FERNANDA MACIEL REBELO

ARSÊNIO, CÁDMIO, CHUMBO E MERCÚRIO EM LEITE HUMANO-ANÁLISE, AVALIAÇÃO DA EXPOSIÇÃO E CARACTERIZAÇÃO DO RISCO DE LACTENTES

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FERNANDA MACIEL REBELO

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Tese de Doutorado apresentada como requisito parcial à obtenção do título de Doutora em Ciências da Saúde pelo Programa de Pós-Graduação em Ciências da Saúde pela Universidade de Brasília

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“Comece fazendo o que é necessário, depois o que é possível, e de repente você estará fazendo o impossível”.

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LISTA DE ABREVIATURAS E SIGLAS

AAS	*Atomic absortion spectrometry*
ANVISA	Agência Nacional de Vigilância Sanitária
As	Arsênio
ATSDR	*Agency for Toxic Substances and Disease Registry*
BLH	Banco de Leite Humano
BM	*Breast milk*
BMD	*Benchmark dose*
BMDL	*Benchmark dose lower confidence limit*
Cd	Cádmio
Cp6	*Cytosine followed by guanine*
CV	Coeficiente de variação
CVAAS	*Could vapor atomic absortion spectroscopy*
DMA	Dimetilarsênio
EFSA	*European Food Safety Authority*
ER	Erro Relativo
ETAAS	*Electrotermal atomic absortion spectrometry*
EtHg	Etilmercúrio
GSH	Glutationa
Hg	Mercúrio
IARC	*Internacional Agency for Research on Cancer*
IBGE	Instituto Brasileiro de Geografia e Estatística
ICP-MS acoplado	Espectrometria de massas por plasma indutivamente
IAs	Arsênio inorgânico

IPb	Chumbo inorgânico
IQ	*Inteliigence quotient*
JECFA	*Joint FAO/WHO Expert Comittee on Food Aditives*
KED	*Kinetc energy discrimination*
LOD	Limite de detecção
LOQ	Limite de quantificação
MAPA	Ministério da Agricultura Pecuária e Abastecimento
MeHg	Metilmercúrio
MMA	Monometilarsênio
MOE	Margem de exposição
MRL	*Minimal risk level*
NOAEL	*No observed adverse effect level*
NRC	*National Research Council*
OMS	Organização Mundial de Saúde
Pb	Chumbo
PTMI	*Provisional Tolerable Monthly Intake*
PTWI	*Provisional Tolerable Weekely Intake*
RfD	*Reference dose*
ROS	*Reative oxigen species*
SE	Somatória de erro
TAs	*Total arsenic*
THg	Mercúrio total
TWI	*Tolerable Weekly Intake*
WHO	*World Health Organization*

RESUMO

REBELO, Fernanda Maciel. **Arsênio, cádmio, chumbo e mercúrio em leite humano: análise, avaliação da exposição e caracterização do risco de lactentes.** Brasília, 2017. Tese de Doutorado em Ciências da Saúde – Faculdade de Ciências da Saúde, Universidade de Brasília, Brasília, 2017.

O leite materno fornece todos os nutrientes necessários para o desenvolvimento do bebê, entretanto, ele pode conter metais tóxicos que podem causar efeitos adversos a saúde. Este estudo tem como objetivos determinar a concentração dos contaminantes arsênio, cádmio, chumbo e mercúrio em amostras de leite materno provenientes de bancos de leite do Distrito Federal, e caracterizar o risco da exposição dos bebês amamentados a esses contaminantes. As amostras foram coletadas em 8 bancos de leite materno do Distrito Federal, Brasil, a maioria das amostras coletadas nos 2 primeiros meses após o parto. Foram analisadas 224 amostras de leite materno para mercúrio total, 181 para metilmercúrio e 212 amostras para chumbo cádmio e arsênio. A análise de mercúrio total foi feita com digestão ácida em microondas e determinação por espectrometria de fluorescência atômica (LOQ: 0,76 µg/L). A análise de metilmercúrio foi feita a partir de liofilização das amostras, etilação e determinação de metilmercúrio pelo sistema automatizado MERX (LOQ: 0,1 µg/L) Foi validado um método de digestão ácida por microondas e determinação das concentrações de arsênio, cádmio e chumbo por espectrometria de massa por plasma indutivamente acoplado (LOQ: 0,31 µg/L, 0,016 µg/L e 0,08 µg/L para chumbo, cádmio e arsênio, respectivamente). Mais de 80% das amostras continham concentrações de mercúrio total acima do LOQ, com máxima de 8,4 µg/L e média de 2,6 µg/L. Em média, metilmercúrio representou 10,1% do mercúrio total, com proporção maxima de 74,9%. A concentração média de chumbo foi de 6,64 µg/L, com 75,9 % das amostras acima do LOQ, e a de cádmio foi 0,24 µg/L, com 71,4% das amostras acima do LOQ. Apenas 4 amostras continham arsenio acima do LOQ (2,25 a 9,5 µg/L). As ingestões de metilmercúrio, chumbo, cádmio e arsênio foi estimada individualmente, considerando a idade do bebê e peso no momento da coleta do leite. A ingestão média semanal de metilmercúrio foi 0,16 µg /kg pc, o que representou 13,6% do PTWI; em apenas 1 caso, a ingestão ultrapassou 100% do PTWI (119%). A ingestão média semanal de cádmio estimada representou 9% da TWI. Para o chumbo e arsênio, as exposições medianas diárias foram 0,87 µg/kg pc/dia e 0,005 µg/kg pc/dia; as margens de exposição medianas foram de 1,2 e 587, respectivamente, não indicando um potencial risco para a saúde dos lactentes.

Palavras chave: arsênio, chumbo, cádmio, mercúrio, metilmercúrio, leite materno, avaliação da exposição pela dieta, risco.

ABSTRACT

REBELO, Fernanda Maciel. **Arsenic, cadmium, lead and mercury in breast milk: analisys, exposure and risk characterization for infants.** Brasília, 2017. Doctoral Thesis in Health Sciences – Faculty of Health Sciences, University of Brasília, Brasília, 2017.

Breastmilk provides all necessary nutrients for the infant development, however, it may contain toxic metals that may impact the health. The objectives of this study were to determine the levels of arsenic, cádmium, lead and mercury in breastmilk from mothers of the Federal District. The samples were provided by 8 milk banks or directly by the donnors, mostly collected in the first 2 months postpartum. A total of 224 breastmilk samples were analyzed for total mercury, 181 for methylmercury and 212 samples for lead cadmium and arsenic. Total mercury was determined by atomic fluorescence spectrometry after acid digestion in microwave (LOQ of 0.76 µg/L). Methylmercury determination was performed in a dedicated automated system MERX after lyophilization and ethylation of samples (LOQ of 0.1 µg/L). A method for the determination of arsenic, cadmium and lead was validated and included acid digestion by microwave and analyzis by inductively coupled plasma mass spectrometry (LOQs of 0.31, 0.016 and 0.08 µg/L for lead, cadmium and arsenic, respectively). More than 80% of samples contained levels of total mercury above the LOQ, reaching a maximum of 8.4 µg/L, with a mean of 2.6 µg/L. Methylmercury represented 10.1% of the total mercury, with a maximum ratio of 74.9%. Mean lead concentration was 6,64 µg/L, with 75,9 % of the samples above the LOQ. Cadmium mean levels was 0.24 µg/L, with 71.4% of the samples above the LOQ. Only 4 samples contained arsenic above the LOQ (2.25 to 9.5 µg/L). The intakes of methylmercury, lead, cadmium and arsenic were estimated individually, considering the age of the baby and its weight at the time of milk collection. Mean intake of methylmercury was 0.16 µg/kg bw/week, which represented 13.6% of PTWI, with only 1 case having the intake exceeding the PTWI (119%). The mean weekly intake estimated for cadmium, was 0.23 µg /kg bw/ week and represented 9% of the maximum tolerable intake. For lead and arsenic, daily exposures were 0.87 µg/kg bw and 0.005 µg/kg bw; the median margin of exposures were 1,2 and 587, respectively, indicating a low health risk for the infants.

Key words: arsenic, cadmium, lead, mercury, methylmercury, breast milk, risk assessment

I. INTRODUÇÃO

Os metais são ubíquos na natureza, estando presentes em solos, água, plantas e animais. É um importante grupo de contaminantes químicos ambientais a que o homem é exposto diariamente e que pode potencialmente representar um risco para a saúde. A exposição a metais pode ocorrer por via oral, inalação ou dérmica, podendo ser pontual ou durante toda a vida. Na maioria das vezes, a exposição se inicia no útero materno, com a transferência desses compostos via cordão umbilical. Após o nascimento, aumentam-se as fontes de exposição, sendo o leite materno uma importante fonte de exposição nos primeiros meses de vida do bebê (Gurbayet al., 2012; Ettinger et al., 2014).

Crianças são mais vulneráveis e sensíveis aos efeitos de substâncias tóxicas do que adultos, devido ao rápido crescimento, a imaturidade fisiológica dos órgãos e a susceptibilidade do sistema nervoso central no primeiro ano de vida (Isaac et al., 2012). Além disso, absorção destas substâncias pelo trato gastrointestinal é geralmente maior para recém-nascidos e bebês (Chao et al., 2014).

O leite humano é um alimento fundamental para recém-nascidos e bebês, contendo proteínas, gorduras, carboidratos e elementos essenciais, além de enzimas e cofatores que criam uma barreira protetora nos bebês contra fatores ambientais, aumentando os mecanismos de defesa e estimulando o sistema imune das crianças (Grzelak et al., 2014). Alguns estudos sugerem que crianças amamentadas com leite materno possuem melhor função cognitiva e menor probabilidade de se tornarem adultos obesos em comparação com aqueles alimentados com fórmulas nutricionais (WHO, 2009; Cardoso, 2014). A Organização Mundial de Saúde (OMS) recomenda que o bebê tenha como fonte de alimentação exclusiva o leite materno nos primeiros 6 meses de vida (WHO, 2007).

A composição do leite materno não é constante e depende do status nutricional da mãe, sua dieta, e a fase da lactação, além de fatores ambientais a que as mães estão expostas, inclusive aos metais (Grzelak et al., 2014; Isaac et al., 2012;). No leite humano, os elementos traços estão principalmente ligados a proteínas, cujos teores diminuem durante o primeiro mês de lactação.

Os mecanismos de regulação da concentração desses contaminantes no leite incluem sua captação dentro das células epiteliais mamárias e subsequente secreção no lúmen alveolar da glândula mamária (Kelleher and Lönnerdal, 2005; Almeida et al., 2008).

Cabe ressaltar que as fórmulas nutricionais não estão livres de contaminantes tóxicos, assim como a água utilizada para dissolução, além da possibilidade de contaminação microbiológica (Bjorklund et al, 2012; Weisstaub and Uauy, 2012).

O monitoramento do leite materno é uma forma não invasiva de determinar níveis de contaminantes químicos de interesse. Este tipo de monitoramento tem recebido especial atenção nas últimas décadas uma vez que ele provê informações sobre a exposição de mulheres em idade reprodutiva e da exposição perinatal do feto e do bebê em amamentação a estas substâncias simultaneamente (Abballe et al., 2007; Hooper and McDonald, 2000).

II. REVISÃO BIBLIOGRÁFICA[1]

1. Introduction

Metals are ubiquitous in nature, but some comprise a group of contaminants to which exposure, even at relatively low levels may represent a risk to human health. Arsenic ranks first on the National Priorities List of the Agency for Toxic Substances and Disease Registry (ATSDR), which prioritizes substances based on a combination of their frequency, toxicity, and human exposure potential. Lead, mercury and cadmium rank 2nd, 3rd and 7th on this list, respectively (ATSDR, 2015).

Human exposure to metals can occur during occupational activities, mainly through inhalation and dermal routes in mining and industry, and over a lifetime, from water and food consumption and exposure to soil, dust and air (ATSDR, 2007; WHO, 2004; EFSA, 2009a; Carlin et al., 2016). The presence of toxic metals in human milk has been reported worldwide (e.g., Gürbay et al., 2012; Chao et al., 2014; Ettinger et al., 2014), and breastfed babies are particularly vulnerable and sensitive to their toxic effects due to their rapid growth, organ immaturity, and susceptibility of their nervous system during the first year (Isaac et al., 2012). Furthermore, newborns absorb metals to a greater extent than adults and have a lower capacity to excrete compounds in the bile, decreasing body clearance (Oskarsson et al., 1998).

Lactation is a highly complex process that begins about 40 hours after birth, and is triggered by the hormones progesterone, estrogen, prolactin and oxytocin (Gundacher and Zödl, 2005). Breast milk is a fundamental source of nutrients for newborns and babies, as it contains proteins, fats, carbohydrates, and elements essential to the proper functioning of the body. It is also a source of lactoferrin, α-lactalbumin and lisoenzymes, substances that create a protective barrier against environmental factors, increasing defense mechanisms and stimulating the development of immunological systems in

[1] Este texto é uma reprodução do artigo *Rebelo FM, Caldas ED. Arsenic, lead, mercury and cadmium: Toxicity, levels in breast milk and the risks for breastfed infants. Environmental Research 151 (2016) 671–688*, Anexo I deste documento.

children (Grzelak et al., 2014). Breast milk influences the intestinal microflora, ensures the structural and functional maturity of mucous membranes, reduces the risk of allergies and autoimmune disorders, and contributes to the proper development of the gastrointestinal, central nervous, endocrine and immune systems (Leon-Cava et al., 2002). The WHO recommends that babies be exclusively breastfed up to 6 months of age, and for an additional 2 years along with appropriate complementary foods (WHO, 2007).

The composition of human milk is not constant and depends on the nutritional status of the mother, her diet, stage of lactation, socio-demographic status, and lifestyle (Ballard and Morrow, 2013; Garcia-Esquinas et al., 2011; Vieira et al., 2013). The transport of xenobiotics into milk is supposed to follow the same pathways as those of other milk components, with toxic metals entering milk through similar ways to those of essential trace elements (Oskarsson et al. 1998). Trace element regulation mechanisms in milk involve the capturing of metals by specific transporters in the mammary epithelial cells and their subsequent discharge in the alveolar lumen of the mammary glands (Rossipal and Krachler, 1998; Kelleher and Lönnerdal, 2005; Bressler et al., 2007). Studies conducted with rats and mice indicated that lead was almost exclusively found in the casein fraction, the highest proportions of cadmium and methylmercury found in fat, and inorganic mercury in whey fractions (Oskarsson et al., 1998). In human milk, mercury possesses a greater ability to interact with milk proteins, while cadmium and lead are equally distributed between light and low molecular weight components (see review by Gundacker and Zödl, 2005).

This paper briefly summarizes arsenic, lead, mercury and cadmium toxicology, focusing particularly on infants and children, and reviews the literature of studies reporting levels of these toxic substances in human breast milk worldwide. Exposure and risk assessment results of metal intake through breastfeeding are also reviewed, and the risks of exposure to breastfed infants discussed. For the incidence data, a query was conducted on the Pubmed, Science Direct and Google Scholar databases for studies published since 2000 (last search June 2016) using the keywords “human milk”, “breastmilk” and “breast milk”, associated with “metal”, “arsenic”, “lead”, “mercury” or “cadmium”.

Additional papers were identified in published reviews related to contaminants in breast milk.

2. Human exposure and toxicity

2.1 Arsenic

Arsenic (As) occurs naturally in volcanic ashes, volcanic rock, clay, iron oxides, mineral sulfur and organic matter. Human exposure to arsenic occurs primarily through the consumption of water and seafood, particularly shellfish (EFSA, 2009a). Arsenic is found in the environment in organic forms, including monomethylarsenic (MMA), dimethylarsenic (DMA), arsenobetaine, and arsenocholine, as well as in inorganic (IAs) forms (As^{III} and As^{V}). A systematic review conducted by Lynch et al (2014) evaluated over 6500 data on inorganic arsenic and its metabolites in food, including seafood and specific foods for children. Algae was the food with the highest concentration (mean of 1000 µg/kg, n=312, mostly as IAs), followed by rice and its byproducts (130 µg/kg, n = 1126, mostly as IAs), and seafood (130 µg/kg, n= 835; mostly as DMA).

Over 80% of inorganic arsenic is absorbed through the human gastrointestinal tract, and excretion occurs mainly via urine (ATSDR, 2007a). Certain characteristics of arsenic are summarized in Table 1. Studies conducted in Taiwan and other countries showed greater risk of lung, bladder, kidney or skin cancer from exposure to arsenic in drinking water, where it was predominantly present in inorganic form (WHO, 2001). Inorganic arsenic compounds, including arsenic trioxide, arsenite, and arsenate are classified as carcinogenic to humans by the International Agency for Research in Cancer (Group I), with extensive evidence of lung, bladder and skin cancer, and positive association with kidney, liver and prostate cancer (IARC, 2016). Although the mechanisms involved in the carcinogenicity of arsenic are not yet fully understood, it may nevertheless be considered genotoxic, since it induces micronuclei, DNA strand breaks, sister chromatid exchanges, aneuploidy and oxidative stress through the generation of reactive oxygen species during its biotransformation (see revision by Bustaffa et al., 2014.)

Inorganic arsenic and the methylated metabolites MMA and DMA cross the placentary barrier (Vahter, 2008), exert epigenetic effects by methylation of DNA (Reichard et al., 2007), and interact with multiple nuclear receptors (Bodwell et al., 2006). As a result, functional changes may occur leading to the development of other diseases later in life (Vahter, 2008). Vahter (2009) suggested that high levels of methylated arsenic in pregnant women are the result of *de novo* synthesis of choline by phosphatidylethanolamine methyltransferase, which is upregulated during pregnancy to supply fetal needs of choline for cerebral development (Zeisel, 2006). Exposure to arsenic can also cause reproductive toxicity, including increases in fetus mortality, underweight newborns, spontaneous abortions, eclampsia, and birth defects (WHO, 2001). As^{III} is the single form of arsenic which is protonated at physiologic pH, and is transported by the aquaglyceroporins (Liu et al., 2004; Rosen, 2002) present in mammary glands during lactation (Matsuzaki et al., 2005).

Recent epidemiologic studies have found a long latency period for lung cancer and other chronic diseases related to arsenic, even when exposure was limited to a short period during childhood or in the uterus. Exposure during these two periods may also have adverse reproductive outcomes for mothers, and induce changes in cognitive development of children (McClintock et al., 2012).

A limit of 10 µg/L was established by the WHO for arsenic in drinking water (WHO, 2004). However, some regions of the world have naturally high arsenic levels in water compartments which exceed that limit, including Argentina, Bangladesh, Chile, China, Hungary, India, Taiwan, and certain regions of the United States (Hopenhayn-Rich et al., 2000; Nordstrom, 2002; Rahman et al., 2011; McClintock et al., 2012). It is well established that almost all arsenic in drinking water is in inorganic form (JECFA, 2011a; EFSA, 2009a).

In Chile, data from 1950 to 1996 showed high late fetal mortality (OR= 1.7; CI: 1.5-1.9), neonatal mortality (OR= 1.53; CI: 1.4-1.7), and post neonatal mortality (OR = 1.26; CI: 1.2-1.3) in a region with a history of high arsenic levels in water, in comparison with a region with low levels (Hopenhayn-Rich et al., 2000). A epidemiologic study conducted in Bangladesh observed 1152

pregnant women and their babies for a period of 1 year, with urine samples collected after confirmation of pregnancy and in the 30th week of gestation for arsenic analysis (Rahman et al., 2011). Estimated risk of occurrence of lower respiratory tract diseases increased 69% for infants of mothers with higher arsenic concentrations in urine.

The mechanism and factors that may affect the excretion of arsenic in breast milk are not completely known, but fetuses and babies are probably protected by increased methylation of arsenic during pregnancy and breastfeeding (Fängström et al., 2008; Gürbay et al., 2012; Vahter, 2009). In a study conducted in Argentina in an area with high arsenic concentration in water (200 µg/L), the median concentration of arsenic was 34 µg/kg in the placenta, and 9 µg/L in cord blood, with a significant correlation with maternal blood levels (Concha et al., 1998). All arsenic in the blood plasma of newborns and their mothers, and about 90% of the arsenic in the urine of both, was present as DMA, a result also found by other authors (Fängström et al., 2008; Islam et al., 2014), indicating that methylation of arsenic occurred during pregnancy and the metal was transferred to the fetus as DMA. Fängström et al. (2008) indicated that the methylated arsenic metabolites in blood plasma do not pass easily through the mammary glands. The authors found that the arsenic concentrations in breast milk were negatively correlated with %DMA (r_s = -0.19), and positively correlated with %iAs (r_s = 0.16) in maternal urine. Thus, efficient maternal methylation of iAs leads to lower arsenic excretion in breast milk, which contains essentially inorganic arsenic, mainly as As^{III}.

In 2010, the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2011a) concluded that the provisional tolerable weekly intake (PTWI) previously adopted for arsenic (15 µg/kg bw, or 2.1 µg/kg bw/day) was no longer safe for humans, and established a benchmark dose, and a lower confidence level ($BMDL_{0.5}$) of 3 µg/kg bw/day as the reference point for risk assessment (Table 1). This dose corresponds to a 0.5% increase in the incidence of lung cancer associated with dietary exposure to inorganic arsenic over background in northeastern Taiwan (JECFA, 2011a).

2.2 Lead

Lead is a toxic metal widely present in nature, primarily in inorganic form, and is produced in activities such as mining and smelting, and in battery manufacturing (WHO, 2010a). The general population is exposed to lead mainly through food consumption, with about 5-15% of the oral intake being absorbed by the gastrointestinal tract, a rate that is higher in children under 6 years of age (WHO, 2010a). The higher gastrointestinal absorption of lead by children is related to the uptake pathways for essential minerals (e.g. calcium and iron), which are more active than in adults (HERAG, 2007). Inorganic lead compounds are classified by the IARC as potentially carcinogenic to humans (Group 2A), and organic lead compounds are "not classifiable to its carcinogenicity to humans" (Group 3) (IARC, 2016). Organic lead compounds are metabolized to ionic lead both in humans and animals, when the toxicity associated with inorganic lead is expected to be exerted (IARC, 2016). Table 1 summarizes some characteristics of lead.

Erythrocytes have high affinity for lead, and over 90% of what is absorbed is bound in the blood stream just after exposure. With age, lead is deposited in bone tissue, with a half-life of 10–30 years (WHO, 2010a). For the adult population, the cardiovascular and renal systems are the most critically affected by lead exposure, while for infants and children the effects on the central nervous system are the most critical (Sanders et al., 2009; EFSA, 2010; JECFA, 2011b). Encephalopathy, decreased nerve conduction, and cognitive deficits may occur in children with blood lead concentrations lower than the level that would induce similar effects in adults (ATSDR, 2007b). The particular vulnerability of fetuses and infants to the neurotoxicity of lead may be due in part to the immaturity of the blood-brain barrier, and to the lack of the high-affinity lead-binding protein in astroglia, which trap divalent lead ions in adults (Lindahl et al., 1999; EFSA, 2010; Schnaas et al., 2006). The various molecular, intracellular and cellular mechanisms that cause lead neurotoxicity also include the induction of oxidative stress, and interference in enzyme calcium dependents (eg. nitric oxide reductase), which amplify apoptosis of neurons (Nemsadze et al., 2009).

Gulson et al. (1997), using lead isotopic ratios of immigrant women arriving in Australia and of the local population, showed that mobilization of lead from bone contributed significantly to blood lead levels during the last trimester of pregnancy, a critical time for the development of the central nervous system, exceeding the normal exchange of bone lead stores observed in the non-pregnant condition. These increases were detected among subjects with blood levels <5 μg/dL, and were attributed to a low daily calcium intake, as calcium may reduce mobilization of skeletal mineral stores to supply calcium needs during pregnancy and lactation (Gulson et al., 1998, 2003). Lead skeleton mobilization was even higher during the post-pregnancy period, and was the major source of lead in breast milk, in addition to the diet and other exogenous factors (Gulson et al., 2003).

Various studies have shown the transfer of lead from the mother to the fetus via placenta prenatally, and via breast milk postnatally. In Mexico, Ettinger et al. (2004) found lead concentration in breast milk to be significantly correlated with the levels in umbilical cord and maternal blood lead at delivery, and with maternal blood lead and patella lead at 1 month postpartum. In another study with the same group (Ettinger et al., 2014), the mean mother milk:plasma ratio was 7.7; infant blood lead level (3.4 ± 2.2 μg/dL) increased by 1.8 μg/dL per 1 μg/L milk lead ($p < 0.0001$, $R^2 = 0.3$). Li et al. (2000) also found a significant correlation between lead levels in cord blood and breast milk with those in maternal blood in China.

In a cohort study with 175 children conducted in Mexico, Schnaas et al. (2006) found that lead exposure during the early third trimester of pregnancy can affect child intellectual development, with the strongest effects of lead being on the intelligence quotient (IQ) occurring within the first few micrograms of blood lead levels. IQ tests include a variety of tasks that probe cognitive abilities including memory, verbal and spatial reasoning, planning, learning, and comprehension and use of language (EFSA, 2010). The authors hypothesized that prenatal lead exposure would have a more powerful and lasting impact on child development than postnatal exposure.

Furthermore, a number of cross-sectional and prospective epidemiological studies have related lead blood levels to neurobehavioral effects on infants and children chronically exposed to lead (WHO, 2010a; Miranda et al., 2007; Counter et al., 2008; Roy et al., 2009). Most studies report a 2 to 4 point IQ deficit for each 10 µg/dL increase in blood lead within the range of 5-35 µg/dL (WHO, 2010a).

Lanphear et al. (2005) examined data collected from 1,333 children who participated in seven international population-based longitudinal cohort studies initiated prior to 1995 and were followed from birth or infancy until 5–10 years of age. There was an inverse correlation between blood lead concentration and IQ scores, and the authors concluded that environmental lead exposure in children who have maximal blood lead levels < 10 µg/dL was associated with intellectual deficits. No threshold for these effects was identified, and the dose-response relationship was steeper at low lead exposure than at higher exposure levels.

Based on the various available studies, the JECFA (2011b) and the EFSA (2010) concluded that the previous PTWI of 25 µg/kg bw/day for lead was associated with a decrease of at least 3 IQ points in children, with no evidence of a threshold for critical lead-induced effects. A $BMDL_1$ of 0.50 µg/kg bw/day was established for neurodevelopmental effects in children (Table 1).

2.3 Mercury

Mercury (Hg) is a metal naturally found in the environment in inorganic, organic and elemental (Hg^0) forms. Elemental mercury is used in chlorine gas production and in caustic soda for industrial use, as well as electrical equipment, lamps, thermometers, pressure gauges, barometers, and dental amalgams. Inorganic mercury occurs as salts of its divalent and monovalent cationic forms, mainly chlorine and sulfur (Poulin and Gibb, 2008).

Amalgam fillings are the most important source of exposure to mercury vapor (Hg^0) by the general population, and an association between meconium Hg and IHg in the placenta and the number of dental amalgam fillings has been reported (Ask et al., 2002; Gundacker et at., 2010). The major effect from

chronic exposure to IHg is kidney damage, and may include morphological changes, renal tubular damage, regeneration of the tubular epithelium, and proximal tubular necrosis (WHO, 2003).

Methylmercury (MeHg) is formed in nature by methylation of inorganic mercury mainly by reducing sulfate aquatic bacteria and fungi. The MeHg has a lipophilic property and can be absorbed by plankton, which is eaten by fish and shellfish with greater concentrations ocurring at higher trophic levels of the food chain (Polak-Juszczak, 2012; Poully et al., 2013). While less than 15% of IHg is absorbed by the gastrointestinal tract, about 95% of MeHg ingested is absorbed, and diffuses to various tissues of the body, including kidney and brain (CDC, 2009). Various studies show that the consumption of fish and other foods of marine origin contributes significantly to mercury levels in human hair, including children and their mothers (Gundacher et al., 2010; Castano et al., 2015).

Ethylmercury (EtHg), an organic mercury compound, is the major component of Thimerosal, a preservative present in various vaccines administered to expecting women and babies, mainly in developing countries. Thimerosal is injected intramuscularly, with approximately 100% absorption (Dorea et al., 2013), and a half-life in blood of 20 days in adults and 7 days in infants, much lower than that for methylmercury (about 70 days; Clarkson et al., 2003). EtHg, as well as MeHg, have been detected in blood samples of babies and neonates immediately after vaccination (Pichichero et al., 2008). Animal models demonstrate that EtHg is less neurotoxic than MeHg, but more studies are needed to demonstrate whether repeated doses of EtHg in combination with different MeHg background exposures have consequences in fetuses and infants, particularly due to possible additive and synergistic effects (Dorea et al., 2013).

While inorganic mercury is usually free in plasma, MeHg tends to bind to hemoglobin in red blood cells (RBCs), with about 1% bound to glutathione (GSH) (Oliveira et al., 2014). MeHg can enter mammalian cells using a molecular mimicry mechanism. After forming a stable bond with cysteine, the MeHg-Cys complex is transported by the L-type large neutral amino acid

transporter (LAT-1), which is important for the high Hg levels found in the brain after exposure (Farina et al., 2011).

The mechanisms involved in the neurotoxicity of MeHg are not completely understood, but Farina et al. (2011) identified three interrelated events that are important for MeHg-induced neurotoxicity: Ca2+ dyshomeostasis, glutamate dyshomeostasis, and increased reactive oxygen species (ROS) generation (oxidative stress). *In vivo* studies show that MeHg can alter the expression of genes involved in small GTPase signaling pathways regulating cell growth and proliferation, and can induce mitotic arrest and caspase-dependent apoptosis in developing brains (see review by Antunes dos Santos et al., 2016). In a cohort study with 138 mother-infant pairs, Cardenas et al. (2015) showed that in utero exposure to mercury can affect leukocyte composition and may disrupt the epigenome even at low levels. Furthermore, exposure to both arsenic and mercury in utero may interact jointly to affect the epigenome by hypermethylating relevant CpG regions (cytosine followed by guanine) having the potential to influence neurodevelopment and other childhood health outcomes.

MeHg crosses the blood brain barrier and the placenta, and may affect the neurological development of fetuses. Mercury concentrations in cord blood correlate well with fetal brain mercury concentrations during the third trimester, indicating methylmercury exposure during late pregnancy (Poulin and Gibb, 2008; WHO, 2010b). Mercury levels are higher in umbilical cord-blood than in the blood of mothers (Stern & Smith, 2003). Oskarsson et al (1998) reported a higher plasma clearance and a larger distribution volume for methylmercury in lactating mice than in non-lactating mice, probably due to the increased biliary excretion, greater blood/plasma volume and lower plasma protein content during lactation. The milk mercury excretion in mice over 9 days was approximately 4 and 8% of the administered dose of methylmercury and inorganic mercury, respectively.

Sakamoto et al. (2002) showed a lower risk of MeHg exposure by infants during lactation among the high fish-consuming Japanese population. The geometric mean of red blood cells (RBC)-Hg in umbilical cords was about 1.4

times higher than in mothers, with a strong correlation between these two parameters. All the infants showed declines in RBC-Hg during a 3-month breast-feeding period, probably due to the low Hg transfer through breast milk, and the rapid growth of infants after birth. The authors concluded that the risk was especially high during gestation but may decrease during breast-feeding.

Studies to investigate the outcome of prenatal exposure to MeHg and adverse neurological effects on children have reached different conclusions. In a study conducted on Faroe Island (North Atlantic), whose population has a high consumption of pilot whale meat, mothers' exposure to mercury was assessed through mercury concentration in cord blood and hair (Grandjean et al., 1997). Tests applied to 917 children of about 7 years indicated neuropsychological dysfunctions mainly related to language attention and memory, with the association remaining even after the exclusion of children whose mothers' hair mercury concentrations were above 10 μg/g. In general, a delay in development at 6 months was observed in children with higher levels of mercury. On the other hand, a study conducted with 771 mother-child pairs in the Seychelles Islands (Indian Ocean) found no adverse neurodevelopmental outcomes at 66 months of age associated with prenatal or postnatal MeHg exposure and a high fish consumption diet (Davidson et al., 1998). A follow-up study was conducted with this Seychelles population (up to 19 years old) and no correlation was found with effects on the neurological (Myers et al., 2003; Davidson et al., 2011) and auditory functions (Orlando et al., 2014). A cohort study conducted with 492 Italian babies with low levels of mercury (1 μg/g in hair, 0.33 μg/L in breast milk) found that fish consumption and mothers' IQs were significantly associated with neurodevelopment performance of babies at 18 months, but not with mercury exposure (Valent et al., 2013).

In a study conducted in the Amazon region of Brazil, Marques et al. (2014) found higher levels of MeHg in the hair of fishing village children in comparison with those living in the vicinity of tin-ore kilns and smelters who had higher neurodevelopment delays due to high lead exposure, as discussed above. A deficit in neurodevelopment was found in children with higher levels of EtHg in hair. However, another study conducted by the same group evaluating 194 children living near a tin mine in the same region (Marques et

al., 2015) found that hair EtHg and maternal consumption of fish were not associated with low neurodevelopment scores.

Based on the available epidemiological studies, including those conducted by Grandjean et al. (1997) and Davidson et al. (1998), the JECFA established a PTWI of 1.6 µg/kg bw for MeHg in childbearing-aged women due to the possibility of pregnancy and to protect the fetus (JECFA, 2004). In 2010, the JECFA withdrew the previously established PTWI of 5 µg/kg bw for THg, and established a PTWI of 4 µg/kg bw for IHg (JECFA, 2011a).

2.4. Cadmium

The predominant commercial use of cadmium is in the production of batteries, dyes, coatings, plastic stabilizers, and ironless alloys (CDC, 2009). Cadmium in food may originate from contaminated soil which, in turn, may have been contaminated by irrigation water, with deposition originating from air pollution, or from phosphate or manure fertilizer. The highest mean concentrations can be found in edible offal, legumes, cereals and potatoes (0.02 to 0.13 mg/kg; EFSA, 2009b). Tobacco leaves accumulate high levels of cadmium from the soil, and cigarette smoke is the major source of exposure for smokers (CDC, 2009; ATSDR, 2012). Recent studies have also shown that jewelry and toys can be a source of exposure to cadmium (Guney and Sagury, 2012).

Cadmium is classified by IARC as carcinogenic to humans (Group 1), and causes lung cancer in exposed workers (Table 1), with some evidence of prostate cancer (IARC, 2016). The gastrointestinal tract absorbs 5 to 10% of ingested cadmium, but several factors may affect absorption, including vitamin D, calcium or iron deficiency, metal-metal interactions with iron, lead and chromium, and metal-protein interactions such as metalotinoein interaction with glutathione (ATSDR, 2012; CDC, 2009). Cadmium absorption may increase with iron deficiency, which may contribute to higher absorption of cadmium by women (CDC, 2009). The placenta may act as a partial barrier to fetal exposure to cadmium, as the concentration in cord blood is about half of that in maternal blood; cadmium levels in human milk are 5–10% of the levels in blood (ATSDR,

2012). Cadmium and lead absorption increases in early childhood and with iron deficiency, given the increase in the number of carriers shared by all 3 metals in the duodenum (Sreedharan and Mehta, 2004). Kippler et al. (2009) found a significant positive association between cadmium concentration in erythrocytes and in breast milk (BM), and a breast milk-plasma ratio of approximately 3-4, indicating no barrier against cadmium transport from plasma to breast milk. BM-Cd was positively associated with manganese ($r(s)=0.56$; $p<0.01$) and iron ($r(s)=0.55$; $p<0.01$) in breast milk, but not with plasma ferritin. On the other hand, BM-Cd was negatively associated with BM-Ca ($r(s)=-0.17$; $p=0.05$), indicating that cadmium inhibits the transport of calcium to breast milk. The authors concluded that cadmium shares common transporters with iron and manganese for transfer to breast milk, but inhibits secretion of calcium to breast milk.

Absorbed cadmium accumulates mainly in the kidney and liver, with an estimated half-life of 6 to 38 years, and 4 to 19 years, respectively, and no direct metabolism is known (ATSDR, 2012). The kidney is the critical target and shows the earliest sign of cadmium toxicity. However, the accumulation of cadmium in the kidney with no apparent toxic effects occurs due to the formation of cadmium-thionein or metallothionein, which is considered non-toxic (ATSDR, 2012). Cadmium can disrupt signaling cascades and lead to a variety of toxic effects, mainly due to its physicochemical similarity with calcium ion (Ca^{2+}), which may disrupt Ca-mediated signaling pathways, possibly through significant changes in the activation of calmodulin and calmodulin-dependent protein kinase II in cell death pathways, such as apoptosis, necrosis or autophagy (Choong et al., 2014).

In 2010, the JECFA withdrew the PTWI for cadmium of 7 µg/kg bw/week set by the Committee in 1988, and established a monthly intake (PTMI) of 25 µg/kg bw due to its long half-life in the body (JECFA, 2011b), corresponding to a weekly intake of 5.8 µg/kg body weight. In 2009, the EFSA recommended a tolerable weekly intake (TWI) of 2.5 µg/kg body weight in order to ensure a high level of protection for all consumers, including exposed and vulnerable subgroups of the population (EFSA, 2009b). This decision was confirmed in 2011 (EFSA, 2012a).

Table 1. Some characteristics of arsenic, lead, mercury and cadmium

	IAs	Pb	IHg	MeHg	Cd
IARC classification[a]	Group 1	Group 2B	Group 3	Group 2B	Group 1
PTWI, µg/kg bw/week or PTMI, µg/kg bw/month	-	-	PTWI: 4[d]	PTWI: 1.6[e]	PTWI: 2.5[b] PTMI: 25[c]
BMDL, µg/kg bw/day	3.0[d]	0.5[f] (developmental toxicity in children)	-	-	-
Oral absorption	over 75%[g]	Adults: 3-15% Children: 30-50%[f,j]	Up to 20%; increases in a milk diet[i]	>90%[i]	5%[h]
Half-life (plasma)	3-4 hs[g]	20-40 days[j]	20-66 days[i]	44-88 days[i]	3-4 months[m] ~ 12 yrs (kidney)[h]
Cross the placenta	Yes[g]	Yes[f,j]	Poorly[i]	Yes[i]	Yes[h]
Neurotoxic	Yes[g]	Yes[f,j]	Inconclusive[i]	Yes[e,i]	Inconclusive[h]
Genotoxic	Yes[d,g]	weak, indirect[f]	Inconclusive[i]	Inconclusive[i]	Indirect[h,l]
Embryotoxic	Yes[g]	Inconclusive[k]	Inconclusive[i]	Yes[e,i]	No[h]

Group 1 – carcinogenic to humans; Group 2A- probably carcinogenic to humans; Group 2B: possibly carcinogenic to humans; Group 3- not classifiable as to its carcinogenicity to humans; PTWI: provisional tolerable weekly intake; PTMI: provisional tolerable monthly intake; BMDL – benchmark dose lower bound; [a]IARC, 2016; [b] EFSA, 2012a; [c]JECFA, 2011b; [d]JECFA, 2011a; [e]JECFA, 2004; [f]EFSA, 2010; [g]ATSDR, 2007b; [h] ATSDR, 2012; [i] UNEP, 2008; [j] ATSDR, 2007a; [k]CDC, 2010; [l]EFSA, 2009; [m] Järup & Akesson, 2009

3. Presence of arsenic, lead mercury and cadmium in breast milk

Monitoring breast milk is a non-invasive form of detecting environmental contaminants, having the advantage of allowing the exposure of both the mother and the lactating baby to be assessed at the same time (Hooper and McDonald, 2000; Abballe et al., 2008; CDC, 2010). Two metal analysis techniques are mainly used for different matrices, including milk: atomic absorption spectrometry (AAS) using either flame, cold vapor hydride generator (CVAAS) or electrothermal AAS in graphite furnace (ETAAS), and inductively coupled plasma with mass spectrometry detection (ICP-MS). In most methods, the milk is submitted to microwave acid digestion under controlled temperature and pressure (Kosanovic et al., 2008; Sardans et al., 2010; Amarasiriwardena et al., 2013).

