TOX/2022/05

Committee on Toxicity of Chemicals in Food, Consumer products and the Environment

Discussion paper on the effects of lead on maternal health

Background

1. The Scientific Advisory Committee on Nutrition (SACN) last considered maternal diet and nutrition in relation to offspring health in its reports on 'The influence of maternal, fetal and child nutrition on the development of chronic disease in later life' (SACN, 2011) and on 'Feeding in the first year of life' (SACN, 2018). In the latter report, the impact of breastfeeding on maternal health was also considered.

2. In 2019, SACN agreed to conduct a risk assessment on nutrition and maternal health focusing on maternal outcomes during pregnancy, childbirth and up to 24 months after delivery; this would include the effects of chemical contaminants and excess nutrients in the diet.

3. SACN agreed that, where appropriate, other expert Committees would be consulted and asked to complete relevant risk assessments e.g. in the area of food safety advice. This subject was initially discussed during the horizon scanning item at the January 2020 meeting with a scoping paper being presented to the Committee in July 2020. This included background information on a provisional list of chemicals proposed by SACN. It was noted that the provisional list of chemicals was subject to change following discussion by COT who would be guiding the toxicological risk assessment process: candidate chemicals or chemical classes can be added or removed as the COT considered appropriate. The list was brought back to the COT with additional information in September 2020. Following a discussion at the COT meeting in September 2020, it was agreed that papers on a number of components should be prioritised and to this end, papers on iodine, vitamin D and dietary

supplements have been or will be presented to the Committee. The remaining list of compounds were to be triaged on the basis of toxicity and exposure.

4. Following discussion of the first prioritisation paper on substances to be considered for risk assessment by the COT, the Committee decided that each of the heavy metals (lead, mercury, cadmium and arsenic) should be considered in separate papers. The following paper discusses the risks posed to maternal health by lead in the diet and the environment.

Introduction

5. The Merck Index (12th Edition, 1996) describes lead (Pb) as a bluish-white-to-silvery grey Group 14 metal, with atomic number 82 and a relative atomic mass of its most abundant isotope of 208. It occurs naturally in the Earth's crust at an abundance of about 0.002%, chiefly as lead sulphide (PbS), It is very soft and malleable and has a long history of use in domestic articles such as drinking vessels and plates and in water and drainage pipes (plumbing, from "plumbum", the Latin word for lead). More recently it has been used in paints, ceramic pigments and as the "anti-knock" agent tetraethyl lead in petrol. Due to its long-known toxicity, many of these uses have been substituted with less toxic alternatives but lead is still used in various applications such as car batteries and as radiation shielding in the nuclear industry.

6. The Joint FAO/WHO Committee on Food Additives (JECFA) (FAO/WHO, 2011) state that lead contamination of food arises mainly from the environment or from food processing, handling and packaging. Atmospheric lead can contaminate food through deposition on agricultural crops. Water is another source of lead contamination of food. Although lead exists in both organic and inorganic forms, only inorganic lead has been detected in food.

Previous evaluations

7. The European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM Panel) identified developmental neurotoxicity in young children, and cardiovascular effects and nephrotoxicity in adults as the critical effects

for the risk assessment of lead. The respective BMDLs (Benchmark Dose lower limits) derived from blood lead levels in µg/L (corresponding dietary intake values in µg/kg b.w. per day) were: developmental neurotoxicity $BMDL_{01}$ (BMDL for a 1% change in toxic response), 12 (0.50); effects on systolic blood pressure (SBP) $BMDL_{01}$, 36 (1.50); effects on prevalence of chronic kidney disease $BMDL_{10}$, 15 (0.63). EFSA concluded that even low exposures to lead in pregnant women, infants and children can adversely affect neurodevelopment (EFSA, 2010, updated 2013).

8. The CONTAM Panel concluded that a margin of exposure (MOE) of 10 or greater (of the corresponding dietary intake values) would be sufficient to ensure that there was no appreciable risk of a clinically significant effect on SBP or kidney disease and that at MOEs of greater than 1.0 the risk would be very low.

9. In young children, EFSA concluded that a MOE of 10 or greater (of the corresponding dietary intake values) should be sufficient to ensure that there was no appreciable risk of a clinically significant effect on IQ. At lower MOEs, but greater than 1.0, the risk was likely to be low, but not such that it could be dismissed as of no potential concern. With regard to the developing fetus, EFSA stated that "…The relative sensitivity of the fetus to the effects of lead on neurodevelopment is not known. The CONTAM Panel therefore made the assumption that the developing fetus is as least as sensitive to this effect of lead as a young child. "

10. JECFA (2011) stated that the previously established provisional tolerable weekly intake (PTWI) of 25 µg/kg bw, associated with a decrease of at least 3 IQ points in children and an increase in systolic blood pressure of approximately 3 mmHg (0.4 kPa) in adults could no longer be considered health protective, and it was withdrawn.

11. The International Agency for Research on Cancer (IARC) classified lead compounds as probably carcinogenic to humans (Group 2A) on the basis of limited evidence of carcinogenicity in humans and sufficient evidence in animals. Organic lead compounds were considered not to be classifiable as to their carcinogenicity to humans (Group 3) because there was inadequate evidence for carcinogenicity in humans and animals. The mechanisms of lead induced cancers in experimental systems are complex, appearing to involve oxidative stress, interaction with zinc

finger proteins, induction of apoptosis, altered cell signalling pathways and interactions with cellular genetic machinery by high affinity lead-binding proteins (IARC, 2006).

12. The COT last evaluated lead in food in 2014 as part of the infant diet. (Link to PDF). Of relevance to this current discussion paper on the maternal diet, were:

- That the general population is exposed to lead through food, drinking water, air, soil and dust. Food and water are the major sources of exposure to lead.
- In general, exposure to lead has decreased substantially over recent decades.
- Lead absorption is higher in a fasting state than when people have recently eaten. Inadequate intakes of calcium, iron and zinc have been shown to increase lead absorption, and elevated levels of fat in the diet may result in higher blood lead concentrations.
- Absorbed lead is transported in the blood bound to proteins, predominantly in erythrocytes, but also in plasma. It is then deposited in soft tissues and bone, where it tends to accumulate with age.
- Maternal bone is mobilised during pregnancy and lactation to meet the demands of fetal mineralisation, and this leads to release of lead from the mother's bone.
- Adverse effects can arise from long term dietary exposures at levels below those which cause acute toxicity due to lead accumulation in tissues.
- The kidney and cardiovascular systems are adversely affected by lead exposure in adults. Neurotoxicity has been identified at lower levels of exposure, and the developing brain appears to be more vulnerable than the mature brain.
- It has not been possible to demonstrate a threshold level of exposure below which the neurodevelopmental effects of lead do not occur.

- EFSA and JECFA both derived Benchmark Dose Lower limits for lead for a (a one-point difference in IQ), derived from pooled analysis of multiple cohort studies of exposures in infants and children ($BMDL_{01}$). In the statement, the COT based its risk characterisation on the EFSA $BMDL_{01}$, which is between the EFSA BMD and the lower 90% confidence limit for the BMD_{01} calculated by JECFA, and corresponded to a dietary exposure of 0.5 µg/kg bw/day. COT agreed with the EFSA evaluation that a MOE greater than 1 could be regarded as being of low concern for health.

Hazard Identification

13. This section focusses on summarising papers that have been published since the COT last reviewed lead (as part of the infant diet) in 2014, but also includes summaries of papers which had been included in that statement.

ADME

14. Lead absorption has been measured in a number of studies, and in adult humans is approximately 10% of the ingested dose (Rabinowitz et al., 1976). Lead absorption from the gastrointestinal tract appears to be higher in infants and children than in adults, with an average lead absorption in infants of about 42 % of intake (Ziegler et al., 1978). This is supported by animal studies which indicate that gastrointestinal absorption rates for lead are greater in the very young than in older animals (Forbes *et al.*, 1972; McMichael et al., 1986). Approximately 95 % of lead in adult tissues and 70 % in children resides in mineralised tissues such as bones and teeth. This reflects changing turnover rates throughout an individual's lifetime, with a slower turn-over of lead in the bones of adults than those of children. The lead which has accumulated in adult bone can replenish lead eliminated from blood by excretion, long after the external exposure has ended. It can also be a source of lead transfer to the fetus when the maternal skeleton is resorbed for the production of the fetal skeleton

15. Bolan et al (2021) examined the intestinal permeability of arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg), as influenced by gut microbes and chelating agents using an in vitro gastrointestinal/Caco-2 cell intestinal epithelium

model. The results showed that, for lead, in the presence of gut microbes or chelating agents, there was a significant decrease in the permeability of -7.9% as measured by apparent permeability coefficient value (P_{app}). Chelating agents reduce intestinal absorption of metals and metalloids by forming complexes thereby making them less permeable. In the case of gut bacteria, the decrease in the intestinal permeability may be associated to a direct protection of the intestinal barrier or indirect intestinal sequestration by the gut bacteria through adsorption on bacterial surface. Thus, both gut microbes and chelating agents can be used to decrease the intestinal permeability of toxic metals and metalloids, thereby mitigating their toxicity.

16. Silbergeld et al. (2009) reported that the primary site of lead storage is in bone. This is not a physiological sink since the lead can be mobilized back into the circulation in response to normal or pathological changes in mineral metabolism. Bone lead may be a significant source of target organ exposure under certain conditions, such as pregnancy, kidney disease, and menopause.

17. Rădulescu and Lundgren (2019) reviewed the recent pharmacokinetic models for lead. Absorption takes place via ingestion, inhalation and to a lesser extent through the skin. The effectiveness of the gastrointestinal absorption depends on the individual's prior quantitative (since food consumption decreases absorption of water-soluble lead) and qualitative (due to interactions with other elements in the diet) food intake. The efficiency of gastrointestinal absorption of water-soluble lead is also age-dependent and is higher in children than in adults. The authors cited several older studies regarding the different distributions of lead in human soft tissues, highlighting that the major organ is the liver (Barry, 1975; Gross et al. (1975); Schroeder, H. A. & Tipton, I. H. 1968; Barregård et al. 1999; and Gerhardsson et al. 1995), but their main interest was uptake and damage to developing neural tissues.

18. Gulson et al. (1997, from abstract) performed a longitudinal study in an urban environment of Australia with European female immigrants of child-bearing age (18 to 35 years). High-precision lead isotopic compositions and lead concentrations were measured in maternal blood and urine prenatally, monthly during gestation, and postnatally for 6 months, as well as in infant blood and urine for 6 months. Pregnant subjects served as their own controls to compare changes during gestation with

those before conception. Environmental samples were taken quarterly. There were 13 conceptions in immigrant subjects, with 7 births, and 3 conceptions and 2 births in the Australian control group. Blood lead (bPb) levels had a geometric mean of 30 mg/l (range 19 to 200 mg/l) and 20 % increases in bPb were detected during pregnancy. The skeletal contribution to bPb level was 31 % ± 19 % (mean ± SD). Changes in isotopic composition and bPb for Australian pregnant controls were negligible. The ratio of cord/maternal bPb levels varied from 0.54 to 1.05, and the ratio for the isotopic composition was 0.993 to 1.002. These results showed that mobilisation from long-term stores (i.e., bone) contributed significantly to bPb levels during pregnancy. Even after 800 days of residence in Australia, the contribution of European skeletal lead to blood lead in nonpregnant subjects could be about 50 %.

