## SCIENTIFIC OPINION

# Scientific Opinion on Lead in Food[1]

**EFSA Panel on Contaminants in the Food Chain (CONTAM)**[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

## ABSTRACT

Lead occurs primarily in the inorganic form in the environment. Human exposure is mainly via food and water, with some via air, dust and soil. In average adult consumers, lead dietary exposure ranges from 0.36 to 1.24, up to 2.43 µg/kg body weight (b.w.) per day in high consumers in Europe. Exposure of infants ranges from 0.21 to 0.94 µg/kg b.w. per day and in children from 0.80 to 3.10 (average consumers), up to 5.51 (high consumers) µg/kg b.w. per day. Cereal products contribute most to dietary lead exposure, while dust and soil can be important non-dietary sources in children. Lead is absorbed more in children than in adults and accumulates in soft tissues and, over time, in bones. Half-lives of lead in blood and bone are approximately 30 days and 10-30 years, respectively, and excretion is primarily in urine and faeces. The 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. The respective BMDLs derived from blood lead levels in µg/L (corresponding dietary intake values in µg/kg b.w. per day) were: developmental neurotoxicity $BMDL_{01}$, 12 (0.50); effects on systolic blood pressure $BMDL_{01}$, 36 (1.50); effects on prevalence of chronic kidney disease $BMDL_{10}$, 15 (0.63). The CONTAM Panel concluded that the current PTWI of 25 µg/kg b.w. is no longer appropriate as there is no evidence for a threshold for critical lead-induced effects. In adults, children and infants the margins of exposures were such that the possibility of an effect from lead in some consumers, particularly in children from 1-7 years of age, cannot be excluded. Protection of children against the potential risk of neurodevelopmental effects would be protective for all other adverse effects of lead, in all populations.

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**KEY WORDS**

lead, occurrence, dietary exposure, food consumption, risk assessment, adults, children, margin of exposure

**SUMMARY**

Lead is an environmental contaminant that occurs naturally and, to a greater extent, from anthropogenic activities such as mining and smelting and battery manufacturing. Lead is a metal that occurs in organic and inorganic forms; the latter predominates in the environment. Control measures have been taken to regulate lead in paint, petrol, food cans and pipes in Europe since the 1970s. Human exposure to lead can occur via food, water, air, soil and dust. Food is the major source of exposure to lead. The primary techniques for analysing lead in food samples are based on atomic absorption spectrometry, atomic emission spectrometry and mass spectrometry after digestion of organic material with concentrated acids.

Following a call for data, 14 Member States and Norway submitted approximately 140,000 results of lead concentrations in various food commodities and tap water. A total of 94,126 results covered the period from 2003 to 2009 and were suitable for calculating lead concentrations in the various food categories. The lead level in approximately two thirds of the samples was below the limit of detection or limit of quantification. Sampling adjustment factors were applied when aggregating food sub-category averages to category averages in order to fit the information structure of the European Food Safety Authority (EFSA) Concise European Food Consumption Database.

Mean and 95$^{th}$ percentile lead dietary exposures were calculated separately for each country recorded in EFSA Concise European Food Consumption Database for the whole and subgroups of the population, including infants, children and vegetarians, using a deterministic approach. The Panel on Contaminants in the Food Chain (CONTAM Panel) also performed a probabilistic exposure assessment using lower bound and upper bound values for the non-quantifiable samples. This approach resulted in similar exposure values for average consumers as the deterministic approach. To maintain consistency with its opinions on other heavy metals, the CONTAM Panel therefore decided to use the deterministic approach for its assessment of dietary exposure to lead. Lead dietary exposure for average adult consumers in 19 European countries ranged from 0.36 to 1.24 µg/kg body weight (b.w.) per day (lower bound for country with lowest average exposure – upper bound for country with highest average exposure) and from 0.73 to 2.43 µg/kg b.w. per day for high consumers, respectively. Overall, cereals, vegetables and tap water were the most important contributors to lead exposure in the general European population. More specifically, the following food groups were identified as the major contributors to lead exposure: cereal products, followed by potatoes, cereal grains (except rice), cereal-based mixed dishes and leafy vegetables and tap water. Considerable variation between and within countries in the contribution of different food categories/groups exists.

The available evidence for women of child-bearing age and vegetarians does not indicate a dietary exposure that is different from that of the general adult population. Lead levels in breast milk are highly variable but exposure of infants is estimated to be 0.21 µg/kg b.w. per day on average or 0.32 µg/kg b.w. per day for high consumers. For infants fed with ready-to-consume infant formula, the average exposure estimates range from 0.27 to 0.63 µg/kg b.w. per day, based on lower bound and upper bound assumptions, respectively; for high consumers, lead exposure estimates range from 0.40 to 0.94 µg/kg b.w. per day. For children aged 1-3 years mean lead dietary exposure estimates range from 1.10 to 3.10 µg/kg b.w. per day based on lower bound and upper bound assumptions, respectively; for high consumers, lead exposure estimates range from 1.71 to 5.51 µg/kg b.w. per day. For children aged 4-7 years mean lead dietary exposure estimates range from 0.80 to 2.61 µg/kg b.w. per day based on lower bound and upper bound assumptions, respectively; for high consumers, lead exposure estimates range from 1.30 to 4.83 µg/kg b.w. per day.

Compared to dietary exposure, non-dietary exposure to lead is likely to be of minor importance for the general population in the European Union (EU). House dust and soil can be an important source of exposure to lead for children.

Absorption of lead from the gastrointestinal tract depends on host characteristics and on the physicochemical properties of the ingested material. Absorption of ingested soluble lead compounds appears to be higher in children than in adults. Absorption is lower in the presence of food. Absorption of inhaled sub-micron sized particles occurs in the respiratory tissues whereas larger-sized particles are transferred into the pharynx and are then swallowed. Absorbed lead is transported in the blood primarily within erythrocytes and then transferred to soft tissues, including liver and kidneys, and to bone tissue, where it accumulates with age. Maternal transfer of lead occurs through the placenta and subsequently during breast feeding. Enhanced mobilisation of lead from bones occurs during pregnancy. Half-lives for inorganic lead in blood and bone are approximately 30 days and between 10 and 30 years, respectively, and excretion primarily is in urine and faeces.

Most of the information on human exposure to, and the health effects of, lead is based on blood lead (B-Pb) data. Lead levels in bone and teeth provide information on past exposure to lead.

Due to its long half-life in the body, chronic toxicity of lead is of most concern when considering the potential risk to human health. Studies with rodent and non-human primate models have demonstrated that chronic low-level exposure to lead causes neurotoxicity, particularly learning deficits in the developing animal. Evidence, albeit limited, for lead-induced increases in blood pressure and nephrotoxicity in experimental animals has been reported. Lead may be a weak indirect genotoxic metal. There is extensive experimental evidence that at high doses lead can induce tumours at a number of different sites in rodents. The International Agency for Research on Cancer classified inorganic lead as probably carcinogenic to humans (Group 2A) in 2006.

In humans, the central nervous system is the main target organ for lead toxicity. In adults, lead-associated neurotoxicity was found to affect central information processing, especially for visuospatial organisation and short-term verbal memory, to cause psychiatric symptoms and to impair manual dexterity. There is considerable evidence demonstrating that the developing brain is more vulnerable to the neurotoxicity of lead than the mature brain. In children, an elevated blood lead level is inversely associated with a reduced Intelligence Quotient (IQ) score and reduced cognitive functions up to at least seven years of age. There is some evidence that this subsequently leads to a reduced adult grey matter volume, especially of the prefrontal cortex. The dose-effect relationship between blood lead levels and IQ indicates a nonlinear curve that reflects a greater relative impact at lower lead concentrations. A number of studies in adults have identified an association between blood lead concentration, elevated systolic blood pressure (SBP) and chronic kidney disease (CKD), at relatively low B-Pb levels.

Lead in blood is considered to be the best indicator of the concentration of lead in soft tissues, reflecting recent and, to some extent, past exposure, whereas bone lead in vivo reflects the long-term uptake and body burden. A non-specific biomarker for lead exposure is the inhibition of haem metabolism.