Table 2 summarizes the data for arsenic, lead, mercury and cadmium in breast milk reported by the 75 studies reviewed by this study. Figure 1 shows the distribution of the studies according to region and metal analyzed. A larger number of studies were conducted in Europe (23), and a lower number in North America (3 studies), with lead the most analyzed metal. In the majority of studies, more than one metal was analyzed in the samples.

The analytical variability and validity of the reported results were not assessed, with the exception of one study conducted in Nigeria (Adesiyan et al., 2011), where the results reported in µg/dL were too high, probably due to a typing or unit error. It is important to be aware that inaccuracies involved in the analytical methods affect the results, particularly at low concentrations (CDC, 2010). Furthermore, positive sample percentages (Table 2) are highly dependent on the limit of detection (LOD) or limit of quantification (LOQ) of the method used, mainly when incidences are low, and may not be comparable. Also, it was not clear in most studies how the samples reported as non-detected or below the LOD/LOQ were treated in estimations of the means. In addition to uncertainty regarding the analytical method, extremely high values found in certain studies may be due to contamination during sample collection and storage, mainly for lead, which is the most abundant toxic metal in the environment.

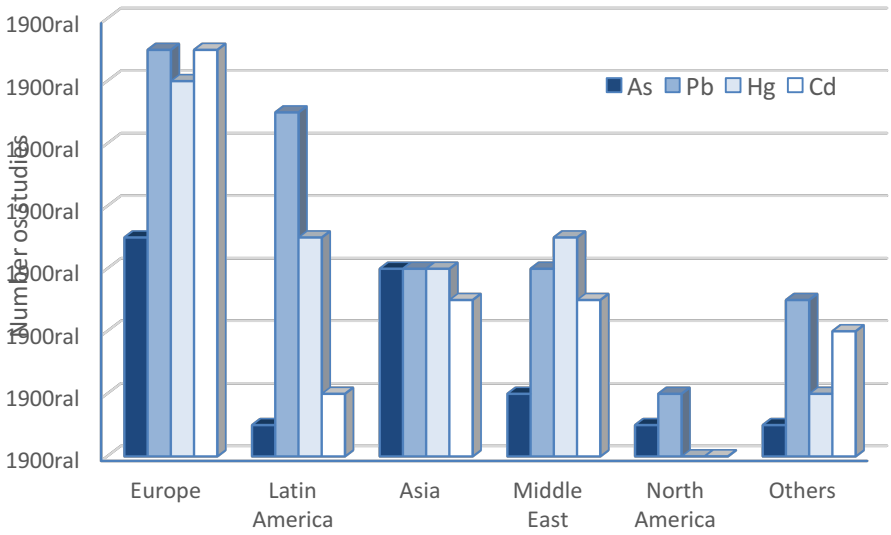


Figure 1. Summary of the number of studies that analyzed arsenic, lead, mercury and cadmium published since the year 2000, according to the region. Others include Indonesia, Tanzania and African countries, Australia and Faroe Island. Most of the studies analyzed more than one metal. Literature search on Pubmed, Science Direct and Google Scholar databases (last on June 2016) using the keywords “human milk”, “breast milk” and “breast milk”, associated with “metal”, “arsenic”, “lead”, “mercury” or “cadmium”. Additional papers were identified in published reviews related to contaminants in breast milk.

3.1. Arsenic

For this review, 18 studies published since 2000 that measured levels of arsenic in breast milk were retrieved, six conducted in Asia, six in Europe, and none in Latin America (Table 2). The techniques used to analyze arsenic in milk included CVAAS, ETAAS and ICP-MS, which has the lowest LOD (0.007 to 0.3 µg/L) (Felip et al., 2014; Miklavcic et al., 2013; Björklund et al., 2012; Fängström et al., 2008; Almeida et al., 2008). Separation of the different arsenic metabolites [As(III), As(V), MA, and DMA] was performed by high performance liquid chromatography coupled to hydride generation and ICP-MS (Fängström et al., 2008).

The highest levels of arsenic in breast milk were found for a district in West Bengal, India (up to 149 µg/L; Samanta et al., 2007), a region with levels of arsenic in water higher than 50 µg/L. Higher levels were found in samples from women who had higher levels of arsenic in urine, hair, and nails. In this population, when breast milk was not sufficient or available, infants drank tube well water as early as the first month after birth, as well as cow/goat milk diluted with water, which increased exposure to arsenic from an early age. The authors found the levels of arsenic in breast milk much lower than in urine (mean of 438 µg/L), which is a much more efficient arsenic excretion route than lactation. Indeed, Fängström et al. (2008) considered the excretion of arsenic through breast milk to be low and concluded that exclusive breastfeeding protects the infant from exposure to arsenic. A similar conclusion was reached by Carignan et al. (2015) in the United States, an area with low levels of arsenic in the water (< 1 µg/L). Fängström et al. (2008) also found that arsenic levels in urine were significantly lower in exclusively breastfed children than in those consuming other foods.

Higher mean levels of arsenic were found in colostrum (3.6 – 14 µg/L; Almeida et al., 2008), decreasing considerably in intermediate and mature milk (Almeida et al., 2008; Islam et al., 2014). Islam et al. (2014) found that arsenic in human milk was weakly correlated with maternal urine levels at 1 and 6 months postpartum ($r = 0.13$ and 0.21, respectively; $n= 29$ and 25) and did not correlate with infants' urine levels. Fängström et al. (2008) however, found a significant association between the TAs in milk and the levels in the urine of 2-3 month-old babies ($rs = 0.64$, $p < 0.001$), as well as with arsenic in maternal blood and saliva. Arsenic was essentially present in breast milk as As^{III}, in addition to As^{V}, DMA and MMA, and was the only form present at total arsenic levels ≤ 1 µg/L. The Fängström et al. study was the only one to identify the forms of arsenic present in breast milk, an important piece of information as inorganic arsenic is the only toxicological relevant form of arsenic for humans (IARC, 2016).

3.2 Lead

There are a large number of published studies that have investigated the levels of lead in human breast milk. The first studies date from the early 1980´s and had the objective of collecting data from different countries to establish an environmental background level for metals in human fluids (Iyengar, 1984). A WHO-sponsored multicenter study conducted in several countries on four continents found average concentrations of lead in human milk ranging from 2.0 to 16.8 µg/L, and values between 2 and 5 µg/L were considered a reference for populations not occupationally exposed to lead (WHO, 1989).

In the present review, we were able to retrieve 43 studies that analyzed lead in breast milk samples collected in different regions of the world, most of which also included analyses of the other metals (Table 2). The number of samples analyzed in these studies varied from less than 50 in Italy (Abballe et al., 2008) to over 300 in Mexico (Ettinger et al., 2006) and Saudi Arabia (Al Saleh et al., 2003; 2015). In most studies, lead was analyzed by ETAAS, with a wide range of reported LODs (0.04 to 3.4 µg/L) (Marques et al., 2014 and 2013; Winiarksa-Mieczan, 2014; Goudarzi et al., 2013; Chao et al., 2014; Gürbay et al., 2012; Garcia-Esquinas et al., 2011; Abbale et al., 2008; Chien et al., 2006a; Leotsinidis et al., 2005; Ursynova & Masanova, 2005; Al-Saleh et al., 2003). The study with the lowest LOD (0.01 µg/L) used isotopic dilution ICP-MS (Ettinger et al., 2014), while the other ICP-MS LODs ranged from 0.03 to 3 µg/L (Cardoso et al., 2014; Felip et al., 2014; Amarasiriwardena et al., 2013; Bjorklund et al., 2012; Örün et al., 2011; Almeida et al., 2008; Koyashiki et al., 2010; Sowers et al., 2002). The highest mean lead levels were found in Turkish milk colostrum samples (391 µg/L from Gürbay et al., 2012; Table 2).

Lead levels in colostrum are usually higher than in mature milk due to their greater protein content (Rothenberg et al., 2000). Chien et al. (2006b) found a significant decline in lead levels during lactation among Taiwanese mothers, with the mean of 9.9 µg/L in colostrum dropping to 2.3 µg/L in mature milk at 2 months postpartum (Table 2), with an estimated lead half-life of 33-35 days. Another study from the same research group found that milk from mothers who consumed traditional Chinese herbs, which can contain over 300

μg/g of lead, had significantly higher levels of lead than milk from non-consumers (Chien et al., 2006a). Ettinger et al. (2006) also found a significant decrease in breast milk lead levels 1 to 7 months postpartum in Mexico (Table 2).

Örün et al. (2011) reported a 2-month postpartum sample in Ankara that contained 1515 μg/L, but no individual or environmental factor was identified that could justify such a high level (mean level was 20.6 μg/L). A significant correlation was found among mothers with a history of anemia, and the higher level of lead in breast milk. Another study, conducted ten years earlier in the same city, found a much lower level in colostrum (14.6 μg/L; Turan et al., 2001), but higher than that found in Greece (0.48 μg/L; Leotsinidis et al., 2005 (Table 2). In a study conducted in Saudi Arabia, Al-Saleh et al. (2003) found a significant correlation between lead levels in breast milk (n= 362) with average duration of lactation for all births and fish consumption (lower consumers had higher levels). The milk of mothers living in agricultural areas had higher lead levels than those in urban regions, although the difference was not significant.

In nature, lead occurs mostly in ore deposits along with other minerals, particularly zinc, accounting for about 20% of total primary lead supplies. Mining, smelting and refining of lead are known to cause contamination of the surrounding environment (ATSDR, 2007a), and to impact levels of the metal in the human body. In fact, two studies conducted in the north of Brazil showed significantly higher lead levels in milk from women living near a tin smelter compared with those living in a fishing village (Marques et al., 2013; 2014; Table 2). Marques et al. (2014) also found higher Pb levels in breast milk associated with longer residence periods in a contaminated region, and a significant association of higher levels with neurodevelopmental delays in 24-month old children living near tin ore smelters. Isaac et al. (2012) found higher lead mean levels in breast milk of women living in industrial areas of Southern India (21.5 μg/L) compared with those in non-industrial areas (13.2 μg/L), showing the impact of environmental contamination of lead by industrial activity. In China, mean level of lead in colostrum from occupationally exposed women were about 15 times higher than the mean for non-exposed women (4.7 and 52.7 μg/L, respectively; Li et al., 2000; Table 2).

3.3 Mercury

A total of 34 studies published since 2000 that analyzed mercury in breast milk (THg) were retrieved for this review, five of which also analyzed MeHg and/or IHg (Table 2). The most widely used technique to analyze THg was CVAAS, with limit of detection ranging from 0.06 to 5 µg/L (Boishio & Henschel, 2000; Al-Saleh et al., 2003; Costa et al., 2005; Bose O'Reilly et al., 2008; Abballe et al., 2008; Gundacher et al., 2010; Vieira et al., 2013; Valent et al 2013; Iwai-Shimada et al., 2015). MeHg was analyzed by gas chromatography coupled with electron capture detector (Miklavcic et al., 2013; Valent et al., 2013; Iwai-Shimada et al., 2015) or MERX^{TM}, which uses atomic fluorescence spectrophotometry (Vieira et al., 2013).

Seven studies were conducted in Latin America, six of which in Brazil, mostly in the Amazon region, where THg in breast milk reached 104 µg/L (mean of 59.4 µg/L; Santos et al., 2015). Overall, breast milk samples from high fish consumers in the Amazon (riverine community) had higher mercury levels compared to an urban population in the same region. Vieira et al. (2013) found this difference significant for both THg (2.3 and 0.36 µg/L, respectively) and MeHg (0.87 and 0.12 µg/L). Among urban mothers with low fish consumption (and with relatively higher dental amalgam fillings), the proportion of IHg in milk was higher (85%) than for riverine communities (62%). In another study conducted earlier in the same region, the levels were about 6 µg/L (Boishio & Henshel, 2000), similar to those found in two studies conducted in the Federal District (DF) of Brazil (Costa et al., 2005; Cunha et al., 2013), located in the Midwest region of the country and with a low fish consuming population. Cunha et al. (2013) found no significant correlation between fish consumption and THg milk levels, although a significant increase was found after the mothers had eaten a meal with salmon (day 75 postpartum). Although the levels of THg found in one Amazonian study and those found in the DF study were similar, most of the mercury present in the DF milk was most likely present as IHg, while in the Amazon the MeHg found was the predominant form, reflecting the high fish consumption in this region. Much lower THg levels were found by Cardoso et al. (2014) among mothers living in the Brazilian state of Minas Gerais (mean <0.2 µg/L), also a low fish consuming region. Costa et al. (2005)

also found that THg levels in breast milk in the Federal District correlated well with the number of amalgam fillings of the mothers.

In a study also conducted with a Mediterranean population (Miklavčič et al., 2013), the levels of THg in breast milk were similar in Croatia, Slovenia and Italy (0.2 ng/g; Table 2). Although Slovenian women consumed the least amount of fish (mean consumption of 25 g/day), they had the highest number of amalgam fillings, which may have contributed to the total excreted mercury. The levels in Greece (39 g fish/day) were 3 times higher than in the other countries (0.6 ng/g), but only 7% was present as MeHg, although this percentage ranged from 47 to 60% in the other countries (Table 2). These results were unexpected as fish consumption is the main external source of MeHg.

An extensive study conducted by Valent et al. (2013) confirmed that in Italy (2.3 servings of fish/week) most of the mercury in breast milk was present as MeHg (mean of 58%). This percentage was similar to the one found in Japan (Iwai-Shimada et al., 2015), a high fish-consuming population (about 71 g/day, in average), with higher mercury concentrations detected in breast milk (mean of 0.81 µg/L). These authors found a correlation between THg or MeHg in breast milk and fish consumption only when the levels were adjusted for the milk lipid content.

Cunha et al. (2013) found no significant changes in the THg levels 15 to 90 days postpartum, all mature milk samples. In Sweden, Bjornberg et al. (2005) found a significant decrease in THg between day 4 (colostrum) and 6 weeks after delivery (median of 0.29 and 0.14 µg/L, respectively), remaining unchanged thereafter (Table 2). At 13 weeks, THg in breast milk was significantly associated with IHg in maternal blood (r_S = 0.61; p =0.006) and MeHg in infant blood (r_S = 0.55; p = 0.01). The authors concluded that exposure to mercury was higher before birth than during breastfeeding, and that MeHg seems to contribute more than IHg to postnatal infant exposure via breast milk.

Gundacker et al. (2002) found higher THg levels in the breast milk of Austrian mothers under 60 kg and in those who had premature infants. Similar to what was reported by Cunha et al. (2013), frequent consumption of cereals correlated well with higher mercury levels. In a later study, Gundacker et al.

(2010) found that all mercury detected in breast milk from Austrian mothers was in inorganic form (Table 2).

In Mexico, Gaxiola-Robles et al. (2013) found a significant correlation between breast milk THg (80.8 % of positive samples; mean levels of 2 to 3 µg/L), fish consumption and exposure to tobacco (active and passive smokers). These correlations were not confirmed in studies conducted in Turkey with a population with lower incidence of positive sample (18-44 %) but higher levels of THg (mean of 3.4 and 20.6 µg/L; Yalçin et al., 2010; Örün et al., 2012).

3.4 Cadmium

Twenty nine studies published since 2000 that analyzed cadmium in breast milk were found in the databases, ten conducted in Europe and none in North America (Table 2). Cadmium was predominantly analyzed by ETAAS, with LODs ranging from 0.01 to 0.5 µg/L (Winiarksa-Mieczan, 2014; Goudarzi et al., 2013; Chao et al , 2013; Gürbay et al., 2012; Garcia-Esquinas et al., 2011; Abbale et al., 2008; Leotsinidis et al., 2005; Ursynova & Masanova, 2005; Al Saleh et al., 2003) or by ICP-MS, with LODs in the range of 0.0027 to 0.3µg/L (Cardoso et al., 2014; Felip et al., 2014; Björklund et al., 2012; Örün et al., 2011).

In most studies, mean levels were below 2 µg/L, with the maximum mean and highest levels found for Turkey (4.6 and 43 µg/L; Gürbay et al., 2012 and Örün et al., 2011 Table 2). In Brazil, Gonçalves et al. (2010) found a significant correlation between cadmium levels in colostrum and the consumption of rice, carrots and chayote, while Cardoso et al. (2014) found correlations between cadmium concentration profiles in mature breast milk (0.77 µg/L), soil (4.50 mg/kg) and water (12.5 µg/L).

Cadmium levels in breast milk decreased over the postpartum period (Chao et al., 2013; Leotsinidis et al., 2005), being higher among smoking women (Rahimi et al., 2009), as expected, and housewives, probably due to exposure to dust particles during housekeeping activities (Örün et al., 2011). Honda et al (2003) found that cadmium in breast milk was significantly

correlated with urinary concentration, reflecting mothers' body burden, and inversely correlated with calcium concentration in breast milk, an indication that it affects calcium secretion in this body fluid.

Table 2. Levels of arsenic, lead, mercury and cadmium in breast milk reported in studies published since 2000.

Country; Reference	Metal	N	% positive	Mean, median* or geometric mean** (range); µg/L or ng/g	Observation
Asia					
Bangladesh; Fängström et al., 2008	As	79	-	1.8 (0.25 – 19.0)	Mature milk
Bangladesh; Kippler et al., 2009	Cd	123	-	0.14* (<0.05 – 1)	2 months pp
Bangladesh; Islam et al., 2014	As	29	-	1.12 (0.5 – 8.9)	30 days pp
		25	-	0.78 (0.5 – 2.32)	180 days pp
		19	-	0.7 (0.5 – 1.68)	270 days pp
China; Li et al., 2000	Pb	165	-	4.7	Colostrum, non occupational
		12	-	52.7	Colostrum, occupational
China; Li et al., 2014	THg	195	-	0.97 (0.42-8.40)	Colostrum
India; Sharma & Pervez, 2005	As	120	82.5	0.6±0.1 – 5.2 ±3.8	Mean range of various groups
	Pb		87.5	0.1±0.0 – 22.3 ±18.5	
	THg		87.5	0.1±0.0 – 16.7 ±11.1	
	Cd		82.5	0.1±0.1 – 3.8 ±12.9	
India; Samanta et al., 2007	As	226	17.3	17 (<LOD - 49)	Area with high levels of arsenic in water
		10	50	3.5 (<LOD – 5)	Area with levels of arsenic within WHO limits
India; Isaac et al, 2012	Pb	25	84	13.21± 5.2 (9.0 - 21.0)	Non-industrial area
			88	21.5±4.5 (15 - 25.5)	Industrial area
Japan; Honda et al.,	Cd	68		0.28±1.82** (0.28-1.22)	5-8 days pp

Country; Reference	Metal	N	% positive	Mean, median* or geometric mean** (range); µg/L or ng/g	Observation
2003					
Japan; Sakamoto et al., 2012	As	9	-	1.4 (0.4-1.8)	3 months pp
	Pb			0.29 (0.18-0.20)	
	THg			0.47 (0.28-0.77)	
	Cd			0.14 (0.06-0.22)	
Japan; Iwai-Shimada et al., 2015	THg	27	-	0.81 (0.14-1.87)	30 days pp
	MeHg		-	0.45 (0.06- 1.2)	
Korea; Li et al., 2014	THg	195	-	0.97* (0.42-8.40)	
Taiwan; Chao et al., 2014	As	45	-	1.50 ± 1.50	1 to 4 days pp
				0.68±1.09	5 to 10 days pp
				0.27±1.26	30 to 35 days pp
				0.16±0.24	60 to 65 days pp
	Pb	45	-	13.2±3.6 (6.7-22.4)	1 to 4 days pp
			-	8.92±2.60 (3.52-14.7)	5 to10 days pp
			-	11.7±2.58 (0.76-11.7)	30 to 35 days pp
			-	2.93±1.70 (0.45-7.8)	60 to 65 days pp
	Cd	45	-	1.37±0.94	1 to 4 days pp
			-	0.65±0.36	5 to 10 days pp
			-	0.49±0.25	30 to 35 days pp
			-	0.34±0.19	60 to 65 days pp
Taiwan ; Chien et al., 2006a	Pb	35	-	8.59±10.9	Chinese herb mothers (9)
				9.94/2.34	Colostrum/mature
		37	-	6.84±2.68	Non consumers (7)

Country; Reference	Metal	N	% positive	Mean, median* or geometric mean** (range); µg/L or ng/g	Observation
Taiwan ; Chien et al., 2006b	THg	56	100	2.02 (0.24 – 9.45)	Colostrum - urban population
		12	100	2.04 (0.26-8.62)	Colostrum - fishing villages
Europe					
Finland; Kantola & Vartiainen, 2001	Cd	165	-	0.095 ±0.12	Samples collected in 1987
		74	-	0.040±0.06	1993-1995 samples
Austria; Gundacker et al., 2002	Pb	116		1.63±1.66	6.6 ± 6 days pp
	THg	116		1.59 ± 1.2	
Austria; Gundacker et al., 2010	THg	21	62	0.2 (0.1 – 2) 100% inorganic	2-8 weeks pp
Croatia, Slovenia, Greece, Italy; Miklavcic et al., 2013	As	123	-	0.2 (0.4-11.9)	Croatian
		287	-	0.04 (0.04-2.9)	Slovenes
		30	-	0.8 (0.3-4.8)	Greek
		602	-	0.3 (0.04-12)	Italians
	THg	125	-	0.2	Croatian
		284	-	0.2	Slovenes
		44	-	0.6	Greek
		605	-	0.2	Italians
	MeHg	26	100	56% of the mean THg	Croatian
		7	100	47% of the mean THg	Slovenes
		21	100	7% of the mean THg	Greek
		224	100	60% of the mean THg	Italians
Cyprus; Kunter et al.	As	50	-	0.73±0.58 (0.03-1.97)	

Country; Reference	Metal	N	% positive	Mean, median* or geometric mean** (range); µg/L or ng/g	Observation
2016	Pb		-	1.19±1.53 (0-4.9)	
	THg			0-0.01	
	Cd			0.45±0.23 (0.12-0.08)	
Germany; Sternowsky et al., 2002	As	187	17.6	0.15* (<0.3-2.8)	2 to 90 days pp From 36 mothers
Greece; Leotsinidis et al., 2005	Pb	180	58,5	0.48±0.60 (<0.2-2.36)	Colostrum
		95	63.6	0.15±0.25 (<0.2-0.94)	Intermediate milk
	Cd	180	89	0.19±0.15 (<0.01-0.70)	Colostrum
		95	91.9	0.14±0.12 (<0.01-0.49)	Intermediate milk
Italy; Abballe et al., 2008	THg	29	-	2.6 - 3.0	Venice
		10	-	3.53	Rome
	Pb	29	-	0.97-1.1	Venice
		10	-	0.85	Rome
	Cd	39		< 0.5	Venice and Rome
Italy; Valent et al., 2013	THg	492	-	0.33 (0 - 28.3)	Mature milk
	MeHg	182	-	0.17 (0.01 - 1.09)	
Italy ; de Felip et al., 2014	As	63	0	< 3	Samples were mixed in 7 pools, according to the region
	Pb		100	2.59-5.99	
	THg		0	< 0.3	
	Cd		0	< 0.1	
Poland; Winiarksa-Mieczan, 2014	Pb	320	-	6.33±4.61 (0.49-12.0)	All milk types
	Cd	320	-	2.1 (0.21-7.4)	
Poland; Olszowski et al., 2016	Cd	51	-	0.11±0.07 (0.01-0.33)	

Country; Reference	Metal	N	% positive	Mean, median* or geometric mean** (range); µg/L or ng/g	Observation
Portugal; Almeida et al., 2008	As	34	-	7.8±2.2 (3.6-14.0)	Colostrum
	Pb	34	-	1.55±1.38 (0.06-5.43)	Colostrum
	As	19	-	5.8±1.1 (4.2-7.8)	Intermediate milk
	Pb	19	-	0.94±1.05 (0.07-4.03)	Intermediate milk
Spain; Garcia-Esquinas et al., 2011	Pb	100	93	15.56 (12.92-18.72)	Mature milk
	THg	100	98	0.53 (0.45-0.62)	Mature milk
	Cd	100	96	1.31 (1.15-1.48)	Mature milk
Sweden; Björnberg et al., 2005	THg	19	-	0.29* (0.06-2.1)	Colostrum
		20	-	0.14* (0.07-0.37)	6 weeks
		19	-	0.2* (0.06-0.4)	13 weeks
Sweden; Björklund et al., 2012	As	60	-	0.55±0.70 (0.04-4.6)	Mature milk
	Pb	60	-	1.5±0.9 (0.74-6.40)	Mature milk
	Cd	60	-	0.09±0.04 (0.02-0.27)	Mature milk
Turkey; Turan et al., 2001	Pb	30	100	14.6±5.5 (8.8-35.4)	Colostrum
	Cd		100	1.7±1.7 (1.2-9)	
Turkey; Yalçin et al., 2010	THg	44	-	3.42±1.66 (0.35-6.9)	All milk types
Turkey; Örün et al., 2011	Pb	144	95	20.6 (<LOQ-1515.0)	2 months pp
	Cd	144	60	0.67 (<LOQ-43.0)	
Turkey; Örün et al., 2012	THg	144	18	25.8±44.6 (1.7-236) positive samples	Mature milk
Turkey; Gürbay et al., 2012	As	64	0	< 7.6	2-5 days pp
	Pb	64	93.8	391 ±269 (4.35-1020)	

Country; Reference	Metal	N	% positive	Mean, median* or geometric mean** (range); µg/L or ng/g	Observation
	Cd	64	1.6	4.62 (LOQ = 0.34)	
Slovakia; Ursinyova & Masanova, 2005	Pb	158	-	4.7 (nd – 24.4)	4 days pp
	Cd		-	0.43 (nd – 1.7)	
	THg		-	0.94 (nd – 4.74)	
Latin America					
Brazil; Boishio and Henshel, 2000	THg	44		5.7 ± 5.9 (nd – 24.8)	Amazonian riverines
Brazil; Anastacio et al., 2004	Pb	38	-	2.8±2.5	Mature milk
Brazil; Costa et al., 2005	THg	23	86.9	5.73±5.43	Federal District
Brazil; Koyashiki et al., 2010	Pb	92	-	2.9±1.1 (1.0-8.0)	Mature milk
Brazil; Gonçalves et al., 2010	Cd	80	100	2.3 (0.02-28.1)	Colostrum
Brazil; Andrade et al., 2013	Pb	70	-	1.46 ± 1.28 (0.01-4.82)	Up to 6 months pp
Brazil; Cunha et al., 2013	THg	142	93.7	6.7±6.45 (<0.76-22.7)	Federal District, 15 to 90 days pp; 18 mothers
Brazil; Marques et al., 2013	Pb	37	-	12.6±8.16 (0.9-29.4)	Close to a tin mine; 15 days up to 12 pp
		45	-	4.30±4.01 (0-16.2)	Fishing village; 1 to 24 pp
Brazil; Vieira et al.	THg	82	-	0.36 (0.09-3.74)	Amazonian urbans

Country; Reference	Metal	N	% positive	Mean, median* or geometric mean** (range); µg/L or ng/g	Observation
2013	MeHg	45	-	0.12 (0.01-0.47)	
	THg	75	-	2.3 (0.12-6.48)	Amazonian riverines
	MeHg	46	-	0.87 (0.11-3.40)	
Brazil; Cardoso et al., 2014	Pb	58	-	0.260 (<0.05–0.69)	Minas Gerais
	THg		-	<0.200 (<0.20–6.11)	
	Cd		-	0.770 (<0.05-6.57)	
Brazil; Marques et al., 2014	Pb	51	-	8.2 (0.9 – 29.4)	Amazonian tin ore smelters and kilns
		45	-	2.5 (0.7 -16.2)	Amazonian fishing village
Brazil; Santos et al., 2015	THg	15	100	59.41 (4.56-104.1)	Amazonian riverine
Ecuador; Counter et al., 2004	Pb	90	-	4.6 (0.4 – 20.5)	Women occupationally exposed
Ecuador; Counter et al., 2014	Pb	22	-	3.73±7.3 (0.049 - 28.4)	Women occupationally exposed
México; Amarasiriwardena et al., 2013	Pb	200	-	(0.2 – 6.7)	Mature milk
Mexico; Ettinger et al., 2004, 2006	Pb	310		1.4±1.1 (0.2-8.0)	1 month pp
		224		1.2±1.0 (0.2-6.8)	4 month pp
		195		0.9±0.8 (0.2-4.8)	7 month pp
Mexico; Gaxiola-Robles et al., 2013, 2014	THg	108	80.6	2.52 (0.03-24.9)	
		36	80.6	1.96±2.01	1st gestation

Country; Reference	Metal	N	% positive	Mean, median* or geometric mean** (range); µg/L or ng/g	Observation
		36	88.9	2.61±4.32	2nd gestation
		36	88.9	3.00±3.23	3rd gestation
Mexico; Gaxiola-Robles et al., 2014	As	108	24	0.01*(0.01-13.8)	7 days pp
Mexico; Ettinger et al., 2014	Pb	81	-	0.8±0.7 (0.6-39.8)	Mature milk
Middle east					
Iran; Rahimi et al., 2009	Pb	44		10.4±9.7 (3.2-24.7)	Industrial area
	Cd	44		2.4±1.5 (0.62-6.3)	
Iran Behrooz et al., 2012	THg	34		0.12±0.06 (nd-1.73)	Industrial area
		18		0.15±0.22 (nd-1.21)	Coast area
		28		0.86±0.26 (0.02- 5.86)	Industrial and agricultural area
Iran; Goudarzi et al, 2013	Pb	37	-	7.11±3.96 (3.06-19.5)	First 6 weeks pp
	THg	37	-	0.92±0.54 (0.0-2.7)	
	Cd	37	-	1.92±1.04 (0.45-5.87)	
Iran; Okati et al., 2013	THg	82	-	0.43 (0.0-2,45)	Under 6 months of lactation
Saudi Arabia; Al-Saleh et al., 2003	Pb	168	94,8	25.1±38.8 (<1.2-355)	Urban area
		194		37.3±50.3 (<1.2-490)	Agricultural area
	THg	168	87	4.15±5.05 (<0,2- 47.2)	Urban area
		194		2.19±2.61 (<0.2 – 25.62)	Agricultural área
	Cd	150	95.1	1.18±1.14 (<0.123-11.7)	Urban área
		194		2.16±19 (<0.123– 9.2)	Agricultural área

Country; Reference	Metal	N	% positive	Mean, median* or geometric mean** (range); µg/L or ng/g	Observation
Saudi Arabia; Al-Saleh et al., 2013; 2015	THg	331	97.3	0.97±0.665 (0.18-6.44)	3-12 months pp
United Arab Emirates; Abdulrazzaq et al., 2008	As	205	-	0.89±0.078 (0.001-0.283)	From 38 mothers. 3 months pp
	Pb	205	-	0.019±0.055 (0-0.55)	
	THg	205	-	0.008±0.025 (0-0.023)	
	Cd	205	-	0.003±0.008 (0-0.115)	
United Arab Emirates ; Kosanovic et al., 2008	As	120	-	0.196+0.032 (0.02–0.65)	-
	Pb	120	-	1.51+0.32 (0.025–2.41)	
	THg	120	-	0.115+0.05 (0.04–0.18)	
	Cd	120	-	0.27+0.04 (0.023–1.19)	
Palestine; Shawahna et al., 2016	Pb	89	100	4.0* (2-12)	15 to 210 days pp
North America					
Canada; Hanning et al., 2003	Pb	25	-	2.1±1.7	Mature milk
United States; Sowers et al., 2002	Pb	15	-	6.1±1.0	45 days pp
		15	-	5.6±1.1	3 months pp
		15	-	5.9±1.0	6 months pp
		15	-	4.3±1.6	12 months pp
United States; Carignan et al., 2015	As	9	55.6	0.31* (< 0.22–0.62)	1.7 – 7 months pp
Other regions					
Indonesia, Tanzania	THg	46	71.7	1.87 (<1 – 149)	Mining area (occupational and

Country; Reference	Metal	N	% positive	Mean, median* or geometric mean** (range); µg/L or ng/g	Observation
and Zimbabwe; Bose-O'Reilly et at., 2008					non-occupational)
Ghana; Bentum et al., 2010	As	20	60	1.54±1.94 (nd-6.22)	-
	Pb	20	40	4.8±9.0 (nd-32.0)	-
	Cd	20	40	1.3±2.9 (nd-12.3)	-
Nigeria; Adesiyan et al., 2011	Pb	180	-	83.1 - 87.1	Values reported as µg/dL[a]
	Cd	180	-	94.8 - 97.8	
Egypt; Moussa, 2011	Pb	30	-	1.7±.085 (0.26-3.33)	Nasr city
			-	5.92±.296 (4.2-7.74)	Helwan
			-	5.11±.25 (3.41-6.88)	El Khanka
	Cd	30	-	0.638 ±0.032 (0.485-0.865)	Nasr city
			-	1.84 ±0.092 (1.02-2.54)	Helwan
			-	2.56 ±0.12 (1.25-3.86)	El Khanka
Australia; Gulson et al., 2001	Pb	72		0.55** (0.09 to 3.1)	First 6 months pp; samples from 9 mothers
Faroe Island; Needham et al., 2011	Pb	15	-	8.5*	-
	THg	15	-	2.31*	
	Cd	15	-	0.25*	

pp: post partum; nd: non detected; [a.] most likely the unit is not correct

4. Risk assessment of infants to arsenic, lead, mercury and cadmium through breast milk

The process of assessing risk to a chemical may be divided into four steps: 1. hazard identification; 2. hazard characterization; 3. exposure assessment and; 4. risk characterization. The outcome of the first two steps indicates the most critical adverse effects and establishes the health-based guidance values, respectively. They are mostly based on laboratory animal data, but may also include human epidemiological studies, especially for metals. For mercury and cadmium, which have a threshold dose (no-observed-adverse-effect level, NOAEL), values may be expressed as PTWI (JECFA, 2011a,b), tolerable weekly intake (TWI; EFSA, 2009a), reference dose (RfD; Rice, 2004) or minimal risk level (MRL; ATSDR, 2016). As discussed above, the previous PTWI for arsenic and lead were found not to be protective of human health (non-threshold dose), and BMDLs were established for different toxicological endpoints for these metals (Table 1).

In the exposure assessment step, the concentration of a substance (mean, median or other value) is multiplied by the consumption of the food in question (generally the mean consumption), and the product is then divided by the body weight of a given population (IPCS, 2009). When the chronic exposure involves more than one food, the total intake is the summation of the intakes of each food.

$$\text{Intake} = \frac{\text{consumption X concentration}}{\text{body weight}}$$

In the risk characterization step for cadmium and mercury, a conclusion regarding a potential risk to human health may be reached by comparing the estimated intake with the health-based guidance value, and expressing it as either a percentage or a hazard index (HI). Risk may exist when the percentage is higher than 100 or if the HI is greater than 1. For arsenic and lead, risk characterization may be performed by estimating the

margin of exposure (MOE), which is defined as a reference point derived from the dose response relationship, such as a BMDL, divided by the estimated human intake. A MOE should be as high as possible so as not to represent a public health concern (EFSA, 2005). It is important to emphasize, however, that the MOE is not a quantification of risk for a chemical, but gives an indication of the level of concern (Benford, 2016).

The uncertainties of the risk assessment depend on the quality of the data used in each step of the process (IPCS, 2009). Uncertainties regarding the PTWI, RfD or BMDL arise from the toxicological database and the dose-response models used in the estimations (Rice, 2004). Uncertainties in exposure assessments normally regard food consumption, body weight, and the concentration data used (whether the sample is representative of the population, the number of samples analyzed, the analytical method used, and how the non-detected samples are considered in the estimation of the mean).

Some of the studies shown in Table 2 also estimated exposure and assessed the risk of infants to arsenic, lead, mercury and/or cadmium through breastfeeding. In order to investigate a wider exposure scenario, when this information was not available, intakes were also estimated using the incidence data provided in some studies, with a milk consumption of 750 mL and a body weight of 5.5 kg, as given by da Costa et. al (2010) for a 2-3 month infant. The objective was to estimate a range of exposure levels for each metal in the various regions (low to highest exposure levels). Figure 2 summarizes the mean/median intakes of arsenic, cadmium, lead and mercury by one- to six-month infants from different regions estimated from the studies. Details of the studies are discussed below. All intakes were expressed in µg/kg/week to facilitate comparison between metals. Additionally, exposure assessments for arsenic, lead and/or mercury conducted by the EFSA for the European population and by the Committee on Toxicity of the UK Food Standards Agency (COT) are also discussed.

In the context of this review, risk characterization was conducted when not available in the studies. Figure 2 also indicates the toxicological parameters

used in the risk characterization process – PTWI for MeHg and cadmium and BMDL for arsenic and lead.

4.1 Arsenic

In breast milk, arsenic is present essentially as IAs (Fängström et al., 2008), and the levels shown in Table 2 for total arsenic were assumed to correspond to IAs levels for risk assessment purposes. Only two of the studies estimated arsenic exposure from breastfeeding.

Carignan et al. (2012) estimated a median exposure of 0.04 µg/kg/day (5.6 kg bw; 810 mL of milk/day) for 1- to 3- month American infants (0.28 µg/kg/week), much lower than that estimated for infants fed with formula (0.22 µg/kg/day), even when the water used to prepare the formula contained arsenic below 1 µg/L. The EFSA estimated a mean IAs intake of 0.04 µg/kg bw/day for 3-month European infants (6.1 kg, 800 mL milk; EFSA, 2014). Exposure reached 2 µg/kg bw/day for toddlers, the most critically exposed population to arsenic through the diet in Europe, mainly from the consumption of milk and dairy products. A lower median arsenic intake (0.02 µg/kg bw/day, or 0.14 µg/kg bw/week) was estimated by Sternowsky et al. (2002) for 3-month German infants (6 kg; 790 mL/day). The authors considered the exposure to be safe, as it was much lower than the PTWI of 15 µg/kg bw/week. Our estimation of arsenic intake from the consumption of intermediate milk of Portuguese mothers (Almeida et al., 2008; Table 2) yielded a much higher value (5.5 µg/kg bw/week). Using the approach currently employed to characterize the risk of exposure to arsenic and a BMDL0.5 of 3 µg/kg bw/day (or 21 µg/kg bw/week), a median MOE of 75 was calculated for the American breastfed infants, which could reach 3.8 for Portuguese babies.

