /13/ revealed mean concentrations of lead in mature milk ranging from 0.2 to 8.0 L<sup>-1</sup>, at 1, 4, and 7 months postpartum.

#### Lead Levels in Colostrum and Mature Milk

From the 112 studies analyzed, 11 meeting our inclusion criteria are summarized in Table 1. The key aspects of study quality (i.e., sample size, analytical technique, detection limit, milk time) were also reported. In colostrum samples, mean levels of the metal ranged from 0.48 to 14.60  $\mu$ g L<sup>-1</sup> for mothers who were not occupationally exposed to lead. Chien et al /22/, Kïrel et al /23/, Ursinyova and Masanova /24/, and Gundacker et al /28/ reported mean levels of lead in milk samples that were within WHO reference values (2–5 5  $\mu$ g L<sup>-1</sup>) /18/. The values reported in a study by Leotsinidis et al /19/ were below this range. Turan et al / 29/ found values much higher than those considered to be within the acceptable range. Overall, the mean lead levels in mature milk samples varied from 0.15 to 6.1  $\mu$ g L<sup>-1</sup>. According to the reference values established by the WHO /18/, only concentrations observed by Sowers et al /27/ in unexposed populations in the United States were above 5  $\mu$ g L<sup>-1</sup>.

Four studies shown in Table 1 did not present information for the date on which the collections were made. The number of samples collected in these studies varied widely from 15 to 310 samples. Regarding the analytical technique applied, Chien et al /22/, Kïrel et al /

Koyashiki et al /21/, Ettinger et al /13/, Anastacio et al /25/, and Sowers et al /27/ used inductively coupled plasma mass spectrometry (ICPMS) for analyzing lead in mature milk samples. From the 11 papers analyzed, the detection limits for various analytical techniques ranged from 0.1  $\mu$ g L<sup>-1</sup> for ICP-MS to 2.08  $\mu$ g L<sup>-1</sup> for GFAAS, and in three studies, the data were not presented.

#### **Correlation between Lead Concentrations in Milk and Blood**

Several studies reported a significant correlation between the concentration of lead in maternal milk and blood, as it can be observed in Table 2. Kirel et al /23/ found a good correlation between lead levels in maternal milk and blood during lactation. Koyashiki et al / 21/, Ettinger et al /13/, Anastacio et al /25/, and Sowers et al /27/ found a significant but modest correlation between maternal blood lead levels and mature milk samples.

**Milk/Blood Lead Ratio**—The ratio was obtained by dividing the means of lead level in milk by the means in lead concentration in maternal blood. This ratio establishes the transfer efficiency means of lead in maternal blood to milk and provides an indication of the concentration of this metal in milk when compared with the concentration presented in maternal blood. Among the 11 articles analyzed, only 7 determined the levels of lead in two biological matrices (milk and blood). Table 3 presents the milk/maternal blood ratio in these studies. A ratio  $\geq 1$  can be assumed to represent a significant dose to the child /18/. The milk/ blood ratio in the analyzed studies ranged between 0.01 and 0.43. In a review performed by Dorea /19/, the blood/milk ratio varied between 0.02 and 8.3, and in a study conducted by Gulson et al /5/, the ratio from the revised studies ranged between 0.02 and 0.42. Only in the study conducted by Li et al /30/ was this ratio > 1.

# DISCUSSION

The objective of this systematic review was to gather and discuss the main studies available in literature, on lead levels in human milk. One difficulty faced in the selection of articles was the heterogeneity of methods used in the different studies. This difficulty, however, is common in systematic reviews. As new inclusion criteria are added, a gain in specificity is achieved but also a loss in sensitivity, compromising the objective of showing the scenario of publications on a specific subject. Because of the substantial heterogeneity and the limitations in methodology in the original studies, we considered a quantitative pooling inappropriate. Hence, the qualitative systematic review was conducted on the evidence available. Although multiple search strategies were used to identify relevant studies, some publications may have been missed.

