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I, Meghan Widestrom, hereby submit this original work as part of the requirements for the degree of Master of Science in Industrial Hygiene (Environmental Health).

It is entitled:

Evaluation of Long-Term Lead Exposure and Potential Health Effects to Mother and Child Following Bone Turnover While Breastfeeding

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Evaluation of Long-Term Lead Exposure and Potential Health Effects to Mother and Child Following Bone Turnover While Breastfeeding

A thesis submitted to the Graduate School of the University of Cincinnati

in partial fulfillment of the requirements for the degree of

Master of Science

in the Department of

Environmental and Public Health Sciences

by

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Abstract

Introduction Lead (Pb) is a naturally occurring element that is used in many different work environments and can also be found in the home through environmental exposures. Adverse health outcomes associated with Pb exposure are well established and include a variety of effects at low levels of exposure, including cardiovascular, kidney, neurological effects, as well as reproductive and developmental effects in pregnant women and their offspring. The pharmacokinetic behavior of Pb is well characterized. However, what remains unknown is how lifetime Pb exposures and occupational Pb exposures interact to influence bone Pb levels during pregnancy and lactation. **Methods** An existing PBPK model as migrated to R software to evaluate the effects of bone loss on Pb levels during lactation. The breast compartment was added to the existing model and a substantial literature search was performed to characterize these parameters. **Results** The model currently models bone reabsorption and changes blood lead (PbB) concentrations during lactation. There was a steady increase in PbB levels from birth through the beginning of lactation followed by a drastic increase in PbB levels during the lactation period. Following the cessation of breastfeeding, PbB dropped to pre-lactation concentrations. The model suggests that changes in PbB for a woman born in 2000 who experiences relatively high Pb exposure and breastfeeds for a period of 180 days at the age of 28 show a 54% increase in maternal blood Pb concentrations, increasing from a baseline level of 2.5 µg/dL pre-lactation to 3.9 µg/dL during lactation. **Discussion** Due to significant efforts from the US Environmental Protection Agency and US Centers for Disease Control and Prevention, the average human exposure to lead has drastically declined over the last 40 years. This resulted in average PbB levels that were much lower than anticipated. Even with the increase in PbB levels during lactation, average exposure to Pb would result in PbB levels below 1µg/dL and would not be anticipated to cause health effects to woman or child.

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Background

Lead (Pb) is a naturally occurring element that is used in many different work environments and can also be found in the home through contaminated drinking water or other environmental exposures such as the deterioration of lead-based paint in the home or workplace.[1] Adverse health outcomes associated with Pb exposure are well established and include a variety of effects at low levels of exposure including cardiovascular, kidney, neurological effects, and reproductive and developmental effects in pregnant women with blood Pb levels below 5 µg/dl.[2-3] Pb can also cause a fluctuation in hormone production which can impact the regularity of menstrual periods and result in menopause at a younger age. Pb can also cross the placenta to the fetus and cause reduced neurodevelopment, low birth weight, premature birth, and even miscarriage.[4] Children are at a much greater risk than adults of developing adverse neurologic effects from Pb exposure. This is because blood levels as low as 5 µg/dL can cause learning deficiencies, decreased IQ, and behavioral issues in children.[5]

When Pb enters the body, it mimics the behavior of calcium ions (Ca^{+2}). Consequently, Pb accumulates in phosphate-rich tissues, particularly in the bones and teeth. Once in bone it has a half-life of 25-30 years.[4] However, bones are not static, as they are constantly undergoing remodeling throughout life and in response to physiologic changes. The skeletal system adapts to physical stress by increasing bone deposition; conversely, factors such as decrease physical activity or stress (e.g., microgravity), increasing age and increased the metabolic demands of pregnancy and lactation result in the mobilization of calcium and phosphate from bones. As bones lose density, contaminants such as Pb that are accumulated in bone redistributed back into the blood, increasing human blood lead levels (PbB). Postpartum breastfeeding increases the rate of bone loss. Some studies have shown that women can lose anywhere from 3 to 5 percent of

their bone mass during breastfeeding.[6] Studies have shown that bones release Pb from multiple compartments at different rates, and the resulting PbB have the potential to add to existing PbB reflective of ongoing environmental exposures. This is particularly concerning for women of reproductive age, especially minority women who have had high Pb exposure histories from childhood, some of whom work in Pb-related industries.

Annually, as many as 1.5 million workers are exposed to Pb in the workplace in the U.S.[7] Additionally, the National Institute for Occupational Safety and Health (NIOSH) Adult Lead Epidemiology and Surveillance (ABLES) program estimates a national prevalence rate of 15.8 adults per 100,000 employed having blood lead levels>10 µg/dL in 2016 based on 26 U.S. states reporting these data.[8] Over 90% of the total BLL that were >10 µg/dL in adults identified in the NIOSH ABLES program had occupational exposures, including in four industry sectors (manufacturing, construction, services, and mining).[8] The Occupational Safety and Health Administration's (OSHA) standard 1910.1025 was developed to protect workers who have occupational exposure to Pb. Within this standard OSHA has developed a medical removal program that requires employees with blood Pb levels over 50 µg/dL to be removed from leaded environments until blood Pb levels drop below 40 µg/dL.[9] OSHA has not implemented requirements for workplaces to remove pregnant or postpartum women at a lower blood Pb levels; however, in 2010 the Centers for Disease Control and Prevention (CDC) established guidelines surrounding screening and management of pregnant and lactating women who are currently or have previously been exposed to Pb. These guidelines recommend counseling and identification of exposure sources for pregnant or lactating women with blood Pb levels over 5 µg/dL.[10]

The pharmacokinetic behavior of Pb is well characterized.[11-14] However, what remains unknown is how lifetime Pb exposures and occupational Pb exposures interact to influence bone Pb levels during pregnancy and lactation. This is an important because the whole-body kinetic behavior of Pb is determined largely by the balance between bone Pb uptake and release.[11] A quantitative physiological based pharmacokinetic (PBPK) model for Pb exposure has existed for over thirty years and is validated and well characterized.[11-15] A PBPK model is a computer modeling that quantifies concentrations of a chemical or drug in key tissues and organs as a function of time as a set of integrated differential equations using physiological information such as blood flow and tissue composition.[16] The key body compartments for Pb include red blood cells (RBCs) and plasma, kidney, liver, bone, other well-perfused tissues, and poorly perfused tissues. The PBPK model describes the fractional absorption, accumulation, and elimination of Pb as a function of age, starting from birth, and includes critical life stages inclusive of hyperbolic growth during childhood, accelerated growth beginning at puberty through adolescence, early adulthood when bone growth ceases and remodeling predominates, and menopause. The model is parameterized for either males or females, and accounts for physiological differences between sexes for each life stage. However, the model does not include parameters for pregnancy and lactation.