The COT reported that arsenic was above the limit of quantitation in 7% of 91 breast milk samples from the UK analyzed in the SUREmilk pilot studies, with a maximum concentration of 4.0 µg/kg (COT, 2004). The maximum estimated intakes ranged from 0.64 µg/kg bw/day for infants under 2 months to 0.15 µg/kg bw/day at 8-10 months. Mean intakes were not reported. The

Committee acknowledged that there were no appropriate safety guidelines for arsenic, and concluded that exposure to inorganic arsenic should be As Low As Reasonably Practicable (Achievable), which is known as the ALARP (ALARA) principle, applicable to compounds with no identified threshold of effect. A maximum MOE of 4.7 could be estimated for UK infants under 2 months.

The highest mean level of arsenic reported in the studies in Table 2 was found in India (19 µg/L; Samanta et al., 2007). Using this level, and a milk consumption of 750 mL for a 5.5 kg 2-3 month baby, we estimated an arsenic intake of 2.6 µg/kg bw/day (or 18.2 µg/kg/week), much higher than that reported in Europe and the USA, and a MOE of 1.2. As pointed out before, this high exposure level reflects the high arsenic levels found in the water sources in the region, although the estimated intake based on concentration levels found in Bangladesh (Table 1), also a region with high arsenic levels in water, was much lower (up to 1.7 µg/kg bw/week). The estimated mean intake from limited data in Japan (9 milk samples; Table 1) was 1.3 µg/kg/week.

The EFSA (2005) considered that a MOE of 10,000 or higher for genotoxic compounds, if based on the BMDL10 from an animal study, would be of low concern from a public health point of view and might be considered as a low priority for risk management actions. This level allows for 100-fold for specie differences (10-fold) and human variability (10-fold), and an additional 100-fold for additional uncertainties (inter-individual human variability in cell cycle control and DNA repair, and effects that can occur below the reference point). In its evaluation of arsenic, the EFSA (2014) did not estimate a MOE nor did it discuss a level above which the exposure would be considered of low health concern. In this review, an attempt was made to estimate this level taking two points into consideration: 1) the additional carcinogenic risk in the BMDL10 related to a MOE of 10,000 (10%) is 20 times higher than the extra risk in the BMDL0.5 established for arsenic (0.5%), and 2) the BMDL0.5 was based on human studies, so uncertainty due to specie differences (10-fold) can be disregarded. A MOE value that may be used in the risk characterization of arsenic exposure would be 10,000 ÷ 20 ÷ 10, or 50. Therefore, a MOE of 50 or higher for arsenic, based on the BMDL0.5 from a human study, would be of low concern from a public health point of view.

In this paper, the estimated MOEs, based on mean or median intakes of breast milk by 2-3 months infants, were above 50 for American infants, as well was for exposures lower than 0.06 µg/kg bw/day, which correspond to a consumption of 750 mL breast milk (5.5 kg infant) containing less than 0.44 µg/L of arsenic (Table 2). Higher arsenic levels, which would lead to MOE lower than 50, were found in breast milk samples from all Asian countries, in some European countries (Greece, Portugal and Sweden), in the United Arab Emirates, and in Ghana (Table 2). Figure 2 shows the arsenic intakes for 1 to 6 month infants through breast milk estimated for USA, Japan, Portugal and India (from 0.28 to 18.2 in µg/kg bw/week).

4.2 Lead

Nine studies shown in Table 2 included exposure assessments of breastfed infants to lead. None of the studies used the MOE to characterize risk which, in the context of this paper, was done using a BMDL1 of 0.5 µg/kg bw/day (3.5 µg/kg bw/week). Al Saleh et al. (2003) estimated a mean intake of 34.3 µg/kg bw/week by infants in Saudi Arabia (850 mL, 5-6 kg bw), and reported that 46.7% of the infants had weekly lead intake levels exceeding the PTWI of 25 µg/kg bw/week. Chien et al. (2006a) found higher daily intakes of lead in breastfed Taiwanese infants at birth (median of ~1.8 µg/kg bw/day; 400 mL milk), which decreased to below 0.3 µg/kg bw/day (2.1 µg/kg bw/week) after 3 months (760 mL milk). Two of the 72 infants (2.6%) had a HI greater than 1. The estimated MOEs were 0.1 and 1.7 for the Saudi Arabian and Taiwanese infants, respectively.

Three studies were conducted in Europe. Leotsinids et al. (2005) estimated lead intake of Greek infants assuming a consumption of 100 to 150 mL/kg bw/day of colostrum and intermediate milk, respectively. The 90th percentile of the intakes were 1.0 and 1.1 µg/kg bw/day, respectively, much lower than the PTWI, which corresponded to 3.6 µg/kg bw/day. The authors estimated a median intake for intermediate milk of 0.49 µg/kg bw/week. Ursinyova & Masanova (2005) estimated mean lead intake of 5.4 µg/kg bw/week for Slovakian breastfed infants using a daily milk consumption

equivalent to 1/6 of the infants' body weight. The intake from the consumption of milk for two of the 158 mothers exceeded the PTWI. The estimated mean MOEs for Greek and Slovakian breastfed infants were 7 and 0.64, respectively.

Using lead levels found in the 2-5 day breast milk samples, Gürbay et al. (2012) estimated the intake of 3-month Turkish breastfed infants (750 mL/day) ranging from 22.9 to 5356 µg/week (mean of 2052 µg/week). Considering a body weight of 5.5 kg, a mean intake of 373 µg/kg bw/week can be estimated. This intake, however, is probably overestimated since metal levels, including lead, decrease in mature milk (Chao et al., 2014; Chien et al., 2006). Winiaska-Mieczan (2014) estimated that the weekly intake of lead by Polish infants decreased from 2.9-2.8 µg/kg bw at 1-3 months to 0.84 µg/kg bw at 12 months using the recommended volume of powdered milk for infants as a parameter for breast milk consumption. The authors expressed these values as % of the BMDL of 3.5 µg/kg bw/week (84 to 24%), and concluded that although the intakes did not exceed the “admissible levels”, they were nevertheless high. It is important to emphasize however that this BMDL is not an admissible level of lead exposure, but is a level that corresponds to a 1 IQ point decrease in cognitive ability in children (EFSA, 2010). A MOE of 1.2 may be estimated for 1-3 month Polish infants.

In the UK, the COT (2004) reported that lead was above the LOQ in 7% of 114 breast milk samples analyzed, with a maximum concentration of 2.6 µg/kg, and a maximum intake ranging from 0.42 µg/kg bw/day for infants below 2 months of age to 0.1 µg/kg bw/day at 8-10 months, lower than the JECFA PTWI in effect at that time. The Committee concluded that this exposure does not raise toxicological concerns.

In a study conducted in Brazil (State of Rondônia, Amazonian region), Marques et al. (2013) estimated a median exposure to lead in the first 6 months of breastfeeding (140 mL milk/kg bw/day) of 3 µg/kg bw/day for rural infants, and of 7.5 µg/kg bw/day (52.5 µg/kg bw/week) for infants living in the vicinity of tin smelters. Our calculations indicate MOEs of 0.16 and 0.07 for rural and smelter neighboring infants, respectively. In another study conducted in the

country however, mean lead levels were much lower (0.26 µg/L; Table 2), and we estimated a MOE of 14.

In its dietary risk assessment of lead for 3-month breastfed infants, the EFSA (2010) calculated MOEs of 2.4 for average consumers, which decreased (higher risk) in infants fed with formula and in children up to 7 years (MOE <1). In its evaluation, the EFSA concluded that the risk from lead exposure for infants can be significant when the MOE is lower than 1; risk is likely to be low when the MOE is between 1 and 10; and a MOE of 10 or greater indicates no appreciable risk of a clinically significant effect on IQ. Most of the calculated MOEs were either below 1 or between 1 and 10, indicating a potential risk to breastfed infants. Figure 2 shows the mean lead intakes by 1- to 6- month infants through breast milk discussed above.

4.3 Mercury

In most of the studies shown in Table 2, only THg was analyzed in the breast milk samples. Currently, the PTWIs for mercury are for IHg (4 µg/kg bw) and for MeHg (1.6 µg/kg bw), which is relevant for pregnant women and infants (JECFA, 2011b). The mean ratio of MeHg to THg in breast milk varies widely (from 0 to 0.6, mostly around 0.5; Table 2), and is considered to be greater in populations with higher fish consumption, reaching over 0.8 in some countries (Valent et al 2013; Miklavcic et al., 2013). For the purpose of this review, when MeHg was not measured, it was considered to represent 50% of the THg present.

Two Brazilian studies conducted risk assessments of exposure of breastfed infants to mercury, both in the Federal District. Costa et al. (2005) estimated a THg mean and maximum intake (150 g milk/bw/day) of 0.86 and 3.46 µg/kg bw/day, respectively. The authors stated that 56.3 % of the samples would indicate intakes higher than the reference value set by the WHO in 1991 for THg (0.5 µg/kg bw/day). Based on our previous assumption, MeHg mean intake in this study corresponded to 0.43 µg/kg bw/day, or 3 µg/kg bw/week, representing 188% of the PTWI.

In the assessment conducted by Cunha et al. (2013), 18 nursing mothers provided samples 15 to 90 days post-partum (142 samples) during 2003 and 2004, the same period as in the study by Costa et al., yielding similar THg mercury concentrations (Table 2). Infant weights were measured at 30, 60, and 90 days, and consumption volumes were estimated from the time the infant spent breastfeeding at each sampling point, assuming a milk flow of 13.5 mL/min. The intakes exceeded the THg PTWI (5 µg/kg bw/week) at least once during the period for 77.8 % of the samples, with one sample reaching over 800% of the PTWI. Only four mothers did not provide samples that would lead to an exceedance of the PTWI at any sampling time. The estimated mean intake of THg was 6.4 µg/kg bw/week, or 3.2 µg/kg bw/week of MeHg (200% of the PTWI).

The study by Santos et al. (2015) in the Brazilian Amazon provided the highest mean levels of THg among all the studies in Table 2 (59.4 µg/L). Based on this level and a daily milk consumption of 750 mL for a 5.5 kg baby, we estimated a mean THg intake of 56.7 µg/kg bw/week, or 28.4 µg/kg bw/week for MeHg. Another study conducted in the same region found a much lower mean THg level (5.7 µg/L; Boishio & Henshel, 2000), and we estimated an intake of 5.4 µg/kg bw/week, and 2.7 µg/kg bw/week of MeHg, which corresponds to 170% PTWI.

The EFSA (2012b) conducted an assessment for MeHg in European infants under six months of age (6.1 kg bw) using contamination data from Miklavcic et al. (2013) and Valent et al. (2013) (Table 2). The mean intakes ranged from 0.09 to 0.62 µg/kg bw/week (800 mL milk consumption), and from 0.14 to 0.94 µg/kg bw/week for high consumers (1200 mL milk), and did not exceed the TWI of 1.3 µg/kg bw/week.

Iwai-Shimada et al. (2014) estimated intakes for Japanese one-month-old infants (4 kg bw and 800 mL milk) ranging from 0.08 to 1.68 µg/kg bw/week for MeHg (median of 0.63 µg/kg bw/week). The authors compared the intakes of MeHg with the Japanese and EFSA TWI (2 and 1.3 µg/kg bw/week, respectively), the JECFA (1.6 µg/kg bw/week), and a reference dose (RfD) from USEPA of 0.1 µg/kg bw/day. For the more restricted situation (USEPA),

exposure exceeded the RfD in 12 of the 27 cases, with the median intake corresponding to 40% of the JECFA PTWI.

Chien et al. (2006b) estimated a mean THg intake of 3 μg/kg bw/day for newborn Taiwanese babies. Assuming that 50% of mercury is present as MeHg, the Monte Carlo simulation showed that HI for mercury was greater than one for 12.9% of urban babies, and for 18.8% of fishing village babies (MRL of 0.3 μg/kg bw/day). The mean MeHg intake represented 660% of the PTWI.

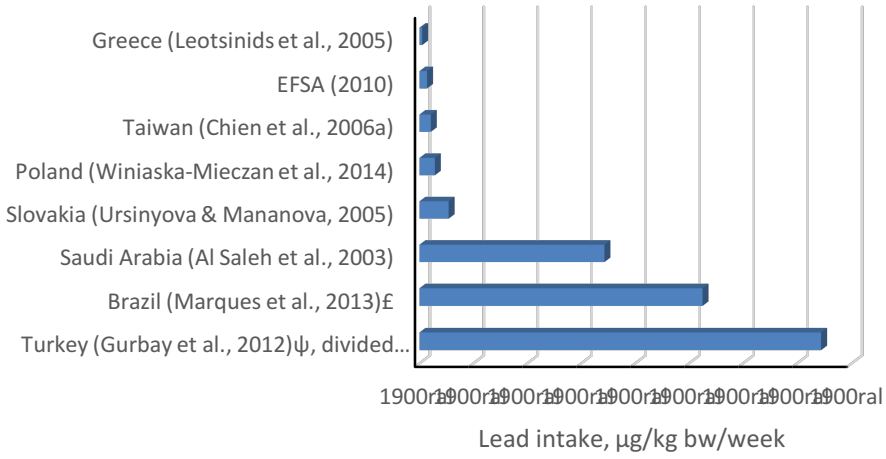
Behrooz et al. (2012) estimated a mean THg intake of 0.065 μg/kg bw/day for Iranian infants based on the actual infant birth weights and a daily milk intake of one-sixth of the infants' weight. Okati et al. (2013) found a similar result for 7 kg Iranian infants (1050 mL milk/day), with a mean of 0.064 μg/kg bw/day. These THg intakes corresponded to 0.22 μg/kg bw/week of MeHg (14% of the PTWI). In Saudi Arabia, Al Saleh et al. (2003) estimated a much higher mercury intake for 5-6 kg infants (3.25 μg/kg bw/week), with 17.1% of the infants exceeding the THg PTWI. The calculated mean MeHg intake represented 100% of the PTWI.

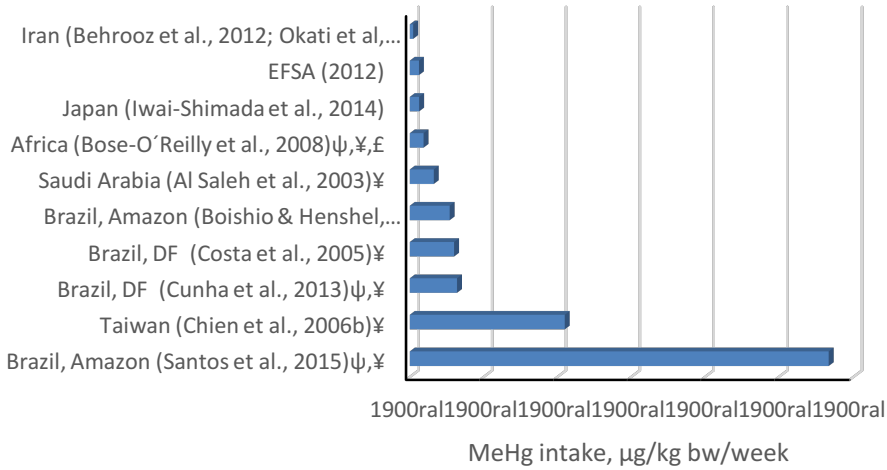
A study conducted by Bose-O´Reilly et al. (2008) involved women with a very high mercury burden in four different gold mining areas in Indonesia, Tanzania and Zimbabwe. The authors estimated that the THg intake by a 3-month infant (6 kg, 850 mL milk/day) exceeded the RfD of 0.3 μg/kg bw/day in 47.8 % of the cases, with the highest intake being 21.2 μg/kg bw/day (7100 %RfD). The authors stated that no conclusion regarding a possible health risk of environmental mercury could be reached given the clear benefits of breastfeeding in developing countries. Based on the mean THg level (1.7 μg/L; Table 2) the estimated mean intake of MeHg was 0.93 μg/kg bw/week, representing 58% of the PTWI. Figure 2 summarizes the intakes of MeHg by 1 to 6-month infants through breast milk discussed in this review. It is important to emphasize that the intakes may be overestimated for low fish consumption populations.

4.4 Cadmium

Four of the studies reported in Table 2 conducted exposure assessments for cadmium (and for lead, as discussed above) through breastfeeding. In Greece, the estimated 90th percentile of cadmium intakes from the consumption of colostrum and intermediate milk were 0.32 and 0.52 μg/kg bw/week, respectively; median values were 0.10 and 0.18 μg/kg bw/week (Leotsinidis et al.; 2005). Ursinyova & Masanova (2005) estimated (milk consumption equal to 1/6 the body weight) a mean cadmium intake of 0.5 μg/kg bw/week for Slovakian newborn infants (0.02-1.99 μg/kg bw/week). In Poland, the mean exposures at 1, 6 and 12 months were 1.8, 2.1 and 0.82 μg/kg bw/week, respectively (Winiarska-Mieczan, 2014). In both studies, the authors compared the exposure with the TWI of 2.5 μg/kg bw/week set by the EFSA, which was not exceeded in any of the cases. Mean intake of cadmium by Saudi infants through breastfeeding (850 mL, 5.5 kg) estimated by Al Saleh et al. (2003) was 1.8 μg/kg bw/week, with 2.6 % of the infants (n=344, 5 months old, on average) having intakes higher than the PTWI of 7 μg/kg bw/week.

The highest mean level of cadmium in breast milk of the studies in Table 2 was found in a study conducted in Turkey (Gürbay et al., 2012) (4.6 μg/L). Using this level and a daily milk consumption of 750 mL for a 2-3 month baby (5.5 kg), we estimated a mean cadmium intake of 4.4 μg/kg bw/week for Turkish breastfed infants. This level is higher than the EFSA TWI (176%), but lower than the PTMI set by the JECFA, which corresponds to 5.8 μg/kg bw/week. These two contradictory risk conclusions demonstrate that risk assessment results need to be seen in light of the conservativeness of the parameters used and the uncertainties involved in the estimations. Figure 2 summarizes the intakes of cadmium by 1- to 6-month infants through breast milk discussed in this review.





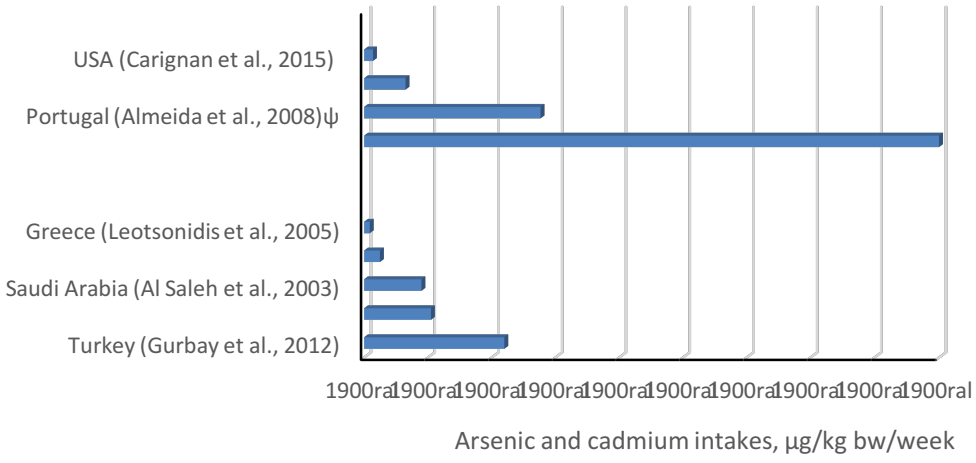


Figure 2. Mean intakes of lead, mercury, arsenic and cadmium by 1 to 6 months infants through breast milk; ψ estimated from the concentration data provided (Table 2), assuming 750 mL daily consumption and 5.5 kg bw baby; ¥. calculated assuming that 50% of THg is present as MeHg; £. population living near mining areas. EFSA: European Food Safety Authority; JECFA: FAO/WHO Joint Expert Committee on Food Additives

5. Summary and Conclusions

Arsenic, lead, mercury, and cadmium are toxic metals ubiquitous in nature, to which exposure can be a public health concern. These metals cross the placenta and the blood brain barrier, and are excreted through breast milk. Exposure to lead and mercury has been related to neurotoxic problems later in life, although studies to discriminate intrauterine and postnatal effects are still needed. Currently, there is no safe dose of exposure established for lead or arsenic.

Monitoring breast milk is a non-invasive way of determining human exposure to metals and other contaminants. This review covers 75 studies that assessed arsenic, lead, mercury and/or cadmium levels in breast milk samples collected worldwide, with about one-third of the studies conducted in Europe. Mean or median levels of arsenic in intermediate and mature breast milk from non-occupational mothers were higher in India, reflecting high levels of this metal in the water sources of the region, and for methyl mercury in the Brazilian Amazon. Cadmium levels in breast milk were the lowest among the metals, mostly below the LOQ of the method. Lead was the metal most investigated and most detected in the studies.

Risk assessments conducted using current methods and toxicological parameters indicate that the risks for breastfed babies in most regions cannot be excluded, mostly due to arsenic, lead and mercury. Arsenic intakes led to MOEs below 10 in most studies. However, bottle-fed infants, who consume milk powder diluted in water, had higher arsenic intakes. Therefore, breastfeeding is protective for the babies, mainly in areas with high levels of arsenic in water. All the Brazilian studies indicated MeHg intakes exceeding the safety exposure parameter, reaching 1700 % PTWI in a Brazilian Amazon riverine community, most likely due to high fish consumption, including piscivorous fish, which may contain high MeHg levels due to the bioconcentration in the aquatic food chain. Although the benefits of a high fish consumption diet are widely recognized due to its high-quality protein, fatty acids and other essential nutrients (IOM, 2005), women of child-bearing age and nursing mothers should avoid consuming piscivorous fish (USFDA, 2014).

The highest mean levels of lead in breast milk were found in Turkey, with an intake that led to a MOE of 0.01, with a potential for neurotoxic effects. The same conclusion may also be reached for infants from other regions, including Saudi Arabia, Brazil and Slovakia (MOE <1). Cadmium intakes were also higher in Turkey, representing 173 % of the TWI established by the EFSA, but were below the PTWI established by the JECFA.

It is clear from most studies that breastfeeding exposes infants to more than one metal simultaneously, and most likely reflects the intrauterine exposure. Although the risk assessments discussed in this review were for each metal separately, it is important to point out that co-exposure to metals, in addition to other environmental contaminants, acting through the same mechanism and/or targeting the same organ, may lead to combined adverse effects with greater health impact on infants and children (Cardenas et al., 2015, Govarts et al., 2016)

The presence of environmental contaminants in human milk and the potential risks to the infants have been long recognized by researchers and health authorities worldwide. However, the World Health Organization and national governments strongly recommend breastfeeding, as it is accepted that the risks are outweighed by the benefits of breast milk consumption (WHO, 2007a; Mead, 2008; VKM, 2013). This conclusion, however, does not preclude the responsibility of health authorities and researchers from continuing to monitor the levels of these metals in breast milk, particularly in regions with high levels of contamination, either by natural sources (as for arsenic in areas with high levels in water) or anthropogenic sources (as for lead in mining areas). Risk communication initiatives to reduce exposure among women of childbearing age by health authorities include:

- Women should be advised to avoid the consumption of predatory fish during pregnancy and when breastfeeding to decrease MeHg exposure.
- Women should be aware that arsenic exposure is much lower for breastfeeding babies than for babies fed with bottles;

- Women should be removed from polluted and mining areas and should avoid smoking to decrease exposure of the fetus and infants to lead and cadmium, among other contaminants.

III. OBJETIVOS

Geral:

Determinar a concentração de arsênio, cádmio, chumbo e mercúrio em amostras de leite materno coletadas em bancos de leite do Distrito Federal, e caracterizar o risco de exposição dos bebês a esses elementos pela amamentação.

Específicos:

1. Determinar as concentrações de mercúrio total e metilmercúrio nas amostras de leite materno;
2. Caracterizar o risco da ingestão de metilmercúrio pelos bebês amamentados;
3. Validar metodologia para digestão ácida e análise de amostras de leite materno para os metais arsênio, cádmio e chumbo;
4. Determinar as concentrações de arsênio, cádmio e chumbo nas amostras de leite materno;
5. Caracterizar o risco da ingestão de arsênio, cádmio e chumbo pelos bebês amamentados.

IV. ESTRUTURA DA TESE

Neste documento, os métodos utilizados, os resultados obtidos, discussão e conclusão serão apresentados em formato de artigos, em dois capítulos distintos.

Capítulo 1: Mercury in breast milk from women of Federal Distrit, Brazil and dietary risk assessment for methyl-mercury

Este capítulo atende aos objetivos específicos 1 e 2 do estudo

Capítulo 2: Arsênio, chumbo e cádmio em leite humano por ICP-MS – validação do método, análise das amostras e avaliação de risco da exposição dos lactentes.

Este capítulo atende aos objetivos específicos 3, 4 e 5 do estudo.

1. Mercury in breast milk from women of Federal Distrit, Brazil and dietary risk assessment for methyl-mercury

Abstract

Breast milk provides all necessary nutrients for the baby, however, it may contain toxic compounds. Mercury is a toxic metal ubiquos in nature that is excreted in breast milk and could affect infant neuro development. In this study, 224 breast milk samples provided by eight human milk banks in the Federal District of Brazil were analyzed for total mercury (THg), of which 181 were also analysed for methyl mercury (MeHg), the most absorbed form of this metal by the gastrointestinal tract in humans. Samples were acid digested in a microwave oven and THg determined by atomic fluorescence spectrometry (LOQ of 0.76 µg/L). Samples were lyophilized, ethylated and MeHg determined on a MERX automated system (LOQ of 0.10 µg/L). Most of the samples were collected 1-2 months postpartum, with 38.3% during the first month Over 80% of the samples had THg values above the LOQ, reaching a maximum of 8.40 µg/L, with average of 2.6 µg/L. In average, MeHg accounted for 10.1% of THg, with a maximum of 74.9%. Weekly intakes for MeHg were estimated individually, considering the baby age and weight estimated by WHO curve of growth at the time of milk collection. Mean weekly intake was 0.16 ± 0.22 µg/kg bw, which represented 13.6 % of the PTWI. In two thirds of the cases weekly intakes of MeHg represented less than 10% of the PTWI. Only in one case, the intake exceeded 100 % of the PTWI (1.90 µg/kg bw, 119% of PTWI). These results indicate no health concern for the breastfed babies, a conclusion that can be extended to the consumers of breastmilk donated to the milk banks, primarily imature and low weight babies.

Key Words: Breast milk, mercury, methylmercury, risk assessment

1.1. Introduction

Breast milk provides almost all the necessary nutrients for the baby, protecting against a variety of diseases, mainly during the first 6 months of life (Haroon et al, 2013; Grezlak et al, 2014). However, milk may contain toxic compounds to which the mother has been exposed to, including mercury. Human exposure to mercury has been an important health concern worldwide since the event of Minamata disease in the middle of the 20th century, that killed over a thoushand people (Watts, 2001). In a recent review, Ha et al. (2017) retrieved 514 relevant papers published since 2012 covering the various aspects of mercury research, from which 75 on its effects on children development

Elemental mercury is derived from natural degassing of the earth surface, and eventually is oxidized to inorganic form (IHg), returning to the surface and water systems through the rain. Furthermore, anthropogenic sources, including mining, industrial activities and deforestation can significantly increase the human burden to this metal (Tokar et al., 2013; Carpi et al., 2014). Methyl mercury (MeHg) is mainly formed in the aquatic environment and sediment by methylation of inorganic mercury by reducing bacteria (Correia & Guimarães, 2017), and the main source of exposure to MeHg is through fish consumption (JECFA, 2011). The main source of IHg for the general population is food, in addition to amalgam fillings (Ask et al., 2002; Gundacker et at., 2010), and rice, which has been shown to contain both the organic and inorganic forms (Zhu et al., 2015; Strickman and Mitchell, 2017). However, while less than 15% of IHg is absorved by the gastrointestinal tract, over 95% of ingested MeHg is absorbed, and diffuses in various body tissues, including brain (CDC, 2009). MeHg crosses the blood-brain and placental barriers and may compromise neurological development of fetuses causing irreversible damage (WHO, 2010). Al-Saleh et al (2016a) reported significant associations between MeHg levels of the mother and infant hair and infant neurodevelopment delay assessed by the Denver Developmental Screening Test II, possibily involving a mechanism of MeHg-oxidative stress (Al-Saleh et al., 2016b).

At its Sixty-first Meeting, the Joint FAO/WHO Expert Committee on Food

Additives (JECFA, 2004) concluded that neurodevelopment is a sensitive health outcome to the exposure to MeHg, and the fetus is the most critical population group for the occurrence of neurodevelopmental toxicity as a result of exposure. The Committee established a Provisional Tolerable Weekly Intake (PTWI) of 1.6 mg/kg bw for MeHg in childbearing-aged women due the possibility of pregnancy and to protect the fetus (JECFA, 2004).

Rebelo and Caldas (2016) retrieved 34 studies published since 2000 that analyzed THg in breast milk worldwide. The levels varied substantially among the regions, with the highest found in a Brazilian Amazonian riverine population (104 µg/L; Santos et al., 2015). Only five studies also analysed MeHg, including one in Brazil (Vieira et al., 2013). In a study conducted in the Federal District of Brazil with 18 mothers, THg intake by the infants during lactation excceed the PTWI (5 mg/kg bw) in most cases, what raised a health concern for this population, although the benefits of breast milk were highlighted by the authors (Cunha et al., 2013).

As the Federal District is a region of low fish consumption (POF 2008/2009; IBGE, 2012), the hypothesis of the present study is that most of the mercury present in milk from the Federal District mothers is in inorganic form, which is of less health concern to the fethus and less absorbed by the lactating baby than the organic form. To test this hypothesis, breast milk samples collected from milk banks were analyzed for the content of THg and MeHg. Furthermore, the risk of the lactant babies associated with the exposure to MeHg was assessed.

1.2. Materials and methods

1.2.1. Breast milk samples

The samples analyzed in this study were provided by eight human milk banks in the Federal District from May 2011 to February 2012, as described by Andrade et al. (2013). To be included in the milk donor bank, volunteers should fill the following requiriments: be breast-feeding or milking for her own child; be healthy; not smoking more than 10 cigarettes per day; not use alcohol or illegal

drugs; and provide medical and laboratory exams. Information on the mother's age and the infant's date of birth was also provided by the milk banks. The project was approved by the Ethics Committee of the University of Brasilia (CEP nº 27/11, Anex 2). The samples collected were kept at −18◦C until analyzed. All the glassware used in the analyses was previously acid washed.

1.2.2. Total mercury analysis

THg was determined using a previously validated method described by Cunha et al. (2013). In summary, 1 mL aliquot of the homogenized milk sample was digested with 2 mL of Suprapur nitric acid (65 %; Merck, USA) in a Microwave (DGT-100 Provecto Systems, Brazil), the digest diluted to 25-mL with nanopure water and THg quantified by atomic fluorescence spectrometry (PSA 10.023 Merlin system; PS Analytical, Kemsig, Sevenoaks, UK) using a 2 % stannous chloride solution as a reduction agent. The performance of the method was confirmed with certified skim milk powder reference material containing 9.4±1.7 ng/g THg (BCR®-150; Institute for Reference Material and Measurements, Belgium) with recoveries between 95% and 105%. The limits of detection (LOD) and quantification (LOQ), estimated based on the instrument response of a blank solution, were 0.26 and 0.76 µg/L, respectively.

1.2.3. Methyl mercury analysis

An aliquot of the breast milk samples (5 mL) was lyophilized (Liotop – K105), and samples analysed following the validated method described by Vieira et al. (2013). In summary, 5 mL of 25% KOH methanolic solution was added to a known amount of lyophilized milk sample (0.2 g) in a teflon tube and let at 70ºC for 6h, with gentle stirring every hour. The samples were kept for 48h in the dark, centrifuged, and 50 µL taken for ethylation with 50 µL of tetra ethyl sodium borate (1%, from Brooks Rand Labs; Seattle, USA) and 200 µL of acetate buffer (pH 4.5; 2 mol/L). The mixture was diluted up to 40 mL with ultra-pure water (milli-Q, Millipore, Cambridge, MA, USA). MeHg was analyzed on a MERX automated MeHg system (Brooks Rand Labs) equipped with an auto-

sampler, a purge and trap unit, a packed column GC/pyrolysis unit, and a Model III atomic fluorescence spectrophotometer. Samples were analyzed in duplicate. A certified material was analyzed with each batch for quality control (IAEA Biological Reference Materials of Terrestrial Origin for Determination of Trace and Minor Elements; Human hair, IAEA 085), with recoveries between 85% and 105%. The LOQ was stablished based on the lower level of calibration curve and corresponded to 0.1 µg/L MeHg.

1.2.4. MeHg intake by infants and risk characterization

Consumption of human-milk by the infants at the time the milk was collected was estimated based on Costa et al. (2010), and body weight was estimated based data from WHO Child Growth Standards (WHO, 2006). As no information about sex was provided, a mean milk consumption and body weight between boys and girls was assumed. MeHg intake, in µg/kg bw/week was calculated for each breast milk sample and child according to Eq. 1.

$$Intake = \frac{Consumption\ (L)\ x\ concentration\ (\mu g/L)}{body\ weight\ (kg)} \qquad \text{Eq. 1}$$

The risk from exposure to MeHg was assessed according to Eq. 2, and expressed as % PTWI of MeHg (1.6 µg/kg bw; JECFA, 2011). Risk may exist when the % is higher than 100:

$$\%\ PTWI\ = \frac{Intake \times 100}{PTWI} \qquad \text{Eq. 2}$$

1.2.5. Statistical Analysis

All data obtained were analysed using SPSS version 22, IBM software. Kolmogorov-Smirnov and Shapiro-Wilk were used to test for normality of the

distributions. Spearman Test was used for correlation analysis in not normal distributions, with significance at $p \leq 0.05$.

1.3. Results

1.3.1. Studied population

The 224 breast milk samples analyzed in this study were provided by 213 mothers to the milk bank of the Federal District. In average, the donors were 28.6 ± 6.6 years (15 to 47 years), and mean body weight of the newborn babies was 3.2 ± 0.56 kg (1.2 to 5.3 kg). Most of the samples were collected 1-2 months postpartum, with 38.3% of them during the first month. All the samples were analyzed for THg. Due to limitations of sample volume, only 181 samples were analysed for MeHg.

1.3.2. THg and MeHg levels in breast milk

Table 1 summarizes the results of mercury analysis in the breast milk samples and the individual results are shown in Appendix 1. Over 80% of the samples had THg values above the LOQ (0.76 µg/L), reaching a maximum of 8.40 µg/L, with average of 2.6 µg/L. Levels of MeHg were much lower, with almost half of the 181 samples analyzed containing levels below the LOQ (0.10 µg/L), with a maximum of 2.82 µg/L. In average, MeHg accounted for 10.1% of THg, with a maximum of 74.9. Figure 1 shows the distributions of the levels found and the boxplots of the data.

Table 1. THg and MeHg levels in breast milk samples provided by the bank milk samples of the Federal District, Brazil.

	N (% ≥ LOQ)	Mean ± SD	Median	Min.	Max
THg (µg/L)	224 (84.1)	2.6 ± 1.6[a]	2.36	<0.76	8.40
MeHg (µg/L)	181 (45.6)	0.19 ± 0.28[b]	0.05	<0.10	2.82
% as MeHg	181	10.1 ± 13.5	13.5	0.28	74.91

a. samples < LOQ (0.76 µg/L) were considered at 1/2 LOQ; samples < LOD (0.26 µg/L) were considered at 1/2 LOD; b. samples ≤ LOQ were considered at 1/2 LOQ (0.10 µg/L); SD: standard deviation

The distribuitions shown in Figure 1 were not normal, so Spearman analysis was used for correlation analysis. A weak, but significant correlation was found between concentrations of THg and MeHg (rs=0.157; p=0.034). Table 2 shows also signfificant correlations between months of breastfeeding and concentration of MeHg (p=0.001) and between mother's age and MeHg (p=0.024).

Table 2. Spearman coefficient correlation for MeHg and THg concentrations (p value)

	MeHg levels (µg/L)	**THg levels (µg/L)**	**% PTWI MeHg**
Months of breastfeeding	***0.234 (0.001)***	-0.16 (0.817)	-0.063 (0.398)
Mother's age, years	***0.168 (0.024)***	0.085 (0.217)	0.107(0.154)
% of MeHg	0.662 (0.000)	-0.592 (0.000)	0.623 (0.000)
MeHg intake (µg/kg bw/week)	0.920 (0.000)	0.137 (0.065)	1.000 (0.000)

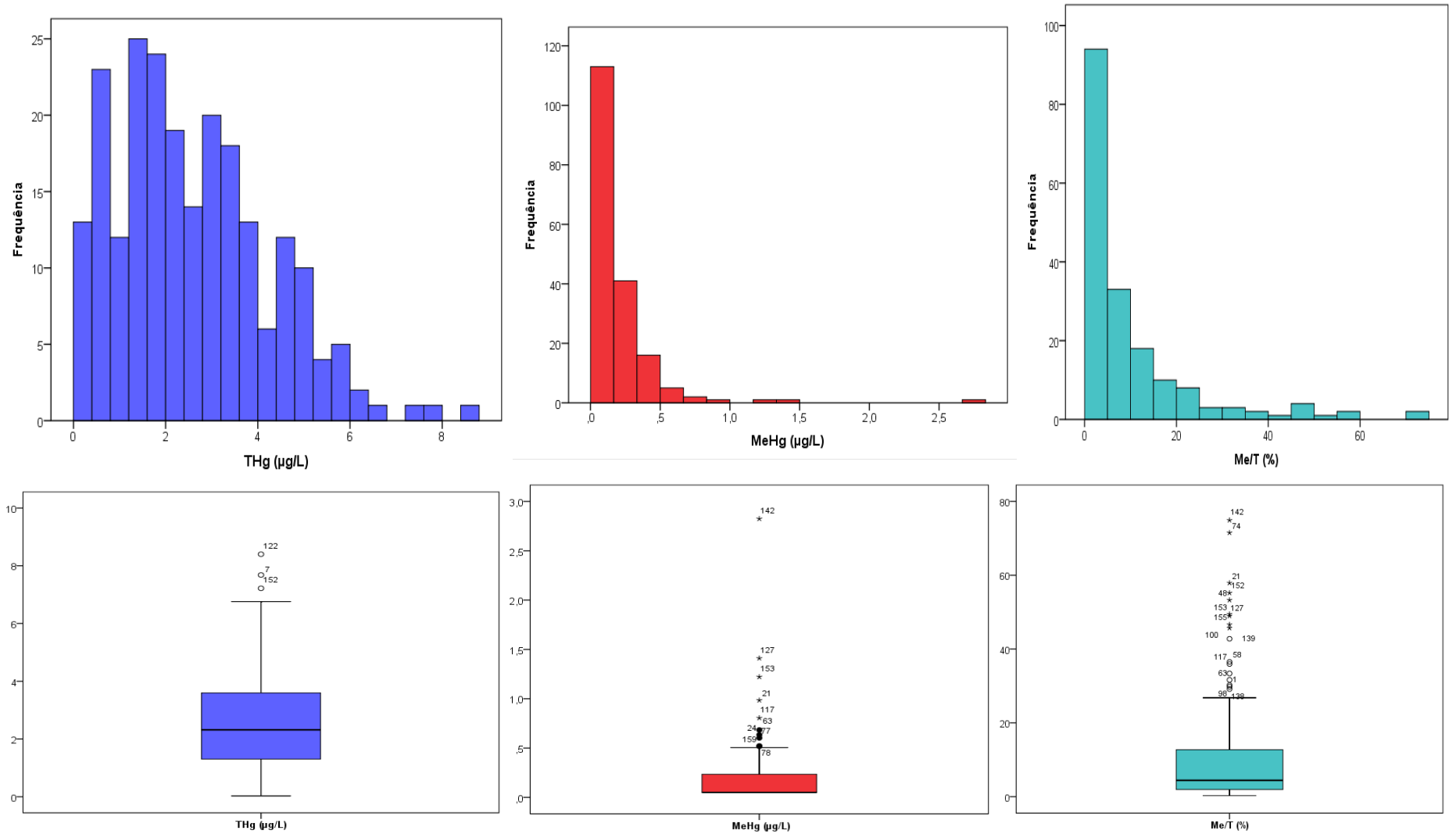


Figure 1. Distributions and boxplots of THg and MeHg and MeHg/THg in breast milk.