The CONTAM Panel identified developmental neurotoxicity in young children and cardiovascular effects and nephrotoxicity in adults as potential critical adverse effects of lead on which to base the risk assessment. Full Scale IQ) score (was chosen as the most sensitive and most relevant endpoint for children. Dose-response analysis of cardiovascular effects identified blood pressure as the most sensitive endpoint, and SBP was the preferred critical endpoint. Nephrotoxicity was analysed for the prevalence of CKD based on a reduction in the glomerular filtration rate (GFR) to values below 60 mL/min. Dose metrics available in published studies were B-Pb concentration measured both concurrently with the examination for health effect and in early childhood, as average or as peak concentration over the lifetime, in units of µg lead per litre (L) blood. When available the CONTAM

Panel used also tibia bone concentration measured concurrently or in the past in units of µg lead/g bone mass.

Using the complete individual data from the seven studies described by Lanphear et al. (2005) the CONTAM Panel determined the 95$^{th}$ percentile lower confidence limit of the benchmark dose (BMD) of 1 % extra risk ($BMDL_{01}$) of 12 µg B-Pb/L as a reference point for the risk characterisation of lead when assessing the risk of intellectual deficits in children measured by the Full Scale IQ score. A 1 % increase of SBP annually or on average in the whole population was considered a public health issue, since it would result in an increased risk of cardiovascular morbidity and coronary heart disease (CHD) mortality in a population. Assuming an average SBP of 120 mmHg and critical Benchmark Response level of 1 %, the dose associated with an increase of SBP by 1.2 mmHg, then this corresponds to a $BMD_{01}$. BMD and $BMDL_{01}$ values were based on the slope estimates derived from the five selected studies on blood and tibia bone lead concentration. Longitudinal data allowed the calculation of a $BMD_{01}$ for the mean annual increase of SBP by 1 % in an individual, whereas cross-sectional data allowed only calculation of the $BMD_{01}$ on a population-based increase of the means. The CONTAM Panel determined four $BMDL_{01}$ values for SBP ranging from 15 to 71 µg/L (longitudinal 27 and 71 µg/L, cross-sectional studies 15 and 21 µg/L). Given the strong overlap of the study results and the absence of any obvious design deficiencies in the studies, the CONTAM Panel calculated a mean $BMDL_{01}$ for SBP of 36 µg/L from the four studies and a $BMDL_{01}$ = 8 µg/g for tibia bone lead concentrations. For CKD, no model was acceptable when using the criterion that the fitted model should not be significantly different from a statistical point-of-view from the full model at a p-value not smaller than 0.10. Considering the high precision of the incidence rates due to the large sample size using the cross-sectional data from the National Health and Nutrition Examination Survey (1999-2006) that criterion was reduced to a value of 0.01. Two models gave an acceptable fit: the probit model and the multistage model gave the same values, i.e. BMD10= 16 µg/L and $BMDL_{10}$=15 µg/L, respectively.

The relationship between dietary lead intake and blood lead levels in adults was estimated using the Carlisle and Wade (1992) model that takes into account dietary lead and lead from soil, dust and air, although in adults it was concluded that direct exposure via soil and dust is negligible. Using this model, BMDL dietary lead intake values in adults of 1.50 µg/kg b.w. per day and 0.63 µg/kg b.w. per day were derived for the cardiovascular and kidney effects, respectively. The relationship between dietary lead intake and blood lead levels in children up to age seven was estimated using the Integrated Exposure Uptake Biokinetic (IEUBK) model for lead in children. Using the IEUBK model, a $BMDL_{01}$ dietary intake value of 0.50 µg/kg b.w. per day for developmental neurotoxicity was derived.

The CONTAM Panel concluded that the provisional tolerable weekly intake (PTWI) of 25 µg/kg b.w. set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and endorsed by the Scientific Committee of Food is no longer appropriate and that as there was no evidence for a threshold for a number of critical endpoints including developmental neurotoxicity and nephrotoxicity in adults, it would not be appropriate to derive a PTWI. The CONTAM Panel does consider it appropriate to calculate margins of exposure to support the risk characterisation.

Estimates of dietary exposure to lead based on lower bound assumptions and upper bound assumptions for the level of reporting for average adult consumers in Europe are lower than the BMDL intake value for effects on SBP (1.50 µg/kg b.w. per day), but vary from above to below the BMDL intake value for effects on the prevalence of CKD (0.63 µg/kg b.w. per day). The respective MOEs range from 1.2 to 4.2 and from 0.51 to 1.81, respectively. Hence, if exposure were closer to the upper bound estimates, the possibility of an effect on some consumers cannot be excluded.

The limited available evidence does not indicate a different average dietary exposure or risk for vegetarians from the adult population, consumer groups with higher lead exposure levels include high consumers of game meat (1.98 to 2.44 µg/kg b.w. per day) and high consumers of game offal (0.81 to 1.27 µg/kg b.w. per day). The estimated dietary exposures of these groups are also within, or at the higher end of the range of the respective BMDL intake values.

Breast-fed 3-month old infants are predicted to have a lead exposure that is below the $BMDL_{01}$ intake value of 0.50 µg/kg b.w. per day for neurodevelopmental effects. Lead exposure based on lower bound assumptions in both average and high 3-month old infant consumers of infant formula is below the $BMDL_{01}$, but may exceed this level, based on upper bound estimates. Therefore, the possibility of a risk to infants cannot be excluded.

Estimated exposure in children up to age seven exceeds the $BMDL_{01}$ intake level of 0.50 µg/kg b.w. per day for neurodevelopmental effects. The MOE in average 1 to 3 year old child consumers ranged from 0.16 to 0.45, and was only slightly higher in 4 to 7 year old children. Therefore, the MOE is such that the possibility of an effect in some children cannot be excluded. It was not possible to estimate the potential numbers of children who might be affected, as even in average consumers the MOE was <1.

Women of 20 to 40 years of age were used as a surrogate for pregnant women to calculate the risk of lead exposure in utero on neurodevelopment in the offspring. Estimates of exposure were at or above the BMDL for neurodevelopmental effects, and the CONTAM Panel concluded that it was not possible to exclude a risk to the developing fetus through exposure of some pregnant female consumers.

The CONTAM Panel concluded that the risk of clinically important effects on either the cardiovascular system or kidneys of adult consumers, at current levels of lead exposure is low to negligible. In infants, children and pregnant women, there is potential concern at current levels of exposure to lead for effects on neurodevelopment. Protection of children and women of child-bearing age against the potential risk of neurodevelopmental effects should be protective for all other adverse effects of lead, in all populations.

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## BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Lead occurs naturally in the environment, but its industrial use (e.g. mining, smelting, processing, use in plumbing solders and alloys, pigments, batteries, ceramics, etc.) has resulted in increased levels in soil, water and air. Emissions from waste incinerators were important contributors to lead in the environment, as was leaded fuel in the past. However, with the introduction of unleaded fuel in the mid 1980's, lead has considerably decreased in the environment all over Europe. Lead accumulation in soils and surface waters depends on many factors, including pH, mineral composition and amount and type of organic material. Lead in soil is transferred to food crops.

Inorganic lead compounds were classified by the International Agency for Research on Cancer (IARC) as probably carcinogenic to humans (Group 2A) in 2006. In 1992, the European Commission's Scientific Committee for Food (SCF) expressed an opinion4 on lead in food and drink in which it endorsed the 1986 Joint FAO/WHO Expert Committee on Food Additives (JECFA) Provisional Tolerable Weekly Intake (PTWI) of 25 µg/kg b.w. (re-confirmed by JECFA in 1999). Furthermore, SCF concluded that the mean level of contamination of foodstuffs would not seem to be of concern, but expressed a wish to undertake a re-examination of the toxicity of lead. In 2004, the European Commission carried out an updated exposure assessment with the data collected in the framework of SCOOP5 task 3.2.11. SCF opinion and SCOOP report served as a basis for setting and updating maximum levels for lead in foodstuffs.

Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs6 contains the most recent maximum levels for lead in foodstuffs. However, these levels continue to be constantly reviewed by the Commission. An updated scientific basis is therefore of great importance.

## TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002 the European Commission asks the European Food Safety Authority for a scientific opinion on the risks to human health related to the presence of lead in foodstuffs.

In particular, the opinion should consider any new developments regarding the toxicity of lead since the SCF opinion of 1992 in order to assess whether the PTWI of 25 µg/kg b.w. is still appropriate. The opinion should contain an updated exposure assessment for lead, in particular addressing exposure from food (incl. drinking water) and from other non-dietary sources (e.g. air), the exposure situation for specific groups of the population (e.g. infants and children, people following specific diets, smokers, etc.) and an indication of the age group in which children would be most exposed to the toxic effects of lead take into account available biomonitoring data when assessing the exposure and compare the results with the calculated exposure.