19. Gulson et al. (2000) compared lead isotopic ratios and lead concentrations in 53 spot urine and fifty-nine 24-hour urine samples, from 13 subjects covering the interval from pre-pregnancy through to 180 days postpartum, to estimate the amount of lead excreted in urine and renal clearance relative to blood. Lead excreted in 24-hour urine samples ranged from 0.8 to 5.9 μg Pb with an arithmetic mean of 2.2 ± 1.1 μg (geometric mean 1.90 μg). This compares with 0.9 - 10 μg of extra lead per day estimated to be released into blood from the skeleton during pregnancy and postpartum. There were no differences in excretion rates between the trimesters of pregnancy or between pregnancy and postpartum periods. The renal clearance relative to blood ranged from 0.8 to 10 g/h (arithmetic mean: 3.2 ± 1.9 g/h; geometric mean: 2.7 g/h). Renal clearance relative to blood was higher in trimesters 2 and 3 compared with 150 - 180 days postpartum ($p = 0.004$ and 0.006, respectively). This indicated that for lead, unlike for calcium, there was no increased gastrointestinal absorption during pregnancy and postpartum. Most of the subjects had low levels of lead in their 24-hour urine samples and the authors recognised potential contamination to be a problem with such samples.

20. Omeljaniuk et al. (2018) studied the concentration of cadmium (Cd) and Pb in blood and placental tissue in women who had miscarried. The study consisted of a group of 83 women who miscarried and a control group of 35 women in the first trimester of pregnancy and after childbirth. The concentrations of Cd and Pb in whole blood and fragments of placental tissue were determined. The average concentration

of Cd (2.730 ± 2.07 µg/L) and Pb (35.54 ± 11.0 µg/L) in the blood of women with miscarriage was higher than those in the blood from the control group (Cd 1.035 ± 0.59 µg/L; Pb 27.11 ± 4.6 µg/L). The average Cd (214.4 ± 514 µg/L) and Pb (199.6 ± 348 µg/L) content in the placenta of women with miscarriage was higher than those in the placenta of women from the control group (Cd 127.4 ± 85 ng/L; Pb 26.35 ± 7.9 ng/L).

21. Coiplet et al. (2020) evaluated the relevance of a questionnaire to screen pregnant women for lead exposure. A cross-sectional, multicentre study was carried out on a population of 792 pregnant women from February 2018 to May 2020. A total of 596 women had a blood lead test: 68.5 % had blood lead levels below 10 µg/L. The estimated prevalence above 25 µg/L was 4 % (95 % confidence interval (CI) [2.6 – 5.9]) and 1.3 % had levels above 50 µg/L (95 % CI [0.6 – 2.6]). Multivariate analysis showed that three risk factors significantly increased the probability of blood lead levels above 25 µg/L: the use of traditional cosmetics (adjusted odds ratio [aOR]: 3.90; 95 % CI [1.65 – 9.21]; p = 0.002), degraded old housing (aOR: 2.67; 95 % CI [1.19 – 6.038]; p = 0.018), and (marginally) eating bread more than twice a day (aOR: 2.40; 95 % CI [0.96 – 6.11]; p = 0.060). The authors concluded that a three-question tool could quickly screen for the risk of lead exposure in a population and could be used to trigger lead blood tests and special vigilance during pregnancy follow-up.

Toxicity

Reviews of toxicity of lead

22. Flora et al. (2012) and Wani et al. (2015) reviewed the toxicity of lead. The central nervous system, erythropoietic system and the kidneys are the most affected but overall, all bodily systems are adversely affected by the presence of this metal.

23. The US Agency for Toxic Substances and Disease Registry (ATSDR,1999) stated that exposure to lead affects multiple health outcomes and physiological systems, including the following: hypertension, the gastrointestinal system, anaemia, nephropathy, vitamin D metabolism, decreased growth, the immune system, the nervous system, behavioural/cognitive/IQ effects (and as a result, multiple social

effects, including increased risk of violence and drug abuse), nerve conductive effects, hearing loss, effects on reproduction and development and death from encephalopathy.

24. The acute effects of lead, from intense exposure of short duration, manifest at blood levels of 1000 – 1200 μg/l with muscle pain, fatigue, abdominal pain, headache, vomiting, seizures, and coma. Chronic lead poisoning from low level, repeated exposure leading to blood levels of 40 – 60 μg/l gives clinical signs of persistent vomiting, encephalopathy, lethargy, delirium, convulsions and coma.

25. Both the central nervous system and the peripheral nervous system are affected by lead exposure. The effects on the peripheral nervous system are more pronounced in adults while the central nervous system is more prominently affected in children. Lead exposure causes encephalopathy, the major symptoms of which include dullness, irritability, poor attention span, headache, muscular tremor, loss of memory and hallucinations. More severe effects occur at very high exposures and include delirium, lack of coordination, convulsions, paralysis, coma and ataxia. Fetuses and young children are especially vulnerable since the developing nervous system absorbs a higher fraction of lead. The proportion of systemically circulating lead gaining access to the brain of children is significantly higher as compared to adults (Needleman *et al.*, 2004). Exposure to lead also causes peripheral neuropathy, with reduced motor activity due to myelin sheath loss. This seriously impairs impulse transmission, causing muscular weakness, especially of the exterior muscles, fatigue and lack of muscular coordination (Sanders et al., 2009).

26. Garza et al. (2007) reviewed the molecular basis for the toxicity of lead. The toxic mechanism of lead is caused by its ability to substitute for other polyvalent cations, particularly divalent cations, such as calcium and zinc that are essential in processes, such as metal transport, energy metabolism, apoptosis, ionic conduction, cell adhesion, inter- and intracellular signalling, diverse enzymatic mechanisms, protein maturation, and genetic regulation. Membrane ionic channels and signalling molecules are the most relevant molecular targets contributing to lead's neurotoxicity and the developing central nervous system is particularly susceptible. At critical times in development, lead may have a disorganizing influence with long-lasting

effects that may continue into teenage years and beyond. Paediatric lead poisoning is more common than adult lead poisoning, and its effects may occur at reduced blood levels with subclinical symptoms.

27. The remainder of this discussion paper will discuss the toxicity of lead in the context of maternal health and pregnancy outcomes. For further information on the general toxicity of lead, if required, see UK government information provided on the NHS website.

Reproductive toxicology

High blood pressure

28. Wells et al. (2011) sought to determine the association between low-level lead exposure and blood pressure during late pregnancy. Initial and maximum systolic and diastolic blood pressures were taken during labour and delivery in 285 women admitted to hospital in Baltimore, Maryland. Umbilical cord blood lead was measured using inductively coupled plasma mass spectrometry. Multivariable models were adjusted for age, race, median household income, parity, smoking during pregnancy, pre-pregnancy body mass index, and anaemia. These models were used to calculate benchmark dose (BMD) values. Geometric mean cord blood lead was 0.66 µg/dL (95 % confidence interval, 0.61 - 0.70). Comparing blood pressure measurements between those in the highest and those in the lowest quartile of lead exposure, there was a 6.87 mmHg (1.51 - 12.21 mmHg) increase in systolic blood pressure and a 4.40 mmHg (0.21 - 8.59 mmHg) increase in diastolic blood pressure after adjustment for confounders. Corresponding values for maximum blood pressure increase were 7.72 (1.83 - 13.60) and 8.33 (1.14 - 15.53) mmHg. BMDL values for a 1-standard deviation increase in blood pressure were < 2 µg/dL blood lead for all blood pressure end points, showing a significant association between low-level lead exposures and elevations in maternal blood pressure during labour and delivery

Pregnancy outcomes

29. Taylor et al. (2015) studied the associations of prenatal blood lead levels with pregnancy outcomes in a large cohort (n = 4285) of mother – child pairs in Bristol, UK. Whole blood samples were analysed by inductively coupled plasma dynamic reaction cell mass spectrometry. Outcomes were adjusted for covariates including maternal height, smoking, parity, sex of the baby and gestational age. Birthweight, head circumference, crown – heel length and preterm delivery rates were assessed. The mean blood lead level (B-Pb) was 3.67 ± 1.47 μg/dl. BPb of ≥ 5 μg/dl significantly increased the risk of preterm delivery (OR: 2.00; 95 % CI: 1.35 – 3.00) but not of having a low birthweight baby (OR: 1.37; 95 % CI: 0.86 – 2.18) in multivariable binary logistic models. Increasing B-Pb was significantly associated with reductions in birth weight (OR: 13.23; 95 % CI: 23.75 - 2.70), head circumference (OR: 0.04; 95 % CI: 0.07 - 0.06) and crown – heel length (b 0.05; 95 % CI: 0.10 - 0.00) in multivariable linear regression models. The authors concluded that there was evidence for adverse effects of maternal BPb on the incidence of preterm delivery, birthweight, head circumference and crown–heel length, but not on the incidence of low birthweight, in this group of women.

30. Jelliffe-Pawlowski et al. (2006) investigated associations between magnitude and timing of maternal pregnancy blood lead levels on birth weight, and total days of gestation, and associations with related clinical diagnoses of low birth weight (LBW), preterm, and small-for-gestational-age (SGA) birth. A retrospective study of 262 mother–infant pairs was undertaken, and one-way ANOVA and regression statistics were used to measure the relationship between maternal pregnancy blood lead levels and birth outcomes, with controls for key maternal and new-born factors. Women with pregnancy blood lead of ≥ 10 μg/dl tended to give birth earlier and their babies were at substantially increased risk for preterm and SGA birth. By holding other explanatory factors constant, each unit increase in blood lead above 10 μg/dl was found to be associated with a decrease of 0.3 days of gestation. Compared to women with lower lead levels, women with blood lead of ≥ 10 μg/dl were at a three-fold increased risk for preterm birth (OR: 3.2; 95 % CI: 1.2 – 7.4) and greater than four-fold increased risk for having an SGA infant (OR: 4.2; 95% CI: 1.3 – 13.9). Second trimester maximum blood lead of ≥10 μg/dl was associated with a steep decrease in total days of gestation (a decrease of 1 day per each unit increase

above 10 μg/dl). These data provided evidence of the adverse effects of maternal pregnancy blood lead levels of ≥10 mg/dl.

31. Hertz-Picciotto (2000) described a prospective study that investigated the link between lead exposure and spontaneous abortion in pregnant women in Mexico City with low-to-moderate-level lead exposures. Blood samples were collected during their first trimester, and the rate of spontaneous abortions ascertained by week 20. Incidence-density-matched controls were used to compare outcome and comparable timing of exposure measurements and to take into account changes in blood lead levels caused by pregnancy. A dose–response relationship between blood lead and risk of spontaneous abortion was found: the OR for spontaneous abortion was 1.8 (95 % CI: 1.1 – 3.1) for every 5 μg/dL increase in blood lead. Thus, low-to-moderate lead exposures may increase the risk for spontaneous abortion, but the author cautioned that further research was needed to confirm the association, to delineate the role of maternal vs. paternal exposures, and to assess increases in menstrual variability as an explanation for this finding.

32. Ou et al. (2020) studied the effects of blood lead levels (BLLs) on spontaneous abortion in a case-control study at Peking Union Medical College Hospital from 2016 - 2018. The case group comprised 150 spontaneous abortion cases requiring suction and curettage within 12 weeks of gestation. The control group (n = 150) was matched in age, gravidity, parity, and gestational age. Age, gravidity, parity, gestational age and the number of lead exposures, smokers, alcoholic beverage drinkers, and coffee drinkers were not significantly different between the two groups. The mean BPbs were 27.17 μg/L and 17.28 μg/L for the case and control groups, respectively (p = 0.000). The odds ratios for spontaneous abortion comparing 5 - 9, 10 - 14, 15 - 24, 25 - 39, and ≥ 40 μg/L with a reference category of < 5 μg/L blood lead were 1.58 (Range 0.23 - 10.90), 3.13 (Range 2.11 - 9.08), 4.63 (1.45 - 14.83), 6.33 (1.95 - 20.56), and 22.56 (4.91 - 103.66), respectively, demonstrating a significant trend (P1 = 0.64, P2 = 0.02, P3 = 0.01, P4 = 0.02, and P5 = 0.00).The conclusion reached by the authors was that during early pregnancy, when BPb is above 10 μg/L, the chance of spontaneous abortion increases compared to BPbs below 5 μg/L.