Among the different studies, a diversity of epidemiologic design and analytic methodologies to measure lead in breast milk could be observed, making data comparison difficult and not allowing for a precise evaluation of estimates. Hence, determining whether these gaps are a result of analytic limitations or if the data truly reflect different types of exposure is difficult. Several studies are limited by the small number of samples, non-adjustment for confounding variables, and short follow-up periods. Other factors include differences in the age groups studied, parity, lactation stage, as well as bias in selecting the participants. Not every author followed international definitions and parameters as inclusion criteria, which also brought difficulty in comparing these studies. All these factors were fundamental for establishing the criteria for the selection of studies for this review.

The articles analyzed in the present review were characterized, in their majority, as crosssectional studies. Note that only the study by Ettinger et al /13/ had a longitudinal design.

Detection levels are comparable, and most studies have reported these limits, which can be seen in Table 1. Nevertheless, the ways in which values lower than the detection limit are reported and used in statistical analysis were not presented in all papers. These numbers must be reported, either as *non-detectable* or by using half their values in statistic calculations. Another possibility would be to divide the limit of detection value by the square root of 2 or, in a more liberal way, use the detection limit value itself in these calculations /31/. However, these options must be carefully and clearly described and the studies have not presented the option used, with the exception of Koyashiki et al /32/, which used half the detection limit for statistics calculations.

Considering that the concentrations of lead in milk did not necessarily present a normal distribution, the best measures of central tendencies to be used would be the median and the geometric mean /33/, which are less influenced by high values than the arithmetic mean. Nevertheless, a great portion of the studies analyzed in the present review presented the arithmetic mean as their central measure.

In the articles conducting analyses of lead in milk, the highly sensitive techniques like ICP-MS and GFAAS could guarantee results that would present good accuracy and precision.

The sample preparation steps are usually the most common source of analytic error and usually increase the overall imprecision of the method /34/. We must also consider that because of the low levels of metal in milk, one problem was the contamination of samples during collection or analytical determination as milk is a substance that can be easily contaminated. Much of the older data and values > 3 parts per billion of lead in breast milk reported during the last 15 years have been questioned /4·5·18/. Using a comparisons of ratios expressed as percentage of lead concentration in milk to that in blood constitutes a method to validate results. Data showing a milk/blood ratio of > 15% should be treated with caution, however, because of possible contamination during the collection of the samples or during laboratorial processing and analysis /4<sup>-5</sup>/.

When determining lead in a biological matrix, fat content in milk is also a complicating factor because it changes during the course of lactation. A methodological challenge would be to establish the most appropriate time for the milk to be used as a good exposure biomarker. According to Needham and Wang /31/, for monitoring purposes, mature milk must be collected only when its fat content is set, which happens 2 weeks after birth. Therefore, mature milk would be more appropriate when compared to colostrum, which might explain the greater variability in lead concentrations found in the studies of colostrum presented in Table 1.

Lead in breast milk from mothers with current ongoing exposure to lead or mothers exposed by the redistribution of cumulative maternal bone lead stores could pose a potential health risk to the infant. Ettinger et al. /14/ found, however, that even among a population of women with relatively high cumulative lifetime exposures to lead as assessed by bone lead levels, breast milk levels were low. Nevertheless, breast milk was found to exert a strong influence on infant blood lead levels over and above the influence of maternal blood lead. Thus, the question arises of what exactly is the risk to child health? Maternal blood lead levels < 10 µg L<sup>-1</sup> are considered no cause for concern as the amount of lead in breast milk is only  $\leq$ 5% of that in her blood. Within the range of lead concentrations reported in the studies reviewed here, the effects are expected to be small /14<sup>,35</sup>/. Thus, breast-feeding should not be discouraged.

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# CONCLUSION

This review has shown that human milk could be a feasible biological matrix for use as a biomarker for lead exposure, with the goal of evaluating the risk to children's health using a non-invasive biological procedure. Although the lead concentrations were highly variable in this review, the health risk for the infant from breast mild appears to be small at such low levels. To increase the accuracy, consistency, and reproducibility of future studies, we recommend that collection protocols and analytical methods for lead assessment in human milk be harmonized to provide a scientific basis for inter-laboratory comparisons and to improve risk assessment strategies to address different exposure scenarios.