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[4] SCF Opinion of 19 June 1992, Thirty second series, 1994, p.7, available at: http://ec. europa. eu/food/fs/sc/scf/reports/scf_reports_32.pdf.

[5] SCOOP Report of task 3.2.11: "Assessment of the dietary exposure to arsenic, cadmium, lead and mercury of the population of the EU Member States", March 2004, available at: http://ec. europa. eu/food/food/chemicalsafety/contaminants/scoop_3-2-11_heavy_metals_report_en. pdf. The SCOOP task was carried out in the framework of scientific cooperation with Member States under Council Directive 93/5/EEC, OJ L 52, 4.3.1993, p.18-21.

[6] OJ L 364, 20.12.2006, p.5.

**ASSESSMENT**

## 1. Introduction

Lead is a soft, dense, and ductile metal. It is a natural environmental contaminant, but its ubiquitous occurrence results, to a great extent, from anthropogenic activities including mining and smelting, soldering, battery manufacturing, ammunition, metal water pipes, and particularly the use in the past of lead in paint and petrol. In the 1970s, the evidence of lead-associated adverse effects led to the implementation of control measures, in both Europe and the U.S. (Federal Clean Air Act, 1970), which significantly decreased the exposure levels of the general population. In time, leaded paints, leaded petrol (Directive 98/70/EC, U.S. EPA, 1973; 1996) and lead solder in food cans and pipes were strictly regulated or banned. Directive 98/70/EC states that Member States had to prohibit the marketing of leaded petrol within their territory by 1 January 2000 at the latest. By way of derogation, a Member State could be allowed to permit the marketing of leaded petrol until 1 January 2005 at the latest, provided that the lead content of leaded petrol did not exceed 0.15 g/L.

Human exposure to lead can occur via food, water, soil, dust and air. Lead exists both in organic and inorganic forms. In the environment, inorganic lead predominates over organic lead. Exposure to the latter is generally limited to occupational settings. Organic and inorganic lead differ in terms of both toxicokinetics and toxicodynamics. Organo-lead compounds, such as tri-alkyl-lead and tetraalkyl-lead compounds, are more toxic than inorganic forms of lead (U.S. ATSDR, 2007; UNEP, 2008). To some extent, organic lead compounds are metabolised to inorganic lead both in humans and in animals (IARC, 2006).

Lead[7] can accumulate in the body, primarily in the skeleton. From the skeleton, it is released gradually back into the blood stream, particularly during physiological or pathological periods of bone demineralisation such as pregnancy, lactation and osteoporosis, even if lead exposure has already ceased. Lead can be transferred from the mother to the fetus/infant in utero and through breast milk.

Lead affects virtually every system in the body, including the blood, the cardiovascular, renal, endocrine, gastrointestinal, immune and reproductive systems. Nevertheless, the most critical target for lead appears to be the central nervous system (CNS), particularly the developing brain, where it has the potential to cause impaired cognitive development and intellectual performance in children even at low exposure levels. Lead is classified as a class 2A carcinogen by the International Agency for Research on Cancer (IARC, 2006).

In order to provide a scientific basis for the European Commission to review the maximum levels of lead in foodstuffs, the European Food Safety Authority (EFSA) was asked for an updated risk assessment on lead in food.[8]

### 1.1. Previous risk assessments

#### 1.1.1. Non-cancer endpoints

The first evaluation of lead by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) dates back to 1972, when a provisional tolerable weekly intake (PTWI) was set to 3 mg of lead per person, corresponding to 50 μg/kg body weight (b.w.). This value referred to all sources of lead exposure and applied just to the adult population. The PTWI was based on the assumption that an

[7] In this Opinion, the term "lead" refers to inorganic lead, unless specified otherwise.
[8] This Opinion takes into consideration the relevant literature published until December 2009.

average person absorbs 60 to 70 µg of lead per day (1 µg/kg b.w. per day) and was allocated as follows: 20 µg from air, 10 µg from water and 40 µg from food (WHO, 1972).

At the JECFA meeting in 1978 (WHO, 1978), the PTWI of 50 µg/kg b.w. for adults was maintained.

In 1985, the U.S. Environmental Protection Agency (U.S. EPA) considered deriving an oral reference dose (RfD) for lead, but judged it inappropriate, given the evidence that some adverse health effects (e.g. alterations of some blood enzyme levels and of children's neurobehavioural development) occurred at blood lead levels so low as to be essentially without a threshold (U.S. EPA, 2004).

In 1986, the JECFA addressed the evaluation of the risks associated with the exposure to lead from all sources, specifically as it impacts on infants and children. For this sensitive group the PTWI was set to 25 µg/kg b.w. (WHO, 1986). This value was set based on the observation that a mean total daily intake of 3 to 4 µg/kg b.w. of lead by infants and children did not cause an increase in steady state blood lead levels (B-Pb). These calculations were based on the contemporary blood levels and the existing limits of analytical detection. The tolerable daily intakes (TDI) from all lead sources were 36 µg for a 0 to 6 month-old infant and 54 µg for a 0.5 to 2 year-old child. Based on the assumption that food contributes about 50 % of the total daily intake of lead, the TDI of lead from dietary sources would range from 18 to 27 µg.

In 1991, the U.S. Centers for Disease Control and Prevention (CDC, 1991) decided to lower the "blood lead level of concern" in children from 250 µg of lead/L to 100 µg/L, as some children had displayed adverse effects even "at B-Pb as low as 100 µg/L" (CDC, 1991). It should be noted that the CDC's advisory level for individual intervention in children did not represent a safe level or a toxic threshold, but rather was meant to trigger the implementation of community prevention measures. Based on the CDC's "Blood Lead Level of concern", the U.S. Food and Drug Administration (FDA) set a provisional total tolerable intake level (PTTIL) for lead at 6 µg/day for small children (under the age of six years), 25 µg/day for pregnant women, and 75 µg/day for other adults (US FDA, 1993). The PTTIL was defined as the total daily lead intake from all sources (air, soil and dust, water and food) and one that provides a reasonable margin of protection against the known adverse effects of lead.

In 1990, the European Commission's Scientific Committee for Food (SCF) endorsed the 1986 JECFA PTWI of 25 µg/kg b.w. for infants and children. In its opinion[9] on lead in food and drink in 1992, the SCF concluded that whilst the mean level of contamination of foodstuffs did not seem to be cause for alarm, there was a need for a re-examination of the toxicity of lead.

In 1993, the JECFA re-evaluated lead, based on the assessment of an International Programme on Chemical Safety Task Group, which was published as an Environmental Health Criteria monograph in 1995 (WHO/IPCS, 1995). JECFA re-confirmed the PTWI of 25 µg/kg b.w. for infants and children and extended it to the overall population irrespective of the age group (WHO, 1993). In the same year, WHO proposed a health-based guideline value for lead in drinking water of 0.01 mg/L (WHO, 2003).

In 1999, at its fifty-third meeting, the JECFA specifically evaluated the impact of lead exposure via dietary sources on children's Intelligence Quotient (IQ). An oral intake of 1 µg/kg b.w. per day was estimated to result in an increase of 10 µg/L in B-Pb (upper estimate for infants). This relationship was considered applicable for the first 10 years of life (WHO, 2000b). On the basis of a quantitative risk assessment, the Committee concluded that the concentrations of lead currently found in food would have negligible effects on the neurobehavioural development of infants and children. The Committee agreed to retain the PTWI of 25 µg/kg b.w. (WHO, 2000b).

[9] SCF Opinion of 19 June 1992, Thirty second series, 1994, p.7, available at: http://ec. europa. eu/food/fs/sc/scf/reports/scf_reports_32. pdf

In 2004, the European Commission carried out an updated exposure assessment with the data collected in the framework of SCOOP (EU Scientific Cooperation) task 3.2.11. According to this report, although very high levels were present in specific food items from some Member States, in general, none of the most consumed foodstuffs were high in lead. The mean intake of the adult population in the Member States was estimated to be 42 µg/day (17 % of the PTWI). Based on available data, from Germany and France, children, especially those aged 4 to 6 years, had a lower absolute intake. However, due to their lower body weights, their body exposure (1 µg/kg b.w. per day: 35 % of PTWI) was higher than that of adults (0.6 µg/kg b.w. per day: 19 % of PTWI). In the same year, EFSA published an opinion on lead as an undesirable substance in animal feed (EFSA, 2004) in which it was concluded that although the lead content of commercial feed commodities was generally too low to induce any toxicity, incidental intoxications of cattle and sheep from waste disposal were regularly reported.