33. Relationships between cord blood lead level (mean 3.9 [SD 3.6] µg/dl) and birth weight and length were reported in a study conducted in Brazil (Zentner et al., 2006). In this study, an inversely proportional correlation was observed between Pb concentrations in umbilical cord blood and newborn weight and length, suggesting that Pb may have a negative influence on the growth of children even at low levels of exposure. The authors suggested that effects of lead on fetal thyroid function may have been involved in the observed effects but cautioned that further data would be required to test this hypothesis.

34. Chen et al. (2006) reported that maternal BPb concentrations ≥20 µg/dL had a higher risk giving birth to a SGA child (RR = 2.15). The subjects were classified into three groups according to serum Pb. L-Pb (low Pb, <1.18 µg/dL), M-Pb (medium Pb, 1.18–1.70 µg/dL), and H-Pb (high-Pb, ≥1.71 µg/dL). The rate of SGA was 6.2 % in subjects with L-Pb, 8.7 % in subjects with M-Pb, and 10.1 % in subjects with H-Pb, respectively. The rate of SGA infants was elevated only in subjects with H-Pb in the first trimester. The authors further reported that maternal exposure during pregnancy elevates risk of SGA in female offspring only.

35. Koi et al. (2019) investigated relationships between the concentrations of macroelements (Ca), microelements (Cr, Cu, Fe, Mn, Mo, Ni, Sn, Sr, V, Zn) and heavy metals (Ag, Cd, Pb) in 81 placentas, 67 fetal membranes and 22 umbilical cords from 83 Polish mothers aged from 17 to 44. They also examined relationships between the concentrations of these metals and maternal age, gestational age, placenta parameters (breadth, length, weight) and newborn parameters (length, weight and Apgar score). There was a relationship between placental width and placental lead. The levels of Pb, Ni, Mo, and V were found to be significantly higher in the umbilical cord than in the placenta and fetal membrane. This contrasted with the observations of Sakamoto *et al.* (2010). and Zhou *et al.* (2017)., who found higher Pb concentrations in maternal blood compared to the umbilical cord blood, implying a possible passive transport of the elements from mother to fetus. The latter authors postulated limited placental protection from Pb.

36. Hernandez-Avila et al. (2002) determined the bone lead burden with *in vivo* K-X-ray fluorescence of the tibia (cortical bone) and the patella (trabecular bone). The mean bone Pb levels were 9.8 and 14.4 µg/g bone mineral for the tibia and patella,

respectively. Birth length of newborns decreased as tibia Pb levels increased. Patella Pb was positively and significantly related to the risk of a low head circumference score; this score remained unaffected by inclusion of birth weight. The authors estimated the increased risk to be 1.02 per µg lead/g bone mineral. Odds ratios did not vary substantially after adjusting for birth weight and other determinants of head circumference.

37. Women's exposure to lead might cause several disorders such as higher prevalence of menstrual disturbance and even at low exposure levels associated with preterm birth and reduced birth weight. Prenatal lead exposure can affect maternal health and infant birth outcomes. Vigeh et al. (2011) reported that blood Pb level was significantly higher in mothers who delivered preterm babies than in those who delivered full-term babies (4.46 ± 1.86 and 3.43 ± 1.22 µg/dL, respectively). Logistic regression analysis demonstrated that a 1 unit elevation in BPb levels led to an increased risk of preterm birth.

38. Lamichhane et al. (2018) reported that maternal lead exposure is associated with poor birth outcomes. They have evaluated whether associations between prenatal Pb and birth outcomes differed by maternal GST genes and infant sex. The genotyping of GST-mu 1 (GSTM1) and theta-1 (GSTT1) polymorphisms was studied and did not find a statistically significant association between prenatal BPb levels and birth outcomes; in stratified analyses, the association between higher BPb during early pregnancy and lower birth weight was significant in males of mothers with GSTM1 null. The results were similar for head circumference model, but the level of significance was borderline. Head circumference model showed a significant three-way interaction among BPb during early pregnancy, GSTM1, and sex. For combined analysis with GSTM1 and GSTT1, GSTM1 null and GSTT1 showed a significant inverse association of BPb with birth weight and head circumference in males.

39. Lamadrid-Figueroa et al. (2007) studied 207 pregnant Mexican women during the 1st trimester of at least their second pregnancy, recruited between 1997 and 2004. Inclusion in the study sample was unrelated to observable characteristics such as number of abortions, number of pregnancies, blood Pb levels, age, schooling, weight and height. Lead was measured in whole blood and plasma by inductively

coupled plasma mass spectrometry. History of miscarriage in previous pregnancies was obtained by interview. The incidence rate of spontaneous abortion was defined as the proportion of previous pregnancies that resulted in miscarriage. The mean number of miscarriages was 0.42 (range 0 to 4); mean Pb concentrations were 62.4 and 0.14 μg/L in whole blood and plasma respectively. Mean plasma/blood Pb ratio was 0.22 %. A 0.1 % increment in the plasma/blood Pb ratio lead was associated with a 12 % greater incidence of spontaneous abortion (p = 0.02). Women in the upper tertile of the plasma/blood Pb ratio had twice the incidence rate of those in the lower tertile (p = 0.02).

40. Borja-Aburto et al. (1999) evaluated the risk of spontaneous abortion from low or moderate Pb exposures. A total of 668 women were enrolled during their first trimester, and the mean BPb levels were 12.03 μg/dL for spontaneous abortion cases and 10.09 μg/dL for controls (*P* = 0.02). Odds ratios for spontaneous abortion comparing 5–9, 10–14, and ≥15 μg/dL with the referent category of <5 μg/dL of BPb were 2.3, 5.4, and 12.2, respectively. After multivariate adjustment, the odds ratio for spontaneous abortion was 1.8 for every 5 μg/dL increase in BPb load. However, Vigeh et al. (2011) did not find significant difference between spontaneous abortion cases and ongoing pregnancies (3.51 ± 1.42 and 3.83 ± 1.99 μg/dL, respectively). They also suggested that in apparently healthy women, low BPb levels (mean <5 μg/dL) in early pregnancy may not be a risk factor for spontaneous abortion. Based on available data, it can be inferred that long-term lead exposure adversely affects fetal viability as well as fetal and early childhood development, as lead is reported to cross the placenta readily

41. Hu et al. (2006) investigated the impact of prenatal lead on neurodevelopment using repeated measures of fetal dose as reflected by maternal whole blood and plasma lead levels. Lead in maternal plasma and whole blood was measured during each trimester in 146 pregnant women in Mexico City, at delivery and in infants at 12 and 24 months of age. The Bayley Scales of Infant Development were applied. Multivariate regression was used, adjusting for covariates and 24-month blood lead, to compare the impacts of the fetal lead dose. Maternal lead in first-trimester blood was 7.1 +/- 5.1 mg/dL (mean ± SD) and 14 % of values were ≥10 mg/dL. Both maternal plasma and whole blood lead during the first trimester (but not in the

second or third trimester) were significant predictors ($p < 0.05$) of poorer Mental Development Index (MDI) scores. In models combining all three trimester measures and using standardized coefficients, the effect of first-trimester maternal plasma lead was greater than the effect of first-trimester maternal whole blood lead and the effects of second- or third-trimester plasma lead, and values averaged over all three trimesters. A 1-SD change in first-trimester plasma lead was associated with a reduction in MDI score of 3.5 points. Postnatal blood lead levels in the offspring were less strongly correlated with MDI scores. From these results it appears that the adverse effect of lead exposure on neurodevelopment may be most pronounced during the first trimester.

42. Irwinda et al. (2019) evaluated the status of micronutrients and toxic metals in pregnant women and whether their concentration was different between term and preterm birth in Indonesia. Blood samples were taken from pregnant women undergoing birth at 26 – 36 weeks of gestational age for the preterm groups and ≥37 weeks of gestational age for the term group. The exclusion criteria were subjects with multiple pregnancies, intrauterine growth restriction, fetal congenital anomaly, preterm premature rupture of membrane and other comorbidities (hypertension in pregnancy, pre-eclampsia, gestational diabetes mellitus, heart disease, and autoimmune disease). The full-term group had higher placental concentrations of manganese, iron, copper, zinc, selenium, all-*trans* retinoic acid, and 25(OH) vitamin D, as well as lower concentrations of mercury and lead compared to the preterm group. The authors suggested that lead, by virtue of its ability to generate reactive oxygen species (ROS) might contribute to DNA damage and telomere shortening. This could accelerate telomere-dependent senescence of fetal membranes, and thereby cause senescence-associated inflammation, leading to premature parturition. However, the authors conceded that much was unknown regarding the placenta and its homeostasis and protective mechanisms.

43. Karri et al (2004) describe the dangers of traditional remedies, in particular Ayurvedic medicines that are popular in Indian cultures. These preparations are well known to contain sometimes high concentrations of heavy metals, including lead. The authors cited the case of a pregnant 24-year-old woman in the 30th week of gestation who presented with abdominal pain, progressive disorientation, and

seizures. The patient was a recent immigrant from India to Australia and had consumed ayurvedic medicines periodically over the previous 9 years. She had a blood lead concentration of 1070 µg/l and a blood film showing anaemia with prominent basophilic stippling. Chronic lead encephalopathy was diagnosed, and chelation therapy was prescribed. The patient gave birth prematurely. Apgar scores were 4 and 6, indicating fetal distress. The infant presented with signs and symptoms of bilateral diaphragmatic palsy and was intubated. A blood film from the child showed Heinz bodies, indicating haemoglobin damage, and a lead concentration in cord blood of around 1400 µg/L. Radiographs of the long bones showed an increase in the bone density adjacent to the metaphyses. The baby was immediately put on chelation therapy and blood lead levels dropped, but then went on to show delayed developmental milestones with peripheral weakness and bilateral wrist drop.

44. Iwai-Shimada et al. (2019) used inductively coupled plasma mass spectrometry to determine the concentrations of arsenic (As), bismuth (Bi), cadmium (Cd), mercury (total mercury (THg), methylmercury (MHg), inorganic mercury (IHg)), lead (Pb), antimony (Sb) and tin (Sn), and essential trace elements, copper (Cu), selenium (Se) and zinc (Zn), in maternal blood, cord blood and placenta, in Japanese women (N = 594 – 650) (Tohoku Study of Child Development of Japan). Unlike other elements, like antimony, lead was found to be evenly distributed between maternal and cord blood, reflecting the ease of its passage across the placenta: Median concentrations (25th–75th) of Pb in the maternal blood were 10.8 (8.65 – 13.5), Median concentrations (25^{th} – 75^{th} percentile) of Pb, in the cord blood were 9.89 (8.02 – 12.5) ng/g. This finding agreed with the results of an earlier systematic review by Esteban-Vasallo *et al.* (2012). However, Kabamba and Tuakuila (2020), also in a systematic review of toxic metals in maternal and cord blood, found that much greater variability was seen with Cd and Pb. At delivery, total Hg and Pb levels in maternal blood were strong predictors of cord blood levels of these metals. The authors said that understanding the partition, levels and correlations of toxic metals in the maternal/cord blood may help to elucidate the adverse effects of these metals on fetuses and neonates.

45. Increased paternal or maternal lead exposure has long been linked to the risk of a congenital malformation in offspring, although the evidence has been inconsistent across studies. In some studies demonstrating such an association, exposure status was based solely on job title rather than on a lead biomarker. An increased risk of neural tube defects was reported in an historic study of offspring of women residing in an area with high lead levels in water (Bound et al., 1997). Although there was evidence suggesting that lead was one cause of neural tube defects, especially anencephaly, the area involved (Fylde in Lancashire), suffered from high social deprivation and other factors, such as deficiency of folic acid and zinc, may have played a part and been exacerbated by lead.