1.3.3. Intake of MeHg by infants and risk characterization

Mean weekly intake of MeHg was 0.16 ± 0.22 µg/kg bw, with a maximum of 1.90 µg/kg bw; the distribution is shown in Figure 2. Also shown in Figure 2 is the distribution of the risk for each infant/mother case from the exposure to MeHg, expressed in % of the PTWI (1.6 µg/kg bw), as well as the dispersion plot of the % PTWI and months of breastfeeding, which correlation was not significant (Table 2).

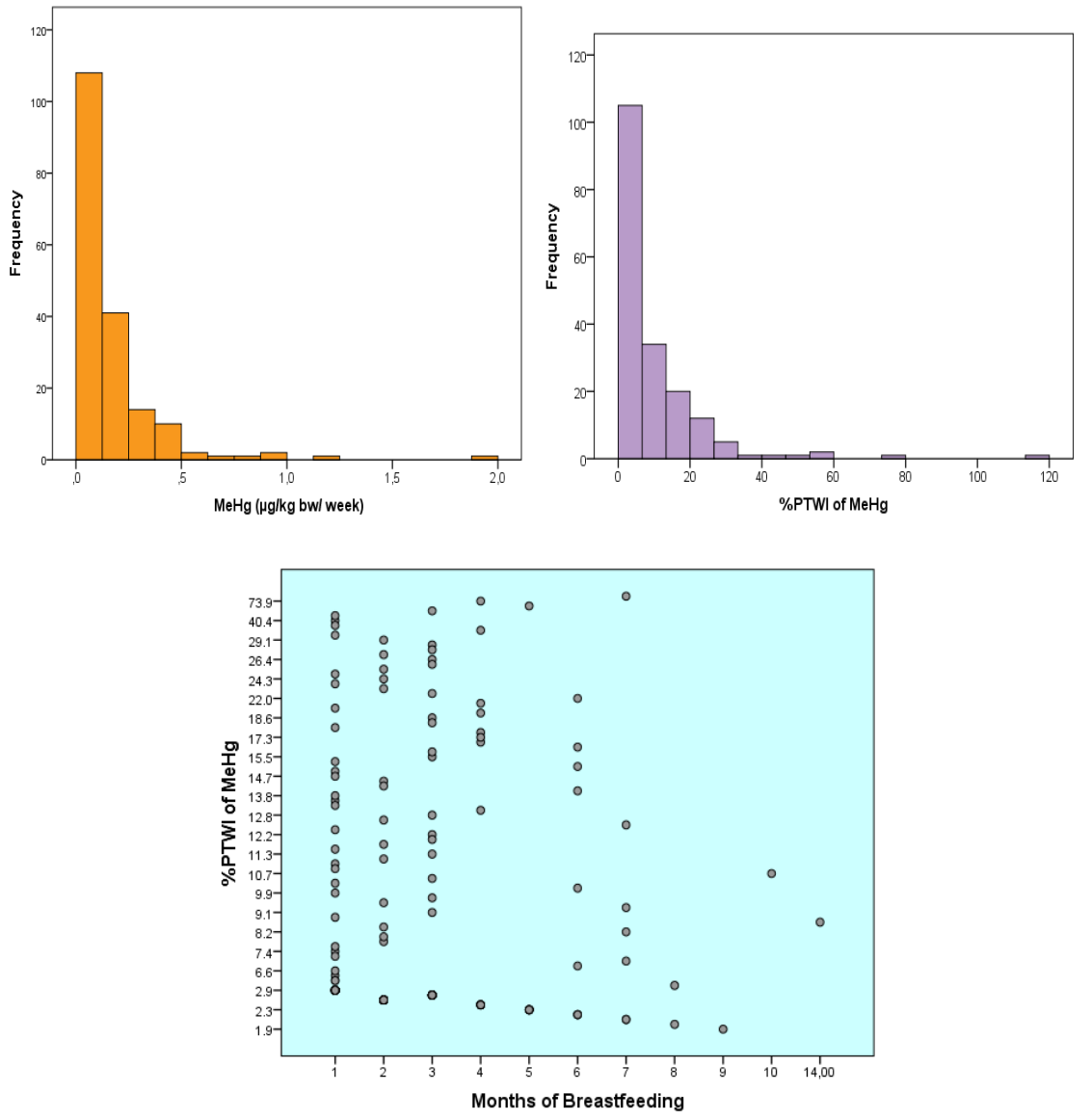


Figure 2. Distribuition of the mean MeHg weekly intake and of %PTWI, and dispersion analysis according to months of breastfeeding

In average, MeHg intake through breastfeeding contributed to 13.6 % of the PTWI, and in two thirds of the cases it represented less than 10% of the PTWI. In only one case, the intake exceeded 100 % of the PTWI (119%; Figure

2), which corresponds to a 7 months old child. There was a significant correlation between % MeHg and MeHg concentration and % PTWI of MeHg (Table 2).

1.4. Discussion

The THg values in breast milk found in this study (mean of 2.6 µg/L) were lower than those obtained in two previous studies conducted in the Federal District, when the number of mothers providing sample were much lower. Costa et al. (2005) analyzed milk samples collected from 23 donors (7 to 30 days postpartum), finding a mean THg level of 5.73 µg/L (<0.43 to 23.1 µg/L). Cunha et al. (2013) found a similar THg mean level in 142 breast milk samples provided by 18 mothers from 15 and 90 days postpartum (6.47 µg/L; <0.76 to 22.7 µg/L). Studies conducted in the Brazilian Amazon found similar (Vieira et al., 2013) or higher levels (Boishio and Henshel, 2000; Santos et al., 2015) than the ones reported in the present study. Overal, the total mercury levels in breast milk from Brazilian mothers are higher than those found in most other regions of the world (see review by Rebelo & Caldas, 2016, and Table 3), probably due to high levels of mercury naturally present in Brazilian soil and water (Lacerda & Gonçalves, 2001; Fadini & Jardim, 2001; Carpi et al., 2013).

Costa et al. (2005) and Cunha et al. (2013) reported a very low frequence of fish consumption among the study participants. Indeed, in the last Brazilian consumption survey (POF 2008-2009; IBGE, 2012), only 7 of 110 Federal District participant women aged 15 to 47 years old reported the consumption of fish (2 non-consecutive days reporting), with an estimated mean consumption of 8.73 g/dia (consumers and non-consumers). Cunha et al. (2013) did not find a significant correlation between fish consumption and THg in breast milk during the 90 days period, but providing a fish meal to the mothers on the 75th day had a significant positive impact on the THg level. Costa et al. (2005) found a significant correlation (r = 0.6087, p = 0.0057) between breast milk THg and the mother number of amalgam fillings, which is an important source of mercury, especially IHg (Gundacher et al., 2010).

Miklavcic et al. (2013) also found a significant correlation between the number of amalgam fillings and IHg in breast milk in Europe.

Cunha et al. (2013) found a signficant correlation between THg levels in breast milk and consumption frequency of fat, grain and vegetable servings ($p<0.02$). Rice is a staple food in Brazil, with a mean consumption of 186 g/day in the Federal District (IBGE, 2012), and can be an important source of mercury exposure (Meng et al., 2010, 2011; Zhu et al., 2015; Strickman and Mitchell, 2017). While it was found that mercury methylated in sediment was the sole source of MeHg in rice grain, IHg was almost entirely from the atmospher (Strickman and Mitchell, 2017). In a recent review, Rothenberg et al. (2014) showed that in most studies, mercury was present in rice primarily as IHg. Furthermore, Temmerman et al. (2009) have shown that atmospheric gaseous mercury is by far the most important source for the accumulation of this element in vegetables.

Due to the relevance of MeHg for the neurological effects of mercury to the fetus and infants, and the much higher gastrintestinal absorption rate of MeHg compared to IHg, it is imperious that speciation of the mercury present in breast milk be performed to evaluate the actual risks that breastfed infants are exposed to. Very few studies have analysed MeHg in breast milk worldwide, and a summary of these studies is shown in Table 3.

In the study conducted by Vieira et al. (2013) in amazonic region, a significant higher level of THg, MeHg and MeHg/THg ratio was found in breastmilk coleccted among the riverine population compared to the urban population ($p< 0.001$), which has a much lower fish consumption (44% consume less than one fish meal/week) than the riverines (54% consume at least 3 fish meals/week). In average, 36% of THg was present as MeHg in the riverine population, while in the urban population this was 12%, similar to what was found in the present study for the low fish consumption Federal District population (10,1%).

Gundacker et al. (2010) reported that Hg in all 21 breast milk samples collected from Austrian women were in the inorganic form. The authors also found that the number of maternal amalgam fillings was associated with THg in

meconium and with IHg in placenta. In a multinational sudy conducted in Europe (Miklavcic et al., 2013), the mean/median THg levels in breast milk reached 0.6 µg/L in Greece (276 g fish/week), with only 7% accounting for MeHg, the lowest ratio among the countries (Table 3). Although Slovenian women consume the lowest fish amount among the populations (178 g/week), MeHg accounted for 47% of THg, in the same range as Croacia and Italy, who were higher fish consumers (280-300 g/week) (Valent et al., 2013; Miklavcic et al., 2013). According to the authors, this apparent contradictory result was related to the type and origine of the fish consumed by each population. Indeed, various studies have shown that mercury concentration in fish depend on the trophic level, with piscivorous fish containing the higher THg concentrations and % MeHg (Berzas Nevado et al., 2010; Maulvault et al., 2015).

Miklavcic et al. (2013) found a significant correlation between the frequency of fish consumption and the levels of THg and MeHg in breast milk ($p= 0.002$ and 0.027, respectively), in addition to cord blood ($p<0.001$), maternal's blood ($p<0.001$) and hair (0.01, respectively). In Japan, which has a high fish consumer population, 54% of mercury found in breast milk was in the organic form, and a significant correlation was found between the lipid-adjusted MeHg in breast milk and Eicosapentaenoic acid (EPA) *plus* docosahexaenoic acid (DHA) in maternal plasma, markers for fish consumption (Iwai-Shimada et al., 2015). The authors found that the median MeHg intake by one month old Japanese infants (4kg bw and 800 mL milk) was 0.63 µg/kg bw/week MeHg, representing 39% of the PTWI.

Considering the mean level of MeHg reported by Vieira et al. (2013; Table 3) and a milk consumption of 750 mL for a 5.5 kg 2-3 month baby, the estimated intake of MeHg for the Amazonian urban and riverine populations were 0.11 and 0.83 µg/kg bw/week, respectively, corresponding to 6.9 and 51% of the PTWI.

Table 3. Studies that evaluated total mercury and methylmercury in breast milk from 2000-2016

Reference	Country	N THg (MeHg)	THg µg/L (range)	MeHg µg/L (range)	% MeHg	Observation
Vieira et al., 2013	Brazil, Amazonia	82 (45)	0.36 (0.09 - 3.7)	0.12 (0.01 - 0.47)	0.12 (0.01 - 0.98)	Urban population
	State	75 (46)	2.3 (0.12 - 6.48)	0.87 (0.11 - 3.4)	0.37 (0.12 - 0.71)	Riveirine population
Gundacker et al., 2010	Austria	21	IHg = 0.2* (0.1 - 2)	not detected	-	2-8 weeks pp
Valent et al., 2013	Italy	492 (182)	0.33 (0 - 28.3)	0.17 (0.01 - 1.9)	58 (1-100)	Mature milk
Miklavcic et al., 2013*	Italy	605 (224)	0.2 (<LOD - 0.8)		60 (15 - 100)	MeHg values are in percentage of THg
	Slovenia	284 (7)	0.2 (<LOD - 0.8)		47 (3 - 71)	
	Croacia	125 (26)	0.2 (<LOD-1)		56 (23 - 100)	
	Greece	44 (21)	0.6 (<LOD - 9.4)	7%	7 (2 - 96)	
Iwai-Shimada et al., 2015	Japan	27 (27)	0.81** (0.14 - 1.87)	0.45** (0.06 - 1.2)	54** (17 - 87)	30 days pp

pp=post-partum; * median; **median (P5-P95);

In a previous study conducted by our research group (Cunha et al. 2013), the intake of THg by Federal District babies during breastfeeding exceeded the PTWI (5 µg/kg bw/week) in most cases (up to 800%), which could indicate a health concern. This THg PTWI was withdrawn by the JECFA in 2010, as it was agreed that MeHg is the relevant toxicological form of mercury for neural adverse effects for the fetus and babies (JECFA, 2011). In attempting to estimate the exposure of the breastfed babies to MeHg within that study, Caldas and Rebelo (2016) assumed that 50% of the THg was present as MeHg. The estimated mean intake of MeHg in the Cunha et al. study was 3.2 µg/kg bw/week, corresponding to 200% of the PTWI. The present study, however showed that a much lower proportion of the THg in breast milk from mothers in the Federal District is present in the organic form, and the intake of MeHg exceeded the PTWI for only one of the 181 infant/mother cases evaluated, with most of the intakes accounting for less than 10% of the PTWI. These results indicate that the risks of neuroeffects due to the MeHg intake through breastfeeding for this population can be excluded.

The breast milk samples analysed in this study were originated from milk banks, which provide breast milk to immature newborn babies and low weight babies that, for some reason, cannot be breastfeed (ANVISA, 2008). Hence, the data provided by the samples analyzed can be extended to this population as well. Considering a consumption of 200 mL milk and 2 kg bw for immature newborn or low weight babies, the maximum %PTWI was 28%, hence confirming our previous conclusion about the safety of consuming breastmilk in the Federal District area.

One main strength of this study was the number of mothers involved and of samples analysed (provided by milk banks), higher than previous studies conducted in the Federal District or other Brazilian regions (provided by the mothers). However, this study had some limitations that should be addressed. One limitation was the lack of body weight of the infants and milk consumption at the time the sample was collected, which were estimated based on published data. Another limitation was the lack of food diet and number of amalgams of the breast milk donnors, especially fish, rice and vegetables. This information would allow some correlations that could explain the large variation among the mercury levels found in the samples. Those informations are not available in milk banks and could not be obtained from the mothers.

1.5. Conclusions

Overall, THg concentrations in breast milk from mothers of the Federal District were lower than what was found in previous studies conducted in the region. The levels of MeHg found in the samples confirm our hypothesis that most of the mercury present in milk from the low fish consumer Federal District mothers was in the inorganic form, probably due to the IHg mercury expected to be present in grain and vegetables ou amalgams fillings. Weekly intake of MeHg represented, in average, 13.6% of the PTWI, with only one exceedance (119% of PTWI), indicating no health concern for the breastfed babies. Consumers of breast milk donated to the milk banks are also not exposed to MeHg levels that could represent a health risk. This conclusion is very important in the context of breast milk banks, in which the quality of the milk are a constant concern. Nevertheless, it is always important to emphasize the importance of monitoring the levels of environmental contaminants in breast milk, an essential food for the baby, mainly in the first 6 months of life.

2. Arsênio, chumbo e cádmio em leite humano por ICP-MS – validação do método, análise das amostras e avaliação de risco da exposição dos lactentes

Resumo

Metais e metalóides tóxicos são encontrados em todos os compartimentos ambientais e são utilizados na produção de vários produtos e equipamentos de uso humano. A exposição a arsênio, cádmio e chumbo pode representar um risco para a saúde, principalmente para o feto e lactentes. Nesse estudo, um método para análise desses contaminantes em leite materno utilizando digestão ácida em microondas e análise por ICP-MS foi validado, com LOQ de 0,31 µg/L para o chumbo, 0,016 µg/L para o cádmio e 0,08 µg/L para o arsênio. No total 212 amostras coletadas em bancos de leite do Distrito Federal foram analisadas, com concentrações médias de 6,64 ± 18,8 µg/L para chumbo (75,9 % de amostras ≥ LOQ) e 0,24 ± 0,41 µg/L para cádmio (71,2 % de amostras ≥ LOQ) e mediana de 0,04 µg/L para arsênio (4 amostras ≥ LOQ). A ingestão média semanal de cádmio estimada representou 9% da ingestão máxima tolerável. Para o chumbo e arsênio, as margens de exposição medianas foram de 1,2 e 587, respectivamente, indicando um baixo risco de exposição para a saúde dos lactentes.

Palavras-chaves: arsênio, cádmio, chumbo, leite materno, ICP-MS, avaliaçao de risco

2.1. Introdução

Os metais e metalóides são ubíquos na natureza, estando presentes em solo, água, plantas e animais, representando grupos importantes de contaminantes químicos ambientais a que o homem é exposto diariamente e que pode potencialmente representar um risco à saúde (ATSDR, 2015). O leite é o principal alimento para o bebê nos primeiros meses de vida, aportando todos os nutrientes necessários para seu crescimento e fortalecendo os laços com a mãe (WHO, 2007). Porém, a presença de contaminantes tóxicos como chumbo, arsênio e cádmio em leite materno tem sido largamente descrita na literatura, levando à preocupação quanto aos potenciais riscos à saúde dos lactentes (Al Saleh et al., 2003 Bose-O'Reilly et al., 2008; Abdulrazzaq et al., 2008; Miklavcic et al., 2013; Islam et al., 2014; Marques et al., 2014; Winiarska-Mieczan, 2014; Carignan et al., 2015).

Os principais métodos de análise para determinação de chumbo, cádmio e/ou arsênio em diferentes matrizes biológicas, inclusive leite, são a espectrometria de absorção atômica (EAA) utilizando atomização em chama (Honda et al., 2003; Gonçalves et al., 2010) ou em forno de grafite (Al Saleh et al., 2003; Marques et al., 2013), e ICP-MS (*Inductively coupled plasma-mass spectrometry*) (Kosanovic et al., 2008; Amarasiriwardena et al., 2013), que é a técnica mais adequada para análise de arsênio. A principal vantagem do ICP-MS é a possibilidade de se analisar simultaneamente todos os metais, o que reduz de maneira importante o tempo de análise. Na maioria dos métodos, o leite é submetido a digestão ácida, principalmente em micro-ondas sob condições de temperatura e pressão controlada (Kosanovic et al., 2008; Sardans et al., 2010; Amarasiriwardena et al., 2013).

O chumbo é o metal tóxico mais abundante na natureza e tem larga utilização industrial (IARC, 2012). Seu alvo primário é o sistema nervoso central (Nemsadze et al., 2009), e cérebros de fetos e bebês apresentam maior sensibilidade aos seus efeitos tóxicos devido à imaturidade da barreira encefálica (Shawanha et al, 2016; Schnaas et al, 2006; Koyashik et al, 2010). A Agência Internacional de Pesquisa em Câncer (IARC) classifica o cádmio e o arsênio inorgânico como carcinogênico a humanos (Grupo I), e a deficiência de ferro pode contribuir para uma maior absorção de cádmio em mulheres durante a gravidez e lactação (CDC, 2009). A exposição humana ao metaloide arsênio se dá, principalmente, pelo consumo de água

contaminada e de frutos do mar, em especial crustáceos (Samanta et al., 2007). O arsênio tem toxicidade reprodutiva importante, podendo levar a morte fetal, baixo peso do feto, aborto e eclampsia (WHO, 2001; Hopenhayn-Rich et al., 2000). Enquanto o cádmio possui uma dose de exposição segura para o homem (tolerable weekly intake, TWI), avaliações conduzidas pelo *Joint FAO/WHO Expert Committee on Food Additives* (JECFA, 2010a,b) e pela Agência Europeia de Segurança dos Alimentos (EFSA, 2010) concluíram, com base no conhecimento disponível, que este parâmetro não pode ser determinado para o chumbo e arsênio. Para estes metais, foram estabelecidos benchmark doses (BMD), que representam níveis de exposição que levam a um efeito adverso específico. O TWI (cádmio) e as BMDs (chumbo e arsênio) são os parâmetros toxicológicos utilizados para caracterizar o risco da exposição humana a estes metais.

Este estudo tem como objetivos a validação de um método analítico para determinação de arsênio, chumbo e cádmio em amostras de leite materno por ICP-MS após digestão ácida em micro-ondas, e avaliar o risco de exposição de lactentes a esses metais pelo consumo de leite materno.

2.2. Materiais e métodos

2.2.1. Reagentes e padrões analíticos

As soluções padrões de chumbo e de cádmio (1000 mg/mL) foram obtidas da Specsol (lotes F13E0369 e F13J0259A, respectivamente), e a de arsênio (1000 µg/mL, High-Purity Standarts (NC, EUA; lote: 0919418). A partir destas soluções foi preparado uma solução mista de metais (0,1 µg/mL para chumbo e arsênio e 0,02 µg/mL para cádmio) preparada com HNO_3 a 2%. O ácido nítrico ultrapuro 65% (Merck & Co, NJ, EUA) utilizado neste estudo foi submetido a um processo de destilação antes de ser utilizado.

Toda vidraria e tubos utilizados nas análises foram submetidos a um protocolo de lavagem, que consiste em lavagem preliminar com água destilada e extran a 5%, imersão em solução com HNO_3 10% por 24 hrs, enxague e imersão em água miliQ por mais 24hrs.

O material de referencia certificado de metais em leite, Skimmed Milk Powder BCR – 150 EC-IRC-IRMM foi obtido da ERM (European Reference Marteirals), e contém valores de referencia para chumbo (1 ± 0,01 µg/g) e cádmio (21,8 ± 1,4 ng/g), mas não para arsênio.

2.2.2 Equipamentos

As amostras de leite foram digeridas na bomba de digestão TFM™-PTFE em forno de microondas Speedwave (Berghof, Microwave digestion system). As condições de operação do microondas estão descritas na Tabela 1.

Tabela 1 – Condições otimizadas de operação do forno de microondas

Etapa	T (ºC)	P(psi)	TA (min)	TI (min)	PO (%)
1	150	30	3	5	80
2	200	30	2	20	90
3	50	25	1	10	0

T= temperatura; P= pressão; TA= tempo para atingir a pressão desejada; TI= tempo; PO= potência (power), em porcentagem da potência total atingida pelo equipamento.

Os metais foram determinados no ICP-MS (*Inductively coupled plasma-mass spectrometry*), marca Perkin Elmer (Nexion 300D, quadrupolo) cujas condições de operação estão descritas na Tabela 2. A análise de arsênio mostrou interferência de poliatômicos com mesma massa, que não foram corrigidas pelo software do equipamento. Dessa forma, para a determinação de arsênio foi necessário a utilização do módulo KED (*Kinetic energy discrimination*). Argônio 5.0, utilizado para carreamento das amostras no sistema e hélio 5.0 utilizado no KED foram obtidos da Air Liquide (Brasil).

Tabela 2. Condições otimizadas de operação do ICP-MS

Parâmetro	Condição de operação
Fluxo de argônio no nebulizador	0,81 L/min
Fluxo de argônio no plasma	18 L/min
Fluxo de hélio na célula de KED	4,7 mL/min
Potência	1600 W
Dwell time	50 ms
Sample flush	45s, 48 rpm
Read delay	25s, 20 rpm
Análise	20 rpm
Wash time	45s, 24rpm

Em cada leitura foram realizadas 60 varreduras e foram realizadas 7 leituras para cada amostra. Diariamente, foi feito um *tunning* de calibração de massas do equipamento com leitura da intensidade dos seguintes isótopos: Be 9, Mg 24, In 115, U 238, CeO 156, Ce 140 e Ce2+ 70. A utilização de padrões internos foi considerada desnecessária após realização de estudo que avaliou o comportamento da amostra e dos padrões internos (Sc 45, Ga 69, Ga 71, Ge 74, Rh 103, Pd 108, In 115, Te 130, Pr 141, Tm 169, Lu 175, Ta 181, Ir 193 e Bi 209). A cada 10 amostras, foi feita a leitura de uma das concentrações da curva de calibração para verificação da manutenção das condições de operação do ICP-MS.

2.2.3. Amostras de leite materno

A amostras de leite foram obtidos de oito bancos de leite humano (BLH) do DF, localizados no Hospital Regional da Asa Norte, Hospital Regional da Asa Sul, Hospital Regional de Brazlândia, Hospital Regional de Planaltina, Hospital Regional do Paranoá, Hospital Regional de Sobradinho, Hospital Regional de Santa Maria e Hospital Regional de Taguatinga. Entre maio de 2011 e fevereiro de 2012, foram coletadas 224 amostras obtidas de 213 doadoras diferentes, representando um nível de confiança de 93,5% em relação ao número total de doadoras da Rede em 2010 (Andrade et al., 2013). Destas, 212 foram analisadas neste estudo.

As doadoras coletaram o leite diretamente nos frascos cedidos pelo laboratório ou alíquotas da amostra doada (leite cru) foram retiradas por funcionárias do BLH e transferidas para tubos falcon de 50 mL cedidos pelo laboratório. As amostras coletadas foram mantidas em freezer (-20ºC) até o momento da análise. O estudo teve a aprovação do Comitê de Ética em Pesquisa sobre Seres Humanos do Departamento de Ciências da Saúde da Universidade de Brasília, sob registro de projeto no CEP nº 27/11, com data de aprovação de 13/04/2011 (Anexo 2).

2.2.4. *Preparação das amostras e análise*

As amostras de leite foram descongeladas e homogeneizadas a temperatura ambiente. Foi retirada uma alíquota de 3 mL da amostra, foram acrescentados 5 mL de ácido nítrico 65% destilado, e as amostras submetidas ao processo de digestão em micro-ondas, segundo as condições mostradas na Tabela 1. Três horas após o término do ciclo de digestão (etapa de resfriamento), as amostras foram transferidas para balões volumétricos certificados de 20 mL, as bombas de digestão lavadas com água milliQ e o conteúdo transferido para o balão volumétrico até completar o volume. As amostras foram transferidas para tubos falcon de 50 mL e armazenadas em geladeira até o momento da análise. Uma alíquota de 2,5 mL da amostra digerida foi retirada, e 5,5 mL de água miliQ adicionada para injeção no ICP-MS.

2.2.5. Validação do método

A validação do método foi realizada utilizando um pool de amostras de leite humano. O efeito matriz e a recuperação foram avaliados pela comparação das contagens (cps) obtidas para cada um dos metais analisados no ICP-MS nas três condições: [1] solução de ácido nítrico a 5% fortificada com concentrações conhecidas dos metais; [2] matriz de leite materno (pool de amostras de leite humano) fortificado com concentrações conhecidas dos metais após procedimento de digestão da amostra e [3] matriz de leite materno fortificada com concentrações conhecidas dos metais antes do processo de digestão da amostra. O experimento foi realizado em triplicata em cada nível de concentração.

O efeito matriz foi avaliado comparando-se a diferença entre o número de contagens obtidas em cada nível na matriz branca fortificada pós-extração com as obtidas no analito em solução, segundo a Equação 1.

Equação 1:

$$Efeito\ matriz, \% = \frac{\text{média de contagens obtidas na fortificação pós} - \text{digestão } x\ 100}{\text{média de contagens obtidas na fortificação do ácido nítrico}}$$

O cálculo foi feito para cada nível de fortificaçao e depois foi aplicado o teste T por ponto para verificação de diferença estatística entre as médias dos dois conjuntos de amostras.

A linearidade foi avaliada por meio de uma curva com pontos em triplicata em matriz extraída (fortificação pós-extração). A homocedasticidade dos dados da curva utilizando testes F e de Cochran Os parâmetros avaliados foram a determinação linear (aceito quando significativo, α=0,05), análise da dispersão de resíduos, desvio padrão dos resíduos, falta de ajuste e soma dos erros residuais.

A recuperação foi estimada pela comparação do número de contagens do analito na presença de matriz (fortificação pós-extração) com o número de contagens na amostra fortificada pré-extração (Equação 2), e permite avaliar as perdas durante o processo de extração. Para o nível de concentração testado, a recuperação média deve ficar entre 70 e 110% (MAPA, 2011),

Equação 2:

$$Recuperação\ \% = \frac{n^{O}\ contagens\ obtidas\ na\ fortificação\ pré - extração\ x100}{n^{O} de\ contagens\ obtidas\ na\ fortificação\ pós - extração}$$

A repetitividade foi avaliada pela análise de amostras fortificadas nos mesmos níveis da curva de calibração, preparados e analisados pelo mesmo analista, nas mesmas condições de trabalho. A repetitividade foi considerada satisfatória quando o coeficiente de variação (CV) foi menor que 20% (MAPA, 2011).

A precisão intermediária foi avaliada pela análise de amostras fortificadas, nas mesmas concentrações, cada uma em triplicata, preparadas e analisadas pelo mesmo analista em dias diferentes, mas com as mesmas condições laboratoriais. A precisão intermediária foi considerada satisfatória quando o CV foi menor que 30% (MAPA, 2011).

A eficiência do procedimento foi avaliada a partir da digestão de 7 alíquotas do material de referência Skin Milk Powder BCR – 150 EC-IRC-IRMM, com concentrações conhecidas de cádmio e chumbo.

O limite de quantificação (LOQ) do método foi definido como a menor concentração testada que apresentou recuperação, e precisão dentro dos valores estabelecidos (CV<20% para repetitividade e CV<30% para precisão intermediária) (MAPA, 2011). Para o nível de concentração testado, a recuperação deve ficar entre 70 e 110% (MAPA, 2011).

Em cada lote de análise foram corridas, ao final, amostras do material de referência, para verificação da adequação das análises realizadas pelo equipamento naquele dia. A recuperação variou de 85 a 110%.

2.2.6. Avaliação da exposição de lactentes a chumbo, cádmio e arsênio e caracterização do risco a saúde

O cálculo da exposição (ingestão) a cada um dos metais analisados foi realizado para cada amostra, de acordo com a Equação 5. O peso corpóreo do bebê no mês em que o leite foi doado foi estimado a partir dos dados da OMS (WHO, 2002). O consumo de leite materno foi estimado a partir do trabalho de Costa et al. (2010).

Equação 3

$$Ingestão\ diária = \frac{concentração\ do\ metal \times consumo\ diário\ de\ leite\ materno}{peso\ corpóreo}$$

A caracterização do risco da exposição a cádmio foi realizada comparando-se a ingestão estimada na Eq. 3, expressa em µg/kg pc/semana, com a ingestão tolerável semanal de 2,5 µg/kg pc (TWI, *tolerable weekly intake;* EFSA, 2012), expressa em termos percentuais. Risco pode existir quando a %TWI é maior que 100

Equação 4

$$\%\,TWI = \frac{Ingestao\ diária\ x\ 7\times 100}{TWI}$$

Nem o chumbo nem o arsênio possuem uma dose estabelecida que pode ser considerada segura. A EFSA estabeleceu uma dose benchmark, limite inferior ($BMDL_1$) de 0,5 µg/kg pc/dia para efeitos neurológicos em crianças, relacionada com a diminuição no coeficiente de inteligência (EFSA, 2010). Para o arsênio, o JECFA estabeleceu uma $BMDL_{0,5}$ de 3 µg/kg pc/dia, relacionada com um aumento de 0,5% da incidência de câncer de pulmão (JECFA, 2011b). Para esses dois metais, a caracterização do risco é estimada a partir da margem de exposição (MOE), na qual se compara a exposição com a BMDL (Equação 5). Quanto menor a MOE, maior o risco de uma população, já que a ingestão se aproxima da dose que causou um determinado efeito (BMDL).

Equação 5

$$MOE = \frac{BMDL}{ingestão\ diária}$$

2.2.7. Análise Estatística

Os dados obtidos foram analisados utilizando o programa estatístico SPSS versão 22, IBM software. Foram realizadas análises descritivas, de normalidade utilizando os testes Kolmogorov-Smirnov e Shapiro-Wilk e teste de Spearman para realizar análise de correlação nas distribuições não normais, com significância com $p \leq 0.05$.

2.3. Resultados

2.3.1 Validação do método analítico

Chumbo

A Tabela 3 mostra o *efeito matriz* para a determinação de chumbo em leite humano. A representação gráfica (Figura 1) e os resultados do teste T em cada nível de concentração (Tabela 3) mostraram que a resposta do equipamento quando o chumbo estava em presença da matriz foi significativamente diferente quando comparado ao meio de ácido nítrico ($p < 0,05$), indicando a necessidade de utilização da curva em matriz para determinação de chumbo em leite humano. Em todos os casos, houve um aumento de sinal na presença da matriz leite, provavelmente relacionada também ao chumbo presente na amostra controle. A Tabela 3 mostra também as médias dos coeficientes de correlação de cada curva analítica em matriz, sendo maior que 0,99 para curva em matriz na faixa de concentração avaliada

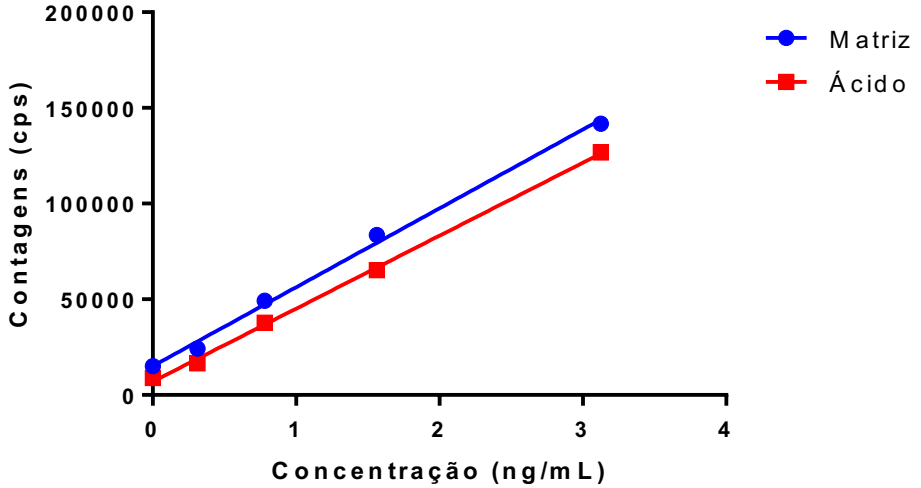


Figura 1. Representação gráfica das curvas de calibração para chumbo em matriz leite e em ácido nítrico 5%.

Tabela 3. Estudo de efeito matriz para determinação de chumbo em leite humano por ICP-MS, leitura no ICP-MS (cps, n=4)

Concentração (µg/L)	Média*, ácido	DP	Média*, matriz	DP	Efeito matriz (%)	P**
0	8978	1204,9	15029	1140,3	+67,3	0,018
0,3125	16586	661,9	24205	1712,8	+45,9	0,031
0,7813	37743	1773,2	49208	1393,0	+30,4	0,001
1,5625	65325	1980,1	83539	709,3	+27,9	0,004
3,1250	126713	2776,9	141748	2176,3	+11,9	0,009
R2	**0,9974**		**0,9978**			

*Média de contagens; ** - p referente a aplicação do teste T para determinação de diferença estatística entre contaminação em ácido e em matriz.

A curva analítica em matriz ajustada de acordo com o método de mínimos quadrados (sem fator de ponderação) foi considerada homocedástica, de acordo com os testes F e teste de Cochrane. A Figura 2 mostra a homocedasticidade da curva, onde os resíduos são aleatórios em toda faixa de concentração.

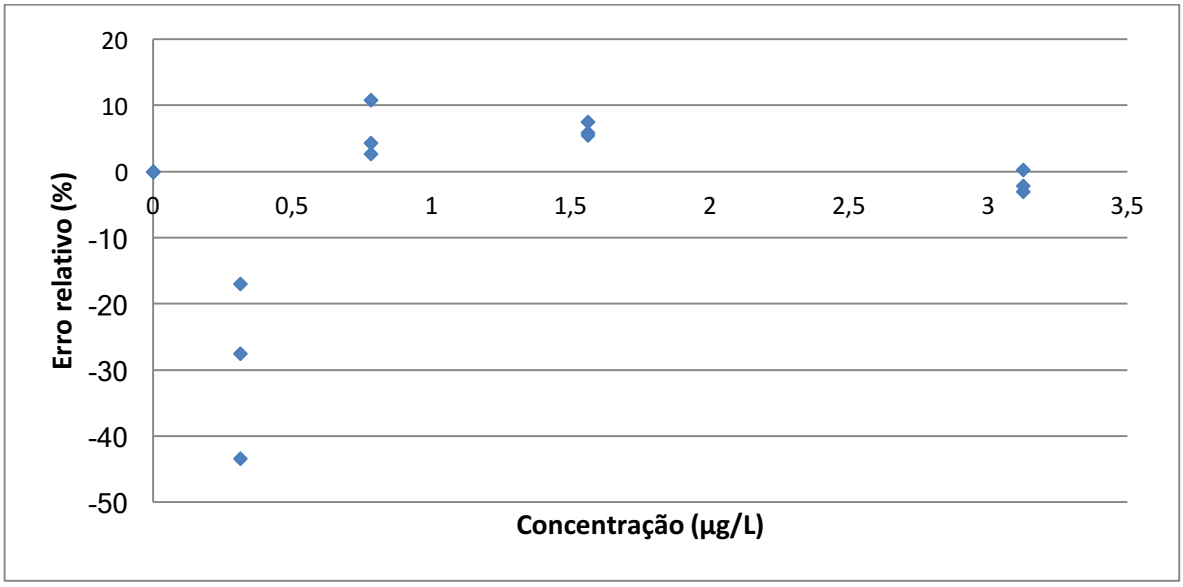


Figura 2. Representação gráfica dos resíduos percentuais da curva de calibração em matriz leite para chumbo ajustada pelo método dos mínimos quadrados, sem ponderação.

A *recuperação, repetibilidade e precisão intermediária* na determinação de chumbo em leite foram avaliadas em 4 níveis de concentração, em triplicata, com preparação separada de cada uma das soluções (Tabela 4). O menor nível de concentração que atendeu aos critérios de aceitabilidade do método foi de 0,31 µg/L (recuperação entre 70-110% e CV< 20% e < 30 % para repetibilidade e precisão intermediária), estabelecido como o LOQ do método para determinação de chumbo em leite.