In 2005, the CDC performed a fifth revision of its statement, and decided not to lower the B-Pb level of concern any further, despite evidence that adverse effects may occur in children at B-Pb <100 µg/L (CDC, 2005). Thus, since 1991, the CDC's B-Pb level of concern has stood at ≥100 µg/L, equivalent to 0.48 µmol/L (CDC, 2005).

The CDC based its decision not to change the level of concern on the apparent lack of a threshold level for lead-induced adverse effects: the setting of a new B-Pb level of concern somewhere below 100 µg/L would therefore have to have been arbitrary. The CDC also considered "as not demonstrated" the feasibility and effectiveness of clinical interventions to reduce B-Pb below 100 µg/L. A final remark was that the inaccuracy intrinsic to laboratory testing would not allow accurate classification of children as having B-Pb concentrations above or below 100 µg/L. The CDC concluded by highly recommending primary prevention - that is, all lead sources in children's environments be controlled or eliminated.

In 2006, the Scientific Committee on Neurotoxicology and Psychophysiology and the Scientific Committee on the Toxicology of Metals of the International Commission on Occupational Health (Landrigan et al., 2006) stated that current exposure standards for lead needed to be reduced urgently, and that for children the action level which triggers community prevention efforts to reduce exposure sources should be reduced immediately to a B-Pb concentration of 50 µg/L in nations worldwide. This level was proposed as a temporary level that may need to be revised downward in future years, if new evidence accumulates on toxicity at lower B-Pb. Also, in the case of female industrial workers of reproductive age, the standard for B-Pb should be reduced to the lowest achievable, preferably to 50 µg/L, a level consistent with the proposed B-Pb standard for children.

In 2007, the Agency of Toxic Substances and Disease Registry (U.S. ATSDR, 2007) published the document "Toxicological profile for lead", in which no minimum risk levels (MRLs) for lead were derived "because a clear threshold for some of the more sensitive effects in humans has not been identified. Instead of MRLs, U.S. ATSDR has developed a framework to guide decisions at lead sites. This approach utilises site-specific exposure data to estimate internal doses as measured by blood lead levels."

On a request from the Food Standards Agency (FSA) in the UK, the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) recently evaluated the results of a survey of metals in foods (FSA, 2009) and assessed whether the levels of any of these could have the potential to put human health at risk (COT, 2008). With respect to lead, the COT noted that the dietary exposure of the general UK population had declined considerably from 1976 to 2000, although it had not changed significantly since then (i.e.7 µg/day in 2000 vs. 6 µg/day in 2006). The latter estimates of exposure (ranging between 0.16 and 0.42 µg/kg b.w. per day in high-level consumers) were below the JECFA PTWI corresponding to 3.6 µg/kg b.w. per day.

The COT estimated that in infants and young children the intake of dietary lead at the PTWI would result in an approximate 36 µg/L increase in the B-Pb, and this in turn in a mean IQ deficit of 0.36 to 1.8 points. This estimation relied on the JECFA's correlation between dietary intake and B-Pb increase (i.e. 1 µg lead /kg b.w. per day results in 10 µg/L B-Pb increase), and it was based on the assumption that each 100 µg/L increase in B-Pb causes the IQ score to decrease by 1 to 5 points (U.S. ATSDR, 2007). The COT noted a number of uncertainties in this estimation relating to the true steepness of the dose-effect relationship at B-Pb <100 µg/L, the nature of the dose-effect relationship below the lowest B-Pb (<10 µg/L) in epidemiological studies, inter-individual variation in children, study differences, and limits to the precision of analytical and psychometric measurements. The Committee concluded that at the estimated current dietary intakes, adverse effects, if any, were likely to be very small. However, since it was not possible to identify a threshold for the association between lead exposure and decrements in intelligence quotient, efforts should continue to reduce lead exposure from all sources (COT, 2008).

In 2008, the United Nations Environment Programme (UNEP, 2008) published an interim review of scientific information on lead, focussing especially on long range environmental transport, in order to inform future discussions of the UNEP Governing Council on the need for global action in relation to lead. Among data gaps, the Committee recognised that "The mechanism of lead toxicity is not well understood. There is a controversy on whether the endogenous exposure from bone-lead is a particular risk because it affects B-Pb, which in turn hits the target organs. Exposure-response relationship is incomplete for many effects."

### 1.1.2. Cancer endpoints

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 (IARC, 2006). 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 (IARC, 2006).

EPA has categorised lead as a probable human carcinogen (Group B2), supported by sufficient data in animals, even though there was inadequate information on humans (U.S. EPA, 2004).

In the 11th Report on Carcinogens, the National Toxicology Program (NTP) of the U.S. National Institutes of Health concluded that "lead and lead compounds are reasonably anticipated to be human carcinogens", based on limited evidence from studies in humans and sufficient evidence from studies in experimental animals (NTP, 2004).

The American Conference of Governmental Industrial Hygienists (ACGIH) has designated inorganic lead and lead compounds as A3 carcinogens, categorised as "confirmed animal carcinogen with unknown relevance to humans" (ACGIH, 2004).

## 1.2. Chemistry

Lead is a naturally occurring element that belongs to Group 4A of the periodic table and has an atomic number of 82 and atomic mass of 207.2 g/mol. It is a silver-bluish white metal that is found in small amounts in the earth's crust although it is rarely found naturally as a metal. Usually it is found combined with two or more other elements to form lead compounds. It is highly malleable, ductile and a relatively poor conductor of electricity. Lead is very resistant to corrosion but tarnishes upon exposure to air (Korn et al., 2006).

The main oxidation states of lead are +2 and +4, although in the environment +2 is the prevalent form. Inorganic lead compounds, such as lead phosphate and lead carbonate, usually contain lead in its

divalent state (+2). The solubility of lead compounds in water is a function of pKa, hardness, salinity and the presence of humic material (U.S. ATSDR, 2007).

Industrially synthesised organic lead compounds, such as alkyl-lead compounds, have been used mainly as fuel additives as anti-knock agents in combustion engines. Tetraethyl lead ($Pb(C_2H_5)_4$) and tetramethyl lead ($Pb(CH_3)_4$) are the most commonly used alkyl-lead compounds. They are both highly volatile, lipid soluble liquids. Human exposure to these compounds is mainly through inhalation of leaded petrol vapours, dermal exposure to leaded petrol and ingestion of lead-contaminated soil, food or water (U.S. EPA, 2000). Once absorbed into the body, these compounds may be dealkylated to divalent lead ions by cytochrome P450 mono-oxygenase activity and this must be considered in total exposure assessment (WHO/IPCS, 1995). Alkyl-lead compounds were included on the EPA's Persistent Bioaccumulative and Toxic (PBT) chemicals programme, aiming at reducing their use and developing safer alternatives.

## 2. Legislation

In order to protect public health, Article 2 of Council Regulation (EEC) No 315/93 of 8 February 1993 laying down Community procedures for contaminants in food[10] stipulates that, where necessary, maximum tolerances for specific contaminants shall be established. The current maximum levels (ML) for lead are laid down in the Annex, Section 3 of Commission Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs[11]. The MLs established for lead reflect the results of a dietary exposure assessment carried out in the SCOOP-task 3.2.11[5] and the outcome of the EC SCF opinion[4]. Maximum levels are generally set for the edible part of the foodstuffs concerned, unless otherwise specified in Section 3 of the Annex of Regulation (EC) No 1881/2006. For dried, diluted, processed and compound foodstuffs, changes of concentration of the contaminants caused by drying, dilution or processing are taken into account. For compound foodstuffs, the relative proportions of the ingredients in the product are taken into account. As regards the exact definition of the respective foodstuff to which the maximum levels apply, the reader is referred to the footnotes of the Annex of Regulation (EC) No 1881/2006.

All MLs for lead are expressed as mg/kg wet weight (Table 1). While the lowest MLs of 0.02 mg/kg are set for infant formula, follow-on formula, as well as raw milk, heat-treated milk and milk for the manufacture of milk-based products, the highest ML of 3.0 mg/kg applies to food supplements. Moreover, performance characteristics for the analytical determination of lead are set in Regulation (EC) No 333/2007[12].

Harmonised levels for lead in drinking water are set by Council Directive 98/83/EC on the quality of water intended for human consumption[13]. The Directive stipulates that Member States shall set limit values of 10 µg/L for lead in water intended for human consumption. This limit will enter into force on 1st December 2013. Until then a limit value of 25 µg/L applies.