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Fig. 1. Study selection process flowchart

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Author/Year	sample collection	Country	Sample	size (n)	Ы	) н <u>д</u> L <sup>-1</sup>	Mother milk time	Detection Limit μg L <sup>-1</sup>	Objective of the study (Comments)
Koyashiki et al.,	LOOC	F	ę		2.90±1.1 <sup>a</sup>	28.2±9.4	E	ICP-MS <sup>e</sup>	Evaluate lead levels in milk and blood from
2010/17/	/007	DTaZII	76		3.0b	27.0b		LD: 1.0 µg	milk Bank donors
			Ę	35	8.59±10.95 <sup>a</sup>	NA			-
Chien et al., 2006 /	1000	₹	71	37	$6.84\pm 2.68^{a}$	NA	colostrum	GFASS	Examine the relationship between lead concentrations in breast milk and the
19/	2007-1007	China		6	2.34 <sup>a</sup>	NA	F	LD: not given	consumption of traditional Chinese nerbs commonly used during pregnancy and loctation Constitution ye control around
			01	7	2.36 <sup>a</sup>	NA			iaviauon. Comounipuon va comuoi group.
				010	1.40±1.1 <sup>a</sup>	$93.0\pm45.0^{d}$			
				010	$1.0^{b}$	83.0b	1		
Ettinger et al.,	1004 1005	Marine			$1.20\pm1.0^{d}$	$90.0\pm40.0^{d}$		ICP-MS <sup>e</sup>	A longitudinal analysis conducted among lactating women at 1, 4, and 7 months
2006/18/	C661-4661	INICAICO		+77	q6.0	82.0b		LD: 0.1	postpartum to evaluate the relations annoig maternal blood, bone, and breast milk lead
				105	0.90±0.8 <sup>a</sup>	81.0±34.0 <sup>a</sup>	1		
				C61	$qL^{0}$	74.0b			
					$0.48\pm0.60^{a}$	VI.V			
Leotsinidis et al.,		ç	05		0.44b	M	colosum	$ m GFAAS^f$	Measuring lead and cadmium concentrations in colostrum and transitory milk samples, from
2005 /16/	7007-0007	Dieece	с <i>к</i>		$0.15\pm 0.25^{a}$		F	LD: 0.20	lactating mothers, to assess if the infant intakes are within recommended levels.
				•	ND <sup>bc</sup>	NA	mature milk		
						-	-	-	
A uthou/V acu	Period of	Contern	Common	(n) ozi	Milk	Maternal blood	Comlo truo (timo)	Analytical	Obioativo of chudy (Commando)
Autuory rear	sample collection	Country	Sampre		Pb	μg L <sup>-1</sup>	Same type (unite)	иссилицие, р.н. µg L <sup>-1</sup>	Objective of study (Comments)
Kirel et al., 2005 / 20/	2001–2002	Turkey	143		2.34±1.0 <sup>a</sup>	28.0±15.0 <sup>a</sup>	colostrum	GFAAS <sup>f</sup> LD: not given	To determine the level of lead exposure in children and adults among the general population; and to determine the lead level in maternal-cord blood and breast-milk samples during prenatal and neonatal periods.