Tabela 4. Recuperação, repetibilidade e precisão intermediária para análise de chumbo em leite por ICP-MS

Concentração (µg/L)	**Recuperação (%), N=3**	**Repetibilidade CV(%), N=3**	**Precisão intermediária CV(%), N =6**
0,31	80,7	12,8	9,1
0,78	107,4	1,8	11,7
1,56	102,4	6,6	10,9
3,12	96,4	2,0	-

CV: coeficiente de variação

Os resultados da análise do material certificado Skin Milk Powder BCR – 150 EC-IRC-IRMM estão mostrados na Tabela 5.

Tabela 5. Análise de chumbo e cádmio no material de referência de leite, que declara conter 1,0 ± 0,04 µg/g de chumbo e 21,8 ± 1,4 ng/g de cádmio.

	Massa (g)	**Pb (µg/g)**	**Recuperação (%)**	**Cd (ng/g)**	**Recuperação (%)**
MR1	0,15303	0,997	99,7	19,03	87,28
MR2	0,15067	0,863	86,3	18,30	83,93
MR3	0,15203	0,844	84,4	24,69	113,27
MR4	0,15109	0,944	94,4	19,37	88,84
MR5	0,15050	0,920	92,0	20,47	93,89
MR6	0,15082	0,997	99,7	20,47	93,90
MR7	0,15010	1,012	101,2	23,82	109,25
	Média	***0,940***	***93,96***	***20,98***	***95,77***
	desvio	***0,07***	***6,74***	***2,45***	***11,22***

Cádmio

A Figura 3 e a Tabela 6 mostram o *efeito matriz* para a determinação de cádmio em leite humano. Tanto a representação gráfica quanto a avaliação feita pelo teste T ($p < 0,05$) mostraram a existência de efeito matriz nas duas concentrações mais altas da curva, indicando a necessidade de utilizar a curva em matriz. A Tabela 6 mostra também os coeficientes de correlação de cada curva analítica em matriz foi > 0,99, mostrando *linearidade* na resposta do ICP-MS na faixa de concentração avaliada.

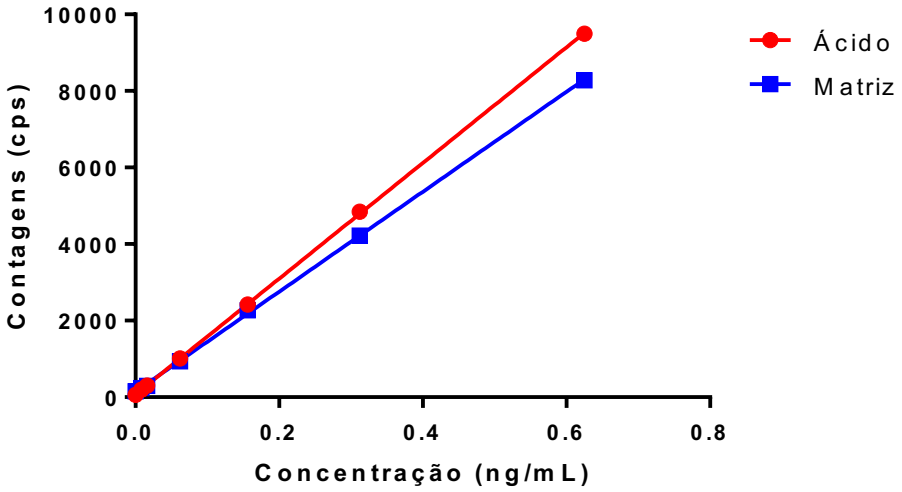


Figura 3. Representação gráfica das curvas de calibração para cádmio em matriz leite materno e em ácido.

A curva analítica foi ajustada de acordo com o método de mínimos quadrados (sem fator de ponderação) e foi considerada homocedástica, de acordo com os testes F (F calc 0,979; F tab 99) e teste de Cochrane (C calc 0,4933; C tab 0,683). A Figura 4 mostra a homocedasticidade da curva, onde os resíduos são aleatórios em toda faixa de concentração.

Tabela 6. Estudo de efeito matriz para determinação de cádmio em leite humano por ICP-MS, leitura no ICP-MS (cps, n=4)

Concentração (µg/L)	Média, ácido	DP	Média, matriz	DP	Efeito matriz (%)	p
0	61,9	6,5	155,6	13,60	+151	0,003
0,008	198,7	16,1	239,1	116,7	+20	0,268
0,016	303,9	19,1	293,4	54,09	-4,5	0,486
0,062	1010	50,4	937,6	49,39	-8,8	0,171
0,156	2419	127	2271	47,94	-6,1	0,148
0,312	4843	192	4223	33,97	-12,8	0,002
0,625	9493	402	8281	167,7	-12,8	0,005
R2	**0,9973**		**0,9992**			

DP: desvio padrão; $p < 0,05$

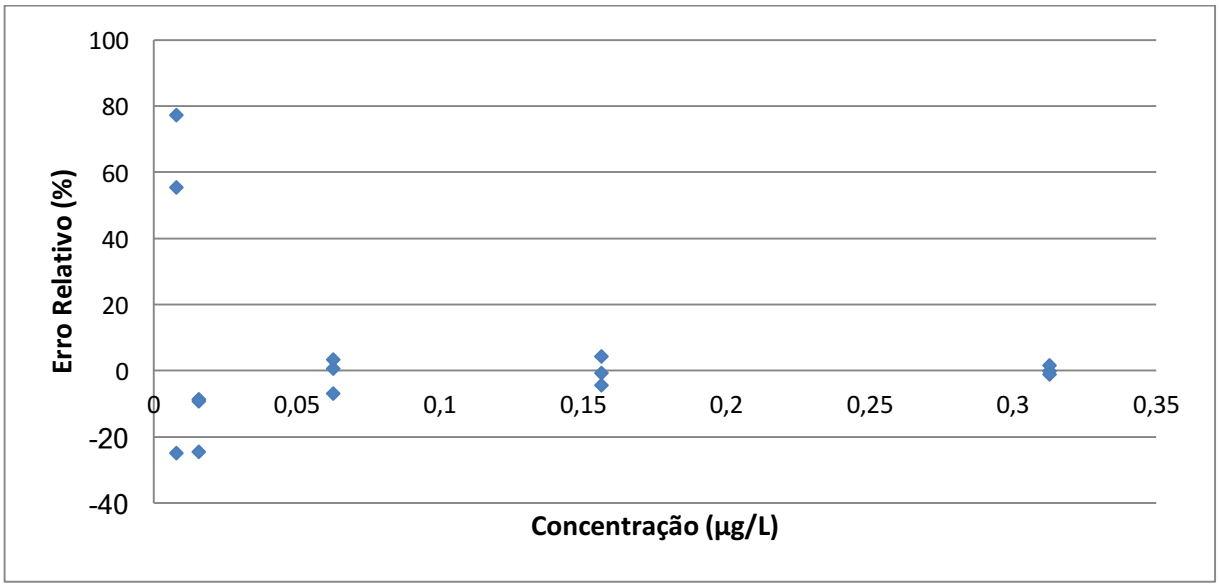


Figura 4. Representação gráfica dos resíduos da curva de calibração em matriz leite para cádmio ajustada pelo método dos mínimos quadrados, sem ponderação

A *recuperação, repetibilidade* e *precisão intermediária* foram avaliadas em 6 níveis de concentração, e os resultados mostrados na Tabela 7. O menor nível de

concentração que atendeu aos critérios de aceitabilidade do método foi 0,016 ng/mL, que representa o LOQ do método.

Tabela 7. Recuperação, repetibilidade e precisão intermediária para análise de cádmio por ICP-MS

Concentração (µg/L)	Recuperação (%), N=3	Repetibilidade CV (%), N=3	Precisão intermediária CV (%), N =6
0,008	434,7	91,2	-
0,016	101,1	7,1	21,3
0,062	88,4	1,0	4,6
0,156	103,7	3,7	3,6
0,312	96,0	1,9	1,6
0,625	91,2	9,7	-

CV: coeficiente de variação

A eficiência do método foi testada a partir da recuperação do material certificado Skin Milk Powder BCR – 150 EC-IRC-IRMM, que declara conter 21,8 ± 1,4 ng/g de cádmio, e os resultados estão mostrados na Tabela 5. A recuperação média foi de 95,8%, com variação entre 83,9% e 113,3%, indicando boa exatidão e precisão do método para determinação de cádmio em leite.

Arsênio

A Figura 4 e a Tabela 8 mostram o *efeito matriz* para a determinação de arsênio em leite humano. Resultados do teste T em cada nível de concentração mostraram que a resposta do equipamento quando o arsênio estava em presença da matriz foi significativamente diferente quando comparado ao meio de ácido nítrico a partir do nível de concentração 0,08 µg/L ($p < 0,05$), indicando a necessidade de uma curva em matriz para análise de arsênio em leite.

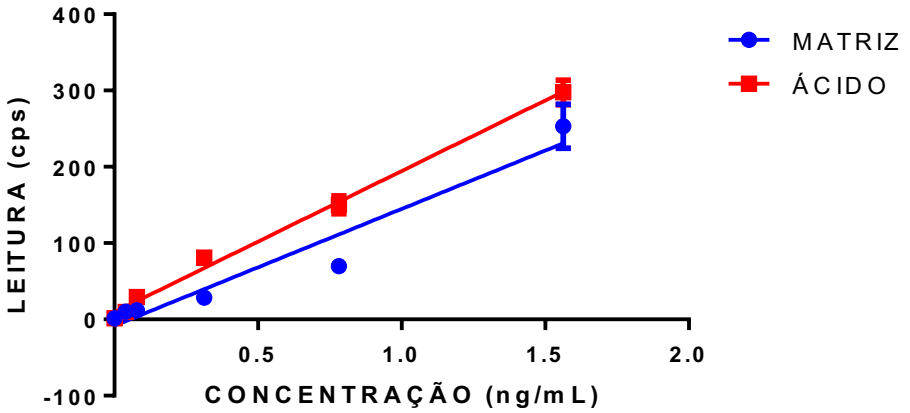


Figura 5. Representação gráfica das curvas de calibração para arsênio em matriz de leite e em ácido.

Tabela 8. Estudo de efeito matriz para determinação de arsênio em leite humano por ICP-MS, leitura no ICP-MS (cps, n=3)

Concentração (µg/L)	Média, ácido	DP	Média, matriz	DP	Efeito matriz (%)	p
0	1,43	0,12	1,6	0,17	+12	0,211
0,04	9,97	3,23	10,3	0,45	+3	0,436
0,08	29,6	3,49	11,8	1,04	-60	0,011
0,31	81,0	9,5	28,6	3,16	-64,6	0,009
0,78	150,2	11,6	69,7	4,50	-54,6	0,003
1,56	297,6	16,1	253,2	28,9	-14,9	0,025

DP: desvio padrão; $p < 0,05$

A curva analítica ajustada de acordo com o método de mínimos quadrados (sem fator de ponderação) foi considerada heterocedástica, de acordo com os testes F (F calc 771,334; F tab 99) e teste de Cochrane (C calc 0,905; C tab 0,683).

Como a curva foi heterocedástica, foram avaliados ajustes para adequação da curva. Como pode ser observado na Tabela 9, o ajuste mais adequado foi o 1/x, que obteve a maior correlação R e o menor falta de ajuste com a menor somatória de erro (SE) e de erro relativo (ER%).

Tabela 9. Avaliação de fatores de ponderação para a curva de arsênio em leite materno

Fator de ponderamento	R	F para FAJ	ER%	SE
1	0,9868	2,04	264,37	15,14
1/x	0,9905	2,24	160,54	15,41
1/x^2	0,9872	2,46	163,97	15,71
1/x^0,5	0,9896	2,12	168,67	15,25
1/y	0,9900	2,46	159,20	15,71
1/y^2	0,9894	3,29	167,68	16,80
1/y^0,5	0,9890	2,16	181,17	15,30
1/Variância	0,9889	3,34	173,70	16,87

FAJ= falta de ajuste; ER% = somatória dos erros relativos; SE= raiz da somatória de erro sobre número de leituras

A Figura 6 mostra a distribuição dos erros relativos das curvas antes e depois do ajuste de heterocedasticidade. Observa-se que antes da ponderação, os erros eram sistematicamente positivos em todos os pontos da curva, chegando a 20% na maioria dos pontos. Após o ajuste com a ponderação 1/x, os erros foram aleatóreos, positivos e negativos, levando a uma somatória de erros menor.

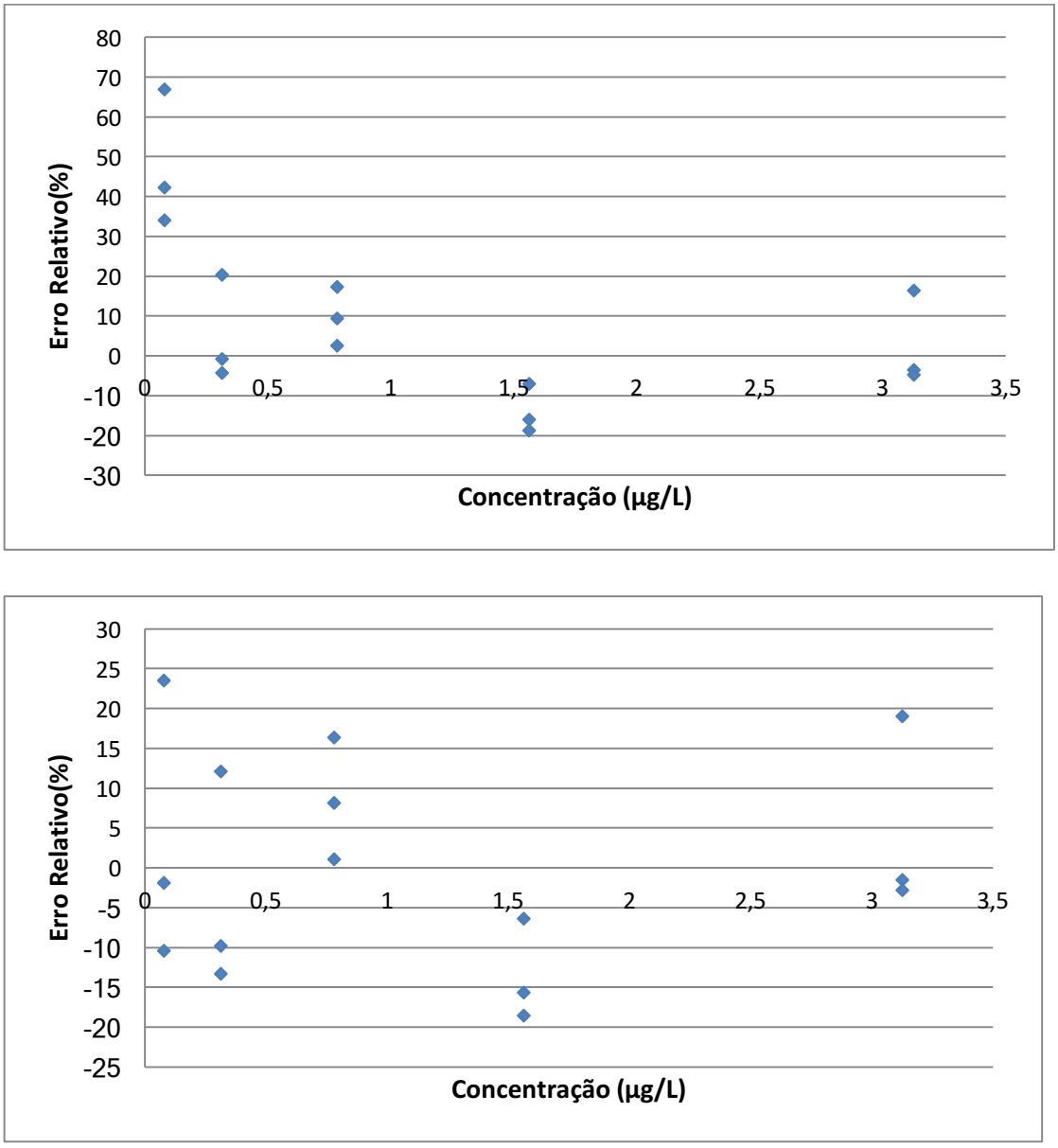


Figura 6. Representação gráfica do erro relativo, da curva de calibração em matriz de leite humano ajustada pelo método dos mínimos quadrados sem (acima) e com ponderação 1/x (abaixo)

A *recuperação, repetibilidade* e precisao intermediária foram avaliadas em 6 níveis de concentração (Tabela 10). O menor nível de concentração atendeu aos critérios de aceitabilidade do método foi de 0,08 µg/L (recuperação entre 70-110% e CV< 30%), estabelecido como o LOQ.

Tabela 10. Recuperação, repetibilidade e precisão intermediária para análise de arsênio por ICP-MS

Concentração, ng/mL	Recuperação, N=3	Repetibilidade CV (%), N=3	Precisão intermediária, CV (%), N =6
0,04	102	61,8	12,2
0,08	75,3	28,7	14,6
0,31	75,6	2,0	21,0
0,78	75,4	5,5	11,2
1,56	77,1	5,5	30,0
3,12	80,5	1,7	-

CV: coeficiente de variação

2.3.2 Dados epidemiológicos da população

As 212 amostras de leite materno analisadas foram doadas por 201 mães. A idade média das doadoras foi de 24,4 ± 11,6 (16 a 47 anos) e o peso médio dos recém-nascidos foi 3,1 ± 0,5 kg (n=151). A maioria das amostras foi coletada nos três primeiros meses após o parto (80,2 %), com apenas 3 doadas após os 9 meses de amamentação. A Tabela 11 mostra a frequência de doação de acordo com o tempo de amamentação.

Tabela 11. Frequência de doações de acordo com o tempo de amamentação

Meses após o parto	Frequência	Porcentagem
1	74	34,9
2	37	17,4
3	58	27,4
4	14	6,6
5	7	3,3
6	9	4,2
7	7	3,3
8	2	0,9
9	1	0,5
10	1	0,5
14	1	0,5
não informado	1	0,5
Total	212	100

2.3.3. Concentração de chumbo, cádmio e arsênio nas amostras de leite materno

As concentrações dos metais nas amostras de leite materno foram determinadas utilizando curvas analíticas em matriz de leite. Para chumbo (0,31; 0,78; 1,56; 3,12 µg/L; R2>0,99) e cádmio (0,016; 0,062; 0,156; 0,312; 0,625; R2>0,99), as curvas foram ajustadas pelo método de mínimos quadrados (sem fator de ponderação). Para arsênio (0,08; 0,31; 0,78; 1,56; 3,12 µg/L; R2>0,99), utilizou-se a ponderação 1/x para ajuste da curva.

A Figura 7 mostra a distribuição e o boxplot das concentrações dos metais analisados e a Tabela 12 resume os resultados encontrados. Os valores individuais estao mostrados no Anexo 2. Chumbo teve 75,6 % de amostras acima do LOQ, presente nas maiores concentrações, chegando a 224,4 µg/L. Apesar de 71,2% das amostras conterem cádmio acima do LOQ, os valores encontrados foram baixos, com média de 0,24 µg/L. Arsênio foi o metal menos detectado, presente acima do LOQ em apenas 4 amostras, com valores entre 2,25 a 9,5 µg/L

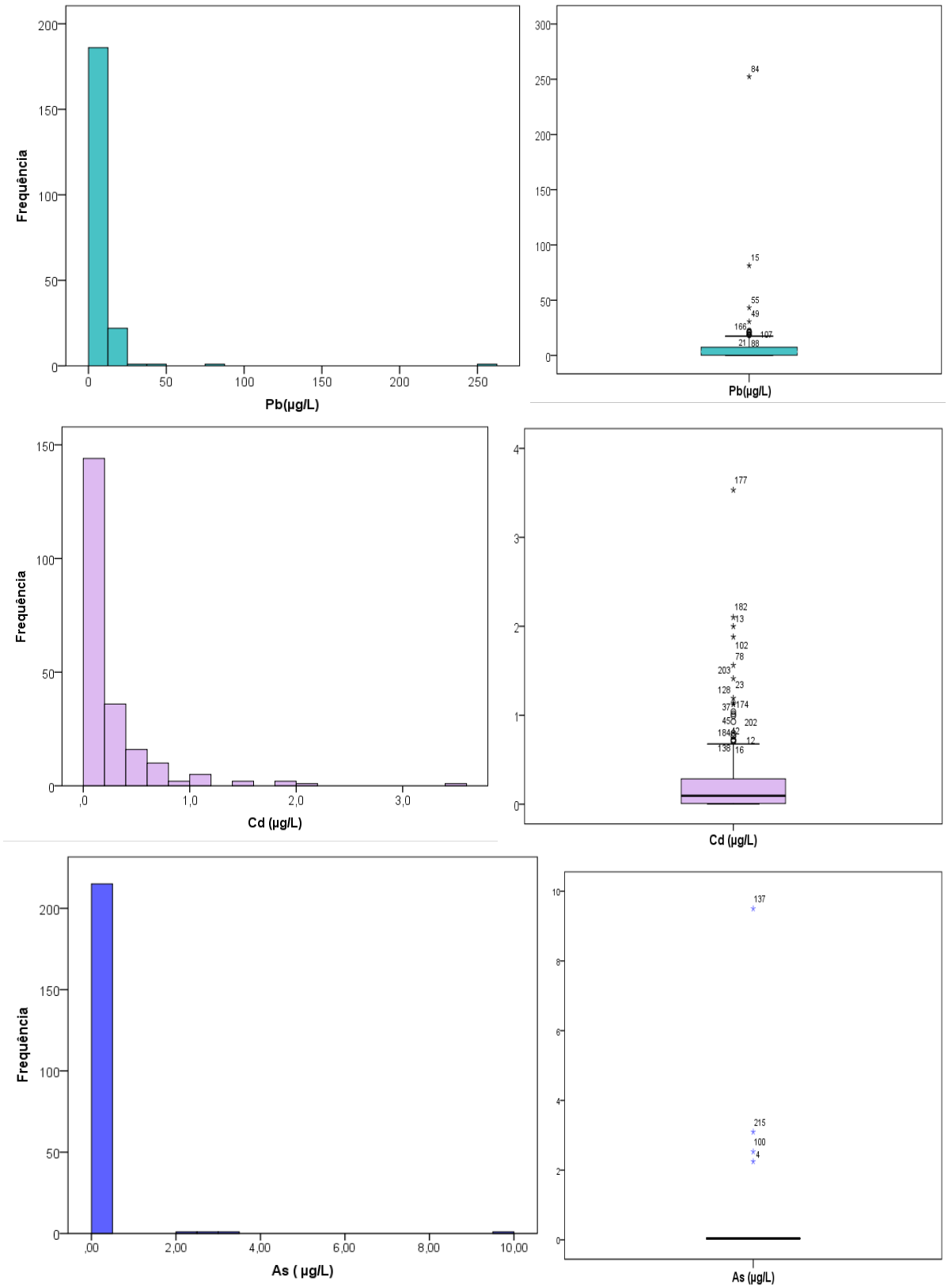


Figura 7. Distribuição e boxplot de concentrações de Pb, Cd e As em leite materno

Tabela 12. Concentrações de chumbo, cádmio e arsênio, obtidas pelas análises de 212 amostras de leite materno coletadas em bancos de leite do Distrito Federal

	% ≥ LOQ*	Min. (LOQ)**	Máx	Média** ± DP	Mediana**
Pb (µg/L)	75,9	<0,31	252,4	6,64 ± 18,8	3,36
Cd (µg/L)	71,2	<0,016	3,53	0,24 ± 0,41	0,11
As (µg/L)	1,9	<0,08	9,50	-***	0,04

* - porcentagem de amostras maiores que o LOQ; **- Amostras com valores abaixo do LOQ foram consideradas como 1/2 LOQ; *** - devido ao pequeno número de amostras acima do LOQ (4/212), o cálculo da média não é adequado; DP: desvio padrão

Análise de Spearman (Tabela 13) mostrou correlação significativa entre as concentrações de chumbo e de cádmio (0,540; p=0,01), entre a idade da mãe e o peso do bebê ao nascer (0,542; p=0,01) e entre a idade e o número de meses amamentando (0,345; p=0,01).

Tabela 13. Análise de correlação entre os parâmetros avaliados utilizando o teste de Correlaçao de Spearman

	Meses ama-mentação	**Peso bebê (kg)***	**Idade mãe (anos)**	**Arsênio (µg/L)**	**Chumbo (µg/L)**	**Cádmio (µg/L)**
Meses ama-mentação	-	0,170 (0,012)	0,345 (0,000)	-0,056 (0,406)	-0,053 (0,444)	-0,009 (0,899)
Peso bebê, kg*	0,170 (0,012)	-	0,542 (0,000)	0,026 (0,701)	-0,037 (0,589)	-0,080 (0,242)
Idade da mãe, anos	**0,345 (0,000)**	**0,542 (0,000)**	-	0,030 (0,660)	-0,092 (0,179)	-0,027 (0,692)
Arsênio, µg/L	-0,056 (0,406	0,026 (0,701)	0,030 (0,660)	-	0,059 (0,311)	-0,029 (0,672)
Chumbo, µg/L	-0,053 (0,444)	-0,037 (0,589)	-0,092 (0,179)	0,059 (0,311)	-	**0,457 (0,000)**
Cádmio, µg/L	-0,009 (0,899)	-0,080 (0,242)	-0,027 (0,692)	-0,029 (0,672)	0,457 (0,000)	-

**ao nascer*

2.3.4 Exposição a chumbo, cádmio e arsênio e caracterização do risco

A Tabela 14 sumariza os resultados da avaliação de risco para Pb, Cd e As. A ingestão semanal média de cádmio foi de 0,23 µg/kg pc, variando de 0,002 a 3,23 µg/kg pc. A Figura 8 mostra a distribuição do %TWI das amostras para o cádmio, com apenas um par bebê/mãe com a ingestão ultrapassando o TWI (125%). Foi encontrada correlação negativa entre o período de lactação e %TWI (-0,234, p=0,01), indicando que o risco da exposição diminui ao longo da lactação.

A ingestão média diária de chumbo foi 0,87 µg/kg pc/dia, variando de 0,014 a 33,0 µg/kg pc/dia, com MOE média de 9,0 (Tabela 14). A Figura 8 mostra também a distribuição da ingestão e MOE para chumbo. De acordo com o EFSA (2010), o risco da exposição ao chumbo pode ser significativo quando a MOE é inferior a 1; é provável que o risco seja baixo quando a MOE estiver entre 1 e 10; e uma MOE de 10 ou superior não indica risco apreciável de um efeito clinicamente significativo, no

caso decréscimo do QI. Utilizando este parâmetro, pode-se inferir que, para a maioria da população em estudo, a ingestão de chumbo pela amamentação representa um baixo risco para o bebê, uma vez que tanto a MOE média quanto a mediana estiveram dentro dessa faixa (1-10). Entretanto, a MOE calculada foi inferior a 1 para 98 pares de amostra/bebê (44,5%), o que pode caracterizar um risco para esta população. Não foram encontradas correlações significativas relacionadas à exposição ao chumbo e margem de exposição com outros parâmetros da população.

Tabela 14. Exposição e caracterização de risco para cádmio, chumbo e arsênio

	Exposição		**%TWI**		**MOE**	
	Faixa	**Média/ mediana**	**Faixa**	**Média/ mediana**	**Faixa**	**Média/ mediana**
Cádmio[a]	0,002 - 3,23 µg/kg pc/semana	0,23/0,09 µg/kg pc/semana	0,01-129	9,1/3,7	-	-
Chumbo[b]	0,014 - 33,0 µg/kg pc/dia	0,87/0,42 µg/kg pc/dia	-	-	0,02-36	9/1,2
Arsênio[c]	0,02-1,3 µg/kg pc/dia	-/0,005 µg/kg pc/dia	-	-	2,3-1203	-/587

a. TWI = 2,5 µg/kg pc/semana; b. BMDL = 0,5 µg/kg pc/dia; c. BMDL = 3 µg/kg pc/dia

Rebelo e Caldas (2016) propuseram que valores de MOE para o arsênio abaixo de 50 indicariam preocupação do ponto de vista da saúde publica. Nesse estudo, apenas 4 das 212 amostras de leite tiveram níveis de arsênio acima do LOQ (0,08 µg/L), com uma mediana de ½ LOQ. A ingestão mediana levou a uma MOE de 587 (Tabela 14), portanto não significando uma preocupação para a saúde do bebê. As MOEs para os 4 casos onde os níveis de arsênio foram positivos, porém, variaram entre 10 e 2, o que indicaria um potencial risco para menos de 2% da população em estudo.

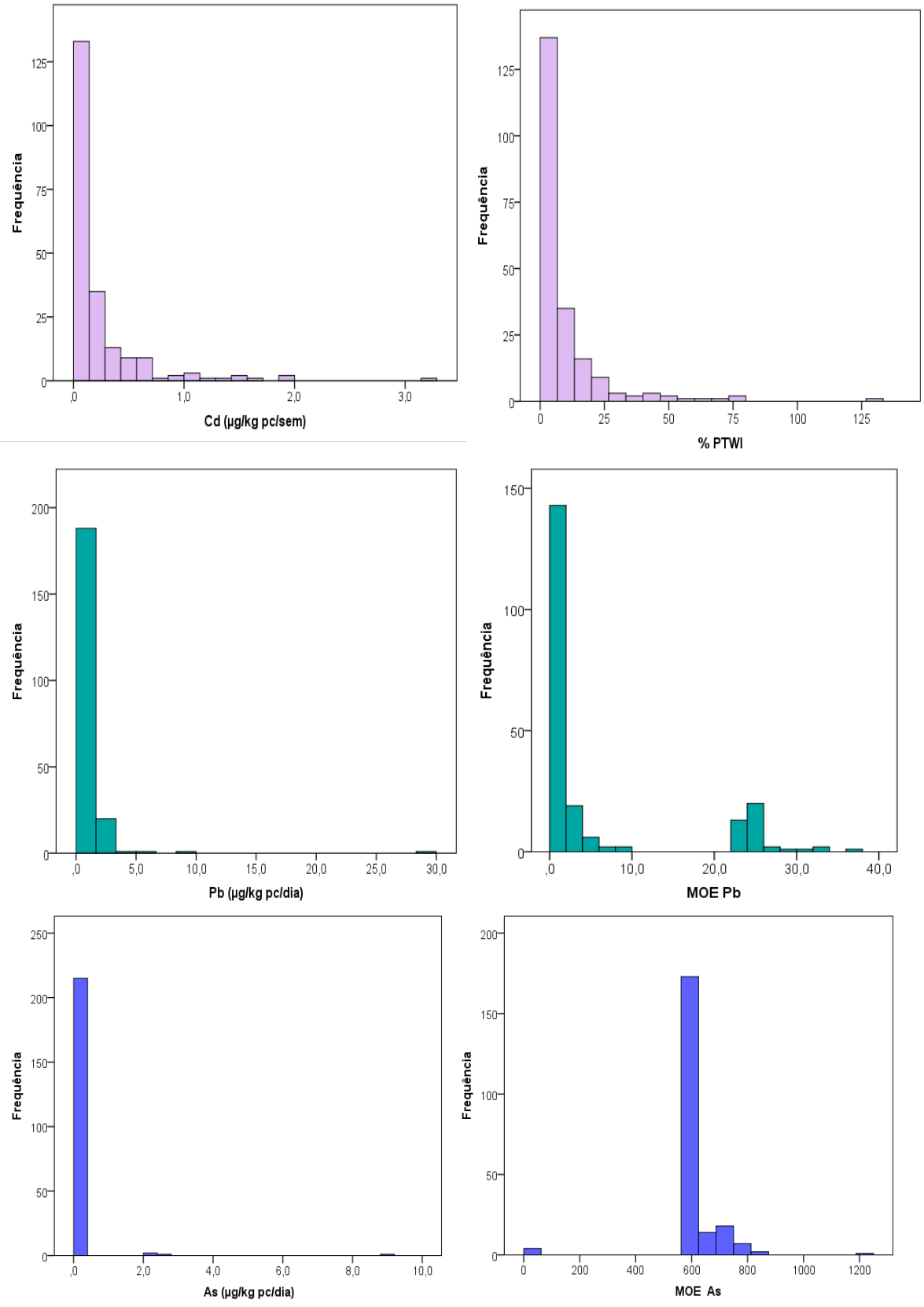


Figura 8. Distribuição da ingestão de chumbo, arsênio (µg/kg pc/dia) e cádmio (µg/kg pc/semana) e as respectivas margens de exposição (MOE) e dose tolerável semanal (%TWI)

2.4. Discussão

Neste estudo, um método por ICP-MS após digestão ácida em micro-ondas para determinação de chumbo, cádmio e arsênio em amostras de leite humano foi validado. A performance do método (exatidão e precisão) também foi aferida pela análise de chumbo e cádmio no material de referência certificado (leite), que não tinha valor certificado para arsênio. O LOQ foi estabelecido como o menor nível no qual o método foi satisfatoriamente validado, sendo de 0,31; 0,016 e 0,08 µg/L para chumbo, cádmio e arsênio, respectivamente. A comparação destes valores com outros reportados na literatura é limitada, já que em vários estudos o limite de detecção (LOD) e/ou quantificação (LOQ) não foi reportado (Abdulrazzaq et al., 2008; Needham et al., 2011) ou não está claro como foi estabelecido (Gundacker et al., 2010; Sakamoto et al., 2012; Ettinger et al., 2014; Cardoso et al., 2014; Carignan et al., 2015; Olszowski et al., 2016).

O LOQ depende da técnica utilizada para determinação do metal. Similar LOQ para chumbo utilizando também digestão micro-ondas/ICP-MS foi reportado por Amarasiriwardena et al. (2013), enquanto Cardoso et al. (2014) reportaram LOQ de 0,05 µg/L após digestão da amostra em temperatura ambiente seguido de aquecimento a 80^{o}C. O LOQ estabelecido para cádmio neste estudo é bem menor que LOD/LOQ reportados na literatura utilizando ICP-MS (Cardoso et al., 2014; Kippler et al., 2009; Sakamoto et al., 2012). Caringan et al. (2015) reportaram um LOD de 0,22 µg/L para arsênio em leite humano por micro-ondas/ICP-MS, bem superior ao LOQ reportado neste estudo, enquanto Miklavcic et al. (2013) reportaram um LOD de 0,04 µg/L utilizando método similar. Métodos mais sensíveis são extremamente importantes para contaminantes ambientais com baixa concentraçãoem leite humano, o que é o caso de arsênio.

O LOQ/LOD do método tem impacto direto no percentual de amostras reportado como positiva num estudo, e também no cálculo da média, apesar de nem todos estudos reportarem como valores abaixo do LOQ/LOD foram tratados para o cálculo da média. Neste caso, assumiu-se a estes valores como ½ LOQ. Desta maneira, as comparações de incidência e médias com outros estudos publicados na literatura também tem suas limitações.

A alta ocorrência de chumbo e cádmio nas amostras de leite humano encontrada neste estudo também tem sido reportada em outros estudos realizados no Brasil e em outras regiões do mundo (> 60% de amostras positivas; Rebelo e Caldas; 2016), o que é esperado devido a grande abundancia natural destes metais no planeta. Os níveis de chumbo encontrado nas amostras de leite analisadas neste estudo (média de 6,57 ± 16,8 µg/L) estão acima daqueles encontrados em amostras do Rio de Janeiro (média de 2,87 µg/L; Anastácio et al., 2004), Ribeirão Preto (SP) (1,46 µg/L; Andrade et al., 2013), Paraná (2,97 µg/L, amostras de banco de leite; Koyashiki et al., 2010), Minas Gerais (0,260 µg/L; Cardoso et al., 2014) e ribeirinhos de Rondônia (4,3 e 2,5 µg/L; Marques et al., 2013; 2014). Níveis médios maiores foram encontrados por Marques et al. (2013; 2014) em populações de Rondônia que vivem próximas a áreas de mineração (10-12 µg/L), porém o valor máximo encontrado foi de 29 µg/L, bem menor que o valor máximo encontrado no DF (252,4 µg/L). A maioria dos estudos conduzidos em outros países também reportam níveis de chumbo em leite humano menores que o reportado neste estudo (Rebelo e Caldas, 2016). Níveis médios acima de 14 µg/L foram reportados na Turquia (Orun et al., 2011; Gurbay et al., 2012; Dursun et al., 2016), Iram (Al-Saleh et al., 2003) e Índia (Isaac et al., 2012).

Os níveis relativamente altos de chumbo em leite humano de mães do DF não podem ser explicados por nenhuma característica ambiental ou antropogênica da região, que não é de mineração ou industrial. Não se pode descartar a possibilidade de que estes altos níveis sejam devidos à contaminação deste metal nos locais onde foram feitas as digestões das amostras (Laboratório de Geocronologia, Universidade de Brasília) e/ou onde foram realizadas as determinações por ICP-MS (Instituto de Criminalística da Polícia Civil do DF), apesar de todas as precauções tomadas para evitar contaminação externa durante os procedimentos. Outra potencial fonte de contaminaçao externa de chumbo pode ter sido os frascos utilizados pelas mães para a coleta de leite. O banco de leite orienta que as mães podem coletar o leite por até 15 dias e armazená-lo no congelador em frasco de vidro com tampa de plástico ou metal, previamente lavados e esterilizados. A coleta do leite em frascos com tampa de metal pode ter levado a uma contaminação externa ao chumbo.