Effects on maternal health

46. In a study conducted in Kosovo (Factor-Litvak et al., 1993), the OR for proteinuria rose above 1 for women with a blood lead level greater than 58 µg/l and was 4.5 (95 % CI: 1.5 – 13.6) for women in the highest decile of pregnancy blood lead level (>400 µg/L).

47. Kahn et al. (2014) provide an example of the detrimental effects of lead on the endocrine system. This group investigated associations between mid-pregnancy blood lead (BPb) and concomitant measures of thyroid function among participants in the Yugoslavia Prospective Study of Environmental Lead Exposure. Women in the second trimester of pregnancy from two towns in Kosovo (one with high levels of environmental lead and one with low) were, recruited to the study. Blood samples and questionnaire data were collected. Concentrations of BPb, free thyroxine (FT4), thyroid-stimulating hormone (TSH), and thyroid peroxidase antibodies (TPOAb) were measured in serum samples. Compared with women from the unexposed town, women from the exposed town had lower mean FT4 (0.91 ± 0.17 vs. 1.03 ± 0.16 ng/dL), higher mean TPOAb (15.45 ± 33.08 vs. 5.12 ± 6.38 IU/mL), and higher mean BPb (20.00 ± 6.99 vs. 5.57 ± 2.01 µg/dL). No differences in TSH levels were found. After adjustment for potential confounders, for each natural log unit increase in BPb, FT4 decreased by 0.074 ng/dL (95 % CI: -0.10, -0.046 ng/dL), and the odds ratio for testing positive to TPOAb was 2.41 (95 % CI: 1.53, 3.82). No association was found between BPb and TSH.

Sun et al (2019) measured the concentrations of heavy metals including lead in urine samples and thyroid hormones in blood samples from 675 women during early pregnancy as part of a cohort study conducted in China. Multivariable linear regressions were applied to explore the associations of maternal urinary heavy metal levels with both maternal thyroid hormones and birth outcomes. Urinary lead had an inverse relationship with FT3. Since there were positive associations of maternal FT3 and FT3/FT4 ratio with birthweight, their analyses suggested that Pb levels and birth size might be mediated by maternal FT3 or FT3/FT4 ratio.

48. Kutlu et al. (2006). determined Pb concentrations in placental samples of smoking, passive smoking and non-smoking mothers. The concentrations of Pb in the placenta obtained from the mothers were similar to those noted in mothers who smoked 25 cigarettes per day (0.258). In the Koi *et al.* study (paragraph 35), none of the patients admitted cigarette smoking, although it was estimated that the percentage of pregnant women exposed to passive tobacco smoking might have been as high as 50 % in some regions of Poland. Exposure to tobacco smoke results in changes to macro- and microstructures of the placenta and the effectiveness of its functioning. This affects the quality of maternal fetus-placenta transport and may have explained the higher Pb concentrations in the umbilical cord compared to the placental tissues.

49. Reviews of the effects of environmental pollution on maternal health, including the effects of lead were published by Varshavsky et al. (2017) and Rzymski et al. (2015). The authors highlighted the potential for lead exposure to lead to pre-eclampsia and maternal hypertension.

50. Poropat et al. (2017, from abstract) performed a systematic review and meta-analysis on the association between preeclampsia and lead poisoning. They found that blood lead concentrations were significantly associated with preeclampsia (N = 6069; p = 0.005). This was despite the studies coming from eight separate countries and having countervailing risks of bias. The authors concluded that blood lead concentrations in pregnant women were a major risk factor for preeclampsia, with an increase of 10µg/l associated with a 1.6 % increase in likelihood of the disease. They recommended that pregnant women who had previously been exposed to lead

should have blood lead concentrations tested routinely, especially after the mid-term of their pregnancy. The authors further recommended that women with blood concentrations higher than 50 µg/l should be actively monitored for preeclampsia and be advised to take prophylactic calcium supplementation and that all pregnant women should be advised to actively avoid lead exposure.

51. Ikechukwu et al. (2012, from abstract) investigated the effect of blood lead and its relationship with calcium and phosphorus in the development of pre-eclampsia in pregnant women in Nigeria. Blood samples were collected from 59 pre-eclamptic, 150 normal pregnant, and 122 non-pregnant women. Blood lead was significantly higher ($p < .001$), whereas serum Ca and P were significantly lower ($p < .001$) in preeclamptic women than in normal pregnant women (60.2 ± 12.8 vs 26.3 ± 8.0 µg/dL for Pb, 1.39 ± 0.33 vs 2.03 ± 0.22 mmol/L for Ca, and 0.76 ± 0.10 vs 0.99 ± 0.13 mmol/L for P, respectively). There was a significant increase ($p < 0.05$) in blood lead and decreases ($p < 0.01$) in serum Ca and P in pregnant women compared with non-pregnant women (35.7 ± 18.0 vs 13.1 ± 6.4 µg/dL for Pb, 1.85 ± 0.33 vs 2.33 ± 0.20 mmol/L for Ca, and 0.93 ± 0.38 vs 1.24 ± 0.26 mmol/L for P). Blood lead concentration was negatively correlated with serum Ca and, P, and positively correlated with systolic and diastolic blood pressures (SBP and DBP respectively) in pregnancy ($r = −0.804$ for Ca, $r = −0.728$ for P, $r = 0.908$ for SBP, and $r = 0.842$ for DBP) and preeclampsia ($p < 0.01$). It appeared from this study that increases in blood lead, paralleled decreases in serum calcium and phosphorus, may be correlated with the development and progression of preeclampsia.

52. Conversely, Liu et al (2019) measured Mn, Se, Cd, Pb, and Hg from red blood cells collected within 24 to 72 hours after delivery from 1274 women from the Boston Birth Cohort (enrolled since 1998). Preeclampsia diagnoses were taken from medical records. A total of 115 (9.0%) women developed preeclampsia. There was a dose–response trend for Mn (P for trend<0.001) and to some extent for Cd (P for trend=0.009) quintiles, but null associations were observed for Se, Pb, and Hg.

53. Kumar (2018) reviewed the literature on the impairment of reproductive health caused by occupational and environmental exposure to lead. Effects manifested mainly as higher prevalence of menstrual disturbance, spontaneous abortion, and threatened abortion in exposed females but cautioned that it can be difficult to assign

observed specific effects to a metal if it is the only one evaluated and results may be inconsistent if levels of other metals or dietary constituents that can modify effects are not considered

Animal studies

54. Basha and Reddy (2015) exposed pregnant rats to 0.2% Pb acetate in drinking water from gestational day (GD) 6 to GD 21 or 0.2% Pb acetate plus 0.02% calcium chloride ($CaCl_2$). The results showed decrease in body weight gain (GD 6–21) of dams, but no changes in offspring body weight at different postnatal days following Pb exposure. Male offspring showed a decrease in synaptosomal acetylcholinesterase (AChE) activity and an increase in acetylcholine (ACh) levels in the cortex, cerebellum and hippocampus on post-nata day (PND) 14, PND 21, PND 28 and in the four-month age group of rats following Pb exposure.. Deficits were also observed in total locomotor activity, exploratory and open field behaviour in selected age groups of Pb-exposed rats. Addition of 0.02% $CaCl_2$. To the maternal drinking water reversed these neurological effects of Pb later in life, suggesting a protective effect of calcium in Pb-exposed animals.

55. Barkur and Bairy (2015) Exposed pregnant albino Wistar strain rats were to 0.2% Pb acetate in drinking water during gestation (G group), lactation (L group), both gestation and lactation (GL group) and one month prior to pregnancy (PG group). The rat pups born in each of these groups were assessed in preweaning neurobehavioral parameters including surface righting reflex, swimming development, negative geotaxis and ascending wire mesh test. The swimming development scores were low in the GL group of rats. The negative geotaxis score in GL and G groups were altered. The day of achievement of ascending wire mesh test was significantly delayed in GL, G and L groups of rats. The conclusion was that low level of Pb exposure during both gestation and lactation caused significant alterations in the early neurobehavioral and sensorimotor reflex development in the absence of concomitant weight loss. Exposure during gestation only, or during lactation only, caused alterations in only some of the measured neurobehavioral outcomes.

56. Mousa et al (2018) investigated the effects of developmental Pb exposure on neurogenesis and on cortical neurons in rats. Pregnant Wistar rats were exposed to 0.5% lead acetate in drinking water throughout pregnancy and to postnatal day (PD) 28. Offspring were grouped as gestational day (GD) 18 and 21 and PD 7, 14, 21, and 28. Brain sections were stained with anti-proliferating cell nuclear antigen (PCNA) or glial fibrillary acidic protein (GFAP) and Golgi-Cox stain. Pb exposure significantly increased PCNA-positive nuclei in the ventricular and subventricular zones of the lateral ventricle at 18 and 21 GDs. Postnatally, the Pb-treated groups showed a significant decrease in PCNA-staining and neuron density compared to the controls, and this was associated with damaged or apoptotic cells in the experimental groups. At PD 21, there was a significant increase in GFAP immunoreactivity in Pb-exposed groups compared with the controls. Dendritic spine density was significantly increased on cortical pyramidal cell apical dendrites but not basal dendrites of Pb-treated groups, leading to the conclusion that developmental Pb exposure in rats induces a toxic effect on neurogenesis and on cortical neurones.

57. Hong et al. (2021) exposed pregnant female rats to lead acetate in drinking water and examined DNA methylation changes in the hippocampus of their offspring in parallel with learning and memory tests using the Morris Water Maze and a Roche NimbleGen's rat DNA methylation 385K Promoter Plus CpG Island Array assay. Until 21 days after birth and the lactation period, the learning and memory abilities of offspring with lead exposure in utero and during lactation were significantly lower than those of the control group. The hippocampus DNA methylation levels of the three types of promoters were found to be increased compared with those of the control group. Metal ion transport, cell connections, the lamellar body, the axon bulge, and methylation of various metal transporters were found to be significantly enriched. Moreover, pathway analysis showed that the hedgehog signalling pathway, neuroactive receptor-ligand interaction with the ligase pathway, and interaction between cytokines were altered in the lead exposed animals compared with the control group.

Mechanistic studies

58. Zhang et al. (2014) investigated interactions between lead and human chorionic gonadotropin (hCG) using spectroscopic techniques, isothermal titration

calorimetry (ITC), molecular docking studies, and enzyme-linked immunosorbent assay (ELISA). Fluorescence measurements showed that lead acetate dynamically quenched the intrinsic fluorescence of hCG through a collisional mechanism. Lead acetate bound to 5 binding sites of hCG through electrostatic effects and hydrophobic forces. UV–vis absorption spectroscopy, circular dichroism spectroscopy, and ELISA indicated that lead acetate changed the secondary structure of hCG by loosening and destruction of the hCG backbone and increasing the hydrophobicity around tyrosine residues. This resulted in decreased bioactivity of the hormone. The authors suggested that their work presented direct interactions of lead with sex hormones, providing a possible mechanism on lead induced reproductive toxicity at the molecular level.