Commission Directive 2003/40/EC of 16 May 2003, establishing the list, concentration limits and labelling requirements for the constituents of natural mineral waters and the conditions for using ozone-enriched air for the treatment of natural mineral waters and spring waters, also sets a maximum limit for lead in natural mineral water of 10 µg/L. Moreover, performance characteristics for the analytical determination of lead are set both in Council Directive 98/83/EC and in Commission Directive 2003/40/EC.

[10] Official Journal L 037, 13/02/1993 P.0001 – 0003.
[11] OJ L 364, 20.12.2006, p.5.
[12] OJ L 88, 29.3.2007, p.29-38.
[13] OJ L 330, 5.12.1998, p.32.

Commission Directive 2008/60/EC of 17 June 2008 laying down specific purity criteria concerning sweeteners for use in foodstuffs, Commission Directive 2008/84/EC of 27 August 2008 laying down specific purity criteria on food additives other than colours and sweeteners, and Commission Directive 2008/128/EC of 22 December 2008 laying down specific purity criteria concerning colours for use in foodstuffs, all provide maximum levels between 0.5 and 20 mg/kg for lead as an impurity in numerous food additives.

Codex Alimentarius has also set a number of standards for lead, such as maximum permissible concentrations for edible fats and oils (0.1 mg/kg), food grade salt (2 mg/kg), corned beef (1 mg/kg) and others.

Lead and cadmium in ceramics are regulated by Council Directive 84/500/EEC[14]. This Directive sets migration limits for the metals, which might be released from decoration or glazing. In addition, it prescribes an analytical method for the determination of the migration of these two elements.

Directive 2009/48/EC of the European Parliament and of the Council of 18 June 2009 on the safety of toys[15] sets migration limits, from toys or components of toys that shall not be exceeded. For lead the migration limits range from 3.4 mg/kg in liquid or sticky toy material to 160 mg/kg in scraped-off toy material.

Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed, sets maximum contents for lead in a number of feed commodities (see Table 2). All levels are based on a product with a moisture content of 12 %. The maximum levels refer to an analytical determination of lead, in which digestion is performed with nitric acid (5 %) for 30 minutes at boiling temperature. Equivalent procedures can be applied when it can be demonstrated that the recovery efficiency is at least as good as that of the recommended procedure.

[14] OJ L 277, 20.10.1984, p.12.
[15] OJ L 170, 30.06.2009, p.1.

**Table 1:** Maximum levels for lead as laid down in Regulation (EC) No 1881/2006.

| Foodstuffs[(a)] | Maximum levels (mg/kg wet weight) |
|---|---|
| Raw milk, heat-treated milk and milk for the manufacture of milk based products | 0.02 |
| Infant formula and follow-on formula | 0.02 |
| Meat (excluding offal) of bovine animals, sheep, pig and poultry | 0.10 |
| Offal of bovine animals, sheep, pig and poultry | 0.50 |
| Muscle meat of fish | 0.30 |
| Crustaceans, excluding brown meat of crab and excluding head and thorax meat of lobster and similar large crustaceans (*Nephropidae* and *Palinuridae*) | 0.50 |
| Bivalve molluscs | 1.5 |
| Cephalopods (without viscera) | 1.0 |
| Cereals, legumes and pulses | 0.20 |
| Vegetables, excluding brassica vegetables, leaf vegetables, fresh herbs and fungi. For potatoes the maximum level applies to peeled potatoes | 0.10 |
| Brassica vegetables, leaf vegetables and the following fungi: *Agaricus bisporus* (common mushroom), *Pleurotus ostreatus* (Oyster mushroom), *Lentinula edodes* (Shiitake mushroom) | 0.30 |
| Fruit, excluding berries and small fruit | 0.10 |
| Berries and small fruit | 0.20 |
| Fats and oils, including milk fat | 0.10 |
| Fruit juices, concentrated fruit juices as reconstituted and fruit nectars | 0.05 |
| Wine (including sparkling wine, excluding liqueur wine), cider, perry and fruit wine | 0.20 |
| Aromatised wine, aromatised wine-based drinks and aromatised wine product cocktails | 0.20 |
| Food supplements | 3.0 |

(a) See the original Regulation for further definitions and explanations of individual food commodities.
ML: maximum level.

**Table 2:** EU legislation on lead in products intended for animal feed.

| Products intended for animal feed | Maximum content in mg/kg (ppm) relative to a feedingstuff with a moisture content of 12 % |
|---|---|
| Feed materials with the exception of: | 10 |
| green fodder(*) | 30 |
| phosphates and calcareous marine algae | 15 |
| calcium carbonate | 20 |
| yeasts | 5 |
| Additives belonging to the functional group of compounds of trace elements except | 100 |
| zinc oxide | 400 |
| manganous oxide, iron carbonate, copper carbonate | 200 |
| Additives belonging to the functional groups of binders and anti-caking agents except | 30 |
| clinoptilolite of volcanic origin | 60 |
| Premixtures | 200 |
| Complementary feedingstuffs with the exception of | 10 |
| mineral feedingstuffs | 15 |
| Complete feedingstuffs | 5 |

(*) Green fodder includes products intended for animal feed such as hay, silage, fresh grass, etc.

# 3. Sampling and methods of analysis

## 3.1. Sampling of foodstuffs

Sampling, sample preparation and analytical procedures play an important role in the reliable determination of contaminants in foodstuffs. Harmonised general criteria for heavy metals were first set in the European Union (EU) in 2001 by Commission Directive 2001/22/EC, which laid down the sampling methods and the methods of analysis for the official control of the levels of lead, cadmium, mercury and monochloropropane-1,2-diol (3-MCPD) in foodstuffs. This Directive has been replaced by Commission Regulation (EC) No 333/2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo[a]pyrene in different foodstuffs. No requirements exist for the number of samples that have to be analysed.

The requirements for sampling methods differ, especially depending on type and weight of lot, in order to obtain samples that are representative for the lot. Each lot to be examined should be sampled separately. Large lots should be sub-divided into defined sub-lots that have to be sampled separately. In as far as it is possible, incremental samples should be taken from various places throughout the lot or sublot. By combining the incremental samples, an aggregate sample is formed, which after homogenisation represents the base material for enforcement, defence and referee purposes (as stated in Commission Regulation (EC) No 333/2007).

The analyst must ensure that samples do not become contaminated during sample preparation. Wherever possible, apparatus and equipment that comes into contact with the sample should not contain those metals to be determined and should be made of inert materials e.g. plastics such as polypropylene, polytetrafluoroethylene (PTFE). These should be acid cleaned to minimise the risk of contamination. High quality stainless steel may be used for cutting edges. There are many satisfactory specific sample preparation procedures that can be used for the products under consideration. Those described in the European Committee for Standardisation (CEN) Standard 'Foodstuffs - Determination of trace elements - Performance criteria, general considerations and sample preparation'[16] have been found to be satisfactory, but others may be equally valid.

## 3.2. Methods of analysis

Commission Regulation (EC) No 333/2007 also contains strict requirements with which the methods of analysis have to comply in order to ensure that control laboratories use procedures with comparable levels of performance. The Regulation follows the "criteria approach". This means that no prescribed fixed official methods have to be followed, but laboratories can use any method of analysis, provided it can be demonstrated in a traceable manner that it strictly fulfils the analytical requirements laid down in the respective legislation. As an exception, for the analysis of lead in wine, Commission Regulation (EEC) No 2676/9017 lays down the method to be used in chapter 35 of its Annex.

As a general requirement, methods for lead analysis used for food control purposes must comply with the provisions of points 1 and 2 of Annex III (characterisation of methods of analysis) to Regulation (EC) No 882/2004 of the European Parliament and of the Council on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules18. While Regulation (EC) No 882/2004 contains the general provisions, the specific requirements for the official control of lead in foodstuffs are laid down in Commission Regulation

[16] Standard EN 13804:2002, 'Foodstuffs — Determination of trace elements — Performance criteria, general considerations and sample preparation', CEN, Rue de Stassart 36, B-1050 Brussels.
[17] OJ L 272, 3.10.1990, p.1. Regulation as last amended by Regulation (EC) No 1293/2005 (OJ L 205, 6.8.2005, p.12).
[18] OJ L 191, 28.5.2004, p.1.