In collaboration with National Aeronautics and Space Administration (NASA), Garcia et al. (2013) adapted the PBPK model developed by O'Flaherty et al. (1993), to evaluate the effects of microgravity-related bone loss on PbB.[15] It has been known since Skylab that spaceflight causes severe bone loss in the lumbar spine/pelvis and lower limbs at a rate of approximately 0.8% [0.5 to 1.1] per month, with a cumulative loss of −5.4% [−6.0, −4.9] from the lower limbs, and −6.2 [−6.7, −5.6] spine/pelvis.[17] By comparison, women undergo a transient loss of bone density in the range of 3-7% during lactation, which is rapidly regained after weaning.[18-19] We therefore

reason that this spaceflight Pb PBPK model could be adapted to represent the effects of pregnancy and lactation on PbB and furthermore, could estimate the transfer of Pb to infants during postnatal. This is accomplished by modifying the model through the addition of a lactating breast compartment that becomes activated only during the during specified period of lactation.

Furthermore, the proposed PBPK model is already structured to model lifelong maternal Pb exposures from birth, including dietary exposures from water, food, childhood oral dirt and dust exposures, environmental inhalation exposures and occupational exposures. Therefore, this model can be parameterized with exposures representative of susceptible populations of Americans, such as urban minority women who have grown up in homes, neighborhoods, schools, and communities where Pb contamination is a known risk. For these women we can model the complex accumulation of Pb in blood and bone tissue over the full course of their lives.

The aim of this study is to create a generalizable model that quantifies the amount of Pb that will be released from the bone during lactation, and eventually gestation.

Purpose and Scope

The objective of this study is to model accumulated of maternal Pb in bone over the course of a reproductive lifetime to determine if bone resorption during breastfeeding can be a health risk for the mother or nursing child.

Specific Aims and Hypothesis

The hypothesis of this study is that bone resorption during breastfeeding, in the context of ongoing occupational and environmental Pb exposures will result in PbB that are significant enough to result in health effects to the child.

The following aims will be completed to accomplish the objectives and test the hypothesis:

1. Adapt the Garcia et al (2013) PBPK model from AcslX software to R software with the addition of body compartment for lactation and an updated model parameters for post-partum females and the rate of bone resorption in breastfeeding women.
2. Evaluate prior research to determine the level at which Pb deposits in bone following workplace and environmental exposure and the longevity of Pb in bone.
3. Use the modified Garcia et al (2013) PBPK model to predict PbB of breastfeeding women following workplace exposure to Pb and predict Pb concentrations in breast milk. Model predictions will be benchmarked to known health effects of Pb levels seen in adult women and infants.

Methods

Similar, to breastfeeding women, prolonged exposure to microgravity is also associated with accelerated bone loss. Garcia et al. (2013) developed a physiologically based pharmacokinetic model (PBPK) to evaluate the effects of bone loss on PbB in microgravity. This model allowed the user to input historic environmental exposures to Pb such as soil, water, and air concentrations and individualize the parameters of exposed individuals such as year of birth, gender, and weight. A major challenge for this project is that the O'Flaherty PBPK model [11-13] is that it was written in Advanced Continuous Simulation (ACSL) Language software and later

updated in acslXtreme® software program Garcia et al. (2013) and Sweeney (2015). The software was discontinued by the manufacturer Aegis Technologies in 2020. For this reason, the PBPK model was migrated to R software, which is open-source software. Structurally, however, the model requires substantial revision to adapt it to the R environment. In this study, to evaluate the effects of bone loss on Pb levels during lactation, the R-adapted Garcia spaceflight model was repurposed and parameterized with applicable physiological numbers to predict the expected PbB levels in breastfeeding women. The first step of this process was the addition of a breast compartment. There are three key elements to incorporating the breasts as a tissue compartment: characterizing the change of blood flow to the breasts during lactation, characterizing the change in tissue volume of the breasts during lactation, and characterizing the elimination rate of Pb from the blood by the breasts during lactation. With knowledge of the approximate daily milk production rate, the daily amount of Pb passed to a nursing infant can be estimated. The inclusion of a breast compartment, therefore, required a substantial literature search to characterize these parameters.

A literature search was performed in PubMed and Web of Science. For anatomy characterization, key PubMed search terms include (breast OR mammary) AND anatomy AND (lactation or lactating) AND blood flow NOT drug, which yielded 57 results. For lactation rate search terms included (breast OR mammary) AND (lactation or lactating) AND (rate OR "lactation rate" OR "milk production") AND human AND anatomy NOT (drug or endocrine) yielding 199 results.

Four scenarios were investigated in this study: women with average occupational and environmental exposure levels to Pb; women with elevated workplace exposures to Pb; women with elevated environmental exposure to Pb; and women with elevated occupational and

environmental exposure levels to Pb. The parameters for each scenario were based on regulatory standards, reports, and a thorough literature review. Finally, a literature review was performed to determine the level at which Pb deposits in bone following workplace and environmental exposure and the longevity of Pb in bone. These values were used as bone Pb variables used in the model.

Results

Model parameters for the breasts and lactation.