59. Ahamed et al. (2009) examined the possible free-radical-mediated mechanism by which lead exposure could give rise to premature birth by determining the placental lead level and oxidant/antioxidant status in women with the preterm and full-term deliveries. Twenty-nine women with preterm deliveries (gestational age 28-37 weeks) and 31 women with full-term deliveries (gestational age >37 weeks) attending a hospital of Lucknow, India were recruited to a pilot study. Placental lead level, thiobarbituric acid reactive species (TBARS) level (a measurement of lipid peroxidation), glutathione (GSH) level, and the activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione S-transferase (GST) were measured in the placental tissue. In the preterm delivery group, significantly higher placental lead levels were recorded than in those of full-term (0.39 μg/g vs. 0.27μg/g; $p<0.05$). TBARS was significantly higher while GSH was significantly lower in the placenta of women with the preterm deliveries as compared to the full-term deliveries ($p<0.05$ in each case). Activity of SOD, CAT, GPx, and GR were significantly higher in the placenta of women with preterm deliveries than those in the full-term group ($p<0.05$ in each case). Placental lead concentrations correlated positively with TBARS ($r=0.34$, $p<0.05$), SOD ($r=0.30$, $p<0.05$) and CAT ($r=0.41$, $p<0.05$), and negatively with GSH ($r=-0.31$, $p<0.05$). The authors accepted that there could be various plausible reasons for increased oxidative stress in preterm delivery, but suggested their results showed that lead-induced oxidative stress may be one of the underlying mechanism(s).

In vitro studies

60. Taupeau (2003) examined the *in vitro* effects of lead on cytochrome P450 aromatase (P450 arom) and on oestrogen receptor b (ERb), in human ovary granulosa cells. Aromatase is required for the bioconversion of androgen to estradiol and ERb mediates oestrogen effects in granulosa cells. Cells were collected from women undergoing in vitro fertilisation and then cultured with 10 μM lead acetate. Lead was shown by atomic absorption spectrometry to accumulate in cells. Aromatase activity was measured by a tritiated water production assay and was found to be significantly reduced in the lead-treated cells. Using semiquantitative RT-PCR and Western blotting, P450 arom and ERb mRNA and protein content were also found to be significantly reduced. By contrast, β-actin transcript and protein content in granulosa cells were not modified by lead exposure, indicating that the variations in mRNA and protein levels were not caused by variations in the overall amount of mRNA and protein. Addition of the protein synthesis inhibitor cycloheximide (10 μg/ml), did not eliminate the effects of lead suggesting that the effects did not require de-novo protein synthesis. The results supported the hypothesis that the action of lead on fertility in women may result, in part, from the down-regulation of P450 arom and ERb gene transcription in ovarian granulosa cells.

Biomarkers of lead exposure

61. Lead inhibits a number of enzymes. One of the major processes affected by the presence of the metal is erythropoiesis due to is inhibition of d-aminolaevulinic acid (ALA) dehydratase (ALAD), which catalyses the first step in protoporphyrin-IX biosynthesis. Elevated blood and urinary ALA are biomarkers for lead exposure. Lead also affects ferrochelatase, which normally imports iron into the complex to form haem. In lead poisoning, the enzyme ferrochelatase catalyses the incorporation of zinc, instead of iron, into protoporphyrin IX, resulting in the formation of zinc protoporphyrin (ZPP) (Braun, 1999). Martin et al. (2007) reviewed data on ZPP. This complex has been used both as a screening and diagnostic test for overexposure to lead for nearly 30 years, since elevations are seen in both chronic and acute lead exposure although they lag behind elevations in whole-blood lead by approximately 8-12 weeks. Therefore, ZPP measurement, in conjunction with whole-

blood lead assays can be used to indicate how long an individual may have been overexposed to lead. However, there is considerable individual variability of ZPP measurements, poor sensitivity at lower ranges of lead exposure, poor specificity and delayed changes in unstable exposure conditions, indicating that this test has limited utility in screening.

62. Kayaaltı et al. (2006) studied whether the maternal ALAD G177C polymorphism (rs1800435) was related to the placental lead levels. The study population comprised 97 blood samples taken from mothers and their placentas. ALAD G177C polymorphism was detected by polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP). Placental lead concentrations were assayed using an atomic absorption spectrometer equipped with a graphite furnace and Zeeman background correction system. The median placental lead levels for ALAD1-1, ALAD1-2 and ALAD2-2 genotypes were 7.54 μg/kg, 11.78 μg/kg and 18.53 μg/kg, respectively. Statistically significant association was found between the maternal ALAD G177C polymorphism and placental lead levels ($p < 0.05$).

63. Lamadrid-Figueroa (2006) assessed the relationship between whole-blood and plasma Pb in a cohort of 237 pregnant women, recruited in Mexico City from 1997 to 1999, Whole-blood and plasma lead concentrations were evaluated at 12, 24, and 34 weeks of gestation by inductively coupled plasma-mass spectrometry. An exponential relationship was found between plasma and whole-blood Pb levels. The association was stronger in the second trimester relative to the first, and increased further in the third trimester. The model predicted increased plasma Pb levels for a given whole-blood Pb value as pregnancy advanced for whole-blood Pb levels greater than approximately 110 μg/L, but not for blood Pb levels less than 100 μg/L. These findings could be due to physiological changes in pregnancy, such as decreased hematocrit, saturation of red cell Pb binding capacity, and increased bone resorption or intestinal absorption. These data suggested that at elevated maternal blood Pb levels the developing fetus may be at greater risk of Pb exposure from increased maternal plasma Pb than was predicted from whole-blood Pb levels.

Hazard Characterisation

64. The dose response modelling and derivation of an HBGV have been reviewed and summarised in the COT statement (2013). These are summarised in brief in the following paragraphs.

Benchmark Dose Modelling

Neurodevelopmental effects

65. The developing brain seems to be more vulnerable to lead exposure than the mature brain. A wide range of neurobehavioural tests have been applied to assess the effects of lead exposure on central nervous system (CNS) functions. The most widely used measure of cognitive ability has been general intelligence (EFSA, 2010).

66. Negative associations between bPb and psychometric performance have been reported in several prospective and cross-sectional studies of children. WHO (1995) concluded that each 100 µg/L increment in bPb concentration was likely to be associated with a deficit of 1 to 3 intelligence quotient (IQ) points (at ages 4 and above).

67. In several more recent studies, both IQ and other outcomes were lower in children with higher bPb levels, the relationship applying even at bPb concentrations less than 100 µg/L. Moreover, decline in IQ for a given increase in bPb concentration was greater at these low concentrations than at higher levels.

68. An analysis by Lanphear et al. (2005), was used by both EFSA and JECFA for dose-response (DR) modelling of neurodevelopmental effects (EFSA, 2010; and FAO/WHO, 2011). This was a pooled analysis of data from seven prospective cohort studies concerning the quantitative relationship between performance on IQ tests and measures of bPb concentration, among children followed from infancy. The primary outcome measure was full-scale IQ, assessed at an age between four years 10 months and 10 years. This was related to four measures of bPb: concurrent bPb (the most recent measurement before IQ was assessed), maximum bPb (the highest concentration of bPb that had been measured at any time before IQ was assessed), average lifetime bPb (the mean of bPb measurements from age 6 months up to the

time that IQ was assessed) and early childhood bPb (the mean of measurements between 6 and 24 months of age). After adjustment for covariates, IQ was inversely related to each of these measures of bPb. (Lanphear et al., 2005).

69. EFSA (2010) had also calculated BMDLs for cardiovascular and renal endpoints. However, whilst noting the BMDLs that had been derived by EFSA for cardiovascular and renal effects, the COT concluded that the toxic effect of lead in infants was most important at low levels of exposure, and which therefore was critical for risk assessment, was its developmental neurotoxicity.

70. Budtz-Jørgensen (2010) was commissioned by EFSA to calculate a BMDL for the association of lead with the development of intellectual function, by modelling of data from the pooled analysis by Lanphear et al. (2005). The benchmark calculations used regression models with full IQ score as the dependent variable, and adjustment for birth weight, Home Observation for Measurement of the Environment (HOME) score (The HOME Inventory is an index that reflects the quality and quantity of emotional and cognitive stimulation in the home environment (Lanphear *et al.*, 2005)), maternal education and maternal IQ, all of which were significantly associated with IQ in the dataset. BMD and BMDL values were calculated for a 1% change in full scale IQ score (a decrease in IQ by 1 point), taking concurrent blood lead, maximum blood lead, average lifetime blood lead and early childhood blood lead as alternative exposure metrics. The dose-response models applied were logarithmic, linear, and a piecewise linear function with breakpoint at 100 µg/L.

71. For the assessment of risk, EFSA took as a point of departure, the $BMDL_{01}$ value of 12 µg/L from the piecewise linear dose-response model for concurrent blood lead. Concurrent blood lead concentration exhibited the strongest relationship with IQ, and the piecewise linear model showed a better fit to the data than the linear model. The logarithmic model generally gave an even better fit than the piecewise linear model, but the differences were small, and EFSA preferred the latter because, taking into account the mathematical properties of the logarithmic model, they considered that it provided "less uncertain estimates of the $BMDL_{01}$". Using the IEUBK toxicokinetic model, the blood lead $BMDL_{01}$ of 12 µg/L was estimated to correspond to a dietary lead exposure in infants and children of 0.5 µg lead/kg bw per day (EFSA, 2010).

72. JECFA (FAO/WHO, 2011) also used data from the Lanphear *et al.* (2005) analysis for dose-response modelling. Models were based on concurrent blood lead levels since they showed the highest correlation with IQ. Initially, six different models were considered – four with linear form and two sigmoidal. From these, a bilinear model (unlike the piecewise linear model used by EFSA, this did not constrain the inflexion in the dose-response relationship to be at a pre-specified blood lead concentration) was chosen to characterise the relationship of blood lead to IQ, since it provided a better fit than four of the other models, and it was considered that it would give better estimates of effect than the one other model with similar fit, when non-dietary exposures to lead were unknown or highly variable. Using this model, the chronic dietary exposure of a 20 kg child corresponding to a decrease of 1 IQ point was estimated to be 0.6 µg/kg bw per day with a 90 % confidence interval of 0.2 - 7.2 µg/kg bw per day.

73. The differences between the EFSA and JECFA analyses are small and reflect inevitable uncertainties in the specification of the mathematical models. The COT noted that both were influenced by an apparently steep dose-response at low levels of lead exposure (blood lead levels less than 75 µg/L), which was based on few data from a single study in Rochester, USA, and may have rendered the BMDL values conservative. In this statement, the COT has based its risk characterisation on the EFSA $BMDL_{01}$, which is between the EFSA BMD_{01} and the lower 90 % confidence limit for the BMD_{01} calculated by JECFA, and corresponds to a dietary exposure of 0.5 µg/kg bw/day.

Cardiovascular effects

74. An association between bPb and elevated blood pressure has been observed both in cross-sectional and in longitudinal studies. It has been estimated that systolic pressure is approximately 1 mm Hg higher for each doubling of bPb, without any clearly identifiable threshold (EFSA, 2010).

75. In the DR modelling for cardiovascular effects, EFSA selected a 1 % change in systolic blood pressure as a benchmark response (BMR). This corresponded to an increase of 1.2 mm Hg from a baseline value of 120 mm Hg in a normotensive adult. This was within the range of observed effects and could have significant

consequences for human health at a population level. EFSA (2010) calculated an average $BMDL_{01}$ of 36 µg/L bPb from two longitudinal and two cross-sectional studies (Glenn et al., 2003; Vupputuri et al., 2003; Nash et al., 2003; Glenn et al., 2006). This value was converted into a corresponding dietary lead exposure of 1.5 µg/kg bw/day.

Renal effects

76. Reduced glomerular filtration rate (GFR) has been observed in association with exposures resulting in average bPb levels <200 µg/L, after allowance for age and other covariates that might contribute to glomerular disease. In subjects with blood lead levels below 100 µg/L, serum creatinine has been estimated to increase by 1.4 mg/L per 10-fold increase in blood lead. (EFSA, 2010).

77. Several factors have been suggested to modify the association between bPb and renal function, although the evidence is inconsistent (FAO/WHO, 2011). Decrements in glomerular filtration rate may contribute to elevations in blood pressure, and elevated blood pressure may predispose people to glomerular disease (U.S. ATSDR, 2007).