Apenas 2 estudos conduzidos no Brasil analisaram cádmio em amostras de leite humano. Amostras de leite coletadas durante o primeiro mês de amamentação

em Minas Gerais apresentaram mediana de 0,77 µg/L, com máximo de 6,57 µg/L (ICP-MS; LOQ 0,05 µg/L) (Cardoso et al., 2014). Gonçalves et al. (2010) detectaram cádmio nas 80 amostras de colostro coletadas de mães goianas (EAA-forno de grafite; LOQ 0,0006 µg/L), com média e mediana de 2,3 e 0,9 µg/L, respectivamente, e máximo de 28,1 µg/L. Níveis mais altos de cádmio e outros metais em colostro comparado com leite maduro são reportados na literatura (Leotsinidis et al., 2005; Chien et al., 2006[a]; Almeida et al., 2008; Chao et al., 2014), provavelmente devido ao alto teor de proteína e gordura nesta fração de leite materno (Dorea, 2004; Leotsinidis et al., 2005). Oskarsson et al. (1998) mostraram que enquanto praticamente todo chumbo presente em leite de rato está ligado a caseína, o cádmio está predominantemente presente na gordura. Os níveis médio e mediano de cádmio encontrados em leite humano neste estudo (< 0,5 µg/L) são comparáveis aos encontrados na maioria dos estudos conduzidos em outras regiões do mundo (Leotsinidis et al., 2005; Kosanovic et al., 2008; Kippler et al., 2009; Sakamoto et al., 2012; Olszawski et al. 2016; Kunter et al., 2016). Assim como para chumbo, níveis mais altos foram reportados na Turquia (Turan et al., 2001; Örün et al., 2011) e também na Nigéria (Edem et al., 2017)

Este estudo encontrou uma correlação significativa entre os níveis de chumbo e cádmio nas amostras de leite humano analisadas. Esta correlação também foi reportada no estudo conduzido por Edem et al. (2017) na Nigéria, que obteve valores médios mais altos para estes metais (28,3 ± 5,1 e 2,81 ± 0,67 µg/L para chumbo e cádmio, respectivamente) que os encontrados neste estudo. Para a população geral, não ocupacional, a principal fonte de exposição a chumbo e cádmio é a dieta, que é impactada pelos níveis ambientais presentes, principalmente decorrente da atividade mineradora e industrial (EFSA, 2010; EFSA, 2012). Leotsinidis et al. (2005) mostrou uma correlação positiva entre consumo de queijo e arroz com níveis de chumbo em leite materno na Grécia e entre consumo de vegetais e nozes com níveis de cádmio. Adicionalmente, o consumo de tabaco durante a gravidez tem sido relacionado positivamente com os níveis de cádmio em leite materno em vários estudos (Chao et al., 2014; Garcia-Esquinas et al., 2011; Örün et al., 2012)

Este é o primeiro estudo que determinou os níveis de arsênio em leite humano no Brasil. Apenas 4 amostras tiveram níveis acima do LOQ, com

concentrações que variaram de 2,25 a 9,5 µg/L. O arsênio é o contaminante menos analisado em leite entre aqueles investigados neste estudo, com 18 estudos publicados desde 2000 (Rebelo e Caldas, 2016). Felip et al. (2014; Italy) and Gurbay et al. (2012; Turkey) não reportaram nenhuma amostra de leite materno positiva para arsênio (LOD 0,7 e 2,5 µg/L, respectivamente). Os níveis médios encontrados por Chao et al. (2012) em leite materno coletados 30 e 60 dias após o parto foram de 0.27 ± 1.26 e 0.16 ± 0.24 µg/L. Graxiola-Robles et al. (2014) encontraram arsênio acima do LOD (0,02 µg/L) em 24% das 101 amostras de leite de mães mexicanas, com mediana e máximo de 0,99 e 13,8 µg/L, respectivamente. O arsênio encontrado no leite materno está essencialmente na forma do arsênio inorgânico, que é a mais tóxica, especialmente como As (III) (Fängstrom et al., 2008). A principal fonte de exposição a arsênio é pelo consumo de água, e níveis altos deste metal ao encontrados em regiões do planeta com alto nível de arsênio na água (acima de 10 µg/L), Bangladesh (Fangstrom et al., 2008; Islam et al., 2014) e Índia (Sharma e Pervez 2005).

Com exceção de uma amostra (129 %TWI), a ingestão de cádmio não ultrapassou o TWI para este metal, não indicando risco para a saúde dos lactentes. Ingestão de cádmio pelo consumo de leite materno inferiores ao TWI também foi relatada em estudos conduzidos regiões diversas do planeta (Al Saleh et al., 2003; Leotsinidis et al., 2005; Ursinyova e Masanova, 2005; Winiarska-Mieczan, 2014).

O método utilizado neste estudo para caracterizar o risco da exposição a chumbo e arsênio, a partir do cálculo da margem de exposição (MOE) foi introduzida a partir das avaliações do JECFA e do EFSA em 2010 que concluíram que a dose tolerável anteriormente estabelecida para estes metais (7 e 15 µg/kg pc/semana para chumbo e arsênio inorgânico, respectivamente), não eram consideradas seguras, já que níveis de exposições bem próximos levavam ao aparecimento de efeitos adversos (EFSA, 2010; JECFA 2011a,b). Esta metodologia, porém, ainda é pouco utilizada por pesquisadores.

As MOEs média e mediana de chumbo estão acima de 1, indicando que a exposiçao representa um risco baixo para a saúde dos lactentes do DF. Resultado similar foi reportado pelo EFSA (2010) na sua avaliação de risco a chumbo por lactentes de 3 meses na Europa (MOE de 1,5).

Para arsênio, o cálculo da MOE a partir do valor mediano estabelecido para amostras negativas (1/2 LOQ) levou a um valor de MOE de 587, o que representa baixo risco (>50). Entretanto, todas as 4 amostras com resultado acima do LOQ apresentaram um MOE abaixo de 50, o que seria considerado uma população em risco. Este resultado representa menos de 2% dos pares mãe-bebê do estudo, e não representa uma preocupação no âmbito de saúde pública.

Esse estudo possui algumas limitações tais como a falta de dados sobre o consumo alimentar, que impossibilitou verificar possíveis correlações entre hábitos alimentares e sua influência na concentração dos metais analisados. Adicionlmente, o hábito de fumar materno é um parâmetro importante que impacta a concentração de cádmio no leite.

2.5. Conclusão

Vários autores têm investigado os níveis de metais tóxicos em leite humano no Brasil, porém este é o primeiro estudo que investigou os níveis de chumbo, cádmio e arsênio em amostras coletadas no Distrito Federal, e o primeiro a determinar arsênio nessa matriz no país. Um método para análise destes metais por ICP-MS após digestão ácida em micro-ondas foi validado, apresentando sensibilidade compatíveis ou melhores que outros publicados na literatura.

Os resultados deste estudo mostraram que a ingestão de cádmio, chumbo e arsênio não representa uma situação de risco à saúde para a maioria da população avaliada. Porém é importante o constante monitoramento desses metais em leite humano, ao mesmo tempo em que se promove a importância o aleitamento materno e seus benefícios para a mãe e o bebê.

V. CONCLUSÕES FINAIS

As concentrações de THg em leite materno de mães do Distrito Federal foram menores que as encontradas em estudos prévios realizados na região. A hipótese inicial de que a maior parte do THg refere-se ao mercúrio inorgânico foi confirmada no estudo, provavelmente, devido ao baixo consumo de peixes e frutos do mar na região. A ingestão semanal de MeHg representou, em média, 13,6% da PTWI, indicando não haver preocupação de saúde para os bebês amamentados, com apenas a ingestão MeHg pelo consumo de uma amostra estando acima do PTWI (119%).

Nesse estudo, foi possível estabelecer e validar um método de digestão e análise de leite materno para chumbo, cádmio e arsênio por ICP-MS, de fácil implementação e com baixos limites de quantificação para o cádmio e o arsênio. A exposição a cádmio e arsênio não significaram um potencial risco a saúde dos bebês. Os níveis de chumbo encontrados nas amostras analisadas foram maiores que aqueles encontrados em outras regiões do Brasil, mas para a maioria da população em estudo, o risco da exposição foi baixo.

Esse foi o primeiro estudo que investigou níveis de mercúrio, metilmercúrio, chumbo, cádmio e arsênio em amostras coletadas no Distrito Federal, e o primeiro a determinar arsênio nessa matriz no País. Mesmo que os riscos da exposição a estes contaminantes foram considerados baixos para a maioria dos casos, é essencial a monitoração contínua de metais tóxicos e outros contaminantes ambientais em leite humano, alimento essencial para o desenvolvimento e proteção dos lactentes. As amostras podem ser provenientes de bancos de leite ou doadas diretamente das mães, desde que tenha um número amostral representativo para a população avaliada. Seria interessante, em estudos posteriores, a aplicação de um questionário que obtenha informações sobre hábitos alimentares, tabagismo e número de restaurações com amálgamas, que podem fornecer importantes informações sobre fontes de exposição a esses contaminantes.

Este estudo tem uma importância adicional, já que as amostras analisadas foram retiradas de porções de leite humano doado aos bancos de leite, que provêm este alimento para recém-nascidos que o necessitavam nos hospitais. Seus resultados serão comunicados aos coordenadores dos bancos que contribuíram com este estudo e, individualmente, a cada mãe doadora.

VI. REFERÊNCIAS BIBLIOGRÁFICAS

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APÊNDICE I – Dados de concentração de chumbo, cádmio, arsênio, mercúrio total e metilmercúrio por amostra

Banco de leite	Identificação	Meses de amamen-tação	Peso ao nascer	As (µg/L)	Pb (µg/L)	Cd (µg/L)	Thg (µg/L)	MeHg (µg/L)
HRAN	356/11	3,00	NR	0,039	7,519	0,291	3,54	
HRAN	404/11	3,00	NR	0,039	11,275	0,677	0,63	
HRAN	40/11	3,00	NR	0,039	17,271	0,172	0,43	
HRAN	451/11	3,00	NR	0,039	8,456	0,520	5,2	
HRAN	549/11	3,00	NR	0,039	6,240	0,008	1,68	
HRAN	549/11	3,00	NR	0,039	1,680	0,412	1,44	
HRAN	598/11	3,00	NR	0,039	0,488	0,008	2,08	
HRAN	681/12	3,00	NR	0,039	3,883	0,289	1,45	
HRAN	283/11	6,00	2.8	0,039	1,164	0,008	0,730	0,221
HRAN	355/11	3,00	3.58	0,039	5,936	0,008	5,100	0,506
HRAN	382/11	1,00	3.1	0,039	3,217	0,243	4,310	0,126
HRAN	439/11	1,00	3.47	2,247	9,973	0,468	0,320	0,102
HRAN	464/11	5,00	NR	0,039	4,676	0,764	3,030	0,100
HRAN	470/11	3,00	2,81	0,039	6,720	0,722	0,350	0,100
HRAN	470/11	1,00	2,81	0,039	81,302	1,999	1,790	0,100
HRAN	481/11	1,00	NR	0,039	17,025	0,286	4,500	0,100
HRAN	484/11	2,00	2.96	0,039	5,526	0,008	1,240	0,152
HRAN	488/11	3,00	2,81	0,039	4,465	0,711	2,640	0,198
HRAN	495/11	1,00	3,38	0,039	9,955	0,159	0,290	0,100
HRAN	505/11	2,00	NR	0,039	0,156	0,116	0,690	0,144
HRAN	508/11	1,00	3.73	0,039	19,181	0,344	3,420	0,100
HRAN	518/11	1,00	NR	0,039	4,721	0,461	1,100	0,100
HRAN	525/11	2,00	NR	0,039	1,759	0,182	3,980	0,100
HRAN	549/11	5,00	3.57	0,039	5,064	0,482	1,440	0,100
HRAN	553/11	1,00	1.84	0,039	7,922	1,140	2,060	0,100
HRAN	562/11	1,00	3,16	0,039	0,156	0,379	1,550	0,229
HRAN	578/11	4,00	2.88	0,039	5,384	0,367	3,620	0,100
HRAN	588/11	1,00	3.12	0,039	0,156	0,008	5,860	
HRAN	592/11	3,00	5.3	0,039	1,964	0,184	1,700	0,984
HRAN	592/11	6,00	5.3	0,039	0,156	0,008	3,850	0,151
HRAN	597/11	1,00	3,24	0,039	5,864	0,008	1,190	0,259
HRAN	600/12	4,00	3.3	0,039	0,156	0,008	3,520	0,634
HRAN	609/11	1,00	3.29	0,039	3,100	0,236	0,040	0,324

HRAN	611/12	14,00	NR	0,039	2,778	0,076	2,940	0,321
HRAN	616/11	1,00	1,72	0,039	0,156	0,378	2,860	0,100
HRAN	619/11	1,00	NR	0,039	0,156	0,139	1,360	0,226
HRAN	635/11	1,00	1.34	0,039	0,156	0,236	1,810	0,436
HRAN	644/11	2,00	3.3	0,039	0,156	0,088	3,000	0,100
HRAN	661/11	3,00	2,35	0,039	12,408	0,994	0,530	0,100
HRAN	666/12	2,00	3.28	0,039	8,351	0,239	4,880	0,100
HRAN	672/11	3,00	2.73	0,039	15,966	0,185	1,800	0,100
HRAN	675/12	2,00	3.6	0,039	0,156	0,134	0,820	0,147
HRAN	677/12	3,00	3.02	0,039	0,352	0,072	2,360	0,160
HRAN	682/11	1,00	NR	0,039	6,812	0,928	1,260	0,100
HRAN	693/11	3,00	3,38	0,039	7,838	0,121	0,140	0,401
HRAN	694/11	2,00	3.31	0,039	0,156	0,064	4,230	0,100
HRAN	699/12	2,00	2,29	0,039	7,866	1,041	1,620	0,100
HRAN	711/12	7,00	3,73	0,039	0,156	0,008	1,360	0,195
HRAN	739/11	1,00	3.9	0,039	30,632	0,448	1,270	0,100
HRAN	740/12	1,00	3,13	0,039			5,690	0,153
HRAN	470/11	1,00	2,81	0,039	4,016	0,008	0,350	0,100
HRAS	202/11	3,00	NR	0,039	4,913	0,142	2,28	
HRAS	218/11	3,00	NR	0,039	12,089	0,481	1,11	
HRAS	277/11	3,00	NR	0,039	6,879	0,094	0,89	
HRAS	359/11	3,00	NR	0,039	43,231	1,562	1,64	
HRAS	359/11	3,00	NR	0,039	8,186	0,071	1,87	
HRAS	115/11	3,00	3.2	0,039	9,566	0,111	3,200	0,183
HRAS	124/11	3,00	1.95	0,039	7,389	0,221	2,900	0,100
HRAS	170/11	4,00	NR	0,039	1,528	0,008	3,030	0,254
HRAS	172/11	2,00	2.87	0,039	0,156	0,151	1,000	0,100
HRAS	180/11	6,00	3.26	0,039	0,156	0,008	1,320	0,300
HRAS	188/11	3,00	2.8	0,039	0,156	0,008	2,700	0,225
HRAS	195/11	4,00	2.7	0,039	5,164	0,008	0,600	0,320
HRAS	206/11	5,00	NR	0,039	0,156	0,008	1,280	0,100
HRAS	239/11	1,00	3,75	0,039	0,855	0,109	2,150	
HRAS	245/11	NR	NR	0,039	0,156	0,170	2,740	0,161
HRAS	247/11	3,00	NR	0,039	1,240	0,060	2,400	
HRAS	277/11	7,00	3.47	0,039	21,125	0,413	0,890	0,169
HRAS	334/11	1,00	3,41	0,039	0,156	0,017	3,590	0,251
HRAS	379/11	3,00	4,06	0,039	8,830	0,139	1,870	0,100
HRAS	395/11	1,00	2.78	0,039	4,246	0,008	2,500	0,100
HRAS	5517/11	7,00	3,22	0,039	4,547	0,081	1,950	0,227
HRAS	90/11	5,00	NR	0,039	0,156	0,022	0,580	0,100

HRBZ	021/11	3,00	NR	0,039	0,156	0,008	3,33	
HRBZ	90/11	3,00	NR	0,039	9,650	0,008	1,1	
HRBZ	001/12	1,00	2,85	0,039	0,156	0,008	0,540	0,197
HRBZ	006/12	1,00	2.67	0,039	1,074	0,008	2,660	0,100
HRBZ	016/11	4,00	3.69	0,039	7,226	0,544	0,390	0,100
HRBZ	101/11	2,00	NR	0,039	12,841	0,278	2,050	0,262
HRBZ	108/11	3,00	2,66	0,039			3,410	0,172
HRBZ	124/11	1,00	2.5	0,039	9,120	0,008	2,690	0,100
HRBZ	126/11	1,00	2.7	0,039			0,980	0,683
HRBZ	126/11	1,00	2.7	0,039	5,349	0,307	2,160	0,100
HRBZ	137/11	3,00	3.8	0,039	252,390	0,008	2,720	0,213
HRBZ	143/11	1,00	3,81	0,039	16,258	0,484	3,360	0,100
HRBZ	161/11	1,00	3.62	0,039	2,206	0,008	2,820	0,113
HRBZ	165/11	1,00	3.3	0,039	0,156	0,349	3,560	0,186
HRBZ	166/12	3,00	3.42	0,039	18,634	0,111	0,590	0,100
HRBZ	173/12	1,00	3,40	0,039	13,517	0,441	0,500	0,100
HRBZ	178/12	2,00	3.36	0,039			2,510	0,100
HRBZ	182/12	3,00	3.97	0,039	2,658	0,138	2,310	0,100
HRBZ	43/11	2,00	2.36	0,039	2,173	0,279	5,150	0,100
HRBZ	44/11	3,00	3.1	0,039	8,979	0,023	0,690	0,100
HRBZ	67/11	1,00	3,12	0,039	6,636	0,080	2,190	0,272
HRBZ	79/11	1,00	NR	0,039	3,357	0,241	2,440	0,298
HRBZ	81/11	1,00	2,67	0,039	0,156	0,008	6,140	0,100
HRBZ	82/11	1,00	3.25	0,039	3,356	0,094	0,050	0,603
HRBZ	96/11	2,00	3.25	0,039	16,187	1,881	2,360	0,100
HRP	002/12	1,00	3.75	0,039	0,156	0,008	1,170	0,522
HRP	009/12	1,00	3,12	2,530	5,658	0,799	3,320	0,100
HRP	106/11	1,00	2.94	0,039	4,728	0,092	4,520	0,100
HRP	108/11	1,00	3,07	0,039	0,156	0,147	0,670	0,207
HRP	113/11	1,00	3.08	0,039	12,548	0,339	6,760	0,100
HRP	129/11	1,00	3,98	0,039	5,053	0,182	4,590	0,174
HRP	136/11	1,00	3.54	0,039	3,008	0,202	1,620	0,100
HRP	143/11	1,00	2.4	0,039	4,329	0,093	2,820	0,388
HRP	163/11	3,00	2,76	0,039	18,515	0,416	4,260	0,462
HRP	212/11	3,00	NR	0,039	0,742	0,232	5,690	0,100
HRP	235/11	2,00	3,19	0,039	1,767	0,074	0,650	0,100
HRP	265/11	1,00	3,04	0,039	3,156	0,008	1,330	0,100
HRP	280/12	3,00	3.04	0,039	1,580	0,341	4,350	0,395
HRP	289/12	1,00	2,96	0,039	2,000	0,038	5,270	0,183
HRP	305/12	1,00	2,83	0,039			0,450	0,100

HRP	307/12	2,00	3,12	0,039	0,156	0,008	8,400	0,225
HRP	313/12	1,00	3,95	0,039	0,767	0,154	5,890	0,100
HRP	369/11	2,00	3,95	0,039			3,740	0,614
HRP	61/11	2,00	3,93	0,039	1,169	0,008	3,030	0,100
HRP	98/11	6,00	2.83	0,039	0,156	0,008	1,600	0,477
HRPA	001/11	3,00	NR	0,039	5,412	0,558	1,14	
HRPA	97/11	3,00	NR	0,039	3,993	0,008	3,23	
HRPA	124/11	3,00	NR	0,039	0,156	0,299	3,04	
HRPA	414/12	3,00	NR	0,039	0,156	0,008	2,28	
HRPa	104/11	2,00	3.57	0,039	2,808	0,061	5,350	0,100
HRPa	130/11	2,00	3.73	0,039	4,247	0,047	0,390	0,348
HRPa	173/11	1,00	3.83	0,039	8,148	0,093	0,590	0,100
HRPa	181/11	1,00	3.09	0,039	20,616	1,192	1,940	0,168
HRPa	187/11	1,00	3.2	0,039	0,156	0,008	3,050	0,124
HRPa	233/11	9,00	2.99	0,039	0,156	0,008	1,640	0,100
HRPa	258/11	1,00	2.93	0,039	5,090	0,008	1,670	0,100
HRPa	259/11	1,00	2.08	0,039	1,055	0,038	3,120	0,100
HRPa	280/11	1,00	3.84	0,039	6,229	0,127	3,990	0,250
HRPa	302/11	2,00	3.01	0,039	1,129	0,083	0,710	0,100
HRPa	36/12	1,00	3.5	0,039	0,156	0,008	1,690	0,100
HRPa	364/12	3,00	2.95	0,039	0,156	0,008	3,860	0,327
HRPa	43/12	1,00	3.9	0,039	6,550	0,120	1,520	0,100
HRPa	75/11	3,00	3.19	0,039	22,229	0,725	3,600	
HRPa	78/11	6,00	2.97	0,039	5,099	0,248	3,270	0,100
HRPa	85/11	2,00	2,76	0,039	1,252	0,263	2,100	0,100
HRPa	92/11	6,00	3,10	0,039	0,156	0,625	1,410	0,329
HRPa	93/11	4,00	3.72	0,039	0,156	0,008	1,250	0,417
HRPa	96/11	1,00	3,16	9,501	2,781	0,135	0,030	0,100
HRS	41/11	3,00	NR	0,039	0,156	0,145	5,02	
HRS	41/11	3,00	NR	0,039	0,156	0,008	5,02	
HRS	73/11	3,00	NR	0,039	21,409	3,532	5,36	
HRS	172/11	3,00	NR	0,039	12,441	1,122	1,63	
HRS	001/11	4,00	NR	0,039	1,009	0,152	1,140	0,100
HRS	002/12	1,00	3.33	0,039	0,156	0,354	2,240	0,805
HRS	109/11	1,00	2.6	0,039	0,156	0,008	2,910	0,100
HRS	113/11	1,00	2.7	0,039	6,901	0,143	4,700	0,100
HRS	140/11	3,00	2.9	0,039	0,156	0,008	7,220	0,479
HRS	142/11	1,00	3.5	0,039	0,156	0,284	1,710	0,100
HRS	162/11	1,00	2.49	0,039	8,114	0,091	2,950	0,100
HRS	173/11	7,00	3.2	0,039	1,628	0,417	4,790	0,100

HRS	174/11	1,00	NR	0,039	0,156	0,008	4,740	0,100
HRS	182/11	1,00	3.35	0,039	0,156	0,008	4,650	0,100
HRS	195/12	5,00	2,54	0,039	4,046	0,008	0,040	0,100
HRS	200/12	7,00	2,94	0,039	0,156	0,419	1,920	0,100
HRS	202/11	4,00	3.21	0,039	0,156	0,084	2,880	1,411
HRS	217/11	1,00	3,13	0,039	9,463	0,338	3,330	0,100
HRS	221/11	1,00	NR	0,039	11,106	0,039	2,280	0,100
HRS	231/11	1,00	3.26	0,039			5,070	0,355
HRS	246/11	1,00	3,94	0,039	0,156	0,008	1,230	0,100
HRS	249/12	2,00	4.1	0,039	2,937	0,478	0,470	0,100
HRS	254/11	2,00	3.36	0,039	4,934	0,067	4,490	0,199
HRS	255/11	1,00	3.65	0,039	4,142	0,167	1,290	0,100
HRS	266/12	3,00	3,17	0,039	18,990	0,121	2,580	0,100
HRS	271/12	3,00	3.03	0,039	0,156	0,114	1,720	0,100
HRS	278/12	1,00	3.53	0,039	20,841	0,073	1,520	0,100
HRS	53/11	3,00	3.78	0,039	0,156	0,073	0,720	0,210
HRS	59/11	2,00	NR	0,039	0,156	0,071	3,490	
HRS	71/11	2,00	2,62	0,039	0,646	0,767	0,600	0,257
HRSM	421/11	3,00	NR	0,039	14,251	0,008	5,14	
HRSM	467/12	3,00	NR	0,039	0,156	0,008	2,97	
HRSM	001/11	6,00	NR	0,039	4,838	0,008	5,140	0,100
HRSM	002/11	6,00	NR	0,039	4,003	0,125	1,450	0,342
HRSM	003/11	7,00	NR	0,039	0,156	0,008	3,770	2,824
HRSM	004/11	1,00	NR	0,039	5,687	0,082	0,390	0,100
HRSM	005/11	1,00	NR	0,039	5,957	2,102	4,680	0,100
HRSM	006/11	3,00	NR	0,039	11,847	0,233	0,770	0,100
HRSM	007/11	1,00	NR	0,039	17,379	0,704	2,690	0,100
HRSM	445/12	3,00	3.23	0,039	10,520	0,063	1,690	0,320
HRSM	461/12	2,00	3,16	0,039	8,016	0,018	3,350	0,100
HRSM	512/12	1,00	3.99	0,039	3,904	0,008	2,920	0,100
HRT	2415/11	3,00	NR	0,039	0,156	0,149	2,03	
HRT	2428/11	3,00	NR	0,039	0,156	0,008	1,58	
HRT	2464/11	3,00	NR	0,039	0,156	0,053	6,1	
HRT	1971/11	10,00	3,01	0,039	1,203	0,008	1,480	0,238
HRT	2104/11	8,00	3,01	0,039	4,048	0,139	3,980	0,100
HRT	2285/11	4,00	3.04	0,039	4,296	0,008	0,670	0,370
HRT	2386/11	8,00	3.6	0,039	6,836	0,175	4,810	0,103
HRT	2386/11	5,00	3.6	0,039	0,156	0,008	2,470	1,221
HRT	2405/11	2,00	2.52	0,039	0,156	0,034	0,450	0,210

HRT	2431/11	2,00	3.54	0,039	0,156	0,008	2,120	0,100
HRT	2464/11	4,00	3,30	0,039	0,156	0,008	6,100	0,335
HRT	2468/11	2,00	NR	0,039	0,156	0,002	3,640	0,100
HRT	2478/11	1,00	2,72	0,039	3,529	0,216	3,220	0,518
HRT	2483/11	1,00	2.75	0,039	0,156	0,040	1,620	
HRT	2483/11	4,00	2.75	0,039	2,871	0,215	3,260	0,100
HRT	2495/11	2,00	2.88	0,039	0,156	1,014	2,630	0,100
HRT	2557/11	1,00	3,49	0,039	17,358	1,414	4,950	0,234
HRT	2584/11	2,00	2,47	0,039	0,156	0,008	0,470	0,100
HRT	2584/11	2,00	2,47	0,039	0,156	0,008	3,660	0,100
HRT	2589/12	7,00	NR	0,039	6,873	0,260	1,620	0,297
HRT	2677/11	2,00	2,55	0,039	0,156	0,008	0,990	0,100
HRT	2678/11	5,00	2.94	0,039	10,643	0,394	1,220	0,100
HRT	2685/11	1,00	2.7	0,039	0,156	0,083	2,800	0,100
HRT	2700/11	2,00	2.36	0,039	7,641	0,008	3,000	0,100
HRT	2702/11	1,00	2.79	0,039	0,156	0,022	3,980	0,128
HRT	2711/11	2,00	NR	0,039	0,406	0,033	5,750	0,465
HRT	2714/12	4,00	NR	0,039	4,655	0,026	2,070	0,100
HRT	2714/12	4,00	NR	0,039	0,156	0,053	2,370	0,100
HRT	2738/12	6,00	4.34	0,039	3,576	0,224	1,430	0,100
HRT	2759/12	3,00	3.6	0,039	12,903	0,221	2,330	0,458
HRT	2767/12	3,00	NR	0,039	0,156	0,257	4,440	0,272
HRT	2777/12	4,00	3.65	0,039	4,353	0,391	1,550	0,331
HRT	2784/12	2,00	3.9	0,039	1,380	0,021	4,010	0,100
HRT	2866/12	2,00	2.78	0,039	7,212	0,095	4,710	0,409
HRT	2934/12	2,00	NR	3,095	3,923	0,008	0,380	0,931
HRT	2989/12	2,00	3.58	0,039	12,673	0,636	4,150	0,437

NR – Não registrado

ANEXO I - Artigo Publicado

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Review article

Arsenic, lead, mercury and cadmium: Toxicity, levels in breast milk and the risks for breastfed infants

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ABSTRACT

Metals are ubiquitous in nature, being found in all environmental compartments, and have a variety of applications in human activities. Metals are transferred by maternal blood to the fetus via the placenta, and exposure continues throughout life. For the general population, exposure comes mainly from water and food consumption, including breast milk. In this paper, we reviewed studies on the toxicity of arsenic, lead, mercury and cadmium, the toxic metals of most concern to human health, focusing on the potential risks to newborns and infants. A total of 75 studies published since 2000 reporting the levels of these metals in breast milk were reviewed. Lead was the metal most investigated in breast milk (43 studies), and for which the highest levels were reported (up to 1515 μg/L). Arsenic was the least investigated (18 studies), with higher levels reported for breast milk (up to 149 μg/L) collected in regions with high arsenic concentrations in water (> 10 μg/L). Data from 34 studies on mercury showed that levels in breast milk were generally higher in populations with high fish consumption, where it may be present mainly as MeHg. Cadmium levels in breast milk were the lowest, with means < 2 μg/L in most of the 29 studies reviewed. Results of risk assessments indicated that the intake of arsenic, lead and mercury by infants through breastfeeding can be considered a health concern in most regions of the world. Although the potential risks to infants are mostly outweighed by the benefits of breast milk consumption, it is essential that contaminants be continuously monitored, especially in the most critical regions, and that measures be implemented by health authorities to reduce exposure of newborns and infants to these metals, and thus avoid unnecessary health risks.

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1. Introduction

Metals are ubiquitous in nature, but some comprise a group of contaminants to which exposure, even at relatively low levels may represent a risk to human health. Arsenic ranks first on the National Priorities List of the Agency for Toxic Substances and Disease Registry (ATSDR), which prioritizes substances based on a combination of their frequency, toxicity, and human exposure potential. Lead, mercury and cadmium rank 2nd, 3rd and 7th on this list, respectively (ATSDR, 2015).

Human exposure to metals can occur during occupational activities, mainly through inhalation and dermal routes in mining and industry, and over a lifetime, from water and food consumption and exposure to soil, dust and air (ATSDR, 2007a, 2007b; WHO, 2004; EFSA, 2009a; Carlin et al., 2016). The presence of toxic metals in human milk has been reported worldwide (e.g., Gürbay et al., 2012; Chao et al., 2014; Ettinger et al., 2014), and breastfed babies are particularly vulnerable and sensitive to their toxic effects due to their rapid growth, organ immaturity, and susceptibility of their nervous system during the first year (Isaac et al., 2012). Furthermore, newborns absorb metals to a greater extent than adults and have a lower capacity to excrete compounds in the bile, decreasing body clearance (Oskarsson et al., 1998).

Lactation is a highly complex process that begins about 40 h after birth, and is triggered by the hormones progesterone, estrogen, prolactin and oxytocin (Gundacher and Zödl, 2005). Breast milk is a fundamental source of nutrients for newborns and babies, as it contains proteins, fats, carbohydrates, and elements essential to the proper functioning of the body. It is also a source of lactoferrin, α-lactalbumin and lisoenzymes, substances that create a protective barrier against environmental factors, increasing defense mechanisms and stimulating the development of immunological systems in children (Grzelak et al., 2014). Breast milk influences the intestinal microflora, ensures the structural and functional maturity of mucous membranes, reduces the risk of allergies and autoimmune disorders, and contributes to the proper development of the gastrointestinal, central nervous, endocrine and immune systems (Leon-Cava et al., 2002). The WHO recommends that babies be exclusively breastfed up to 6 months of age, and for an additional 2 years along with appropriate complementary foods (WHO, 2007).

The composition of human milk is not constant and depends on the nutritional status of the mother, her diet, stage of lactation, socio-demographic status, and lifestyle (Ballard and Morrow, 2013; Garcia-Esquinas et al., 2011; Vieira et al., 2013). The transport of xenobiotics into milk is supposed to follow the same pathways as those of other milk components, with toxic metals entering milk through ways similar to those of essential trace elements (Oskarsson et al., 1998). Trace element regulation mechanisms in milk involve the capturing of metals by specific transporters in the mammary epithelial cells and their subsequent discharge in the alveolar lumen of the mammary glands (Rossipal and Krachler, 1998; Kelleher and Lönnerdal, 2005; Bressler et al., 2007). Studies conducted with rats and mice indicated that lead was almost exclusively found in the casein fraction, the highest proportions of cadmium and methylmercury found in fat, and inorganic mercury in whey fractions (Oskarsson et al., 1998). In human milk, mercury possesses a greater ability to interact with milk protein, while cadmium and lead are equally distributed between light and low molecular weight components (see review by Gundacker and Zödl (2005)).

This paper briefly summarizes arsenic, lead, mercury and cadmium toxicology, focusing particularly on infants and children, and reviews the literature of studies reporting levels of these toxic metals in human breast milk worldwide. Exposure and risk assessment results of metal intake through breastfeeding are also reviewed, and the risks of exposure to breastfed infants discussed. For the incidence data, a query was conducted on the Pubmed, Science Direct and Google Scholar databases for studies published since 2000 (last search June 2016) using the keywords "human milk", "breastmilk" and "breast milk", associated with "metal", "arsenic", "lead", "mercury" or "cadmium". Additional papers were identified in published reviews related to contaminants in breast milk.

2. Human exposure and toxicity

2.1. Arsenic

Arsenic (As) occurs naturally in volcanic ashes, volcanic rock, clay, iron oxides, mineral sulfur and organic matter. Human exposure to arsenic occurs primarily through the consumption of water and seafood, particularly shellfish (EFSA, 2009a). Arsenic is found in the environment in organic forms, including monomethylarsenic (MMA), dimethylarsenic (DMA), arsenobetaine, and arsenocholine, as well as in inorganic (IAs) forms (As^{III} and As^{V}). A systematic review conducted by Lynch et al. (2014) evaluated over 6500 data on inorganic arsenic and its metabolites in food, including seafood and specific foods for children. Algae was the food with the highest concentration (mean of 1000 μg/kg, n=312, mostly as IAs), followed by rice and its byproducts (130 μg/kg, n =1126, mostly as IAs), and seafood (130 μg/kg, n=835; mostly as DMA).

Over 80% of inorganic arsenic is absorbed through the human gastrointestinal tract, and excretion occurs mainly via urine (ATSDR, 2007a). Certain characteristics of arsenic are summarized in Table 1. Studies conducted in Taiwan and other countries showed greater risk of lung, bladder, kidney or skin cancer from exposure to arsenic in drinking water, where it was predominantly present in inorganic form (WHO, 2001). Inorganic arsenic compounds, including arsenic trioxide, arsenite, and arsenate are classified as carcinogenic to humans by the International Agency for Research in Cancer (Group I), with extensive evidence of lung, bladder and skin cancer, and positive association with kidney, liver and prostate cancer (IARC, 2016). Although the mechanisms involved in the carcinogenicity of arsenic are not yet fully understood, it may nevertheless be considered genotoxic, since it induces micronuclei, DNA strand breaks, sister chromatid exchanges, aneuploidy and oxidative stress through the generation of reactive oxygen species during its biotransformation (see revision by Bustaffa et al. (2014)).

Inorganic arsenic and the methylated metabolites MMA and DMA cross the placentary barrier (Vahter, 2008), exert epigenetic effects by methylation of DNA (Reichard et al., 2007), and interact with multiple nuclear receptors (Bodwell et al., 2006). As a result, functional changes may occur leading to the development of other diseases later in life (Vahter, 2008). Vahter (2009) suggested that high

Table 1
Some characteristics of arsenic, lead, mercury and cadmium.

	IAs	Pb	IHg	MeHg	Cd
IARC classification[a]	Group 1	Group 2B	Group 3	Group 2B	Group 1
PTWI, μg/kg bw/week or PTMI, μg/kg bw/month	–	–	PTWI: 4[d]	PTWI: 1.6[e]	PTWI: 2.5[b] PTMI: 25[c]
BMDL, μg/kg bw/day	3.0[d]	0.5[f] (developmental toxicity in children)	–	–	–
Oral absorption	over 75%[g]	Adults: 3–15% Children: 30–50%[f,j]	Up to 20%; increases in a milk diet[i]	> 90%[i]	5%[h]
Half-life (plasma)	3–4 hs[g]	20–40 days[i]	20–66 days[i]	44–88 days[i]	3–4 months[m] ~ 12 yrs (kidney)[h]
Cross the placenta	Yes[g]	Yes[f,i]	Poorly[i]	Yes[i]	Yes[h]
Neurotoxic	Yes[g]	Yes[f,i]	Inconclusive[i]	Yes[e,i]	Inconclusive[h]
Genotoxic	Yes[d,g]	weak, indirect[f]	Inconclusive[i]	Inconclusive[i]	Indirect[h,j]
Embryotoxic	Yes[g]	Inconclusive[k]	Inconclusive[i]	Yes[e,i]	No[h]

Group 1 – carcinogenic to humans; Group 2A- probably carcinogenic to humans; Group 2B: possibly carcinogenic to humans; Group 3- not classifiable as to its carcinogenicity to humans; PTWI: provisional tolerable weekly intake; PTMI: provisional tolerable monthly intake; BMDL – benchmark dose lower bound.

[a] IARC, 2016.
[b] EFSA, 2012a.
[c] JECFA, 2011b.
[d] JECFA, 2011a.
[e] JECFA, 2004.
[f] EFSA, 2010.
[g] ATSDR, 2007b.
[h] ATSDR, 2012.
[i] UNEP, 2008.
[j] ATSDR, 2007a.
[k] CDC, 2010.
[l] EFSA, 2009b.
[m] Järup and Akesson, 2009.

levels of methylated arsenic in pregnant women are the result of *de novo* synthesis of choline by phosphatidylethanolamine methyltransferase, which is upregulated during pregnancy to supply fetal needs of choline for cerebral development (Zeisel, 2006). Exposure to arsenic can also cause reproductive toxicity, including increases in fetus mortality, underweight newborns, spontaneous abortions, eclampsia, and birth defects (WHO, 2001). As^{III} is the single form of arsenic which is protonated at physiologic pH, and is transported by the aquaglyceroporins (Liu et al., 2004; Rosen, 2002) present in mammary glands during lactation (Matsuzaki et al., 2005).

Recent epidemiologic studies have found a long latency period for lung cancer and other chronic diseases related to arsenic, even when exposure was limited to a short period during childhood or in the uterus. Exposure during these two periods may also have adverse reproductive outcomes for mothers, and induce changes in cognitive development of children (McClintock et al., 2012).

A limit of 10 μg/L was established by the WHO for arsenic in drinking water (WHO, 2004). However, some regions of the world have naturally high arsenic levels in water compartments which exceed that limit, including Argentina, Bangladesh, Chile, China, Hungary, India, Taiwan, and certain regions of the United States (Hopenhayn-Rich et al., 2000; Nordstrom, 2002; Rahman et al., 2011; McClintock et al., 2012). It is well established that almost all arsenic in drinking water is in inorganic form (JECFA, 2011a; EFSA, 2009a).

In Chile, data from 1950 to 1996 showed high late fetal mortality (OR = 1.7; CI: 1.5–1.9), neonatal mortality (OR = 1.53; CI: 1.4–1.7), and post neonatal mortality (OR = 1.26; CI: 1.2–1.3) in a region with a history of high arsenic levels in water, in comparison with a region with low levels (Hopenhayn-Rich et al., 2000). A epidemiologic study conducted in Bangladesh observed 1152 pregnant women and their babies for a period of 1 year, with urine samples collected after confirmation of pregnancy and in the 30th week of gestation for arsenic analysis (Rahman et al., 2011). Estimated risk of occurrence of lower respiratory tract diseases increased 69% for infants of mothers with higher arsenic concentrations in urine.