PBPK models calculate blood and tissue concentrations of a chemical using well documented tissue blood flow rates, tissue volumes and chemical partition coefficients for each modeled tissue. The O'Flaherty PBPK model (1998) of life-stage dependent accumulation of Pb in bone, and the subsequent PBPK model of bone resorption published by Garcia and Hayes, did not include a breast compartment to account for postpartum lactation. The general modeling approach uses in this project was to split a portion of the "poorly-perfused" body tissue compartment already present in the preexisting PBPK models to create a breast tissue compartment that represents the totality of lactating breast tissue (i.e., both breasts are modeled together). The addition of a separate breast tissue compartment requires a detailed understanding of the anatomy and physiology of the breasts before (nonparous) and during lactation, as well as the partitioning of Pb into breastmilk. The addition of a separate lactational breast compartment requires that total tissue blood flow and tissue volume remain in balance throughout the time course of the PBPK model. This means that all compartments must sum to a total blood flow equal to 100% of cardiac output, a total volume that is 100% of total body weight. To maintain flow and tissue balance, a breast tissue compartment was removed from the existing poorly-perfused tissue compartment. This choice is based on the fact that the breasts are not considered a richly perfused tissue in the absence of lactation during which time the breasts (combined)

receive only 0.4% (0.004) of cardiac output (5.9 L/min x 0.4% x 60 min/h x 24 h/day = 34 L/day).

The fraction of cardiac output supplying the poorly-perfused tissues compartment was diverted for perfusion of the added lactating breast compartment. For most PBPK models, cardiac output remains constant; however, during lactation cardiac output is increased compared to nonparous females. The reference value for cardiac output in an adult female is 5.9 L/min.[21] Cardiac output in postpartum (mean 17 weeks) breastfeeding mothers is approximately 7.5 L/min.[20] This represents a 1.6-fold increase in cardiac output during lactation. By comparison, post-partum (mean ~25 week) non-nursing mothers who bottle-fed their infants exhibit a cardiac output of ~6 L/min, which is close to the reference value for nonparous adult females.[20] Total age-dependent body mass-scaled tissue blood flow for a nonparous adult female was calculated as

$$QC = (QL+QK+QW+QP+QB) = 5.9\ L/min\ (8496\ L/day\ for\ a\ 60\ kg\ woman)$$

where QC is total cardiac output, and QL, QK, QW, QP, QB are blood flow rates to the liver, kidney, well-perfused tissues, poorly-perfused, and bone tissues, respectively. Tissue perfusion during the lactational period was calculated as,

$$QC = (QL+QK+QW+QP+QB + QBREAST) = 7.5\ L/min\ (10800\ L/day\ for\ a\ 60\ kg\ woman).$$

It is unlikely that the full 1.6-fold increase in cardiac output is attributable solely to changes in breast blood flow. It has been reported that total arterial blood flow rates to the lactating breast measured in 55 nursing mothers have a median of 236 L/day (126 L/day left and 110 L/day right.)[22] (Table 1). Although this volume equates to a total blood flow increase of approximately 7-fold (34 to 236 L/day), it is a small fraction of the overall increase in cardiac output (QC) during lactation, which is approximately 2300 L/day for a 35-year-old nonparous female[21], and

speaks to the elevated metabolic demand associated with breastfeeding. The lactation-modified PBPK Pb model is only activated during the period of postnatal breastfeeding, which we assume lasts for a period of 120 days. This is consistent with research showing that the median duration of any breastfeeding, which is 4 months for exclusive breastfeeding in developed western nations.[23]

A similar modification of the previously existing PBPK model is required to account for the added volume of the lactating breast tissue compartment. For non-lactating women, the mean glandular fraction of the breasts is estimated 42.9% with a median value of 39%, from which a reference value of 40% has been proposed.[21] Overall there is a very high degree of variability among women for both nonparous and lactating breast volume. Despite this variability, there is no correlation between total breast volume and amount of glandular tissue, nor total breast volume and milk production rate.[24]

Total breast volume primarily reflects several types of adipose tissue, including subcutaneous, intraglandular and retromammary fat.[24] Adipose tissue, as well as skin, are both considered to have relatively low metabolic activity (compared to other tissues such as brain, liver, and gut, for example) and are both accounted for as part of the "poorly-perfused" compartment in the existing model. It is that glandular compartment of the breast that must be represented in the revised PBPK model. Ultrasound measurements of the lactating breast indicate that glandular tissue volume is approximately 63 ± 9% (range 46 to 83 %) of breast tissue volume, while fat tissue composes 37 ± 9% (range 16 to 51%) of the lactating breast.[24] Hence, during lactation, glandular tissue volume appears to increase from approximately 40% of breast tissue to roughly 66% of breast tissue, with total breast volume increasing from a reference value of 360 g (range

280 – 400 g) to a lactational volume of 560 to 1800 g, for adult women age 18 – 31 years old (Table 1).

Total body weight in the existing model is calculated as:

$$WBODY = (VL + VK + VW + VP + VBL + VBONE) = 60\ kg\ (reference\ values\ for\ adult\ female)$$

Where WBODY is the body weight at any age, VL, VK, VW, VP and VBONE are the volumes (L) of the liver, kidney, well-perfused tissue, poorly-perfused tissue and bone compartments of the model. To account for changes in glandular breast tissue during lactation, a separate compartment is applied by subtracting the volume of nonlactating glandular breast tissue from the poorly-perfused tissue compartment and applying this mass to volume of the lactating breast compartment using a specific gravity of female breast tissue of 1.05 [21] such that:

$$WBODY = (VL + VK + VW + VP + VBL + VBONE + VBREAST) = 60\ kg$$

with VBREAST representing the volume of lactating glandular breast tissue.

The lactational Pb clearance rate was calculated using the information in Table 1.

Table 1. Human breast milk production and perfusion rates used to the calculate the lactational clearance rate of Pb.