78. EFSA selected as a BMR for renal effects, a 10 % increase in the prevalence of chronic kidney disease. A $BMDL_{10}$ of 15 µg/L bPb was calculated using data from a cross-sectional study conducted in the USA (Navas-Acien et al., 2009). This was converted into a corresponding dietary lead exposure of 0.63 µg/kg bw/day.

Exposure Assessment

Exposure from food

79. The Exposure Assessment Team has provided dietary exposure data on lead for women of childbearing age (16 – 49 years of age), as shown in Appendix 1. In short, the food commodities with the highest measured lead concentrations are green vegetables, miscellaneous cereals and other vegetables, with mean exposure values of 0.0088, 0.0080 and 0.0063 µg/kg bw/day and 97.5th percentile values

0.034, 0.028 of 0.019 μg/kg bw/day respectively. The total exposure via food was measured as 0.12 μg/kg bw/day (mean) and 0.23 μg/kg bw/day (97.5th percentile).

Exposure from drinking water

80. Data on concentrations of lead in water were provided by the Drinking Water Inspectorate (DWI) (for England and Wales), the Drinking Water Quality Regulator (DWQR) for Scotland and Northern Ireland Water. The concentration data from 2019 for lead in the drinking water are given in Table 1.

Table 1. Concentration of lead in tap water sampled in the nations of the United Kingdom in 2019 (μg/l)

Region	N	LB mean	LB SD	UB mean	UB SD
England and Wales*	10967	0.38	0.38	2.15	1.9
Scotland	436	0.34	1.2	0.48	1.2
Northern Ireland	122	0.24	0.79	1.1	0.75

*99th percentile concentration

LB = lower bound: values below the limit of detection assumed to be zero.

UB = upper bound: values below the limit of detection assumed to be the same as the limit of detection

81. The FSA Exposure Assessment Team has provided values for water consumption for women of child-bearing age of 8 (median) and 32 (97.5th percentile) g (ml) of water per kg bodyweight per day. Using the upper bound mean values from Table 3, exposure to lead in drinking water for this group is shown in Table 2.

Table 2. Median and 97.5th percentile exposure values for women of childbearing age to lead from water, using the mean upper bound concentration values (μg/kg bw/day).

Region	N	Median	97.5th percentile

England and Wales	10967	0.017	0.069
Scotland	436	0.0038	0.015
Northern Ireland	122	0.0088	0.035

Exposure from the air

82. Defra provide data on air pollution throughout the UK. An interactive map (Link to map) shows that the majority of the country in 2020 had an average air concentration of <10 ng lead/m^3, with major urban centres in England and Wales having concentrations of 10 – 50 ng lead/m^3 .

83. An internet search reveals that the average breathing rate at rest is 12 – 16 breaths per minute (Link here) and that a healthy female adult has a tidal volume of 0.4 litres (Link here).

84. As a worst-case scenario, if an adult female were to be constantly exposed to an air concentration of 50 ng lead/m^3 then this would result in a daily exposure to 345 – 460 ng of lead from the air. For women with an average body weight of 70.3 kg, (value provided by the FSA Exposure Assessment Team from years 1 – 11 of the rolling National Diet and Nutrition Survey, NDNS) this gives an intake of 4.9 – 6.5 ng/kg bw (0.0049 – 0.0065 μg/kg bw/day).

85. This assumes full absorption of all lead in particles inhaled, but this depends upon particle sizes and since some of the inhaled dose may become trapped in other parts of the nasopharynx, these inhalation values are probably an overestimate, but may contribute in a small way to ingested lead.

Risk characterisation

Food

86. Taking the BMD values from EFSA (2010, updated 2013 and paragraph 7), the Margins of Exposure (MOE) for women of childbearing age from the highest-lead-containing dietary items and the total diet are given in tables 3 and 4.

Table 3. Margins of exposure for lead in the dietary commodities with the highest measured mean lead concentrations (upper bounds) and for the total diet in women

aged 16 to 49 years of age (compared with dietary intake values in µg/kg b.w. per day corresponding to the blood $BMDL_{01}$)

Commodity	Mean lead exposure (µg/kg bw/day	MOE for 0.5 µg/kg bw/day	MOE for 1.5 µg/kg bw/day	MOE for 0.63 µg/kg bw/day
Green vegetables	0.0088	57	170	72
Misc. cereals	0.0080	63	188	79
Other vegetables	0.0063	79	235	100
Total in all food	0.12	4.2	12.5	5.3

$BMDL_{01}$ = 0.5 µg/kg bw/day for developmental neurotoxicity; $BMDL_{01}$ =1.5 µg/kg bw/day for systolic BP; $BMDL_{10}$=0.63 µg/kg bw/day for nephrotoxicity

87. The MOEs for the total diet are higher than those reported by EFSA, where *in utero* developmental toxicity (taken from women of age 20 – 40) had a mean upper bound MOE of 1.3, adult cardiovascular effects 4.2 and nephrotoxicity 1.8.

Table 4. MOEs for lead in the dietary commodities with the highest measured 97.5th percentile lead concentrations (upper bounds) and for the total diet in women aged 16 to 49 years of age (compared with dietary intake values in µg/kg b.w. per day corresponding to the blood $BMDL_{01}$).

Commodity	97.5th percentile lead exposure (µg/kg bw/day)	MOE for 0.5 µg/kg bw/day	MOE for 1.5 µg/kg bw/day	MOE for 0.63 µg/kg bw/day
Green vegetables	0.034	15	44	19

Misc. cereals	0.023	22	65	27
Other vegetables	0.019	26	79	33
Total in all food	0.23	2.2	6.5	2.7

$BMDL_{01}$ =0.5 μg/kg bw/day for developmental neurotoxicity; $BMDL_{01}$ =1.5 μg/kg bw/day for systolic BP; $BMDL_{10}$ =0.63 μg/kg bw/day for nephrotoxicity

88. EFSA reported MOEs for "high consumers", without specifying a percentile of consumption, but give an upper bound value of 0.74 for in utero developmental toxicity (taken from women of age 20 – 40), 2.1 for cardiovascular effects in adults and of 0.86 for nephrotoxicity.

89. Neither the mean nor the 97.5th percentile consumption MOEs for the foods with the highest measure of lead, nor for the total amount of lead in food as a whole as reported by the NDNS, has a value of 1 or lower, indicating that lead in food does not pose an acute risk to women of childbearing age. From the EFSA evaluation, the total exposure to lead in food, giving a MOE of < 10 but > 1 for neurodevelopment may imply that the diet may contain levels of lead that could be of concern for the developing child. Moreover, as noted above, lead may accumulate in tissues, particularly bone and so continued consumption of low concentrations in food may cause persistent low-level toxicity and contribute to developmental damage in a fetus.

Drinking water

90. The MOEs for lead in drinking water are shown in Table 5.

Table 5. MOEs for lead in drinking water using the concentration data provided by the water regulators for England and Wales, Scotland and Northern Ireland and consumption data provided by the FSA Exposure Assessment Team (compared with dietary intake values in μg/kg b.w. per day corresponding to the blood $BMDL_{01}$).

Region	97.5th percentile lead	MOE for 0.5 μg/kg bw/day	MOE for 1.5 μg/kg bw/day	MOE for 0.63 μg/kg bw/day

	exposure (μg/kg bw/day)			
England and Wales	0.069	7.3	22	9.2
Scotland	0.015	33	100	42
Northern Ireland	0.035	14	43	18

$BMDL_{01}$ =0.5 μg/kg bw/day for developmental neurotoxicity; $BMDL_{01}$ =1.5 μg/kg bw/day for systolic BP; $BMDL_{10}$ =0.63 μg/kg bw/day for nephrotoxicity

91. Similarly, to lead in food, the MOEs for intake of lead from drinking water from the four countries of the United Kingdom are all greater than 1, and so acute toxicity is unlikely, but chronic toxicity and accumulation cannot be ruled out. The MOE for developmental neurotoxicity for high consumers of drinking water in England and Wales is >1 but <10 and so cannot be ruled out as of potential concern for the fetus. However, this value is very conservative, being the high-level intake of an upper-bound mean of a 99th percentile concentration value that was provided by the water companies. For the large majority of the population, the concentration will be much lower than this and, therefore, so will the exposure.

Air

92. The inhaled exposure level would have little impact upon total lead exposure. Relative to the $BMDL_{01}$ values derived by EFSA, these intakes from air give MOE s of 102 - 77, 307 – 230 and 129 - 97 for developmental neurotoxicity, systolic blood pressure and nephrotoxicity, respectively, (Link here).

Conclusions

93. Lead is a heavy metal pollutant that is ubiquitous in the environment and is thus present in the diet of the general population, including women of childbearing age, those pregnant and postpartum. Levels have, nonetheless, fallen since the phasing out of lead in petrol, plumbing and paints.

94. Lead in pregnant women can cause increased blood pressure and may be associated with preeclampsia and premature birth.

95. EFSA (2010, updated 2013) derived BMDLs for neurodevelopment, renal function and systolic blood pressure and provided values as both μg/ml in blood and the corresponding dietary intake values in μg/kg b.w. per day.

96. Exposure of women of childbearing age to lead in food at the 97.5th percentile of consumption of commodities with the highest concentrations given by NDNS give MOEs of 2.2, 6.5 and 6.7 for effects of developmental neurotoxicity; elevated systolic BP and nephrotoxicity, respectively, relative to the $BMDL_{01}$.

97. Exposure of women of childbearing age to lead in drinking water in the UK has a MOE of 7.2 or greater.

98. Lead accumulates in the body, so adverse effects can occur from long term dietary exposures at levels below those which cause acute toxicity. The kidney and cardiovascular systems are adversely affected by lead exposure in adults. Neurotoxicity has been identified at lower levels of exposure, and the developing brain appears to be more vulnerable than the mature brain. It has not been possible to demonstrate a threshold level of exposure below which the neurodevelopmental effects of lead do not occur.

99. Toxicity will depend on total exposure to lead from all sources, so it is important to consider combined exposures from food, water, and also non-dietary sources. Calculated MOEs indicate that exposures from air are negligible.

Questions for the Committee

a) Does the Committee think that the current level of exposure to lead from the diet, drinking water and air is a cause for concern for pregnant women or their fetuses?

b) Would the Committee like to expand the exposure section of the paper by including other potential sources of lead, such as soil and dust?

c) Is the Committee happy with the MOE approach for assessing the risks of lead to maternal health and with which BMDL values would they like comparisons to be made if so??

d) Does the Committee have and other comments on this discussion paper?

Secretariat

January 2022

Abbreviations

AGA	Adequate for gestational age
ALA	D-aminolaevulinic acid
ALAD	D-aminolaevulinic acid dehydratase
ATSRD	Agency for Toxic Substances and Disease Registry
BMD	Benchmark Dose
$BMDL_{01}$	Benchmark Dose Lower Limit for !% change in effect
BPb	Blood lead
CAT	Catalase
CI	Confidence interval
COT	Committee on Toxicity
DNA	Deoxyribonucleic acid
DWI	Drinking Water Inspectorate
DWQR	Drinking Water Quality Regulator
EFSA	European Food Safety Authority
ELISA	Enzyme-linked immunosorbent assay
ERb	(O)estrogen receptor b
FSA	Food Standards Agency
FT4	Free thyroxine
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Glutathione
GST	Glutathione-S-transferase
hCG	Human chorionic gonadotrophin

IQ	Intelligence Quotient
ITC	Isothermal titration calorimetry
JECFA	Joint FAO/WHO Committee on Food Additives
kPa	KiloPascals
L	Litre
MDI	Mental development index
mmHg	Millimetres of mercury
MOE	Margin of exposure
mRNA	Messenger ribonucleic acid
NDNS	National diet and nutrition survey
OR	Odds ratio
CYP_{450}	Cytochrome P450
Pb	Lead
RT-PCR	Reverse transcriptase polymerase chain reaction
SBP	Systolic blood pressure
SD	Standard deviation
SGA	Small for gestational age
SOD	Superoxide dismutase
TBARS	Thiobarbituric acid reactive species
TPO Ab	Thyroid peroxidase antibodies
TSH	Thyroid stimulating hormone
ZPP	Zinc protoporphyrin
μg	Microgram

Search terms

The references cited in this discussion paper are of publications found in PubMed searches and references therein, using the following search terms:

Pb AND	Maternal health
	Pre-conception
	Conception
	Post-partum
	Toxicity
	Mechanism
	ADME
	Toxicokinetics
	Absorption
	Distribution
	Metabolism
	Excretion
	Biomarker
	Exposure
	Pre-eclampsia
	Abortion

References

Ahamed M, Mehrotra PK, Kumar P, Siddiqui MK. Placental lead-induced oxidative stress and preterm delivery. Environmental Toxicology and Pharmacology 2009; **27**: 70-74.