(EC) No 333/2007. Annex, Table 5 of the latter sets performance criteria for applicability, limits of detection (LOD) and quantification (LOQ), precision, recovery and specificity. The methods used for the determination should be applicable to those foodstuffs specified in Regulation (EC) No 1881/2006. Requirements for LOD are given as less than one tenth of the maximum level in Regulation (EC) No 1881/2006, unless the maximum level for lead is less than 100 µg/kg. For the latter, less than one fifth of the maximum level applies. The requirements for LOQ are given as less than one fifth of the maximum level in Regulation (EC) No 1881/2006, unless the maximum level for lead is less than 100 µg/kg. For the latter, less than two fifths of the maximum level is required as LOQ. It should be noted that LOD and LOQ will vary with the analytical technique, the sample weight, the laboratory and the food matrix.

Normally, as no extraction step is used in the analysis of heavy metals, the result may be reported for recovery without correction if evidence is provided, ideally by making use of suitable certified reference material to show that the certified concentration, allowing for measurement uncertainty, is achieved (i.e. high accuracy of the measurement). Where the result is reported without correction for recovery, this should be mentioned. Concerning precision, it is required that the $HORRAT_r$[19] and $HORRAT_R$[20] values are less than 2. The requirement for specificity is given as "free from matrix or spectral interferences".

Finally, Commission Regulation (EC) No 333/2007 sets requirements for reporting results and for the assessment of compliance of the lot or sub lots. For this, the analytical result corrected for recovery, if necessary, should be used for checking compliance. The analytical result must be reported as x ± U, where x is the analytical result and U is the expanded measurement uncertainty, using a coverage factor of 2, which gives a level of confidence of approximately 95 %. The lot or sublot is accepted if the analytical result of the laboratory sample does not exceed the respective maximum level as laid down in Regulation (EC) No 1881/2006 taking into account the expanded measurement uncertainty and correction of the result for recovery, if necessary.

The primary techniques for analysing lead in food samples are based on atomic absorption spectrometry (AAS), atomic emission spectrometry (AES) and mass spectrometry (MS). When very high concentrations occur, X-ray fluorescence spectroscopy (XRFS) is also applicable.

Except for XRFS, samples containing organic material must be digested with concentrated acids. This process is enhanced by high temperatures and the use of digestion autoclaves or Parr bombs is highly recommended. This must be followed by dilution and therefore, compared to the analysis solution, LOD will be increased by a factor of ten to hundred, depending on the dilution factor.

Flame AAS (FAAS) and graphite furnace AAS (GFAAS) are the two atomic absorption spectrometry techniques used for measuring trace elements in foodstuffs. Empirical limits of detection for lead in analysis solution are in the range of 0.5 and 0.1 µg/L for FAAS and GFAAS, respectively. Specific analytical efforts may result in lower LODs by a factor of up to 10. In principle, these are single element techniques, although with the compromise of lower sensitivity, multielement methods are also available. The linear range of the AAS technique is quite low (10 to 100) and might require dilution adjustment. The specificity is very high, particularly when state-of -the-art background correction is applied. The capital costs and the operating costs are relatively low. FAAS provides the highest throughput, of about 0.5 minutes per sample (GFAAS ca. 4 min/sample), but FAAS sensitivity is quite low and GFAAS must be used in most cases.

[19] HORRATr:: The observed relative standard deviation calculated from results generated under repeatability conditions ($RSD_r$) divided by the $RSD_r$ value estimated from the Horwitz equation (using the assumption that the repeatability r= 0.66R) (reproducibility). The Horwitz equation is a generalised precision equation which has been found to be independent of analyte and matrix but solely dependent on concentration for most routine methods of analysis.

[20] $HORRAT_R$: The observed relative standard deviation calculated from results generated under reproducibility conditions ($RSD_R$) divided by the $RSD_R$ value calculated from the Horwitz equation.

Inductively coupled plasma atomic emission spectrometry mass spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS) are multielement techniques (up to 75 elements). LODs for lead in analysis solution are in the range of 0.5 and 0.05 µg/L and lower for ICP-AES and ICP-MS, respectively. ICP-AES is based on atomic emission after high atomic excitation using an argon-plasma at about 7000K. The interferences between the emission lines are minimised by use of high-resolution dispersion optics and deconvolution software. Nevertheless, the specificity is less than for the AAS techniques. ICP-MS makes use of high temperature argon-plasma induced ions, which are separated by mass filters such as quadrupole or magnetic sector field. Plasma-generated molecules might interfere with the mass of the analyte, but for lead analysis this is of minor importance. Both techniques provide a very high linear range of about $10^5$ to $10^6$ and a throughput of about three minutes per sample. Due to the high argon gas consumption, the operating costs are high.

In contrast to the methods mentioned above, XRFS does not need digestion and the samples can be measured directly after homogenisation. This technique makes use of the characteristic fluorescence X-rays of the sample material emitted after ionisation by the radiation of an X-ray-tube. Due to the relatively low yield of fluorescence X-rays, XRFS (compared to the other above mentioned methods) is less sensitive with a LOD of around 10 mg/kg. Thus, it is not feasible to apply XRFS to check for compliance of food samples with maximum levels, although this can be used as a fast screening tool for several feed materials, such as premixtures and additives belonging to the Functional Group of Compounds of Trace Elements which have much higher maximum levels for lead. The XRFS is a multielement technique and hence fluorescence lines of lead might interfere with lines of other elements of the sample material or characteristic lines of the X-ray-tube material, but for organic material, this is a minor problem. The throughput is about five minutes per sample. The operating costs are very low.

## 4. Sources, use and environmental fate

### 4.1. Air

Lead enters into the atmosphere mainly from anthropogenic sources, such as metal production, manufacturing industries, electricity and heat production (European Monitoring Environmental Pollution (EMEP/CCC, 2006). Lead levels in ambient air range from 7.6 x $10^5$ µg/$m^3$ in remote areas such as Antarctica, to >10 µg/$m^3$ near stationary sources such as smelters (U.S. ATSDR, 2007).

Long-term measurements of background lead concentrations have demonstrated significant reduction in lead levels in the environment after the phase-out of leaded petrol in many countries (EMEP/CCC, 2006). According to EMEP data (which should be considered as background values for Europe), lead concentrations in air, averaged over a number of EMEP monitoring stations during the period 1990-2003, decreased from about 0.020 to about 0.005 µg/$m^3$ (EMEP, 2005). The extent of these changes varied considerably across Central and North-Western Europe. The lead concentration in air for Germany, UK, Norway and the Czech Republic ranged from 0.003 to 0.033 µg/$m^3$ in 1990, and from 0.003 to 0.010 µg/$m^3$ in 2003 (UNEP, 2008). The latter values were the ones used in the estimation of exposure presented in Table 29.

The annual average lead level in air should not exceed 0.5 µg/$m^3$, which is the guideline value set by WHO (WHO, 2000a). Typical values of atmospheric lead concentration in different areas are illustrated in Table 3.

Atmospheric lead concentrations vary widely, but usually decrease with increasing vertical and horizontal distance from emission sources. They are generally 0.3 to 0.8 times lower indoors than outdoors (U.S. ATSDR, 2007).

The addition of tetraethyl lead to fuels in the 1950s resulted in changes in the emission of lead to the atmosphere. Lead emissions increased until the 1970s, when several regulations on the permissible lead content of petrol were adopted. These changes in legislation led to decreases in lead emissions throughout Europe. Total European atmospheric lead emission has continued to decrease substantially over the past four decades, because lead emissions from other sources have seen comparable, if not more dramatic, reductions, as a result of various economic, technological and process changes. These were a result mainly of the economic transition in Eastern European countries, in addition to the installation of efficient devices to control emissions (von Storch et al., 2003, Pacyna et al., 2009).

Pyrometallurgical processes in the primary non-ferrous metal industries are also an important source of atmospheric lead (Pacyna and Pacyna, 2001). In the atmosphere, lead exists primarily in the form of $PbSO_4$ and $PbCO_3$ (U.S. ATSDR, 2007), but the chemical forms of lead emitted into the atmosphere depend on the source. Lead released from coal combustion enters the atmosphere as $PbCl_2$, PbO, PbS and insoluble mineral particles (Wadge and Hutton, 1987), whereas that produced by oil combustion is mainly in the form of PbO (Kabata-Pendias and Mukherjee, 2007).

The size of a lead particle depends on its source. For example, the particle size of lead from an automobile exhaust varies between 0.1 and 1.0 μm. In the atmosphere, lead particles/aerosols are transformed by chemical and physical processes and are ultimately removed from the atmosphere by dry and wet deposition in terrestrial or aquatic ecosystems (Mishra et al., 2004).