The mechanism and factors that may affect the excretion of arsenic in breast milk are not completely known, but fetuses and babies are probably protected by increased methylation of arsenic during pregnancy and breastfeeding (Fängström et al., 2008; Gürbay et al., 2012; Vahter, 2009). In a study conducted in Argentina in an area with high arsenic concentration in water (200 μg/L), the median concentration of arsenic was 34 μg/kg in the placenta, and 9 μg/L in cord blood, with a significant correlation with maternal blood levels (Concha et al., 1998). All arsenic in the blood plasma of newborns and their mothers, and about 90% of the arsenic in the urine of both, was present as DMA, a result also found by other authors (Fängström et al., 2008; Islam et al., 2014), indicating that methylation of arsenic occurred during pregnancy and the metal was transferred to the fetus as DMA. Fängström et al. (2008) indicated that the methylated arsenic metabolites in blood plasma do not pass easily through the mammary glands. The authors found that the arsenic concentrations in breast milk were negatively correlated with %DMA ($r_s = -0.19$), and positively correlated with %iAs ($r_s = 0.16$) in maternal urine. Thus, efficient maternal methylation of iAs leads to lower arsenic excretion in breast milk, which contains essentially inorganic arsenic, mainly as As^{III}.

In 2010, the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2011a) concluded that the provisional tolerable weekly intake (PTWI) previously adopted for arsenic (15 μg/kg bw, or 2.1 μg/kg bw/day) was no longer safe for humans, and established a benchmark dose, and a lower confidence level ($BMDL_{0.5}$) of 3 μg/kg bw/day as the reference point for risk assessment (Table 1). This dose corresponds to a 0.5% increase in the incidence of lung cancer associated with dietary exposure to inorganic arsenic over background in northeastern Taiwan (JECFA, 2011a).

2.2. Lead

Lead is a toxic metal widely present in nature, primarily in inorganic form, and is produced in activities such as mining and smelting, and in battery manufacturing (WHO, 2010a). The general

population is exposed to lead mainly through food consumption, with about 5–15% of the oral intake being absorbed by the gastrointestinal tract, a rate that is higher in children under 6 years of age (WHO, 2010a). The higher gastrointestinal absorption of lead by children is related to the uptake pathways for essential minerals (e.g. calcium and iron), which are more active than in adults (HERAG, 2007). Inorganic lead compounds are classified by the IARC as potentially carcinogenic to humans (Group 2 A), and organic lead compounds are "not classifiable to its carcinogenicity to humans" (Group 3) (IARC, 2016). Organic lead compounds are metabolized to ionic lead both in humans and animals, when the toxicity associated with inorganic lead is expected to be exerted (IARC, 2016). Table 1 summarizes some characteristics of lead.

Erythrocytes have high affinity for lead, and over 90% of what is absorbed is bound in the blood stream just after exposure. With age, lead is deposited in bone tissue, with a half-life of 10–30 years (WHO, 2010a). For the adult population, the cardiovascular and renal systems are the most critically affected by lead exposure, while for infants and children the effects on the central nervous system are the most critical (Sanders et al., 2009; EFSA, 2010; JECFA, 2011b). Encephalopathy, decreased nerve conduction, and cognitive deficits may occur in children with blood lead concentrations lower than the level that would induce similar effects in adults (ATSDR, 2007b). The particular vulnerability of fetuses and infants to the neurotoxicity of lead may be due in part to the immaturity of the blood-brain barrier, and to the lack of the high-affinity lead-binding protein in astroglia, which trap divalent lead ions in adults (Lindahl et al., 1999; EFSA, 2010; Schnaas et al., 2006). The various molecular, intracellular and cellular mechanisms that cause lead neurotoxicity also include the induction of oxidative stress, and interference in enzyme calcium dependents (eg. nitric oxide reductase), which amplify apoptosis of neurons (Nemsadze et al., 2009).

Gulson et al. (1997), using lead isotopic ratios of immigrant women arriving in Australia and of the local population, showed that mobilization of lead from bone contributed significantly to blood lead levels during the last trimester of pregnancy, a critical time for the development of the central nervous system, exceeding the normal exchange of bone lead stores observed in the non-pregnant condition. These increases were detected among subjects with blood levels <5 μg/dL, and were attributed to a low daily calcium intake, as calcium may reduce mobilization of skeletal mineral stores to supply calcium needs during pregnancy and lactation (Gulson et al., 1998, 2003). Lead skeleton mobilization was even higher during the post-pregnancy period, and was the major source of lead in breast milk, in addition to the diet and other exogenous factors (Gulson et al., 2003).

Various studies have shown the transfer of lead from the mother to the fetus via placenta prenatally, and via breast milk postnatally. In Mexico, Ettinger et al. (2004) found lead concentration in breast milk to be significantly correlated with the levels in umbilical cord and maternal blood lead at delivery, and with maternal blood lead and patella lead at 1 month postpartum. In another study with the same group (Ettinger et al., 2014), the mean mother milk:plasma ratio was 7.7; infant blood lead level (3.4 ± 2.2 μg/dL) increased by 1.8 μg/dL per 1 μg/L milk lead ($p < 0.0001$, $R^2 = 0.3$). Li et al. (2000) also found a significant correlation between lead levels in cord blood and breast milk with those in maternal blood in China.

In a cohort study with 175 children conducted in Mexico, Schnaas et al. (2006) found that lead exposure during the early third trimester of pregnancy can affect child intellectual development, with the strongest effects of lead being on the intelligence quotient (IQ) occurring within the first few micrograms of blood lead levels. IQ tests include a variety of tasks that probe cognitive abilities including memory, verbal and spatial reasoning, planning, learning, and comprehension and use of language (EFSA, 2010). The authors hypothesized that prenatal lead exposure would have a more powerful and lasting impact on child development than postnatal exposure.

Furthermore, a number of cross-sectional and prospective epidemiological studies have related lead blood levels to neurobehavioral effects on infants and children chronically exposed to lead (WHO, 2010a; Miranda et al., 2007; Counter et al., 2008; Roy et al., 2009). Most studies report a 2–4 point IQ deficit for each 10 μg/dL increase in blood lead within the range of 5–35 μg/dL (WHO, 2010a).

Lanphear et al. (2005) examined data collected from 1333 children who participated in seven international population-based longitudinal cohort studies initiated prior to 1995 and who were followed from birth or infancy until 5–10 years of age. There was an inverse correlation between blood lead concentration and IQ scores, and the authors concluded that environmental lead exposure in children who have maximal blood lead levels <10 μg/dL was associated with intellectual deficits. No threshold for these effects was identified, and the dose-response relationship was steeper at low lead exposure than at higher exposure levels.

Based on the various available studies, the JECFA (2011b) and the EFSA (2010) concluded that the previous PTWI of 25 μg/kg bw/day for lead was associated with a decrease of at least 3 IQ points in children, with no evidence of a threshold for critical lead-induced effects. A $BMDL_1$ of 0.50 μg/kg bw/day was established for neurodevelopmental effects in children (Table 1).

2.3. Mercury

Mercury (Hg) is a metal naturally found in the environment in inorganic, organic and elemental (Hg^0) forms. Elemental mercury is used in chlorine gas production and in caustic soda for industrial use, as well as electrical equipment, lamps, thermometers, pressure gauges, barometers, and dental amalgams. Inorganic mercury occurs as salts of its divalent and monovalent cationic forms, mainly chlorine and sulfur (Poulin and Gibb, 2008).

Amalgam fillings are the most important source of exposure to mercury vapor (Hg^0) by the general population, and an association between meconium Hg and IHg in the placenta and the number of dental amalgam fillings has been reported (Ask et al., 2002; Gundacker et at., 2010). The major effect from chronic exposure to IHg is kidney damage, and may include morphological changes, renal tubular damage, regeneration of the tubular epithelium, and proximal tubular necrosis (WHO, 2003).

Methylmercury (MeHg) is formed in nature by methylation of inorganic mercury by reducing sulfate aquatic bacteria. The MeHg has a lipophilic property and can be absorbed by plankton, which is eaten by fish and shellfish with greater concentrations occurring at higher trophic levels of the food chain (Polak-Juszczak, 2012; Pouilly et al., 2013). While less than 15% of IHg is absorbed by the gastrointestinal tract, about 95% of MeHg ingested is absorbed, and diffuses to various tissues of the body, including kidney and brain (CDC, 2009). Various studies show that the consumption of fish and other foods of marine origin contributes significantly to mercury levels in human hair, including children and their mothers (Gundacker et al., 2010; Castano et al., 2015).

Ethylmercury (EtHg), an organic mercury compound, is the major component of Thimerosal, a preservative present in various vaccines administered to expecting women and babies, mainly in developing countries. Thimerosal is injected intramuscularly, with approximately 100% absorption (Dorea et al., 2013), and a half-life in blood of 20 days in adults and 7 days in infants, much lower than that for methylmercury (about 70 days; Clarkson et al., 2003). EtHg, as well as MeHg, have been detected in blood samples of babies and neonates immediately after vaccination (Pichichero et al., 2008). Animal

models demonstrate that EtHg is less neurotoxic than MeHg, but more studies are needed to demonstrate whether repeated doses of EtHg in combination with different MeHg background exposures have consequences in fetuses and infants, particularly due to possible additive and synergistic effects (Dorea et al., 2013).

While inorganic mercury is usually free in plasma, MeHg tends to bind to hemoglobin in red blood cells (RBCs), with about 1% bound to glutathione (GSH) (Oliveira et al., 2014). MeHg can enter mammalian cells using a molecular mimicry mechanism. After forming a stable bond with cysteine, the MeHg-Cys complex is transported by the L-type large neutral amino acid transporter (LAT-1), which is important for the high Hg levels found in the brain after exposure (Farina et al., 2011).

The mechanisms involved in the neurotoxicity of MeHg are not completely understood, but Farina et al. (2011) identified three interrelated events that are important for MeHg-induced neurotoxicity: $Ca2+$ dyshomeostasis, glutamate dyshomeostasis, and increased reactive oxygen species (ROS) generation (oxidative stress). *In vivo* studies show that MeHg can alter the expression of genes involved in small GTPase signaling pathways regulating cell growth and proliferation, and can induce mitotic arrest and caspase-dependent apoptosis in developing brains (see review by Antunes dos Santos et al. (2016)). In a cohort study with 138 mother-infant pairs, Cardenas et al. (2015) showed that in utero exposure to mercury can affect leukocyte composition and may disrupt the epigenome even at low levels. Furthermore, exposure to both arsenic and mercury in utero may interact jointly to affect the epigenome by hypermethylating relevant CpG regions (cytosine followed by guanine) having the potential to influence neurodevelopment and other childhood health outcomes.

MeHg crosses the blood brain barrier and the placenta, and may affect the neurological development of fetuses. Mercury concentrations in cord blood correlate well with fetal brain mercury concentrations during the third trimester, indicating methylmercury exposure during late pregnancy (Poulin and Gibb, 2008; WHO, 2010b). Mercury levels are higher in umbilical cord-blood than in the blood of mothers (Stern and Smith, 2003). Oskarsson et al. (1998) reported a higher plasma clearance and a larger distribution volume for methylmercury in lactating mice than in non-lactating mice, probably due to the increased biliary excretion, greater blood/plasma volume and lower plasma protein content during lactation. The milk mercury excretion in mice over 9 days was approximately 4% and 8% of the administered dose of methylmercury and inorganic mercury, respectively.

Sakamoto et al. (2002) showed a lower risk of MeHg exposure by infants during lactation among the high fish-consuming Japanese population. The geometric mean of red blood cells (RBC)-Hg in umbilical cords was about 1.4 times higher than in mothers, with a strong correlation between these two parameters. All the infants showed declines in RBC-Hg during a 3-month breast-feeding period, probably due to the low Hg transfer through breast milk, and the rapid growth of infants after birth. The authors concluded that the risk was especially high during gestation but may decrease during breast-feeding.

Studies to investigate the outcome of prenatal exposure to MeHg and adverse neurological effects on children have reached different conclusions. In a study conducted on Faroe Island (North Atlantic), whose population has a high consumption of pilot whale meat, mothers' exposure to mercury was assessed through mercury concentration in cord blood and hair (Grandjean et al., 1997). Tests applied to 917 children of about 7 years indicated neuropsychological dysfunctions mainly related to language attention and memory, with the association remaining even after the exclusion of children whose mothers' hair mercury concentrations were above 10 μg/g. In general, a delay in development at 6 months was observed in children with higher levels of mercury. On the other hand, a study conducted with 771 mother-child pairs in the Seychelles Islands (Indian Ocean) found no adverse neurodevelopmental outcomes at 66 months of age associated with prenatal or postnatal MeHg exposure and a high fish consumption diet (Davidson et al., 1998). A follow-up study was conducted with this Seychelles population (up to 19 years old) and no correlation was found with effects on the neurological (Myers et al., 2003; Davidson et al., 2011) and auditory functions (Orlando et al., 2014). A cohort study conducted with 492 Italian babies with low levels of mercury (1 μg/g in hair, 0.33 μg/L in breast milk) found that fish consumption and mothers' IQs were significantly associated with neurodevelopment performance of babies at 18 months, but not with mercury exposure (Valent et al., 2013).

In a study conducted in the Amazon region of Brazil, Marques et al. (2014) found higher levels of MeHg in the hair of fishing village children in comparison with those living in the vicinity of tin-ore kilns and smelters who had higher neurodevelopment delays due to high lead exposure, as discussed above. A deficit in neurodevelopment was found in children with higher levels of EtHg in hair. However, another study conducted by the same group evaluating 194 children living near a tin mine in the same region (Marques et al., 2015) found that hair EtHg and maternal consumption of fish were not associated with low neurodevelopment scores.

Based on the available epidemiological studies, including those conducted by Grandjean et al. (1997) and Davidson et al. (1998), the JECFA established a PTWI of 1.6 μg/kg bw for MeHg in child-bearing-aged women due to the possibility of pregnancy and to protect the fetus (JECFA, 2004). In 2010, the JECFA withdrew the previously established PTWI of 5 μg/kg bw for THg, and established a PTWI of 4 μg/kg bw for IHg (JECFA, 2011a).

2.4. Cadmium

The predominant commercial use of cadmium is in the production of batteries, dyes, coatings, plastic stabilizers, and ironless alloys (CDC, 2009). Cadmium in food may originate from contaminated soil which, in turn, may have been contaminated by irrigation water, with deposition originating from air pollution, or from phosphate or manure fertilizer. The highest mean concentrations can be found in edible offal, legumes, cereals and potatoes (0.02–0.13 mg/kg; EFSA, 2009b). Tobacco leaves accumulate high levels of cadmium from the soil, and cigarette smoke is the major source of exposure for smokers (CDC, 2009; ATSDR, 2012). Recent studies have also shown that jewelry and toys can be a source of exposure to cadmium (Guney and Zagury, 2012).

Cadmium is classified by IARC as carcinogenic to humans (Group 1), and causes lung cancer in exposed workers (Table 1), with some evidence of prostate cancer (IARC, 2016). The gastrointestinal tract absorbs 5–10% of ingested cadmium, but several factors may affect absorption, including vitamin D, calcium or iron deficiency, metal-metal interactions with iron, lead and chromium, and metal-protein interactions such as metallothionein and interaction with glutathione (ATSDR, 2012; CDC, 2009). Cadmium absorption may increase with iron deficiency, which may contribute to higher absorption of cadmium by women (CDC, 2009). The placenta may act as a partial barrier to fetal exposure to cadmium, as the concentration in cord blood is about half of that in maternal blood; cadmium levels in human milk are 5–10% of the levels in blood (ATSDR, 2012). Cadmium and lead absorption increases in early childhood and with iron deficiency, given the increase in the number of carriers shared by all 3 metals in the duodenum (Sreedharan and Mehta, 2004). Kippler et al. (2009) found a significant positive association between cadmium concentration in erythrocytes and in breast milk (BM), and a breast milk-plasma ratio of approximately 3–4, indicating no barrier against cadmium

transport from plasma to breast milk. BM-Cd was positively associated with manganese ($r(s)=0.56$; $p<0.01$) and iron ($r(s)=0.55$; $p<0.01$) in breast milk, but not with plasma ferritin. On the other hand, BM-Cd was negatively associated with BM-Ca ($r(s)=-0.17$; $p=0.05$), indicating that cadmium inhibits the transport of calcium to breast milk. The authors concluded that cadmium shares common transporters with iron and manganese for transfer to breast milk, but inhibits secretion of calcium to breast milk.

Absorbed cadmium accumulates mainly in the kidney and liver, with an estimated half-life of 6–38 years, and 4–19 years, respectively, and no direct metabolism is known (ATSDR, 2012). The kidney is the critical target and shows the earliest sign of cadmium toxicity. However, the accumulation of cadmium in the kidney with no apparent toxic effects occurs due to the formation of cadmium-thionein or metallothionein, which is considered non-toxic (ATSDR, 2012). Cadmium can disrupt signaling cascades and lead to a variety of toxic effects, mainly due to its physicochemical similarity with calcium ion (Ca^{2+}), which may disrupt Ca-mediated signaling pathways, possibly through significant changes in the activation of calmodulin and calmodulin-dependent protein kinase II in cell death pathways, such as apoptosis, necrosis or autophagy (Choong et al., 2014).

In 2010, the JECFA withdrew the PTWI for cadmium of 7 μg/kg bw/week set by the Committee in 1988, and established a monthly intake (PTMI) of 25 μg/kg bw due to its long half-life in the body (JECFA, 2011b), corresponding to a weekly intake of 5.8 μg/kg body weight. In 2009, the EFSA recommended a tolerable weekly intake (TWI) of 2.5 μg/kg body weight in order to ensure a high level of protection for all consumers, including exposed and vulnerable subgroups of the population (EFSA, 2009b). This decision was confirmed in 2011 (EFSA, 2012a).

3. Presence of arsenic, lead mercury and cadmium in breast milk

Monitoring breast milk is a non-invasive form of detecting environmental contaminants, having the advantage of allowing the exposure of both the mother and the lactating baby to be assessed at the same time (Hooper and McDonald, 2000; Abballe et al., 2008; CDC, 2010). Two metal analysis techniques are mainly used for different matrices, including milk: atomic absorption spectrometry (AAS) using either flame, cold vapor hydride generator (CVAAS) or electrothermal AAS in graphite furnace (ETAAS), and inductively coupled plasma with mass spectrometry detection (ICP-MS). In most methods, the milk is submitted to microwave acid digestion under controlled temperature and pressure (Kosanovic et al., 2008; Sardans et al., 2010; Amarasiriwardena et al., 2013).

Table 2 summarizes the data for arsenic, lead, mercury and cadmium in breast milk reported by the 75 studies reviewed by this study. Fig. 1 shows the distribution of the studies according to region and metal analyzed. A larger number of studies were conducted in Europe (23), and a lower number in North America (3 studies), with lead the most analyzed metal. In the majority of studies, more than one metal was analyzed in the samples.

The analytical variability and validity of the reported results were not assessed, with the exception of one study conducted in Nigeria (Adesiyan et al., 2011), where the results reported in μg/dL were too high, probably due to a typing or unit error. It is important to be aware that inaccuracies involved in the analytical methods affect the results, particularly at low concentrations (CDC, 2010). Furthermore, positive sample percentages (Table 2) are highly dependent on the limit of detection (LOD) or limit of quantification (LOQ) of the method used, mainly when incidences are low, and may not be comparable. Also, it was not clear in most studies how the samples reported as non-detected or below the LOD/LOQ were treated in estimations of the means. In addition to uncertainty regarding the analytical method, extremely high values found in certain studies may be due to contamination during sample collection and storage, mainly for lead, which is the most abundant toxic metal in the environment.

3.1. Arsenic

For this review, 18 studies published since 2000 that measured levels of arsenic in breast milk were retrieved, six conducted in Asia, six in Europe, and none in Latin America (Table 2). The techniques used to analyze arsenic in milk included CVAAS, ETAAS and ICP-MS, which has the lowest LOD (0.007–0.3 μg/L) (Felip et al., 2014; Miklavcic et al., 2013; Björklund et al., 2012; Fängström et al., 2008; Almeida et al., 2008). Separation of the different arsenic metabolites [As(III), As(V), MA, and DMA] was performed by high performance liquid chromatography coupled to hydride generation and ICP-MS (Fängström et al., 2008).

The highest levels of arsenic in breast milk were found for a district in West Bengal, India (up to 149 μg/L; Samanta et al., 2007), a region with levels of arsenic in water higher than 50 μg/L. Higher levels were found in samples from women who had higher levels of arsenic in urine, hair, and nails. In this population, when breast milk was not sufficient or available, infants drank tube well water as early as the first month after birth, as well as cow/goat milk diluted with water, which increased exposure to arsenic from an early age. The authors found the levels of arsenic in breast milk much lower than in urine (mean of 438 μg/L), which is a much more efficient arsenic excretion route than lactation. Indeed, Fängström et al. (2008) considered the excretion of arsenic through breast milk to be low and concluded that exclusive breastfeeding protects the infant from exposure to arsenic. A similar conclusion was reached by Carignan et al. (2015) in the United States, an area with low levels of arsenic in the water (<1 μg/L). Fängström et al. (2008) also found that arsenic levels in urine were significantly lower in exclusively breastfed children than in those consuming other foods.

Higher mean levels of arsenic were found in colostrum (3.6–14 μg/L; Almeida et al., 2008), decreasing considerably in intermediate and mature milk (Almeida et al., 2008; Islam et al., 2014). Islam et al. (2014) found that arsenic in human milk was weakly correlated with maternal urine levels at 1 and 6 months postpartum ($r=0.13$ and 0.21, respectively; $n=29$ and 25) and did not correlate with infants' urine levels. Fängström et al. (2008) however, found a significant association between the TAs in milk and the levels in the urine of 2–3 month-old babies ($rs=0.64$, $p<0.001$), as well as with arsenic in maternal blood and saliva. Arsenic was essentially present in breast milk as As^{III}, in addition to As^{V}, DMA and MMA, and was the only form present at total arsenic levels ≤ 1 μg/L. The Fängström et al. study was the only one to identify the forms of arsenic present in breast milk, an important piece of information as inorganic arsenic is the only toxicological relevant form of arsenic for humans (IARC, 2016).

3.2. Lead

There are a large number of published studies that have investigated the levels of lead in human breast milk. The first studies date from the early 1980's and had the objective of collecting data from different countries to establish an environmental background level for metals in human fluids (Iyengar, 1984). A WHO-sponsored multicenter study conducted in several countries on four continents found average concentrations of lead in human milk ranging from 2.0 to 16.8 μg/L, and values between 2 and 5 μg/L were considered a reference for populations not occupationally exposed to lead (WHO, 1989).

Table 2
Levels of arsenic, lead, mercury and cadmium in breast milk reported in studies published since 2000.

Country; Reference	Metal	N	% positive	Mean, median* or geometric mean** (range); μg/L or ng/g	Observation
Asia					
Bangladesh; Fängström et al., 2008	As	79	–	1.8 (0.25–19.0)	Mature milk
Bangladesh; Kippler et al., 2009	Cd	123	–	0.14* (< 0.05–1)	2 months pp
Bangladesh; Islam et al., 2014	As	29	–	1.12 (0.5–8.9)	30 days pp
		25	–	0.78 (0.5–2.32)	180 days pp
		19	–	0.7 (0.5–1.68)	270 days pp
China; Li et al., 2000	Pb	165	–	4.7	Colostrum, non occupational
		12	–	52.7	Colostrum, occupational
China; Li et al., 2014	THg	195	–	0.97 (0.42–8.40)	Colostrum
India; Sharma and Pervez, 2005	As	120	82.5	0.6 ± 0.1 to 5.2 ± 3.8	Mean range of various groups
	Pb		87.5	0.1 ± 0.0 to 22.3 ± 18.5	
	THg		87.5	0.1 ± 0.0 to 16.7 ± 11.1	
	Cd		82.5	0.1 ± 0.1 to 3.8 ± 12.9	
India; Samanta et al., 2007	As	226	17.3	17 (< LOD–49)	Area with high levels of arsenic in water
		10	50	3.5 (< LOD–5)	Area with levels of arsenic within WHO limits
India; Isaac et al., 2012	Pb	25	84	13.21 ± 5.2 (9.0–21.0)	Non-industrial area
			88	21.5 ± 4.5 (15–25.5)	Industrial area
Japan; Honda et al., 2003	Cd	68		0.28 ± 1.82** (0.28–1.22)	5 – 8 days pp
Japan; Sakamoto et al., 2012	As	9	–	1.4 (0.4–1.8)	3 months pp
	Pb			0.29 (0.18–0.20)	
	THg			0.47 (0.28–0.77)	
	Cd			0.14 (0.06–0.22)	
Japan; Iwai-Shimada et al., 2015	THg	27	–	0.81 (0.14–1.87)	30 days pp
	MeHg		–	0.45 (0.06–1.2)	
Korea; Lit et al., 2014	THg	195	–	0.97* (0.42–8.40)	
Thailand; Chao et al., 2014	As	45	–	1.50 ± 1.50	1–4 days pp
				0.68 ± 1.09	5–10 days pp
				0.27 ± 1.26	30–35 days pp
				0.16 ± 0.24	60–65 days pp
	Pb	45	–	13.2 ± 3.6 (6.7–22.4)	1–4 days pp
			–	8.92 ± 2.60 (3.52–14.7)	5 to10 days pp
			–	11.7 ± 2.58 (0.76–11.7)	30–35 days pp
			–	2.93 ± 1.70 (0.45–7.8)	60–65 days pp
	Cd	45	–	1.37 ± 0.94	1–4 days pp
			–	0.65 ± 0.36	5–10 days pp
			–	0.49 ± 0.25	30–35 days pp
			–	0.34 ± 0.19	60–65 days pp
Taiwan; Chien et al., 2006a	Pb	35	–	8.59 ± 10.9	Chinese herb mothers (9)
				9.94/2.34	Colostrum/mature
		37	–	6.84 ± 2.68	Non consumers (7)
Taiwan; Chien et al., 2006b	THg	56	100	2.02 (0.24–9.45)	Colostrum – urban population
		12	100	2.04 (0.26–8.62)	Colostrum – fishing villages
Europe					
Finland; Kantola and Vartiainen, 2001	Cd	165	–	0.095 ± 0.12	Samples collected in 1987
		74	–	0.040 ± 0.06	1993–1995 samples
Austria; Gundacker et al., 2002	Pb	116		1.63 ± 1.66	6.6 ± 6 days pp
	THg	116		1.59 ± 1.2	
Austria; Gundacker et al., 2010	THg	21	62	0.2 (0.1–2) 100% inorganic	2–8 weeks pp
Croatia, Slovenia, Greece, Italy; Miklavcic et al., 2013	As	123	–	0.2 (0.4–11.9)	Croatian
		287	–	0.04 (0.04–2.9)	Slovenes
		30	–	0.8 (0.3–4.8)	Greek
		602	–	0.3 (0.04–12)	Italians
	THg	125	–	0.2	Croatian
		284	–	0.2	Slovenes
		44	–	0.6	Greek
		605	–	0.2	Italians
	MeHg	26	100	56% of the mean THg	Croatian
		7	100	47% of the mean THg	Slovenes
		21	100	7% of the mean THg	Greek
		224	100	60% of the mean THg	Italians
Cyprus; Kunter et al., 2016	As	50	–	0.73 ± 0.58 (0.03–1.97)	
	Pb		–	1.19 ± 1.53 (0–4.9)	
	THg			0–0.01	
	Cd			0.45 ± 0.23 (0.12–0.08)	
Germany; Sternowsky et al., 2002	As	187	17.6	0.15* (< 0.3–2.8)	2–90 days pp From 36 mothers
Greece; Leotsinidis et al., 2005	Pb	180	58,5	0.48 ± 0.60 (< 0.2–2.36)	Colostrum
		95	63.6	0.15 ± 0.25 (< 0.2–0.94)	Intermediate milk
	Cd	180	89	0.19 ± 0.15 (< 0.01–0.70)	Colostrum
		95	91.9	0.14 ± 0.12 (< 0.01–0.49)	Intermediate milk
Italy; Abballe et al., 2008	THg	29	–	2.6–3.0	Venice

Table 2 (continued)

Country; Reference	Metal	N	% positive	Mean, median* or geometric mean** (range); μg/L or ng/g	Observation
		10	–	3.53	Rome
	Pb	29	–	0.97–1.1	Venice
		10	–	0.85	Rome
	Cd	39		< 0.5	Venice and Rome
Italy; Valent et al., 2013	THg	492	–	0.33 (0–28.3)	Mature milk
	MeHg	182	–	0.17 (0.01–1.09)	
Italy; Felip et al., 2014	As	63	0	< 3	Samples were mixed in 7 pools, according to the region
	Pb		100	2.59–5.99	
	THg		0	< 0.3	
	Cd		0	< 0.1	
Poland; Winiarska-Mieczan, 2014	Pb	320	–	6.33 ± 4.61 (0.49–12.0)	All milk types
	Cd	320	–	2.1 (0.21–7.4)	
Poland; Olszowski et al., 2016	Cd	51	–	0.11 ± 0.07 (0.01–0.33)	
Portugal; Almeida et al., 2008	As	34	–	7.8 ± 2.2 (3.6–14.0)	Colostrum
	Pb	34	–	1.55 ± 1.38 (0.06–5.43)	Colostrum
	As	19	–	5.8 ± 1.1 (4.2–7.8)	Intermediate milk
	Pb	19	–	0.94 ± 1.05 (0.07–4.03)	Intermediate milk
Spain; García-Esquinas et al., 2011	Pb	100	93	15.56 (12.92–18.72)	Mature milk
	THg	100	98	0.53 (0.45–0.62)	Mature milk
	Cd	100	96	1.31 (1.15–1.48)	Mature milk
Sweden; Bjornberg et al., 2005	THg	19	–	0.29* (0.06–2.1)	Colostrum
		20	–	0.14* (0.07–0.37)	6 weeks
		19	–	0.2* (0.06–0.4)	13 weeks
Sweden; Björklund et al., 2012	As	60	–	0.55 ± 0.70 (0.04–4.6)	Mature milk
	Pb	60	–	1.5 ± 0.9 (0.74–6.40)	Mature milk
	Cd	60	–	0.09 ± 0.04 (0.02–0.27)	Mature milk
Turkey; Turan et al., 2001	Pb	30	100	14.6 ± 5.5 (8.8–35.4)	Colostrum
	Cd		100	1.7 ± 1.7 (1.2–9)	
Turkey; Yalçin et al., 2010	THg	44	–	3.42 ± 1.66 (0.35–6.9)	All milk types
Turkey; Örün et al., 2011	Pb	144	95	20.6 (< LOQ–1515.0)	2 months pp
	Cd	144	60	0.67 (< LOQ–43.0)	
Turkey; Örün et al., 2012	THg	144	18	25.8 ± 44.6 (1.7–236) positive samples	Mature milk
Turkey; Gürbay et al., 2012	As	64	0	< 7.6	2–5 days pp
	Pb	64	93.8	391 ± 269 (4.35–1020)	
	Cd	64	1.6	4.62 (< 0.34–4.62)	
Slovakia; Ursinyova and Masanova, 2005	Pb	158	–	4.7 (nd – 24.4)	4 days pp
	Cd		–	0.43 (nd – 1.7)	
	THg		–	0.94 (nd – 4.74)	
Latin American					
Brazil; Boischio and Henshel, 2000	THg	44		5.7 ± 5.9 (nd – 24.8)	Amazonian riverines
Brazil; Anastácio et al., 2004	Pb	38	–	2.8 ± 2.5	Mature milk
Brazil; Costa et al., 2005	THg	23	86.9	5.73 ± 5.43	Federal District
Brazil; Koyashiki et al., 2010	Pb	92	–	2.9 ± 1.1 (1.0–8.0)	Mature milk
Brazil; Gonçalves et al., 2010	Cd	80	100	2.3 (0.02–28.1)	Colostrum
Brazil; Andrade et al., 2013	Pb	70	–	1.46 ± 1.28 (0.01–4.82)	Up to 6 months pp
Brazil; Cunha et al., 2013	THg	142	93.7	6.7 ± 6.45 (< 0.76–22.7)	Federal District, 15–90 days pp; 18 mothers
Brazil; Marques et al., 2013	Pb	37	–	12.6 ± 8.16 (0.9–29.4)	Close to a tin mine; 15 days up to 12 pp
		45	–	4.30 ± 4.01 (0–16.2)	Fishing village; 1–24 pp
Brazil; Vieira et al., 2013	THg	82	–	0.36 (0.09–3.74)	Amazonian urbans
	MeHg	45	–	0.12 (0.01–0.47)	
	THg	75	–	2.3 (0.12–6.48)	Amazonian riverines
	MeHg	46	–	0.87 (0.11–3.40)	
Brazil; Cardoso et al., 2014	Pb	58	–	0.260 (< 0.05–0.69)	Minas Gerais
	THg		–	< 0.200 (< 0.20–6.11)	
	Cd		–	0.770 (< 0.05–6.57)	
Brazil; Marques et al., 2014	Pb	51	–	8.2 (0.9–29.4)	Amazonian tin ore smelters and kilns
		45	–	2.5 (0.7–16.2)	Amazonian fishing village
Brazil; Santos et al., 2015	THg	15	100	59.41 (4.56–104.1)	Amazonian riverine
Ecuador; Counter et al., 2004	Pb	90	–	4.6 (0.4–20.5)	Women occupationally exposed
Ecuador; Counter et al., 2014	Pb	22	–	3.73 ± 7.3 (0.049–28.4)	Women occupationally exposed
México; Amarasiriwardena et al., 2013	Pb	200	–	(0.2–6.7)	Mature milk
Mexico; Ettinger et al., 2004, 2006	Pb	310		1.4 ± 1.1 (0.2–8.0)	1 month pp
		224		1.2 ± 1.0 (0.2–6.8)	4 month pp
		195		0.9 ± 0.8 (0.2–4.8)	7 month pp
Mexico; Gaxiola-Robles et al., 2013, 2014	THg	108	80.6	2.52 (0.03–24.9)	
		36	80.6	1.96 ± 2.01	1st gestation
		36	88.9	2.61 ± 4.32	2nd gestation
		36	88.9	3.00 ± 3.23	3rd gestation
Mexico; Gaxiola-Robles et al., 2014	As	108	24	0.01*(0.01–13.8)	7 days pp
Mexico; Ettinger et al., 2014	Pb	81	–	0.8 ± 0.7 (0.6–39.8)	Mature milk
Middle east					
Iran; Rahimi et al., 2009	Pb	44		10.4 ± 9.7 (3.2 – 24.7)	Industrial area

Table 2 (continued)

Country; Reference	Metal	N	% positive	Mean, median* or geometric mean** (range); μg/L or ng/g	Observation
	Cd	44		2.4 ± 1.5 (0.62 – 6.3)	
Iran Behrooz et al., 2012	THg	34		0.12 ± 0.06 (nd–1.73)	Industrial area
		18		0.15 ± 0.22 (nd–1.21)	Coast area
		28		0.86 ± 0.26 (0.02–5.86)	Industrial and agricultural area
Iran; Goudarzi et al., 2013	Pb	37	–	7.11 ± 3.96 (3.06–19.5)	First 6 weeks pp
	THg	37	–	0.92 ± 0.54 (0.0–2.7)	
	Cd	37	–	1.92 ± 1.04 (0.45–5.87)	
Iran; Okati et al., 2013	THg	82	–	0.43 (0.0–2,45)	Under 6 months of lactation
Saudi Arabia; Al-Saleh et al., 2003	Pb	168	94,8	25.1 ± 38.8 (< 1.2–355)	Urban area
		194		37.3 ± 50.3 (< 1.2–490)	Agricultural area
	THg	168	87	4.15 ± 5.05 (< 0,2–47.2)	Urban area
		194		2.19 ± 2.61 (< 0.2–25.62)	Agricultural area
	Cd	150	95.1	1.18 ± 1.14 (< 0.123–11.7)	Urban area
		194		2.16 ± 19 (< 0.123–9.2)	Agricultural area
Saudi Arabia; Al-Saleh et al., 2013; 2015	THg	331	97.3	0.97 ± 0.665 (0.18–6.44)	3 – 12 months pp
United Arab Emirates; Abdulrazzaq et al., 2008	As	205	–	0.89 ± 0.078 (0.001–0.283)	From 38 mothers.
	Pb	205	–	0.019 ± 0.055 (0–0.55)	3 months pp
	THg	205	–	0.008 ± 0.025 (0–0.023)	
	Cd	205	–	0.003 ± 0.008 (0–0.115)	
United Arab Emirates; Kosanovic et al., 2008	As	120	–	0.196 + 0.032 (0.02–0.65)	–
	Pb	120	–	1.51 + 0.32 (0.025–2.41)	
	THg	120	–	0.115 + 0.05 (0.04–0.18)	
	Cd	120	–	0.27 + 0.04 (0.023–1.19)	
Palestine; Shawahna et al., 2016	Pb	89	100	4.0* (2–12)	15–210 days pp
North America					
Canada; Hanning et al., 2003	Pb	25	–	2.1 ± 1.7	Mature milk
United States; Sowers et al., 2002	Pb	15	–	6.1 ± 1.0	45 days pp
		15	–	5.6 ± 1.1	3 months pp
		15	–	5.9 ± 1.0	6 months pp
		15	–	4.3 ± 1.6	12 months pp
United States; Carignan et al., 2015	As	9	55.6	0.31* (< 0.22–0.62)	1.7–7 months pp
Other regions					
Indonesia, Tanzania and Zimbabwe; Bose-O'Reilly et al., 2008	THg	46	71.7	1.87 (< 1–149)	Mining area (occupational and non-occupational)
Ghana; Bentum et al., 2010	As	20	60	1.54 ± 1.94 (nd–6.22)	–
	Pb	20	40	4.8 ± 9.0 (nd–32.0)	–
	Cd	20	40	1.3 ± 2.9 (nd–12.3)	–
Nigeria; Adesiyan et al., 2011	Pb	180	–	83.1–87.1	Values reported as μg/dL[a]
	Cd	180	–	94.8–97.8	
Egypt; Moussa, 2011	Pb	30	–	1.7 ± .085 (0.26–3.33)	Nasr city
			–	5.92 ± .296 (4.2–7.74)	Helwan
			–	5.11 ± .25 (3.41–6.88)	El Khanka
	Cd	30	–	0.638 ± 0.032 (0.485–0.865)	Nasr city
			–	1.84 ± 0.092 (1.02–2.54)	Helwan
			–	2.56 ± 0.12 (1.25–3.86)	El Khanka
Australia; Gulson et al., 2001	Pb	72		0.55** 0.09–3.1)	First 6 months pp; samples from 9 mothers
Faroe Island; Needham et al., 2011	Pb	15	–	8.5*	–
	THg	15	–	2.31*	
	Cd	15	–	0.25*	

pp: postpartum; nd: non detected.

[a] Most likely the unit is not correct.