Human Breast Perfusion and Milk Production Rates						
Source	**Sample**	**Population**	**Geometric mean Lactation rate**	**Breast Blood Flow**	**Breast Mass (each)**	**Glandular tissue**
Geddes et al, 2012[22]	N = 55 lactating mothers	Postpartum 6 to 76 weeks	Left: 382 mL/day (IQR* 123mL) Right:397 mL/day (IQR 117 mL)	Left: 126 L/day Right: 110 L/day (226 L/d total breast)		
ICRP, 2002[21]	"Young adult females"		Day 1: 50 mL Day 2: 100 – 200 mL Day 5: 500 – 600 mL (in text, cites up to 1000 mL/day by day 5. Also cites 700 mL at 1-month and 800 mL/day at 6-months)	0.04% non-pregnant; 3.5% cardiac output (near term pregnant) QC = 340 L/day/kg x 55 kg^3/4 = 6866.745 L/day 6866.745 L/d*0.035 = 240 L/day (2 breasts)	280 to 900 g 250 g each reference	40% (non-lactating)
Ramsay et al 2005[24]	N = 21 lactating mothers	Postpartum 1–6 months lactation	Left: 387 mL/day (SD 101 mL) Right: 407 mL/day (SD 121 mL)	NA	Not provided	63.9% (lactating) (range: 43 to 83%) [fat = 37 ± 9%]] 2:1 during lactation; 1:1 for non-lactating
IAEA reference female = 60 kg. Female cardiac output = 5.9 L/min = 8496 L/day, which is 5/9 L/Kg/hour						

*IQR= Interquartile range

The partitioning of Pb into the breast and breast milk has been partially investigated. A partition factor for Pb to breast milk was not identified. However, several studies that have characterized concentrations in blood and breastmilk investigated the lactational transfer of Pb from maternal

blood to breast milk and showed a blood: milk ratio of 0.012 and 0.01 for 310 and 81 women residing in Mexico City. [25-26] Similar to the calculation of clearance of a chemical in the urine, the rate of clearance in breast milk is calculated as:

$$Cl = \frac{Milk\ flow\ \left(\frac{L}{day}\right) \times\ Milk\ concentration\ (\frac{\mu g}{L})}{Blood\ Concentration\ (\frac{\mu g}{L})} = \frac{0.7\ \frac{L}{day} \times 1.1\ \frac{\mu}{L}}{94\ \frac{\mu}{L}} = 0.0082\ \frac{L}{day}$$

where the rate of daily milk expression is listed in Table 1 for a few of the identified studies and is in the range of 700 mL/day, depending on the age of the infant. Multiple measurements of milk concentration were identified, some of which showed wide ranging values. A number of such studies were previously reviewed by Gulson et al. (1998) who suggested that any study with a breast milk to maternal blood concentration > 15% should be treated with caution.[27] For our PBPK model, two similar studies were considered, both by Ettinger et al. Based on these studies, the clearance of Pb from blood by breast milk is estimated to be in the range of 0.0082 L/day as shown in Table 2.

Table 2. Maternal blood and breast milk Pb concentrations estimated from studies by Ettinger et al and Gulson et al.

Maternal blood and breast milk Pb concentrations					
PbB		Breast Milk	Blood: milk ratio	Clearance (L/day)	Source
µg/dL	µg/L	µg/L			
9.3 ± 4.4	93 ± 44	1.1	0.012	0.0082	Ettinger et al., 2004[25]
7.7 ± 4	77 ± 40	0.8	0.01	0.0072	Ettinger et al., 2014[26]
	29	0.73 ±0.7	0.026	0.01	Gulson et al., 2002[27]

Based on these data, the PBPK model calculates the amount of Pb in the breast compartment based on the formula:

$$\frac{dAmount}{dTime} = QBr\ \times (CB - CBBr) - RABrX$$

Where QBr is blood flow to the breasts (L/day), CB is arterial blood concentration (mg/L), CBBr is the venous blood concentration leaving the breasts (mg/L), and RABrX is the rate of excretion of Pb from the breasts (mg/day). RaBrX is a rat that is calculated as:

$$RaBrX = KBr * (ABr/VBr*PBr)$$

Where KBr is the elimination rate constant of 0.008 L/day, ABr is the amount of Pb in breast tissue, VBr is the volume of breast tissue and PBr is the partition factor for breast tissue, which is calculated as the AUC in milk and blood using the method of Singh et al. 2008.[28]

The parameters for each exposure simulation were compiled following a literature review and are shown in Table 3. Further information was collected regarding historic Pb exposures from food, water, soil, dust, air, and workplace sources.[13] This information was not utilized in the current model but has been provided in Appendix 1 for review.

Table 3. Parameters used in four exposure simulations. Simulations investigated average Pb exposure, elevated occupational exposure, elevated environmental exposure and elevated occupational and environmental exposure. Compiled following a literature review.

Parameters for Average Human Exposure	
Description	**Value**
Mean Pb concentration in air in United States 2020[29]	0.044 μg/m^3
Mean Pb concentration in water in United States[30]	2.8 μg/L
Average Pb concentration in food[31]	0.25 ppm
Average PbB in adults 2015-2016[8]	0.92 μg/dL
Parameters for Elevated Occupational Exposure	
Air exposure: OSHA Permissible Exposure Limit (PEL)[9]	50 mg/m^3
Workplace Air Exposure concentration used	5 mg/m^3
Mean Pb concentration in water in United States[30]	2.8 μg/L
Average Pb concentration in food[31]	0.25 ppm
CDC Recommended PbB level not to exceed while pregnant[8]	5 μg/dL*
Parameters for Elevated Environmental Exposure	
Highest Pb concentration in air in United States 2020[29]	0.3 μg/m^3
EPA Action Level for Pb in drinking water[32]	15 ug/L
FDA Interim Reference Levels (IRL) for adults[33]	12.5 μg/day
Average PbB in adults 2015-2016[8]	0.92 μg/dL
Parameters for Elevated Occupational and Environmental Exposure	
Air exposure: OSHA PEL[9]	50 mg/m^3
EPA Action Level for Pb in drinking water[32]	15 ug/L
FDA IRL for adults[33]	12.5 μg/day
Average PbB in adults 2015-2016[8]	0.92 μg/dL

Additional simulations were performed with the following initial PbB levels; 10 μg/dL, 40 μg/dL and 60 μg/dL.

In addition to exposure parameters, the physiological parameters from the O'Flaherty model (1998) were used in the simulations performed in this study and are summarized in Table 4.