### 4.2. Water

Worldwide reports confirm that there are great variations in lead concentrations in seawaters (Kabata-Pendias and Mukherjee, 2007). The median lead concentration has been reported to be 0.03 μg/L in worldwide ocean waters (see Table 3), and 0.003 μg/L in the North Pacific Ocean. The average concentration of lead in worldwide river waters has been reported to be 0.08 μg/L (Millot et al., 2004). Mean values for lead concentration in waters for the period 1999-2001 from nine European countries ranged from 0.02 to 14 μg/L. Between the years 1990 and 2002, sites in the Czech Republic showed a clear decline in lead (from 1.6 μg/L in 1990 to 0.2 μg/L in 2002), due to a decrease in deposition, while sites in Germany and Latvia showed no clear trend. The values for German sites during these years were in the range from <1 to 2 μg/L with some instances of higher concentrations, whilst lead concentrations for Latvian sites varied from <0.1 to 0.4 μg/L (Skjelkvale and Traaen, 2003). The input of lead to the Baltic Sea has been reduced considerably over the past 20 years. A significant proportion of lead enters the Baltic Sea via rivers or as direct discharges (HELCOM, 2007). In the aquatic environment, lead can occur in ionic form (highly mobile and bio-available), in organic complexes with dissolved humic materials (binding is rather strong and limits availability) (U.S. ATSDR, 2007), attached to colloidal particles such as iron oxide (strongly bound and less mobile when available in this form than as free ions), or attached to solid particles of clay or dead remains of organisms (very limited mobility and availability) (OECD, 1993). The speciation of lead in the aquatic environment is controlled by many factors, such as pH, salinity, sorption and biotransformation processes. Additional factors, such as pollution sources, sediment lead content, temperature, and type and amount of organic matter also have a significant impact on the lead status in waters (Kabata-Pendias and Mukherjee, 2007).

Lead is typically present in acidic aquatic environments as $PbSO_4$, $PbCl_2$, ionic lead and cationic forms of lead hydroxide (U.S. ATSDR 2007). In water, tetraalkyl-lead compounds, such as tetraethyl-lead (which is anthropogenic) and tetramethyl-lead (which may be anthropogenic or may be formed by biological methylation), undergo photolysis and volatilisation. Degradation proceeds from trialkyl species to dialkyl species, and eventually to inorganic lead oxides (Hill, 2005; UNEP, 2008).

The speciation of lead differs in fresh water and seawater. In fresh water, lead may partially exist as the divalent cation ($Pb^{2+}$) at pHs below 7.5, but it complexes with dissolved carbonate to form

insoluble $PbCO_3$ under alkaline conditions. Lead chloride and lead carbonate are the primary complexes formed in seawater (UNEP, 2008). The speciation of lead in water is also dependent on the presence of other ligands.

Some of the lead is removed to bottom sediment, which is the long-term sink for the metal (OECD, 1993). The average background content in bottom sediments has been reported to be around 30 to 45 mg/kg. However, in polluted rivers (especially by industrial and mine discharges), lead contents (in bottom sediments) ranged from 700 to 2,600 mg/kg (Kabata-Pendias and Pendias, 1999). Adsorption decreases with water hardness, and at higher pH, lead precipitates as $Pb(OH)^+$ and $PbHCO_3^+$ into bed sediments. Conversely, at low pH, lead is negatively sorbed (repelled from the adsorbent surface) (UNEP, 2008).

Of the known aquatic releases of lead, the largest ones are from the steel and iron industries and from lead production and processing operations. Urban runoff and atmospheric deposition are significant indirect sources of lead found in the aquatic environment. The direct releases to aquatic environments are considered to be relatively small compared to the releases to the atmosphere and land. Other major sources are domestic wastewater, non-ferrous metal smelting and refining, metal manufacturing processes and dumping of sewage sludge.

The toxicity of lead salts to aquatic organisms is strongly dependent on environmental conditions such as water hardness, pH and salinity. Lead is unlikely to affect aquatic plants at levels that might be found in the general environment (UNEP, 2008).

### 4.3. Soil

The concentration of lead in the top layer of soils varies considerably because of the deposition and accumulation of atmospheric particulates from anthropogenic sources (U.S. ATSDR, 2007). In Europe, lead concentrations in top soils are geographically heterogeneous and vary from below 10 mg/kg up to >70 mg/kg. The median value was estimated by WHO (2007) to be 23 mg/kg (see Table 3). The lead content in uncontaminated top soils of remote areas is generally within the range of 10 to 30 mg Pb/kg.

Investigations carried out in a former mining area of Northern France found a wide range of lead concentrations, ranging from 20 to 3,711 mg/kg (Pruvot et al., 2006).

Concentrations of heavy metals in 50 soil samples (0 to 20 cm) taken from a 10 x 5 km grid downwind of the smelter near Plovdiv, Bulgaria, were 46 to 4,196 mg Pb/kg (Bacon and Dinev, 2005). Lead concentrations in soil beside roadways and in towns are reported to be up to several thousands mg Pb/kg, whereas soils adjacent to smelters and battery factories are reported at up to 60,000 mg Pb/kg (UNEP, 2008). Very high concentrations of lead were also reported for surface soil (0 to 10 cm) in some areas of Upper Silesia, Poland. The range was 64 to 1,540 mg/kg; geometric mean: 293 mg/kg (Kulka and Gzyl, 2008).

Among various natural and anthropogenic sources of lead contamination, the impact of industrial emissions and previously used leaded petrol are considered to have the greatest environmental impact (Kabata-Pendias and Mukherjee, 2007). Metal mining, coal mining and electrical utilities are the industrial sectors that most heavily contribute to releases to land (U.S. ATSDR, 2007).

In general, lead is not very mobile in soil. The downward movement of inorganic lead and lead salts from soil to groundwater, by leaching, is very slow under most natural conditions (U.S. ATSDR, 2007). Soil pH, content of humic acids and the amount of organic matter influence the content and mobility of lead in soils (UNEP, 2008). There is a strong association between lead hydroxide and other hydroxides, especially of Fe and Mn (Kabata-Pendias and Mukherjee, 2007). Furthermore, more acidic conditions (lower pH) increase the solubility of lead. Lead is strongly adsorbed to organic

matter in soil. Lead adsorbed in a soil matrix may enter surface waters as a result of erosion of lead-containing soil particles (U.S. ATSDR, 2007).

The lead content of plants is largely the result of atmospheric deposition. Some plants are capable of taking up lead from soil through their root systems, although this uptake does not appear to be appreciable (U.S. ATSDR, 2007). Only about 0.005 to 0.13 % of lead in the soil solution is available to plants. The absorption of lead by root is passive, so the uptake rate is rather low. Levels of lead in leaves often correlate with the atmospheric concentrations (Kabata-Pendias and Mukherjee, 2007). Elevated lead contents were recorded in various plants in contaminated areas. In the vicinity of natural ore deposits of lead and processing or recycling factories, much higher concentrations of lead were observed even in roots of vegetables (up to 10.7 mg/kg dry mass), while the lead concentrations in soil were in the range of 129 to 1,996 mg/kg dry mass (Gzyl, 1995).

**Table 3:** Recent Pb concentrations in air, water and soil.

| **Air** | **Pb concentration** |
|---|---|
| European countries (outside industrial areas) | 0.003 to 0.010 µg/m$^3$ |
| European countries (proximity to industrial areas) | 0.03 to 0.10 µg/m$^3$ |
| Highly contaminated areas | >10 µg/m$^3$ |
| Water | |
| Seawater | |
| median content in worldwide ocean waters | 0.03 µg/L |
| in the North Pacific Ocean | 0.003 µg/L |
| The Baltic Sea waters | 0.005 - 1.33 µg/L |
| Black Sea Rivers | 0.014 – 1.46 µg/L |
| for 9 European countries | 0.02 - 14 µg/L |
| Soil | |
| Current levels | <10 to >70 mg/kg (median 23 mg/kg) |
| Vicinity of smelters, etc | up to 60,000 mg/kg |
| Surface soil in cont. areas | from 100 to >1,000 mg/kg |

### 4.4. Other sources of human exposure to lead

People may be exposed to lead and chemicals that contain lead via air, drinking water and from eating foods or swallowing dust or dirt that contains lead. Exposure is higher in those living near hazardous waste sites. Drinking water in houses containing lead pipes may contain elevated levels of lead, especially if the water is acidic or "soft" (U.S. ATSDR, 2007). Ingestion of contaminated soil, dust and old lead-based paint, as a result of hand-to-mouth activities, is an important source of lead intake in infants and young children. People of all ages living in areas where houses have been painted with lead paint may be exposed to elevated levels of lead in dust and soil.