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	Period of				Milk	Maternal blood	()	Analytical	
Author/Year	sample collection	Country	Samples	ize (n)	Pb	μg L <sup>-1</sup>	Samle type (time)	tecnnique, DL µg L <sup>-1</sup>	Objective of study (Comments)
Ursinyova &	VIN	Classicie	150		4.70±4.1 <sup>a</sup>	VIV	(FV)too	GFAAS <sup>f</sup>	To determine lead, mercury and cadmium levels
7 24/ 24/	M	SIOVAKIA	001		3.4b	W	colostrum (4a)	LD: 0.65	in breast milk of lactating women.
-					2.80±2.5 <sup>a</sup>	63.0±20.0 <sup>a</sup>			To determine the concentration and distribution of lead, calcium, iron, zinc, and copper in major
Anastacio et al., 2004 <i>/25/</i>	NA	Brazil	38		1.20d	60.09	mature milk	ICP-MS <sup>e</sup> LD: 0.1	fractions (fat, casein, whey) of mature milk from nursing women with low environmental lead exposure.
Hanning et al, 2003 /26/	NA	Canada	25		2.08±1.67 <sup>a</sup>	22.9±12.5 <sup>a</sup>	mature milk	GFAAS <sup>f</sup> LD: 2.08	To describe lead in maternal and cord blood at birth and infant blood at four months of age. Also to analyze lead levels in breast milk at 2 and 4 months lactation.
					6.10±1.0 <sup>a</sup>	$14.0^{a}$			-
Sowers et al.,	1007 2000	V JII	2		5.60±1.1 <sup>a</sup>	$16.0^{a}$	alline control on	ICP-MS <sup>e</sup>	To determine whether breast mulk lead levels are correlated with maternal blood lead levels, bone
2002 /27/	0007-1661	<b>V</b> CO	CI		5.90±1.0 <sup>a</sup>	$17.0^{a}$		LD: not given	uss, or come turnover during reproduction. Data were collected prospectively at 0, 1.5, 3, 6 and 12 months after delivery in lartating women
					4.30±1.6 <sup>a</sup>	$14.0^{a}$			
				48	$1.22\pm0.92^{a}$	NA			
Gundacker et al., 2002 /28/	1999	Austria	•	37	2.48±2.39 <sup>a</sup>	NA	colostrum (2–14d)	GFAAS <sup>f</sup> LD: 0.10	Measure possible changes in levels of lead in breast milk since earlier measurements in 1981 and 1002
				27	$1.29\pm 1.12^{a}$	NA			and 1770.
Turan et al., 2001 / 29/	1995–1996	Turkey	30		14.6±5.5 <sup>a</sup>	NA	colostrum (4d)	GFAAS <sup>f</sup> LD: 0.92	To determine the concentrations of toxic and essential elements in colostrum samples, to evaluate the exposure to environmentally toxic metals.
arithmetic mean;									

*b* median;

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*c* non detected;

d geometric mean;  $^{e}$ Inductively Coupled Plasma Mass Spectrometry;

 $f_{
m Graphite}$  Fumace Atomic Absorption Spectrophotometry;;

DL - detection limit; NA - not applicable

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# Table 2

Studies on the correlation between lead concentrations in maternal milk and blood

0. > 1/2.3/ r = 0.6, p < .0	001 Colostrum
$ul (2004) / 25/$ $r_s = 0.49, p =$	02 Mature milk
(2006) /13/ $r_s = 0.32$ , p <	0001 Mature milk
2002) /27/ $r_s = 0.30^*$	Mature milk
110) $/21/$ $r_s = 0.297, p = 0.$	:.048 Mature milk
$r_s = 0.30^{\circ}$ , $r_s = 0.30^{\circ}$ , $r_s = 0.297$ , $p = 0.10$ ) /21/ $r_s = 0.297$ , $p = 0.297$ ,	048

S-sperman s correlation

\* at six weeks of lactation Koyashiki et al.

# Table 3

Milk/blood ratio estimated in studies conducted in different countries

			Pb (μg L <sup>-1</sup> )		
Reference	Location	Milk	Maternal blood	Milk/Blood	Ratio
Koyashiki et al 2010/21/	Brazil	2.9	28.0	0.11	
		1.4	93.0	0.01	
Ettinger et al 2006 /13/	Mexico	1.2	90.0	0.01	
		0.9	81.0	0.11	
Kirel et al (2005) /23/	Turkey	2.3	28.0	0.08	
Anastacio etal (2004) /25/	Brazil	2.8	63.0	0.04	
Hanning et al (2003) /26/	Canada	2.1	22.9	0.09	
		6.1	14.0	0.43	
		5.6	16.0	0.35	
Sowers et al (2002) /27/	USA	5.9	17.0	0.35	
		4.3	14.0	0.31	