Table 4. Selected Parameters from the O'Flaherty Model (1998) used to simulate PbB in lactating mothers

Physical Parameters		
Name	**Value (units)**	**Description**
AGEA	25 yr.	Age at which bone growth ceases and remodeling becomes the sole bone formation process
WBIRTH	3 kg.	Weight at birth
WCHILDF	22 kg	Maximum weight for early hyperbolic section of growth curve for female children
HALF	3 yr.	Age at which weight is half of maximum weight for early hyperbolic section of growth curve for a male and/or a female child

WADULTF	34 kg	Maximum weight for later logistic section of growth curve for adult females
KAPPA	600	Logistic constant kappa for age dependent changes in body weight
LAMBDAF	0.017 (1/(kg-yr.))	Logistic constant lambda for age dependent changes in body weight for females
QCC	1.241×10^5 L/yr./kg$^{0.74}$	Body weight normalized for cardiac output in the adult
QLC	0.25	Fraction of the cardiac output going to the liver
QKC	0.17	Fraction of the cardiac output going to the kidney
QWC	0.44	Fraction of the cardiac output going to other well-perfused tissues
QBONEC	0.05	Fraction of the cardiac output going to bone
VBLC	0.067 L/kg body weight	Total blood volume
VLC	0.025 L/kg body weight	Liver volume in the adult
VKC	0.0085 L/kg body weight	Kidney volume in the adult
VBLWPC	0.0576 L/kg body weight	Volume of blood in well-perfused tissues
VWC	0.16	Volume of well-perfused tissues
HCTAF	0.41	Hematocrit in adult female
Bone Parameters		
TLAC	0.192	Trabecular bone loss rate during pregnancy (L/yr) (3% per month)
CLAC	0.192	Cortical bone loss rate during pregnancy (L/yr) (3% per month)
D0	5.0×10^{-7} cm/day/0.5×10^{-4} cm	Permeability constant for diffusion within the bone
R0	5.0×10^{-7} cm/day/0.5×10^{-4} cm	Permeability constant for diffusion across the canaliculus-bone interface from bone to canaliculus
P0F	0.01 cm/day/0.5×10^{-4} cm	Permeability constant for an adult female
S	0.000126 cm^2/cm of canalicular length	Constant for surface area of canaliculi
Pb Parameters		
PL	50	Liver to plasma partition coefficient
PK	50	Kidney to plasma partition coefficient
PW	50	Well-perfused tissue to plasma partition coefficient
PP	2	Poorly-perfused tissue to plasma partition coefficient
LEAD	15000 L plasma cleared/L bone formed	Fractional clearance of Pb from plasma into forming bone
BIND	2.7 mg/L of red cell volume	Maximum capacity of sites in red blood cells to bind Pb
KBIND	0.0075 mg/L of red cell volume	Half-saturation concentration of Pb for binding by sites in red blood cells
G	1.2	Linear parameter for unbound Pb in red blood cells

The model provided PbB levels of women following periods of bone loss while breastfeeding. These PbB levels will be referred to as AFTERPbB.

AFTERPbB levels were used to predict the Pb concentrations in breast milk. These concentrations were benchmarked with known health effects of Pb seen in adult women and infants. Additionally, modeled PbB levels were compared to existing regulatory standards and existing research to determine if the mother's PbB levels were high enough to be associated with an increased risk of adverse health outcomes to themselves or their breastfeeding child.

The model was structured similarly to the Department of Defense and O'Flaherty's physiologically based model (Fig 1).[11]

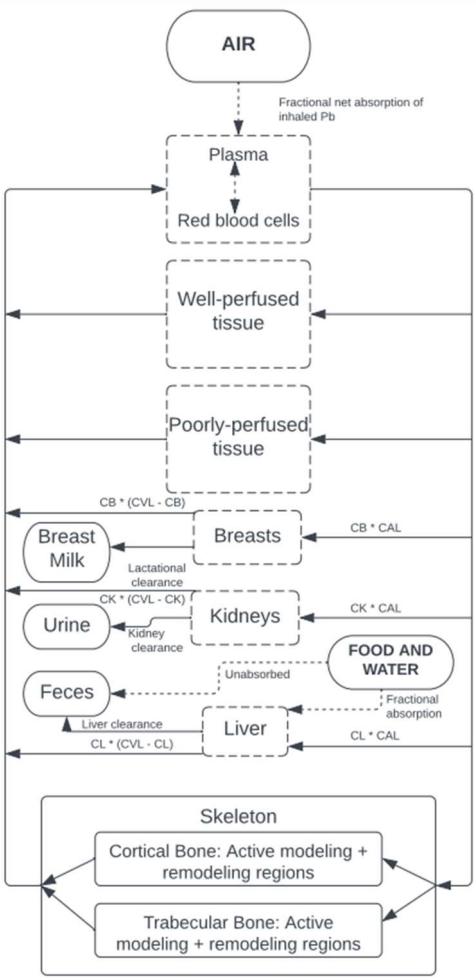

Figure 1. Model structure based on DoD-O'Flaherty model (1998) for Pb. Ovals with text in all capitals represent sources of Pb, ovals with text not in all capitals represent destinations for Pb in the body. Rectangles represent tissue compartments for Pb mass balance; those with dashed lines indicate flow limited equilibrium and those with solid lines indicate processes limited by diffusion. Arrows indicate the movement of Pb in the body; solid arrows indicate Pb distribution via blood flow, dashed arrows indicate uptake and clearance of Pb. The double-sided arrow indicates equilibrium.

The following information was required to parameterize the model to account for lifetime accumulation of Pb in bones. Human bones are made up of two compartments: the cortical bone compartment and the trabecular bone compartment as shown in Figure 2.[37]

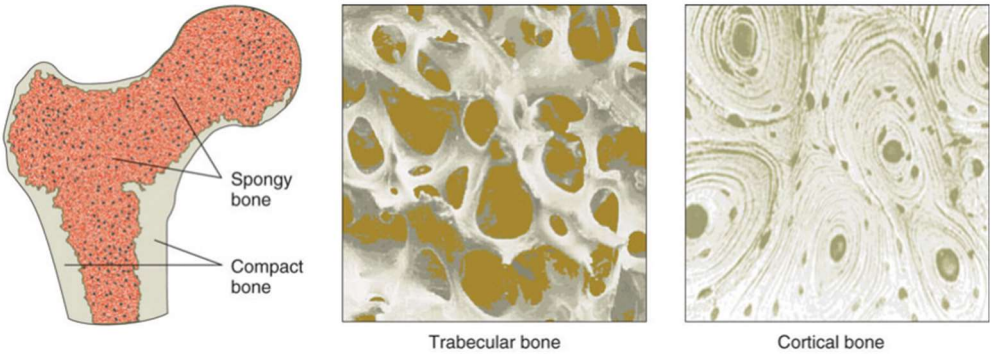

Figure 2. The two main types of bones are trabecular and cortical. Trabecular (spongy) and cortical (compact) bone tissues differ in structure and density.[37]