ATSDR (1999) Toxicological profile for lead (update). (Agency for Toxic Substances and Disease Registry.) U.S. Department of Health and Human Services, Atlanta, GA.

Barregård, L. et al. Cadmium, mercury, and lead in kidney cortex of the general Swedish population: a study of biopsies from living kidney donors. Environmental Health Perspectives 1999 **107**(11), 867.

Barry PS. A comparison of concentrations of lead in human tissues. Occupational and Environmental Medicine 1975 **32**(2): 119–139.

Barry, PS Concentrations of lead in the tissues of children. Occupational and Environmental Medicine 1981 **38** (1), 61–71.

Barkur RR, Bairy LK. Evaluation of passive avoidance learning and spatial memory in rats exposed to low levels of lead during specific periods of early brain development International Journal of Occupational Medicine and Environmental Health 2015 **28**(3):533-44.

Bolan S, Seshadri B, Keely S, Kunhikrishnan A, Bruce J, Grainge I, Talley NJ, Naidu R. Bioavailability of arsenic, cadmium, lead and mercury as measured by intestinal permeability, Scientific Reports. 2021 **11**(1):14675

Borja-Aburto VH, Hertz-Picciotto I, Rojas Lopez M, Farias P, Rios C, Blanco J. Blood lead levels measured prospectively and risk of spontaneous abortion. American Journal of Epidemiology 1999 Sep 15;150(6):590-7.doi: 10.1093/oxfordjournals.aje.a010057. Link to doi

Bound J, Harvey P, Francis B, Awwad F, Gatrell A. Involvement of deprivation and environmental lead in neural tube defects: a matched case-control study Archives of Disease in Childhood. 1997 **76**(2): 107–112. doi: 10.1136/adc.76.2.107 Link to doi

Braun J. Erythrocyte zinc protoporphyrin. Kidney International Supplement 1999 **69**:S57-60. doi: 10.1046/j.1523-1755.1999.055suppl.69057.x Link to doi

Budtz-Jørgensen E.2010. Scientific/technical report submitted to EFSA. An international pooled analysis for obtaining a benchmark dose for environmental lead exposure in children. (Question No. EFSA-Q-2009-01078)

Chen PC, Pan IJ, Wang JD. Parental exposure to lead and small for gestational age births. American Journal of Industrial Medicine. 2006 49(6):417-22. doi: 10.1002/ajim.20313. Link to doi

Coiplet E. Freuchet M, Sunyach C, Mancini J, Perrin J, Courbiere B, 1 Heckenroth H, Pissier C, Hamdaoui N, Bretelle F. Assessment of a Screening Questionnaire to Identify Exposure to Lead in Pregnant women. International Journal of Environmental Research and Public Health 2020, **17**, 9220; doi:10.3390/ijerph17249220. Link to doi

Esteban-Vasallo MD, Aragonés N, Pollan M, López-Abente G, Perez-Gomez B Mercury, Cadmium, and Lead Levels in Human Placenta: A Systematic Review Environmental Health Perspectives. 2012 **120**(10): 1369–1377.

Factor-Litvak P, Stein Z, Graziano J. Increased risk of proteinuria among a cohort of lead-exposed pregnant women. Environmental Health Perspectives. 1993 **101**(5):418-21. doi: 10.1289/ehp.93101418. Link to doi

Flora G, Gupta D, Tiwari A. Toxicity of lead: A review with recent updates. Interdisciplinary Toxicology 2012 **5**(2):47-58. doi: 10.2478/v10102-012-0009-2. Link to doi

Garza A , Vega R, Soto E. Cellular mechanisms of lead neurotoxicity Medical Science Monitor 2006; **12**(3): RA57-65

Gerhardsson, L. et al. Lead in tissues of deseased lead smelter workers. Journal of Trace Elements in Medicine and Biology 1995 **9**(3), 36–143.

Glenn BS, Bandeen-Roche K, Lee BK, Weaver VM, Todd AC, Schwartz BS. Changes in systolic blood pressure associated with lead in blood and bone. Epidemiology. 2006 **17**: 538-544

Glenn BS, Stewart WF, Links JM, Todd AC, Schwartz BS. The longitudinal association of lead with blood pressure. Epidemiology. 2003 **14**: 30-36

Gross, S. B., Pfitzer, E. A., Yeager, D. W. & Kehoe, R. A. Lead in human tissues. Toxicology and Applied Pharmacology 1975 **32**(3), 638–651.

Gulson BL, Jameson CW, Mahaffey KR, Mizon KJ, Korsch MJ, Vimpani G. Pregnancy increases mobilization of lead from maternal skeleton Journal of Laboratory and Clinical Medicine. 1997; **130**(1):51-62. doi: 10.1016/s0022-2143(97)90058-5. Link to doi

Gulson BL, Mizon KJ, Palmer JM, Korsch MJ, Donnelly JB Urinary excretion of lead during pregnancy and postpartum. Science of the Total Environment 2000 ;**262**(1-2):49-55.

Hernandez-Avila M, Peterson KE, Gonzalez-Cossio T, Sanin LH, Aro A, Schnaas L, Hu H. Effect of maternal bone lead on length and head circumference of newborns and 1-month-old infants. Archives of Environmental Health. 2002 **57**(5):482-8. doi: 10.1080/00039890209601441.PMID: 12641193 Link to doi

Hertz-Picciotto I. The evidence that lead increases the risk for spontaneous abortion. American Journal of Industrial Medicine 2000 **38**:300–309.

Hong T, Li S-M, Jia B, Huang Y, Shu K, Yuan KW, Chen L, Li L-X, Liu L, Liu Z-Y. DNA methylation changes in the hippocampus of learning and memory disorder offspring rats of lead exposure during pregnant and lactation period, Annals of Palliative Medicine 2021;**10**(2):1059-10. doi.org/10.21037/apm-19-421 Link to doi

Hu H, Téllez-Rojo MM, Bellinger D, Smith D, Ettinger AS, Lamadrid-Figueroa H, Schwartz J, Schnaas L, Mercado-García A, Hernández-Avila M. Fetal lead exposure at each stage of pregnancy as a predictor of infant mental development. Environmental Health Perspectives. 2006 **114**(11):1730-5.

Irwinda R, Wibowo N, Putri AS. The Concentration of Micronutrients and Heavy Metals in Maternal Serum, Placenta, and Cord Blood: A Cross-Sectional Study in

Preterm Birth. Journal of Pregnancy Volume 2019, Article ID 5062365, 7 pages doi.org/10.1155/2019/5062365. Link to doi

Ikechukwu IC, Ojareva OIA, Ibhagbemien, AJ, Okhoaretor OF, Oluwatomi OB, Akhalufo OS, Oluwagbenga AT, Chigaekwu MN. Blood Lead, Calcium, and Phosphorus in Women With Preeclampsia in Edo State, Nigeria. Archives of Environmental & Occupational Health 2012 67(3) : 63-69. doi: 10.1080/19338244.2011.619212. Link to doi

Iwai-Shimada M, Kameo S, Nakai K, Yaginuma-Sakurai K, Tatsuta N Kurokawa N, Nakayama SF, Satoh H. Exposure profile of mercury, lead, cadmium, arsenic, antimony, copper, selenium and zinc in maternal blood, cord blood and placenta: the Tohoku Study of Child Development in Japan. Environmental Health and Preventive Medicine 2019 **24**:35 -45 doi.org/10.1186/s12199-019-0783-y Link to doi

JECFA (2011) Evaluation of certain food additives and contaminants: seventy-third report of the Joint FAO/WHO Expert(WHO technical report series ; no. 960)1. Meeting (73rd: 2010, Geneva, Switzerland). IV.Series.ISBN 978 92 4 120960 (NLM classification: WA 712)ISSN 0512-3054.

Jelliffe-Pawlowski LL, Miles SQ, Courtney JG, Materna B, Charlton V. Effect of magnitude and timing of maternal pregnancy blood lead (Pb) levels on birth outcomes. Journal of Perinatology 2006; **26** : 154-162.

Kabamba M, Tuakuila J Toxic metal (Cd, Hg, Mn, Pb) partition in the maternal/foetal unit: A systematic mini - review of recent epidemiological studies. Toxicology Letters 2020; **332**:20-26. doi: 10.1016/j.toxlet.2020.06.007. Epub 2020 Jun 20. Link to doi

Kahn LG, Liu X, Rajovic B, Popovac D, Oberfield S, GrazianoJH, Pam Factor-Litvak. Blood lead concentration and thyroid function during pregnancy: results from the Yugoslavia Prospective Study of Environmental Lead Exposure. Environmental Health Perspectives 2014 **122**(10):1134-40. doi: 10.1289/ehp.1307669. Epub 2014 May 27 Link to doi

Kayaaltı Z, Sert S, Kaya-Akyüzlü D, Söylemez E, Söylemezoglu T. Association between delta-aminolevulinic acid dehydratase polymorphism and placental lead levels. Environmental Toxicology and Pharmacology 2016 **41**: 147-151

Karri SK, Saper RB, Kales SN Lead encephalopathy due to traditional medicines Current Drug Safety 2008 Jan;3(1):54-9. doi: 10.2174/157488608783333907 Link to doi

Kot K, Kosik-Bogacka D, Łanocha-Arendarczyk N, Malinowski W, Szymanski S. Mularczyk M, Tomska N, Rotter I. Interactions between 14 Elements in the Human Placenta, Fetal Membrane and Umbilical Cord.Int. Journal of Environmental Research and Public Health 2019, **16**, 1615;

Kumar S. Occupational and Environmental Exposure to Lead and Reproductive Health Impairment: An Overview. Indian Journal of Occupational and Environmental Medicine. 2018 **22**(3): 128–137

Kutlu T, Karagozler AA, Gozukara EM Relationship among placental cadmium, lead, zinc, and copper levels in smoking pregnant women. Biological Trace Element Research 2006;**114**(1-3):7-17.

Lamadrid-Figueroa H, Téllez-Rojo MM, Hernández-Cadena L, Mercado-García A, Smith D, Solano-González M, Hernández-Avila M, Hu H. Biological markers of fetal lead exposure at each stage of pregnancy Journal of Toxicology and Environmental Health A 2006 **69**(19):1781-96.