* is either mean or median

** is geometric mean

In the present review, we were able to retrieve 43 studies that analyzed lead in breast milk samples collected in different regions of the world, most of which also included analyses of the other metals (Table 2). The number of samples analyzed in these studies varied from less than 50 in Italy (Abballe et al., 2008) to over 300 in Mexico (Ettinger et al., 2006) and Saudi Arabia (Al-Saleh et al., 2003, 2015). In most studies, lead was analyzed by ETAAS, with a wide range of reported LODs (0.04–3.4 μg/L) (Marques et al., 2014; 2013; Winiarska-Meiczan, 2014; Goudarzi et al., 2013; Chao et al., 2014; Gürbay et al., 2012; Garcia-Esquinas et al., 2011; Abballe et al., 2008; Chien et al., 2006a; Leotsinidis et al., 2005; Ursinyova and Masanova, 2005; Al-Saleh et al., 2003). The study with the lowest LOD (0.01 μg/L) used isotopic dilution ICP-MS (Ettinger et al., 2014), while the other ICP-MS LODs ranged from 0.03 to 3 μg/L (Cardoso et al., 2014; Felip et al., 2014; Amarasiriwardena et al., 2013; Björklund et al., 2012; Örün et al., 2011; Almeida et al., 2008; Koyashiki et al., 2010; Sowers et al., 2002). The highest mean lead levels were found in Turkish milk colostrum samples (391 μg/L from Gürbay et al. (2012); Table 2).

Lead levels in colostrum are usually higher than in mature milk due to their greater protein content (Rothenberg et al., 2000). Chien et al. (2006b) found a significant decline in lead levels during lactation among Taiwanese mothers, with the mean of 9.9 μg/L in colostrum dropping to 2.3 μg/L in mature milk at 2 months postpartum (Table 2), with an estimated lead half-life of 33–35 days. Another study from the same research group found that milk from mothers who consumed traditional Chinese herbs, which can contain over 300 μg/g of lead, had significantly higher

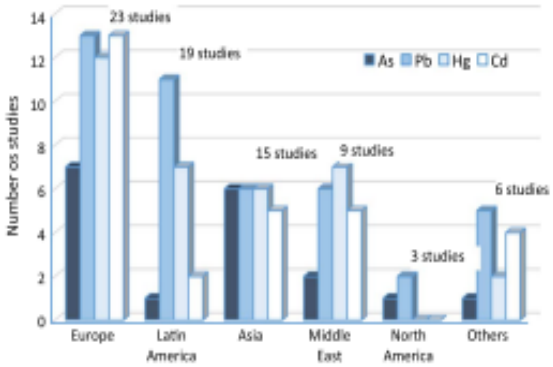

Fig. 1. Summary of the number of studies that analyzed arsenic, lead, mercury and cadmium published since the year 2000, according to the region. Others include Indonesia, Tanzania and African countries, Australia and Faroe Island. Most of the studies analyzed more than one metal. Literature search on Pubmed, Science Direct and Google Scholar databases (last on June 2016) using the keywords "human milk", "breast milk" and "breast milk", associated with "metal", "arsenic", "lead", "mercury" or "cadmium". Additional papers were identified in published reviews related to contaminants in breast milk.

levels of lead than milk from non-consumers (Chien et al., 2006a). Ettinger et al. (2006) also found a significant decrease in breast milk lead levels 1–7 months postpartum in Mexico (Table 2).

Örün et al. (2011) reported a 2-month postpartum sample in Ankara that contained 1515 μg/L, but no individual or environmental factor was identified that could justify such a high level (mean level was 20.6 μg/L). A significant correlation was found between mothers with a history of anemia, and the higher level of lead in breast milk. Another study, conducted ten years earlier in the same city, found a much lower level in colostrum (14.6 μg/L; Turan et al., 2001), but higher than that found in Greece (0.48 μg/L; Leotsinidis et al., 2005 (Table 2)). In a study conducted in Saudi Arabia, Al-Saleh et al. (2003) found a significant correlation between lead levels in breast milk ($n=362$) with average duration of lactation for all births and fish consumption (lower consumers had higher levels). The milk of mothers living in agricultural areas had higher lead levels than those in urban regions, although the difference was not significant.

In nature, lead occurs mostly in ore deposits along with other minerals, particularly zinc, accounting for about 20% of total primary lead supplies. Mining, smelting and refining of lead are known to cause contamination of the surrounding environment (ATSDR, 2007a), and to impact levels of the metal in the human body. In fact, two studies conducted in the north of Brazil showed significantly higher lead levels in milk from women living near a tin smelter compared with those living in a fishing village (Marques et al., 2013, 2014; Table 2). Marques et al. (2014) also found higher Pb levels in breast milk associated with longer residence periods in a contaminated region, and a significant association of higher levels with neurodevelopmental delays in 24-month old children living near tin ore smelters. Isaac et al. (2012) found higher lead mean levels in breast milk of women living in industrial areas of Southern India (21.5 μg/L) compared with those in non-industrial areas (13.2 μg/L), showing the impact of environmental contamination of lead by industrial activity. In China, mean level of lead in colostrum from occupationally exposed women were about 15 times higher than the mean for non-exposed women (4.7 and 52.7 μg/L, respectively; Li et al., 2000; Table 2).

3.3. Mercury

A total of 34 studies published since 2000 that analyzed mercury in breast milk (THg) were retrieved for this review, five of which also analyzed MeHg and/or IHg (Table 2). The most widely used technique to analyze THg was CVAAS, with limit of detection ranging from 0.06 to 5 μg/L (Boishio and Henschel, 2000; Al-Saleh et al., 2003; Costa et al., 2005; Bose O'Reilly et al., 2008; Abballe et al., 2008; Gundacker et al., 2010; Vieira et al., 2013; Valent et al., 2013; Iwai-Shimada et al., 2015). MeHg was analyzed by gas chromatography coupled with electron capture detector (Miklavcic et al., 2013; Valent et al., 2013; Iwai-Shimada et al., 2015) or MERX™, which uses atomic fluorescence spectrophotometry (Vieira et al., 2013).

Seven studies were conducted in Latin America, six of which in Brazil, mostly in the Amazon region, where THg in breast milk reached 104 μg/L (mean of 59.4 μg/L; Santos et al., 2015). Overall, breast milk samples from high fish consumers in the Amazon (riverine community) had higher mercury levels compared to an urban population in the same region. Vieira et al. (2013) found this difference significant for both THg (2.3 and 0.36 μg/L, respectively) and MeHg (0.87 and 0.12 μg/L). Among urban mothers with low fish consumption (and with relatively higher dental amalgam fillings), the proportion of IHg in milk was higher (85%) than for riverine communities (62%). In another study conducted earlier in the same region, these levels were about 6 μg/L (Boishio and Henshel, 2000), similar to those found in two studies conducted in the Federal District (DF) of Brazil (Costa et al., 2005; Cunha et al., 2013), located in the Midwest region of the country and with a low fish consuming population. Cunha et al. (2013) found no significant correlation between fish consumption and THg levels, although a significant increase was found after the mothers had eaten a meal with salmon (day 75 postpartum). Although the levels of THg found in one Amazonian study and those found in the DF study were similar, most of the mercury present in the DF milk was most likely present as IHg, while in the Amazon the MeHg found was the predominant form, reflecting the high fish consumption in this region. Much lower THg levels were found by Cardoso et al. (2014) among mothers living in the Brazilian state of Minas Gerais (mean < 0.2 μg/L), also a low fish consuming region. Costa et al. (2005) also found that THg levels in breast milk in the Federal District correlated well with the number of amalgam fillings of the mothers.

In a study also conducted with a Mediterranean population (Miklavcic et al., 2013), the levels of THg in breast milk were similar in Croatia, Slovenia and Italy (0.2 ng/g; Table 2). Although Slovenian women consumed the least amount of fish (mean consumption of 25 g/day), they had the highest number of amalgam fillings, which may have contributed to the total excreted mercury. The levels in Greece (39 g fish/day) were 3 times higher than in the other countries (0.6 ng/g), but only 7% was present as MeHg, although this percentage ranged from 47 to 60% in the other countries (Table 2). These results were unexpected as fish consumption is the main external source of MeHg.

An extensive study conducted by Valent et al. (2013) confirmed that in Italy (2.3 servings of fish/week) most of the mercury in breast milk was present as MeHg (mean of 58%). This percentage was similar to the one found in Japan (Iwai-Shimada et al., 2015), a high fish-consuming population (about 71 g/day, in average), with higher mercury concentrations detected in breast milk (mean of 0.81 μg/L). These authors found a correlation between THg or MeHg in breast milk and fish consumption only when the levels were adjusted for the milk lipid content.

Cunha et al. (2013) found no significant changes in the THg levels 15–90 days postpartum, all mature milk samples. In Sweden, Bjornberg et al. (2005) found a significant decrease in THg between day 4 (colostrum) and 6 weeks after delivery (median of 0.29 and 0.14 μg/L, respectively), remaining unchanged thereafter (Table 2). At 13 weeks, THg in breast milk was significantly associated with IHg in maternal blood ($r_s=0.61$; $p=0.006$) and MeHg in infant blood ($r_s=0.55$; $p=0.01$). The authors concluded that exposure to mercury was higher before birth than during

breastfeeding, and that MeHg seems to contribute more than IHg to postnatal infant exposure via breast milk.

Gundacker et al. (2002) found greater THg levels in the breast milk of Austrian mothers under 60 kg and in those who had premature infants. Similar to what was reported by Cunha et al. (2013), frequent consumption of cereals correlated well with higher mercury levels. In a later study, Gundacker et al. (2010) found that all mercury detected in breast milk from Austrian mothers was in inorganic form (Table 2).

In Mexico, Gaxiola-Robles et al. (2013) found a significant correlation between breast milk THg (80.8% of positive samples; mean levels of 2–3 μg/L), fish consumption and exposure to tobacco (active and passive smokers). These correlations were not confirmed in studies conducted in Turkey with a population with lower incidence of positive sample (18–44%) but higher levels of THg (mean of 3.4 and 20.6 μg/L; Yalçin et al., 2010; Örün et al., 2012).

3.4. Cadmium

Twenty nine studies published since 2000 that analyzed cadmium in breast milk were found in the databases, ten conducted in Europe and none in North America (Table 2). Cadmium was predominantly analyzed by ETAAS, with LODs ranging from 0.01 to 0.5 μg/L (Winiarska-Mieczan, 2014; Goudarzi et al., 2013; Chao et al., 2013; Gürbay et al., 2012; Garcia-Esquinas et al., 2011; Aballe et al., 2008; Leotsinidis et al., 2005; Ursinyova and Masanova, 2005; Al-Saleh et al., 2003) or by ICP-MS, with LODs in the range of 0.0027–0.3 μg/L (Cardoso et al., 2014; Felip et al., 2014; Björklund et al., 2012; Örün et al., 2011).

In most studies, mean levels were below 2 μg/L, with the maximum mean and highest levels found for Turkey (4.6 and 43 μg/L; Gürbay et al. (2012), Örün et al. (2011) and Table 2). In Brazil, Gonçalves et al. (2010) found a significant correlation between cadmium levels in colostrum and the consumption of rice, carrots and chayote, while Cardoso et al. (2014) found correlations between cadmium concentration profiles in mature breast milk (0.77 μg/L), soil (4.50 mg/kg) and water (12.5 μg/L).

Cadmium levels in breast milk decreased over the postpartum period (Chao et al., 2013; Leotsinidis et al., 2005), being higher among smoking women (Rahimi et al., 2009), as expected, and housewives, probably due to exposure to dust particles during housekeeping activities (Örün et al., 2011). Honda et al. (2003) found that cadmium in breast milk was significantly correlated with urinary concentration, reflecting mothers' body burden, and inversely correlated with calcium concentration in breast milk, an indication that it affects calcium secretion in this body fluid.

4. Risk assessment of infants to arsenic, lead, mercury and cadmium through breast milk

The process of assessing risk to a chemical may be divided into four steps: 1. hazard identification; 2. hazard characterization; 3. exposure assessment and; 4. risk characterization. The outcome of the first two steps indicates the most critical adverse effects and establishes the health-based guidance values, respectively. They are mostly based on laboratory animal data, but may also include human epidemiological studies, especially for metals. For mercury and cadmium, which have a threshold dose (no-observed-adverse-effect level, NOAEL), values may be expressed as PTWI (JECFA, 2011a, 2011b), tolerable weekly intake (TWI; EFSA, 2009a), reference dose (RfD; Rice, 2004) or minimal risk level (MRL; ATSDR, 2016). As discussed above, the previous PTWI for arsenic and lead were found not to be protective of human health (no-threshold dose), and BMDLs were established for different toxicological endpoints for these metals (Table 1).

In the exposure assessment step, the concentration of a substance (mean, median or other value) is multiplied by the consumption of the food in question (generally the mean consumption), and the product is then divided by the body weight of a given population (IPCS, 2009). When the chronic exposure involves more than one food, the total intake is the summation of the intakes of each food.

$$\text{Intake} = \frac{\text{consumption } X \text{ concentration}}{\text{body weight}}$$

In the risk characterization step for cadmium and mercury, a conclusion regarding a potential risk to human health may be reached by comparing the estimated intake with the health-based guidance value, and expressing it as either a percentage or a hazard index (HI). Risk may exist when the percentage is higher than 100 or if the HI is greater than 1. For arsenic and lead, risk characterization may be performed by estimating the margin of exposure (MOE), which is defined as a reference point derived from the dose response relationship, such as a BMDL, divided by the estimated human intake. A MOE should be as high as possible so as not to represent a public health concern (EFSA, 2005). It is important to emphasize, however, that the MOE is not a quantification of risk for a chemical, but gives an indication of the level of concern (Benford, 2016).

The uncertainties of the risk assessment depend on the quality of the data used in each step of the process (IPCS, 2009). Uncertainties regarding the PTWI, RfD or BMDL arise from the toxicological database and the dose-response models used in the estimations (Rice, 2004). Uncertainties in exposure assessments normally regard food consumption, body weight, and the concentration data used (whether the sample is representative of the population, the number of samples analyzed, the analytical method used, and how the non-detected samples are considered in the estimation of the mean).

Some of the studies shown in Table 2 also estimated exposure and assessed the risk of infants to arsenic, lead, mercury and/or cadmium through breastfeeding. In order to investigate a wider exposure scenario, when this information was not available, intakes were also estimated using the incidence data provided in some studies, with a milk consumption of 750 mL and a body weight of 5.5 kg, as given by da Costa et al. (2010) for a 2–3 month infant. The objective was to estimate a range of exposure levels for each metal in the various regions (low to highest exposure levels). Fig. 2 summarizes the mean/median intakes of arsenic, cadmium, lead and mercury by one- to six-month infants from different regions estimated from the studies. Details of the studies are discussed below. All intakes were expressed in μg/kg/week to facilitate comparison between metals. Additionally, exposure assessments for arsenic, lead and/or mercury conducted by the EFSA for the European population and by the Committee on Toxicity of the UK Food Standards Agency (COT) are also discussed.

In the context of this review, risk characterization was conducted when not available in the studies. Fig. 2 also indicates the toxicological parameters used in the risk characterization process – PTWI for MeHg and cadmium and BMDL for arsenic and lead.

4.1. Arsenic

In breast milk, arsenic is present essentially as IAs (Fängström et al., 2008), and the levels shown in Table 2 for total arsenic were assumed to correspond to IAs levels for risk assessment purposes. Only two of the studies estimated arsenic exposure from breastfeeding.

Carignan et al. (2015) estimated a median exposure of 0.04 μg/kg/day (5.6 kg bw; 810 mL of milk/day) for 1- to 3- month American infants (0.28 μg/kg/week), much lower than that estimated for

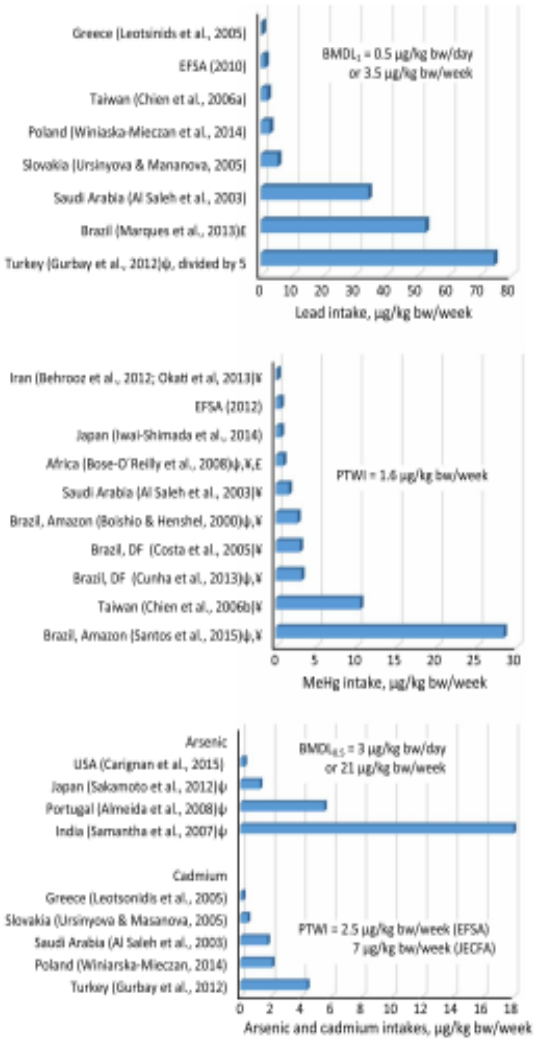

Fig. 2. Mean intakes of lead, mercury, arsenic and cadmium by 1–6 months infants through breast milk; ψ estimated from the concentration data provided (Table 2), assuming 750 mL daily consumption and 5.5 kg bw baby; ¥. calculated assuming that 50% of THg is present as MeHg; £. population living near mining areas. EFSA: European Food Safety Authority; JECFA: FAO/WHO Joint Expert Committee on Food Additives.

infants fed with formula (0.22 μg/kg/day), even when the water used to prepare the formula contained arsenic below 1 μg/L. The EFSA estimated a mean IAs intake of 0.04 μg/kg bw/day for 3-month European infants (6.1 kg, 800 mL milk; EFSA, 2014). Exposure reached 2 μg/kg bw/day for toddlers, the most critically exposed population to arsenic through the diet in Europe, mainly from the consumption of milk and dairy products. A lower median arsenic intake (0.02 μg/kg bw/day, or 0.14 μg/kg bw/week) was estimated by Sternowsky et al. (2002) for 3-month German infants (6 kg; 790 mL/day). The authors considered the exposure to be safe, as it was much lower than the PTWI of 15 μg/kg bw/week. Our estimation of arsenic mean intake from the consumption of intermediate milk of Portuguese mothers (Almeida et al., 2008; Table 2) yielded a much higher value (5.5 μg/kg bw/week). Using the approach currently employed to characterize the risk of exposure to arsenic and a $BMDL_{0.5}$ of 3 μg/kg bw/day (or 21 μg/kg bw/week), a median MOE of 75 was calculated for the American breastfed infants, and could reach 3.8 for Portuguese babies (mean).

The COT reported that arsenic was above the limit of quantitation in 7% of 91 breast milk samples from the UK analyzed in the SUREmilk pilot studies, with a maximum concentration of 4.0 μg/kg (COT, 2004). The maximum estimated intakes ranged from 0.64 μg/kg bw/day for infants under 2 months to 0.15 μg/kg bw/day at 8–10 months. Mean intakes were not reported. The Committee acknowledged that there were no appropriate safety guidelines for arsenic, and concluded that exposure to inorganic arsenic should be As Low As Reasonably Practicable (Achievable), which is known as the ALARP (ALARA) principle, applicable to compounds with no identified threshold of effect. A maximum MOE of 4.7 could be estimated for UK infants under 2 months.

The highest mean level of arsenic reported in the studies in Table 2 was found in India (19 μg/L; Samanta et al., 2007). Using this level, and a milk consumption of 750 mL for a 5.5 kg 2–3 month baby, we estimated an arsenic intake of 2.6 μg/kg bw/day (or 18.2 μg/kg/week), much higher than that reported in Europe and the USA, and a MOE of 1.2. As pointed out before, this high exposure level reflects the high arsenic levels found in the water sources in the region, although the estimated intake based on concentration levels found in Bangladesh (Table 1), also a region with high arsenic levels in water, was much lower (up to 1.7 μg/kg bw/week). The estimated mean intake from limited data in Japan (9 milk samples; Table 1) was 1.3 μg/kg/week.

The EFSA (2005) considered that a MOE of 10,000 or higher for genotoxic compounds, if based on the $BMDL_{10}$ from an animal study, would be of low concern from a public health point of view and might be considered as a low priority for risk management actions. This level allows for 100-fold for specie differences (10-fold) and human variability (10-fold), and an additional 100-fold for additional uncertainties (inter-individual human variability in cell cycle control and DNA repair, and effects that can occur below the reference point). In its evaluation of arsenic, the EFSA (2014) did not estimate a MOE nor did it discuss a level above which the exposure would be considered of low health concern. In this review, an attempt was made to estimate this level taking two points into consideration: 1) the additional carcinogenic risk in the $BMDL_{10}$ related to a MOE of 10,000 (10%) is 20 times higher than the extra risk in the $BMDL_{0.5}$ established for arsenic (0.5%), and 2) the $BMDL_{0.5}$ was based on human studies, so uncertainty due to specie differences (10-fold) can be disregarded. A MOE value that may be used in the risk characterization of arsenic exposure would be 10,000 ÷ 20 ÷ 10, or 50. Therefore, a MOE of 50 or higher for arsenic, based on the $BMDL_{0.5}$ from a human study, would be of low concern from a public health point of view.

In this paper, the estimated MOEs, based on mean or median intakes of breast milk by 2-3 months infants, were above 50 for American infants, as well was for exposures lower than 0.06 μg/kg bw/day, which correspond to a consumption of 750 mL breast milk (5.5 kg infant) containing less than 0.44 μg/L of arsenic (Table 2). Higher arsenic levels, which would lead to MOE lower than 50, were found in breast milk samples from all Asian countries, in some European countries (Greece, Portugal and Sweden), in the United Arab Emirates, and in Ghana (Table 2). Fig. 2 shows the arsenic intakes for 1–6 month infants through breast milk estimated for USA, Japan, Portugal and India (from 0.28 to 18.2 in μg/kg bw/week).

4.2. Lead

Nine studies shown in Table 2 included exposure assessments of breastfed infants to lead. None of the studies used the MOE to characterize risk which, in the context of this paper, was done using a $BMDL_1$ of 0.5 μg/kg bw/day (EFSA, 2010; 3.5 μg/kg bw/week). Al Saleh et al. (2003) estimated a mean intake of 34.3 μg/kg bw/week by infants in Saudi Arabia (850 mL, 5–6 kg bw), and reported that 46.7% of the infants had weekly lead intake levels

exceeding the PTWI of 25 μg/kg bw/week. Chien et al. (2006a) found higher daily intakes of lead in breastfed Taiwanese infants at birth (median of ~1.8 μg/kg bw/day; 400 mL milk), which decreased to below 0.3 μg/kg bw/day (2.1 μg/kg bw/week) after 3 months (760 mL milk). Two of the 72 infants (2.6%) had a HI greater than 1. The estimated MOEs were 0.1 and 1.7 for the Saudi Arabian and Taiwanese infants, respectively.

Three studies were conducted in Europe. Leotsinids et al. (2005) estimated lead intake of Greek infants assuming a consumption of 100–150 mL/kg bw/day of colostrum and intermediate milk, respectively. The 90th percentile of the intakes were 1.0 and 1.1 μg/kg bw/day, respectively, much lower than the PTWI, which corresponded to 3.6 μg/kg bw/day. The authors estimated a median intake for intermediate milk of 0.49 μg/kg bw/week. Ursinyova and Masanova (2005) estimated mean lead intake of 5.4 μg/kg bw/week for Slovakian breastfed infants using a daily milk consumption equivalent to 1/6 of the infants' body weight. The intake from the consumption of milk for two of the 158 mothers exceeded the PTWI. The estimated mean MOEs for Greek and Slovakian breastfed infants were 7 and 0.64, respectively.

Using lead levels found in the 2-5 d breast milk samples, Gürbay et al. (2012) estimated the intake of 3-month Turkish breastfed infants (750 mL/day) ranging from 22.9 to 5356 μg/week (mean of 2052 μg/week). Considering a body weight of 5.5 kg, a mean intake of 373 μg/kg bw/week can be estimated. This intake, however, is probably overestimated since metal levels, including lead, decrease in mature milk (Chao et al., 2014; Chien et al., 2006a, 2006b). Winiarska-Mieczan (2014) estimated that the weekly intake of lead by Polish infants decreased from 2.9 to 2.8 μg/kg bw at 1-3 months to 0.84 μg/kg bw at 12 months using the recommended volume of powdered milk for infants as a parameter for breast milk consumption. The authors expressed these values as % of the BMDL of 3.5 μg/kg bw/week (84–24%), and concluded that although the intakes did not exceed the "admissible levels", they were nevertheless high. It is important to emphasize however that this BMDL is not an admissible level of lead exposure, but is a level that corresponds to a 1 IQ point decrease in cognitive ability in children (EFSA, 2010). A MOE of 1.2 may be estimated for 1–3 month Polish infants.

In the UK, the COT (2004) reported that lead was above the LOQ in 7% of 114 breast milk samples analyzed, with a maximum concentration of 2.6 μg/kg, and a maximum intake ranging from 0.42 μg/kg bw/day for infants below 2 months of age to 0.1 μg/kg bw/day at 8–10 months, lower than the JECFA PTWI in effect at that time. The Committee concluded that this exposure does not raise toxicological concerns.

In a study conducted in Brazil (State of Rondônia, Amazonian region), Marques et al. (2013) estimated a median exposure to lead in the first 6 months of breastfeeding (140 mL milk/kg bw/day) of 3 μg/kg bw/day for rural infants, and of 7.5 μg/kg bw/day (52.5 μg/kg bw/week) for infants living in the vicinity of tin smelters. Our calculations indicate MOEs of 0.16 and 0.07 for rural and smelter neighboring infants, respectively. In another study conducted in the country however, mean lead levels were much lower (0.26 μg/L; Table 2), and we estimated a MOE of 14.

In its dietary risk assessment of lead for 3-month breastfed infants, the EFSA (2010) calculated MOEs of 2.4 for average consumers, which decreased (higher risk) in infants fed with formula and in children up to 7 years (MOE < 1). In its evaluation, the EFSA concluded that the risk from lead exposure for infants can be significant when the MOE is lower than 1; risk is likely to be low when the MOE is between 1 and 10; and a MOE of 10 or greater indicates no appreciable risk of a clinically significant effect on IQ. Most of the calculated MOEs in the present study were either below 1 or between 1 and 10, indicating a potential risk to breastfed infants. Fig. 2 shows the mean lead intakes by 1- to 6-month infants through breast milk discussed above.

4.3. Mercury

In most of the studies shown in Table 2, only THg was analyzed in the breast milk samples. Currently, the PTWIs for mercury are for IHg (4 μg/kg bw) and for MeHg (1.6 μg/kg bw), which is relevant for pregnant women and infants (JECFA, 2011b). The mean ratio of MeHg to THg in breast milk varies widely (from 0 to 0.6, mostly around 0.5; Table 2), and is considered to be greater in populations with higher fish consumption, reaching over 0.8 in some countries (Valent et al., 2013; Miklavcic et al., 2013). For the purpose of this review, when MeHg was not measured, it was considered to represent 50% of the THg present.

Two Brazilian studies conducted risk assessments of breastfed infants to mercury, both in the Federal District. Costa et al. (2005) estimated a THg mean and maximum intake (150 g milk/bw/day) of 0.86 and 3.46 μg/kg bw/day, respectively. The authors stated that 56.3% of the samples would indicate intakes higher than the reference value set by the WHO in 1991 for THg (0.5 μg/kg bw/day). Based on our previous assumption, MeHg mean intake in this study corresponded to 0.43 μg/kg bw/day, or 3 μg/kg bw/week, representing 188% of the PTWI.

In the assessment conducted by Cunha et al. (2013), 18 nursing mothers provided samples 15–90 days postpartum (142 samples) during 2003 and 2004, the same period as in the study by Costa et al., yielding similar THg mercury concentrations (Table 2). Infant weights were measured at 30, 60, and 90 days, and consumption volumes were estimated from the time the infant spent breastfeeding at each sampling point, assuming a milk flow of 13.5 mL/min. The intakes exceeded the THg PTWI (5 μg/kg bw/week) at least once during the period for 77.8% of the samples, with one sample reaching over 800% of the PTWI. Only four mothers did not provide samples that would lead to an exceedance of the PTWI at any sampling time. The estimated mean intake of THg was 6.4 μg/kg bw/week, or 3.2 μg/kg bw/week of MeHg (200% of the PTWI).

The study by Santos et al. (2015) in the Brazilian Amazon provided the highest mean levels of THg among all the studies in Table 2 (59.4 μg/L). Based on this level and a daily milk consumption of 750 mL for a 5.5 kg baby, we estimated a mean THg intake of 56.7 μg/kg bw/week, or 28.4 μg/kg bw/week for MeHg. Another study conducted in the same region found a much lower mean THg level (5.7 μg/L; Boischio and Henshel, 2000), and we estimated an intake of 5.4 μg/kg bw/week, and 2.7 μg/kg bw/week of MeHg, which corresponds to 170% PTWI.

The EFSA (2012b) conducted an assessment for MeHg in European infants under six months of age (6.1 kg bw) using contamination data from Miklavcic et al. (2013) and Valent et al. (2013) (Table 2). The mean intakes ranged from 0.09 to 0.62 μg/kg bw/week (800 mL milk consumption), and from 0.14 to 0.94 μg/kg bw/week for high consumers (1200 mL milk), and did not exceed the TWI of 1.3 μg/kg bw/week.

Iwai-Shimada et al. (2015) estimated intakes for Japanese one-month-old infants (4 kg bw and 800 mL milk) ranging from 0.08 to 1.68 μg/kg bw/week for MeHg (median of 0.63 μg/kg bw/week). The authors compared the intakes of MeHg with the Japanese and EFSA TWI (2 and 1.3 μg/kg bw/week, respectively), the JECFA (1.6 μg/kg bw/week), and a reference dose (RfD) from USEPA of 0.1 μg/kg bw/day. For the more restricted situation (USEPA), exposure exceeded the RfD in 12 of the 27 cases, with the median intake corresponding to 40% of the JECFA PTWI.

Chien et al. (2006b) estimated a mean THg intake of 3 μg/kg bw/day for newborn Taiwanese babies. Assuming that 50% of mercury is present as MeHg, the Monte Carlo simulation showed that HI for mercury was greater than one for 12.9% of urban babies, and for 18.8% of fishing village babies (MRL of 0.3 μg/kg bw/day). The mean MeHg intake represented 660% of the PTWI.

Behrooz et al. (2012) estimated a mean THg intake of 0.065 μg/kg bw/day for Iranian infants based on the actual infant birth weights and a daily milk intake of one-sixth of the infants' weight. Okati et al. (2013) found a similar result for 7 kg Iranian infants (1050 mL milk/day), with a mean of 0.064 μg/kg bw/day. These THg intakes corresponded to 0.22 μg/kg bw/week of MeHg (14% of the PTWI). In Saudi Arabia, Al-Saleh et al. (2003) estimated a much higher mercury intake for 5–6 kg infants (3.25 μg/kg bw/week), with 17.1% of the infants exceeding the THg PTWI. The calculated mean MeHg intake represented 100% of the PTWI.

A study conducted by Bose-O'Reilly et al. (2008) involved women with a very high mercury burden in four different gold mining areas in Indonesia, Tanzania and Zimbabwe. The authors estimated that the THg intake by a 3-month infant (6 kg, 850 mL milk/day) exceeded the RfD of 0.3 μg/kg bw/day in 47.8% of the cases, with the highest intake being 21.2 μg/kg bw/day (7100% RfD). The authors stated that no conclusion regarding a possible health risk of environmental mercury could be reached given the clear benefits of breastfeeding in developing countries. Based on the mean THg level (1.7 μg/L; Table 2) the estimated mean intake of MeHg was 0.93 μg/kg bw/week, representing 58% of the PTWI. Fig. 2 summarizes the intakes of MeHg by 1–6-month infants through breast milk discussed in this review. It is important to emphasize that the intakes may be overestimated for low fish consumption populations.

4.4. Cadmium

Four of the studies reported in Table 2 conducted exposure assessments for cadmium (and for lead, as discussed above) through breastfeeding. In Greece, the estimated 90th percentile of cadmium intakes from the consumption of colostrum and intermediate milk were 0.32 and 0.52 μg/kg bw/week, respectively; median values were 0.10 and 0.18 μg/kg bw/week (Leotsinidis et al., 2005). Ursinyova and Masanova (2005) estimated (milk consumption equal to 1/6 the body weight) a mean cadmium intake of 0.5 μg/kg bw/week for Slovakian newborn infants (0.02-1.99 μg/kg bw/week). In Poland, the mean exposures at 1, 6 and 12 months were 1.8, 2.1 and 0.82 μg/kg bw/week, respectively (Winiarska-Mieczan, 2014). In both studies, the authors compared the exposure with the TWI of 2.5 μg/kg bw/week set by the EFSA, which was not exceeded in any of the cases. Mean intake of cadmium by Saudi infants through breastfeeding (850 mL, 5.5 kg) estimated by Al Saleh et al. (2003) was 1.8 μg/kg bw/week, with 2.6% of the infants (n=344, 5 months old, on average) having intakes higher than the PTWI of 7 μg/kg bw/week.

The highest mean level of cadmium in breast milk of the studies in Table 2 was found in a study conducted in Turkey (4.6 μg/L; Gürbay et al., 2012. Using this level and a daily milk consumption of 750 mL for a 2-3 month baby (5.5 kg), we estimated a mean cadmium intake of 4.4 μg/kg bw/week for Turkish breastfed infants. This level is higher than the EFSA TWI (176%), but lower than the PTMI set by the JECFA, which corresponds to 5.8 μg/kg bw/week. These two contradictory risk conclusions demonstrate that risk assessment results need to be seen in light of the conservativeness of the parameters used and the uncertainties involved in the estimations. Fig. 2 summarizes the intakes of cadmium by 1- to 6-month infants through breast milk discussed in this review.

5. Summary and conclusions

Arsenic, lead, mercury, and cadmium are toxic metals ubiquitous in nature, to which exposure can be a public health concern. These metals cross the placenta and the blood brain barrier, and are excreted through breast milk. Exposure to lead and mercury has been related to neurotoxic problems later in life, although studies to discriminate intrauterine and postnatal effects are still needed. Currently, there is no safe dose of exposure established for lead or arsenic.

Monitoring breast milk is a non-invasive way of determining human exposure to metals and other contaminants. This review covers 75 studies that assessed arsenic, lead, mercury and/or cadmium levels in breast milk samples collected worldwide, with about one-third of the studies conducted in Europe. Mean or median levels of arsenic in intermediate and mature breast milk from non-occupational mothers were higher in India, reflecting high levels of this metal in the water sources of the region, and for methyl mercury in the Brazilian Amazon. Cadmium levels in breast milk were the lowest among the metals, mostly below the LOQ of the method. Lead was the metal most investigated and most detected in the studies.

Risk assessments conducted using current methods and toxicological parameters indicate that the risks for breastfed babies in most regions cannot be excluded, mostly due to arsenic, lead and mercury. Arsenic intakes led to MOEs below 10 in most studies. However, bottle-fed infants, who consume milk powder diluted in water, had higher arsenic intakes. Therefore, breastfeeding is protective for the babies, mainly in areas with high levels of arsenic in water. All the Brazilian studies indicated MeHg intakes exceeding the safety exposure parameter, reaching 1700% PTWI in a Brazilian Amazon riverine community, most likely due to high fish consumption, including piscivorous fish, which may contain high MeHg levels due to the bioconcentration in the aquatic food chain. Although the benefits of a high fish consumption diet are widely recognized due to its high-quality protein, fatty acids and other essential nutrients (IOM, 2005), women of child-bearing age and nursing mothers should avoid consuming piscivorous fish (USFDA, 2014).

The highest mean levels of lead in breast milk were found in Turkey, with an intake that led to a MOE of 0.01, with a potential for neurotoxic effects. The same conclusion may also be reached for infants from other regions, including Saudi Arabia, Brazil and Slovakia (MOE < 1). Cadmium intakes were also higher in Turkey, representing 173% of the TWI established by the EFSA, but were below the PTWI established by the JECFA.

It is clear from most studies that breastfeeding exposes infants to more than one metal simultaneously, and most likely reflects the intrauterine exposure. Although the risk assessments discussed in this review were for each metal separately, it is important to point out that co-exposure to metals, in addition to other environmental contaminants, acting through the same mechanism and/or targeting the same organ, may lead to combined adverse effects with greater health impact on infants and children (Cardenas et al., 2015; Govarts et al., 2016).

The presence of environmental contaminants in human milk and the potential risks to the infants have been long recognized by researchers and health authorities worldwide. However, the World Health Organization and national governments strongly recommend breastfeeding, as it is accepted that the risks are outweighed by the benefits of breast milk consumption (WHO, 2007; Mead, 2008; VKM, 2013). This conclusion, however, does not preclude the responsibility of health authorities and researchers from continuing to monitor the levels of these metals in breast milk, particularly in regions with high levels of contamination, either by natural sources (as for arsenic in areas with high levels in water) or anthropogenic sources (as for lead in mining areas). Risk communication initiatives to reduce exposure among women of childbearing age by health authorities include:

- Women should be advised to avoid the consumption of predatory fish during pregnancy and when breastfeeding to decrease MeHg exposure.

- Women should be aware that arsenic exposure is much lower for breastfeeding babies than for babies fed with bottles;
- Women should be removed from polluted and mining areas and should avoid smoking to decrease exposure of the fetus and infants to lead and cadmium, among other contaminants.

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ANEXO II–Aprovação pelo Comitê de Ética em Pesquisa da Universidade de Brasília

Universidade de Brasília
Faculdade de Ciências da Saúde
Comitê de Ética em Pesquisa – CEP/FS

PROCESSO DE ANÁLISE DE PROJETO DE PESQUISA

Registro do Projeto no CEP: **027/11**

Título do Projeto: "Avaliação da exposição de crianças à micotoxinas e metais pesados através do consumo de leite materno".
Pesquisadora Responsável: Patrícia Diniz Andrade
Data de Entrada: 01/04/11

Com base na Resolução 196/96, do CNS/MS, que regulamenta a ética em pesquisa com seres humanos, o Comitê de Ética em Pesquisa com Seres Humanos da Faculdade de Ciências da Saúde da Universidade de Brasília, após análise dos aspectos éticos e do contexto técnico-científico, resolveu **APROVAR** o projeto **027/11** com o título: "Avaliação da exposição de crianças à micotoxinas e metais pesados através do consumo de leite materno", analisado na 3ª Reunião Ordinária, realizada no dia 12 de abril de 2011.

A pesquisadora responsável fica, desde já, notificada da obrigatoriedade da apresentação de um relatório semestral e relatório final sucinto e objetivo sobre o desenvolvimento do Projeto, no prazo de 1 (um) ano a contar da presente data (item VII.13 da Resolução 196/96).

Brasília, 13 de abril de 2011.

Prof. Natan Monsores
Coordenador do CEP-FS/UnB

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