Cortical bone is the dense outer surface of bone that serves as a protective and surrounds bone marrow. Cortical bone is made up of closed packed haversian systems, otherwise known as osteons. Each osteon is made up of lamellae, which form concentric ring of cells that surround the Haversian canal. An illustration of compact bone tissues is shown in Figure 3.[38]

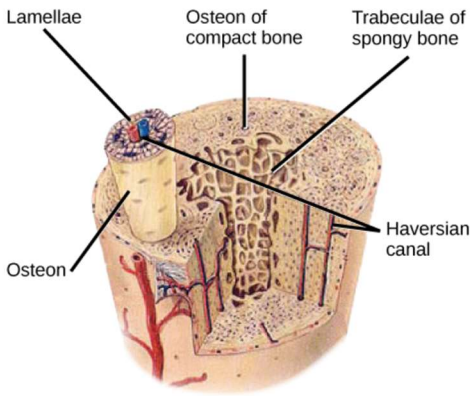

Figure 3. Illustration of compact bone tissues. Osteons are aligned parallel to the Haversian canal that contains the bone's blood vessels and nerve fibers. The inner layer of bones consists of spongy bone tissue.[38]

Cortical bone makes up most bone mass at nearly 80%, however, as humans age the cortical bone loses strength and becomes more porous. The trabecular bone is described as the spongy bone and its compartment is made up of about 20% bone and the remainder is marrow and fat.

The trabecular compartment also undergoes a higher turnover rate than the cortical compartment.[39]

During pregnancy, the demand for calcium within the body increases. If the mother does not have enough calcium through her diet or other supplements, the fetus will draw the calcium needed from the mother's bones. This process can lead to temporary bone mass reduction. Similarly, breastfeeding is also known to affect bone density with many women losing between 3 and 5 percent of their bone density during this time. However, this bone loss is not considered to be permanent, studies have shown that any bone mass lost during pregnancy and lactation will be restored several months after breastfeeding has stopped.[6]

Mammary glands are unique glands that reach maturity during a pregnancy/lactation cycle (PLC) and function by producing, secreting, and supplying milk to newborns. Breast changes such as increased breast volume can occur during pregnancy, however, there is a large variation in these changes between women. Changes in breast size do not reflect the amount of secretory tissue in the breast and do not indicate the lactation potential. Lactation activation and a rapid increase of milk synthesis occur 48-72 hours after the delivery of the placenta. The milk that is secreted during the first days following childbirth is known as colostrum. Colostrum has a higher protein content and is thought to provide newborns with the nutrients they need in their early days.[22] Mature breastmilk is secreted about 5 to 7 days after childbirth and is composed of water, lactose, fat, protein, calcium, sodium, potassium, phosphate, and chloride.[21] The composition of breast milk, as well as the composition of the breast, do not change during the lactation period.[40]

Most blood is supplied to the breasts by two major arteries, the internal mammary artery (IMA) and lateral thoracic artery (LTA). It is estimated that the IMA contributes approximately 70% to mammary blood supply, although this value varies between women. There is no significant

difference in milk production or 24- hour blood flow rate between the left and right breast. A study performed in 2012 found that there was a significant difference in milk productions when breasts were grouped by productivity levels (more productive: 444 mL; less productive: 334 mL; p=0.009), however, there was not a significant difference in blood flow between these productivity levels. Additionally, the mean blood flow velocity of lactating women was found to be double that of adolescent females.[22]

Many studies have shown that Pb can be transferred from blood plasma to breast milk. In a 2014 study, an average milk-to-plasma Pb ratio of 7.7 was found.[25] Furthermore, 97% of the milk-to-plasma ratios were >1. This is concerning because the current recommendations to stop breastfeeding when maternal PbB levels are >40 μg/dL are based on an assumed milk-to-plasma ratio of <1.[39-40] Additionally, infant PbB levels were shown to be significantly associated with maternal breast milk Pb levels.[25]

Model Status

Several steps for model validation were performed to assure proper model function. One of the most important steps is assuring mass balance. For PBPK models, errors and miscalculations almost always show up as progressive changes in mass balance. In computational rounding there is always some very limited fluctuation in mass balance related to rounding errors, usually in the range of 1e-6 or smaller. This model shows fluctuations in the range of 2e-10 as shown in Figure 1. This indicates very consistent performance of the model over the entire period modeled. These results are supported by blood flow and volume balance that are similarly balanced (Figure 4).

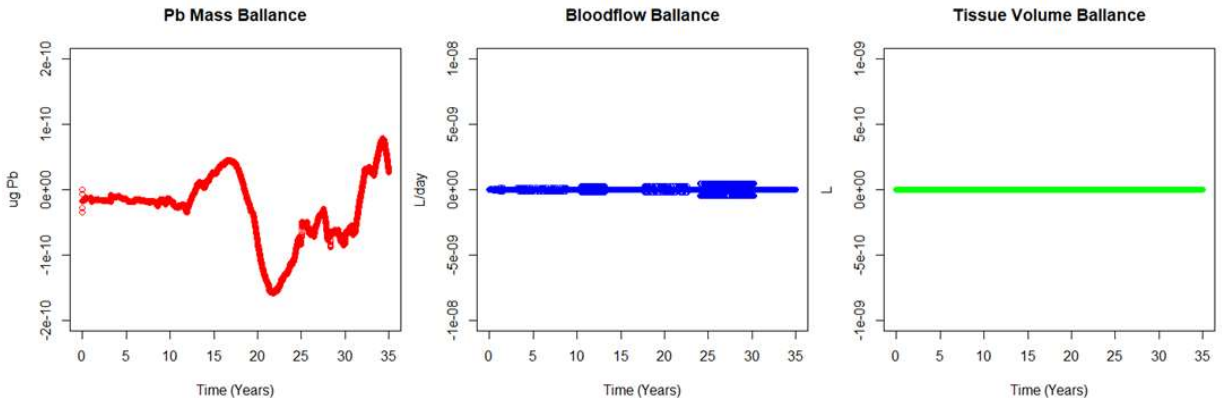

Figure 4. PBPK evaluation of mass, flow and volume balance over the course of a model run that support correct model function. Fluctuations are in the range of 1e-9 or less and amount to propagated rounding errors in the model that resolve with time.