Lamadrid-Figueroa H, Téllez-Rojo MM, Hernández-Avila M, Trejo-Valdivia B, Solano-González M, Mercado-Garcia A, Smith D, Hu H, Wright RO. Association between the plasma/whole blood lead ratio and history of spontaneous abortion: a nested cross-sectional study. BMC Pregnancy Childbirth 2007 **7**:22. doi: 10.1186/1471-2393-7-22. Link to doi

Lamichhane DK, Leem J-H, Park C-S, Ha M, Ha E-H, Kim H-C, Lee J-Y, Ko JK, Kim Y, Hong Y-C. Associations between prenatal lead exposure and birth outcomes: Modification by sex and GSTM1/GSTT1 polymorphism. Science of the Total Environment 2018 **619-620**:176-184. doi: 10.1016/j.scitotenv.2017.09.159. Epub 2017 Nov 29. Link to doi

Lanphear BP, Hornung R, Khoury J, Yolton K, Baghurst P, Bellinger DC, Canfield RL, Dietrich KN, Bornschein R, Greene T, Rothenberg SJ, Needleman HL, Schnaas L, Wasserman G, Graziano J and Roberts R. Low-level environmental lead exposure

and children's intellectual function: An international pooled analysis. Environmental Health Perspectives 2005. 113(7): 894-899

Liu T, Zhang M, Guallar E, Wang G, Hong X, Wang X, Mueller NT, Trace Minerals, Heavy Metals, and Preeclampsia: Findings from the Boston Birth Cohort. Journal of the American Heart Association. 2019 **8**(16): e012436.

Martin CJ, Werntz CL III, Ducatman AM The interpretation of zinc protoporphyrin changes in lead intoxication: a case report and review of the literature. Occupational Medicine 2004 **54**(8): 587–591.

Mousa AMA, Elshahat MA, Renno WM. Effect of developmental lead exposure on neurogenesis and cortical neuronal morphology in Wistar rats Toxicology and Industrial Health 2018 **34**(10):665-678.

Muhle H, Steenland K. Lead and lead compounds. International Agency for Research on Cancer. IARC Monographs 87, 2006.

Nash D, Magder L, Lustberg M, Sherwin RW, Rubin RJ, Kaufmann RB, Silbergeld EK. Blood lead, blood pressure, and hypertension in perimenopausal and postmenopausal women. Journal of the American Medical Association.2003 **289**: 1523-1532.

Navas-Acien A, Tellez-Plaza M, Guallar E, Muntner P, Silbergeld E, Jaar B, Weaver V. Blood cadmium and lead and chronic kidney disease in US adults: a joint analysis. American Journal of Epidemiology.2009 **170**: 1156-1164

Needleman HL. Rabinowitz M. Leviton A. Linn S. Schoenbaum S. The relationship between prenatal exposure to lead and congenital anomalies. Journal of the American Medical Association 1984, **251**, 2956–2959.

Omeljaniuk WJ, Socha K, Soroczynska J, Charkiewicz AE, Laudanski T, Kulikowski M, Kobylec E, Borawska MH. Cadmium and Lead in Women Who Miscarried Clinical Laboratory 2018 64(1):59-67.

Ou J, Peng P, Qiu L, Teng L, Li C, Han J, Liu X. Effect of Lead Exposure on Spontaneous Abortion: a Case-Control Study Clinical Laboratory 2020 **66**(5). doi: 10.7754/Clin.Lab.2019.190940. Link to doi

Poropat AE, Laidlaw MAS, Lanphear B, Ball A, Mielke HW, Blood lead and preeclampsia: A meta-analysis and review of implications, Environmental Research 2018 **160** 12–19. doi:10.1016/j.envres.2017.09.014 Link to doi

Rădulescu A, Lundgren S. A pharmacokinetic model of lead absorption and calcium competitive dynamics Anca Scientific Repots 2019 **9**:14225.

Rzymski P, Tomczyk K, Rzymski P, Poniedziałek B, Opala T, Wilczak M. Impact of heavy metals on the female reproductive system Annals of Agricultural and Environmental Medicine 2015, **22**(2) 259–264.

Sakamoto M, Murata K, Kubota M, Nakai, K.; Satoh, H. Mercury and heavy metal profiles of maternal and umbilical cord RBCs in Japanese population. Ecotoxicological and Environmental. Safety. 2010, 73, 1–6.

Sanders T, Liu Y, Buchner V, Tchounwou PB. Neurotoxic Effects and Biomarkers of Lead Exposure: A Review Reviews of Environmental Health. 2009 ; 24(1): 15–45.

Schroeder, H. A. & Tipton, I. H. The human body burden of lead. Archives of Environmental Health: An International Journal 1968 **17**(6), 965–978.

Silberstein T, Saphier O, Paz-Tal O, Gonzalez L, Keefe DL, Trimarchi JR Trace element concentrations in follicular fluid of small follicles differ from those in blood serum and may represent long-term exposure. Fertilization and Sterilization. 2009 **91**(5):1771-4.

Sun X, Liu W, Zhang B, Shen X, Hu C, Chen X, Jin S, Jiang Y, Liu H, Cao Z, Xia W, Xu S, Li Y. Maternal Heavy Metal Exposure, Thyroid Hormones, and Birth Outcomes: A Prospective Cohort Study. Journal of Clinical Endocrinological Metabolism. 2019 **104**(11):5043-5052.

Taupeau C, Poupon J, Treton D, Brosse A, Richard Y, Machelon V. Lead Reduces Messenger RNA and Protein Levels of Cytochrome P450 Aromatase and Estrogen Receptor b in Human Ovarian Granulosa Cells Biology of Reproduction 2003 **68**, 1982–1988.

Taylor CM, Golding J, Emond AM. Adverse effects of maternal lead levels on birth outcomes in the ALSPAC study: a prospective birth cohort study. British Journal of Obstetrics and Gynaecology 2015;**122**:322–328.

Varshavsky J, Smith A, Wang A, Hom E, Izano M, Huang H, Padula A, Woodruff TJ. Heightened Susceptibility: A Review of How Pregnancy and Chemical Exposures Influence Maternal Health. Reproductive Toxicology. 2020 ; **92**: 14–56. doi:10.1016/j.reprotox.2019.04.004. Link to doi

Vigeh M, Saito H, Sawada S. Lead exposure in female workers who are pregnant or of childbearing age. Indian Health. 2011 **49**(2):255-61. doi:10.2486/indhealth.ms1192. Epub 2010 Dec 16. Link to doi

Vupputuri S, He J, Muntner P, Bazzano LA, Whelton PK, Batuman V. Blood lead level is associated with elevated blood pressure in blacks. Hypertension.2003 **41**: 463-468

Wani AL, Ara A, Usmani JA Lead toxicity: a review, Interdisciplinary Toxicology 2015 **8**(2): 55 - 64.

Wells EM, Navas-Acien A, Herbstman JB, Apelberg BJ, Silbergeld EK, Caldwell KL, Jones RL, Halden RU, Witter FR, Goldman LR. Low-level lead exposure and elevations in blood pressure during pregnancy. Environmental Health Perspectives. 2011 **119**(5):664-9. doi: 10.1289/ehp.1002666. Epub 2011 Feb 2. Link to doi

Zentner LEA, Rondó PHC, Mastroeni SSBS. Lead Contamination and Anthropometry of the Newborn Baby Journal of Tropical Pediatrics, 2006 **52**(5) 369–371.

Zhang H, Liu Y, Zhang R, Liu R, Chen Y. Binding Mode Investigations on the Interaction of Lead(II) Acetate with Human Chorionic Gonadotropin Journal of Physical Chemistry B 2014, **118**, 32, 9644–9650.

Zhou C, Zhang R, Cai X, Xiao R, Yu H. Trace elements profiles of maternal blood, umbilical cord blood, and placenta in Beijing, China. Journal of Maternal, Fetal and Neonatal Medicine 2019 **32**(11):1755-1761

Appendix 1

Lead exposure from food in women of childbearing age

Table 1. Estimated daily exposure to lead from foods consumed by women of childbearing age (16-49 years) using data from the total diet study food groups (Bates *et al*., 2014, 2016; Roberts *et al.,* 2018)

Food Groups	Mean Daily exposure to lead LB to UB (µg/day) *	97.5th Percentile Daily exposure to lead LB to UB (µg/day) *
Bread	0.42	0.95
Miscellaneous Cereals	0.53	1.5
Carcase meat	0-0.098	0-0.41
Offal	0.038	0.70
Meat products	0.15	0.68
Poultry	0.10	0.36
Fish and seafood	0.098	0.47
Fats and oils	0-0.023	0-0.068
Eggs	0-0.035	0-0.17
Sugars and	0.13	0.49
Green vegetables	0.59	2.18
Potatoes	0-0.31	0-0.85
Other vegetables	0.43	1.28
Canned vegetables	0.18	0.91
Fresh fruit	0-0.31	0-1.2
Fruit products	0.26	1.5
Non-alcoholic beverages	0-2.7	0-5.9
Milk	0-0.25	0-0.86
Dairy products	0.16	0.59
Nuts and seeds	0-0.0089	0-0.073

Alcoholic drinks	0.35	2.4
Meat substitutes	0.013	0.19
Snacks	0.037	0.16
Desserts	0.042	0.26
Condiments	0.31	1.1
Tap water	0-0.32	0-1.4
Bottled water	0-0.089	0-0.66
Total	3.8-8.0	7.0-14.0

*Values have been rounded to two significant figures. LB=lower bound; UB=upper bound

Table 2. Estimated exposure (in µg/kg bw/day) to lead from foods consumed by women of childbearing age (16-49 years) using data from the total diet study food groups (Bates *et al*., 2014, 2016; Roberts *et al.,* 2018).

Food Groups	**Mean Exposure to lead LB to UB (µg/kg bw/day)***	**97.5th Percentile Exposure to lead LB to UB (µg/kg bw/day) ***
Bread	0.0061	0.015
Miscellaneous Cereals	0.0080	0.023
Carcase meat	0-0.0014	0-0.0065
Offal	0.00057	0.011
Meat products	0.0022	0.010
Poultry	0.0015	0.0055
Fish and seafood	0.0015	0.0071
Fats and oils	0-0.00034	0-0.0010
Eggs	0-0.00052	0-0.0025
Sugars and	0.0020	0.0081
Green vegetables	0.0088	0.034
Potatoes	0-0.0045	0-0.013
Other vegetables	0.0063	0.019

Canned vegetables	0.0027	0.013
Fresh fruit	0-0.0047	0- 0.018
Fruit products	0.0041	0.024
Non-alcoholic beverages	0-0.039	0-0.091
Milk	0-0.0037	0-0.014
Dairy products	0.0023	0.0087
Nuts and seeds	0-0.00013	0-0.0011
Alcoholic drinks	0.0053	0.037
Meat substitutes	0.00020	0.0027
Snacks	0.00055	0.0025
Desserts	0.00062	0.0039
Condiments	0.0045	0.016
Tap water	0-0.0048	0-0.021
Bottled water	0-0.0013	0-0.0093
Total	0.057-0.12	0.12-0.23

*Values have been rounded to two significant figures. LB=lower bound; UB=upper bound

Food groups with high exposure to lead includes:

1. Green vegetables
2. Miscellaneous cereals
3. Other vegetables

References

Bates, B.; Lennox, A.; Prentice, A.; Bates, C.; Page, P.; Nicholson, S.; Swan, G. (2014) National Diet and Nutrition Survey Results from Years 1, 2, 3 and 4 (combined) of the Rolling Programme (2008/2009 – 2011/2012): Link to PDF

Bates, B.; Cox, L.; Nicholson, S.; Page, P.; Prentice, A.; Steer, T.; Swan, G. (2016) National Diet and Nutrition Survey Results from Years 5 and 6 (combined) of the Rolling Programme (2012/2013 –2013/2014) Link to PDF

Roberts, C.; Steer, T.; Maplethorpe, N.; Cox, L.; Meadows, S.; Page, P.; Nicholson, S.; Swan, G. (2018) National Diet and Nutrition Survey Results from Years 7 and 8 (combined) of the Rolling Programme (2014/2015 – 2015/2016) Link to PDF