The model is adapted into R software and currently models bone reabsorption and changes in PbB during lactation. As expected, there is an increase in PbB from birth through adulthood and a drastic increase in PbB during lactation periods. However, PbB levels return to pre-lactation concentrations immediately following the end of lactation. The model suggests that changes in PbB over time for a woman born in 2000 who experiences relatively high Pb exposures and breastfeeds for a period of 180 days at the age of 28 are shown in Figure 5. The model shows that the bone remodeling during the period of breastfeeding results in a 54% increase in maternal blood Pb concentrations, increasing from a baseline level of 2.5 µg/dL at the age of 28 following relatively high lifetime exposure as specific in the table above and in the absence of lactation, to 3.9 µg/dL during lactation.

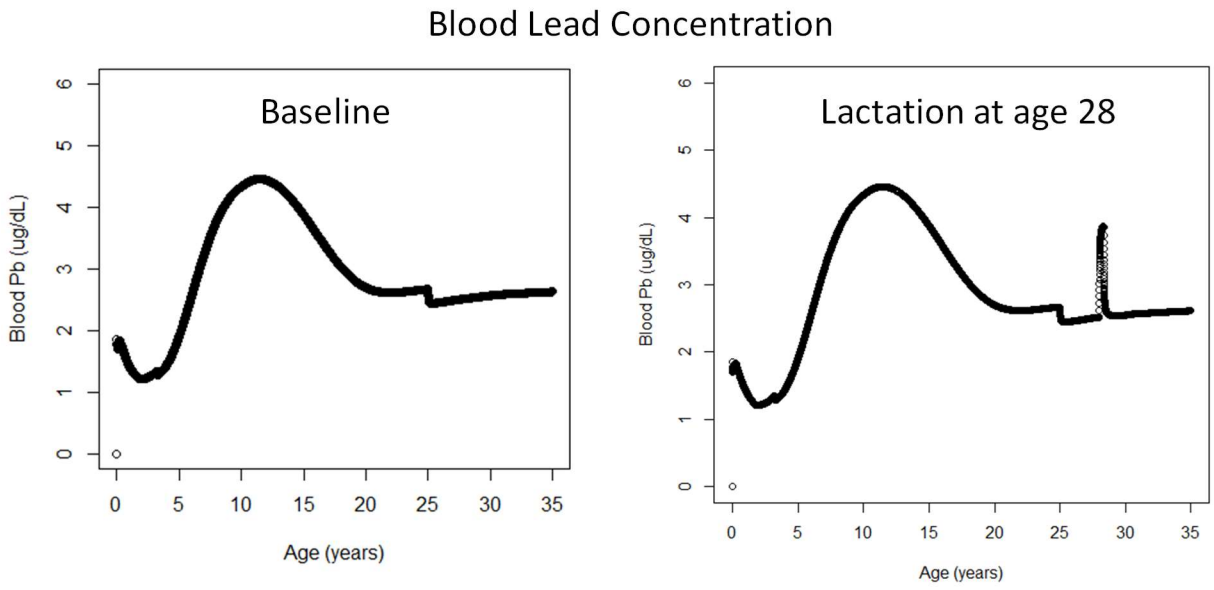

Figure 5. Two trends showing changes in PbB over time following relatively high Pb exposures. Baseline scenario shows PbB trend with no lactation period and Lactation at age 28 shows PbB trend from birth to age 35 including a 180-day period of breastfeeding at the age of 28

Due to the significant amount of time and effort required to create a working model, including defining model parameters and ensuring proper ordering of code, the addition of a breast compartment has not yet been completed. Without the addition of the breast compartment, breast milk Pb concentrations were not able to be obtained. This prevents the comparison of maternal PbB levels with breast milk Pb concentrations.

Discussion

The results from the PBPK modeling show that for women with elevated cumulative Pb exposures from inhalation and multiple oral pathways, PbB levels can exceed 1 µg/dL. Depending on the age and rate of exposure, blood concentration can increase, under current intake estimates, to levels almost as high as 5 µg/dL. In part, these changes reflect hyperbolic bone growth and turnover during maternal childhood and adolescence. During the late teens, bone turnover slows as adult size is reached, and at approximately age 25, bone growth stops,

and bone turnover is only that related to ongoing remodeling (observed as a sharp but small decrease in blood Pb). From age 25 onward until menopause (not modeled), blood Pb remains relatively constant assuming there is no change in intake. The onset of both pregnancy (not modeled) and lactation initiate a rapid increase in bone resorption as the body's demand for calcium and phosphate increases. This appears as a sharp peak in the blood Pb over the period of breastfeeding. In other model runs, it was observed that with very low maternal lifetime Pb exposure, there was very little effect of lactation on PbB levels, and total body burden appeared to drop as a result of renal and hepatic clearance mechanisms. In fact, PbB levels following average Pb exposures remained under 1 μg/dL and would not be expected to result in any negative health effects.[8]

This study served as the first step in determining the relationship between PbB levels and breast milk Pb concentrations. Further work, including the addition of the breast compartment, needs to be completed within the model.

Another factor that may further increase the PbB levels in mother and child is an increased lengths of time that women are breastfeeding their children. This can increase the bone breakdown and contribute to higher PbB levels for mother and child this increasing the likelihood of adverse health effects.[6] The model depicted in the results section of this thesis considers only a 6-month period of breastfeeding, of which the first 6-months is the only source of nutrition for infant, assuming formula is not used. CDC guidance recommends that infants start the switch of solid foods at 6 months of age.

Current environmental Pb levels were lower than anticipated at the beginning of the study. This is likely due to the significant efforts to lower human exposures to Pb by EPA and CDC. Most of these efforts are geared specifically toward lowering childhood exposure to Pb.[5&41] Significant

efforts were made to eliminate Pb from everyday items such as gasoline, paint, food and drink containers, and plumbing systems. Pb was removed from gasoline in 1970 and by 1997 the National Health and Nutrition Examination Survey (NHANES) showed a decrease in average PbB levels for both adults and children of more than 80 percent.[42] This trend has continued into recent years. A study performed in 2019 showed that the geometric mean PbB level of the United States population (ages 1 to 74) has dropped from 12.8 to 0.82 μg/dL.[44] This reduction shows the success of efforts to minimize Pb exposures in the United States.

Although there has been a consistent reduction in the population's exposure to Pb, there are still groups of children with high PbB levels. In 2012, the CDC introduced a population-based blood lead reference value (BLRV) in an attempt to identify children that have been exposed to Pb at a higher rate than other children. The current BLRV is 3.5 μg /dL and was based off the 97.5th percentile of the PbB distribution in U.S. children aged 1- 5 years from the latest NHANES data.[39] The demographic for children at risk of high lead exposures suggest that these exposed children are likely being raised in families with a lower socioeconomic status. The CDC states that young children at the highest risk of Pb exposure include those that live in older homes, particularly homes built prior to the banning of Pb paint in 1978. Older homes are also more likely to have plumbing fixtures that contain Pb. The CDC also states that Non-Hispanic Black or African- American children are at a higher risk of Pb exposure along with children insured through Medicaid and those living in areas with high poverty rates and low-quality housing stock.[44] These high-risk populations are also more likely to have a diet with low nutritional value which has been shown to correlate with higher PbB levels.[46]

The EPA has established the Safe Drinking Water Act (SDWA) and has set legal limits on over 90 contaminants, including Pb, to provide all humans with safe drinking water.[4] However,

citizens of the city of Flint, Michigan were exposed to contaminated drinking water during a period known as the Flint Water Crisis. The Flint Water Crisis started in 2014 when a switch in water supply resulted in contaminated drinking water being distributed to Flint residents. Government officials were slow moving to address the issue and in 2016 the concentration of Pb in Flint's water was still 20 parts per billion.[47]

Media coverage regarding Pb exposure may be giving some populations a false sense of security. There was extensive coverage on the Flint drinking water crisis which succeeded in raising the public's awareness on the issue of Pb exposure; however, in 2015 a significant number of Michigan cities were found to have a higher percentage of children with PbB levels above the BLRV than Flint.[47] Citizens in these cities may be under the impression that there is no risk of Pb contamination in their drinking water because there is a lack of media attention in these areas.

There were some limitations in this study:

1. The model was more complex than originally anticipated at the outset of the project. This has advantages and disadvantages. The major disadvantage was that the model was difficult to adapt to R software because R and AcslX, which was the original model software, are functionally very different. Consequently, it took much longer to adapt than is typical for a PBPK model. The advantage is that the adapted model can be used to capture and model blood and tissue Pb at any life stage, and can account for food water and air intakes from a wide range of sources. These intakes can include Pb in infant formula, baby food and oral Pb exposure in dust and soil for infants and children, and workplace inhalation for adults. This flexibility permits body Pb levels to be investigated for many exposure scenarios and may be particularly useful for investigations related to health inequities related to housing and environmental exposures.

2. The scope of this project was to investigate the effect of breastfeeding on the release of Pb accumulated in bone, and to model breast milk exposure levels. Due to time constraints, the concentration of Pb in breast milk has not been modeled at this time, largely because this requires the addition of a new eliminating compartment into the model for the partitioning of Pb into breast milk without disrupting modeled blood flow, volume, and mass balances.
3. The R-adapted model still requires validation to the original model. This work is currently ongoing and preliminary work is shown as part of the results provided above.
4. The current model as developed by O'Flaherty et al., (1998) and as described by Sweeney (2021) is a deterministic model that produces a predicted body Pb concentration under one specific set of parameters. A future addition to the model would be make the model probabilistic through the use of Monte Carlo modeling methods, which would capture the natural variability of the model input parameters and enable the evaluation of statistical significance.
5. Future work includes the addition of pregnancy as a stage in the model, and the fetus as a separate compartment. To our knowledge, there are only a few PBPK models that have attempted to model chemical exposures in a fetus, and Pb would be particularly challenging.

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Appendices

Appendix A. Historic Pb exposures from food, water, soil, dust, air and workplace sources. Unit is given along with the length of time exposed.

Variable	Value	Unit	Description
RFOODHIGH	12.5	µg/day	FDA IRL for Pb ingestion for adults in US. 2019[33]
RFOOD 2AVG	Range: 0.13 to 6.18 Average: 1.57	µg/kg/day	Rate of ingestion in Pb 2021 for adults[34]
ROTHER	10	µg/day	Rate of ingestion of Pb from other, unspecified oral sources by generic adult[15]
CWATERAVG	2.8	µg/L	Geometric mean concentration of water in United States[30]
CWATERHIGH	15	µg/L	EPA Action Level for Pb in drinking water32
CAIRAVG	0.044	$\mu g/m^3$	Mean Pb concentration in air in US 2020[29]
CAIRWORK	50	mg/m^3	OSHA PEL for workplace[9]
CAIRHIGH	0.3	$\mu g/m^3$	Highest Pb concentration in air in US 2020[29]
CFMLA	median = ND 75% = 5.6 max = 183.6	µg/kg	Pb concentrations in formula[2] 2018[35]
CFLOORDUSTNOW	10	$\mu g/ft^2$	EPA Clearance level for floor dust 2020[36]
CFLOORDUSTOLD	40	$\mu g/ft^2$	EPA Clearance level for floor dust prior to 2020[36]
CWINDUSTNOW	100	$\mu g/ft^2$	EPA Clearance level for windowsill dust 2020[36]
CWINDUSTOLD	250	$\mu g/ft^2$	EPA Clearance level for windowsill dust prior to 2020[36]