Lead can be used as an ingredient in colours and paints which are used for example in toys and these may serve as an additional source of exposure for children. Children's exposure may be a consequence not only of licking and mouthing of toys but also of swallowing small toys or parts of toys. Based on the new migration limits for lead from three different toy materials set in the Directive 2009/48/EC, the German Federal Institute for Rsk Assessment (BfR) calculated a maximal daily intake of 1.3 µg lead per individual toy. If a child is exposed to three toys, the potential oral intake of lead would be 3.9 µg. In this case, assuming a body weight of 7.5 kg, lead exposure from toys would amount to 0.5 µg/kg b.w. per day (BfR, 2009).

It has been estimated that an average child may ingest up to 100 mg of soil/day (WHO, 2007). If the soil contains 100 mg/kg of lead (conservative upper bound), an average child may be exposed to as much as 10 µg of lead/day from this source alone, and, at the median value in Europe of 23 mg/kg, the

exposure would be up to 2.3 µg of lead per day. These were the values used in the estimation of exposure presented in Table 29.

People who live near busy highways or on old orchard land, where lead arsenate pesticides were used in the past, may still be exposed to elevated levels of lead. People may also be exposed to lead through work related activities (e.g. roofers or electronic salvage) or have hobbies in which lead is used, such as making stained glass, fishing weights or toy soldiers, or casting. The relative importance of this exposure is difficult to determine but it could be appreciable in some individuals.

It has been shown that the tobacco plant is able to take up lead from contaminated soils and concentrate it in its harvestable part (Rodríguez-Ortíz et al., 2006). Tobacco could also contain lead deposited on the surface of the leaves. The former use of lead arsenate as a pesticide on tobacco crops contaminated tobacco products with lead as well as arsenic. However, lead arsenate was gradually replaced by organic pesticides, and by the mid 1970s, no domestic production or use was reported in the U.S. (U.S. EPA, 1977). Residual lead in the soil from past applications and from atmospheric deposition remains as a potential source of lead in tobacco. The content of lead in Canadian tobacco decreased from 3.68 µg/g in 1968-1971 to 1.92 µg/g in 1985-1988 (Rickert and Kaiserman, 1994).

In 1977, WHO reported lead to be present in tobacco at concentrations of approximately 2.5 to 12.2 µg/cigarette, of which approximately 2 to 6 % could be inhaled by the smoker. Thus, thirty years ago, lead exposure from 20 cigarettes per day could have amounted to 1 to 15 µg per day. Lead levels in tobacco have since decreased, due to decreasing air lead levels, and current lead content of cigarettes from the UK and the U.S. ranges from 0.4 to 0.9 µg/cigarette (Kazi et al., 2009). This is also reflected in recent U.S. measurements of mainstream cigarette smoke, which have shown that lead exposure from U.S. cigarettes varies between 0.01 and 0.05 µg lead per cigarette smoked (Morton and Laffoon, 2008). Thus, exposure from 20 cigarettes per day has decreased correspondingly to approximately 0.2 to 1.0 µg lead/person per day. These were the values used in the estimation of exposure presented in Table 29.

Recently, Celik et al. (2007) published data on B-Pb levels in smoking (15 cigarettes a day, 13 years) and non-smoking adults. Mean B-Pb levels in non-smokers were 35±1.6 µg/L and in smokers 38±2.4 µg/L. The difference was not statistically significant.

In earlier studies, smokers were reported to show higher B-Pb levels than non-smokers (Bonanno et al., 2001). Using data from the National Human Exposure Assessment Survey (NHEXAS) EPA Region V study collected in 1995-1997, mean B-Pb levels in smokers, non-smokers exposed to environmental tobacco smoke (ETS) and non-smokers without ETS exposure, were 28.5, 20.6 and 18.1 µg/L, respectively (Bonanno et al., 2001). Individuals, including children, may be exposed to lead through the inhalation of ETS. Mannino et al. (2003) employed data from NHANES[21] III (1988-1994) and analysed lead in the blood of children aged 4 to 16 years who were exposed to different levels of ETS. The LOD for lead in this study was 10 µg/L. Serum levels of the nicotine biomarker cotinine were used to classify the children into one of three ETS exposure categories. The geometric mean B-Pb levels were 15, 19 and 26 µg/L for children with low (≤0.050 to 0.104 µg/L), intermediate (0.105 to 0.562 µg/L) and high (0.563 to 14.9 µg/L) serum cotinine levels, respectively. Therefore, Mannino et al. (2003) concluded that ETS could add some 11 µg/L or 75 % to the levels of lead in children's blood. In a recent study by Richter et al. (2009), based on data collected up until 2004, urinary lead excretion was recorded in different age groups with no, low or high exposure to ETS and, in those above 12 years of age, divided into smokers or non-smokers. Based on geometric mean levels of lead in urine adjusted to creatinine, the additional contribution from ETS in the age group 6 to 12 years was around 0.32 µg lead/g creatinine. The levels of this group therefore correspond to 138 %

[21] The National Health and Nutrition Examination Survey (NHANES) is a program of studies designed to assess the health and nutritional status of adults and children in the United States.

of the levels found in the non-ETS exposed group. The corresponding figures for the age groups above 12 years are 0.05 to 0.11 µg/g creatinine, resulting in levels that were around 112 % of those found in the non-ETS exposed groups. The geometric mean adjusted concentration for the age group 6 to 12 years with high exposure to ETS was higher than the concentration found in any other group, including smokers. Thus it was concluded, also on the basis of urinary excretion, that ETS is an important source of exposure to lead in children and young people.

However, the link between ETS and B-Pb levels may be much more complex than suggested by the above studies. A critical analysis of the results published by Mannino et al. (2003) shows that both cotinine and B-Pb levels were strongly related to the poverty index and race or ethnicity. The highest B-Pb levels (52 µg/L and 48 µg/L) occurred in the high cotinine group of inhabitants of houses built before 1946 and in black, non-Hispanic Americans, respectively. In the high cotinine group, the percentage of children with B-Pb >100 µg/L was 4.5 among white non-Hispanics, 16.6 in black non-Hispanics, 17.9 in inhabitants of houses built before 1946 and about 4 in inhabitants living in houses built after 1946. This suggests that poor parents may smoke more cigarettes than wealthier ones, and that in addition, the children of the former were exposed to lead containing dust from their old houses. The authors of the study did not include any information on the concentration of lead in soil. Furthermore, recent measurements of the concentrations of lead in smokers' rooms have found mean lead indoor combined particulate matter (PM) PM2.5 and PM10 values ranging in the EU between 0.03 and 0.13 µg/m$^3$, at least 50 % of which appears to come from smoking (Gemenetzis et al., 2006; Slezakova et al., 2009). These were the values used in the estimation of exposure presented in Table 29. Assuming that children breathe 5 m$^3$ of air daily, a child could potentially be exposed to up to 0.65 µg lead/day from this source. The upper lead concentration in the air of 0.13 µg/m$^3$ multiplied by the air: B-Pb coefficient of 19.2 (WHO/IPCS, 1995) indicates a possible worst case increase in B-Pb of approximately 2.5 µg/L from ETS. The CONTAM Panel recognises that this estimate is likely to be very conservative.

Airborne lead may contribute significantly to occupational exposure and inhalation has been found to be a dominant pathway for lead exposure of workers in industries producing, refining, using or disposing of lead and lead compounds (UNEP, 2008). Despite improvements achieved in workplace environments during the last decades, occupational exposure remains a greater source of exposure than environmental sources.

Herbal preparations used in traditional Asian medicine in China and Tibet, and in Indian traditional systems of medicine such as Ayurveda, may contain significant amounts of lead and other heavy metals. The heavy metals in these preparations have been deliberately incorporated to "improve" their "therapeutic effect" and are not a result of unintentional contamination. There are reports from many parts of the world of intoxications caused by such preparations (Ernst 2002; Tait et al., 2002; CDC, 2004). Martena et al. (2010) monitored lead in 292 traditional Asian herbal preparations on the Dutch market and found that 123 contained lead at levels above the LOQ (0.36 mg/kg). One hundred and five of these samples originated from the Indian Ayurveda traditional medicine system, whereas 13 were of Chinese and 5 of Tibetan origin. The calculated human exposure to lead from these preparations ranged from 0.03 to 192 mg/week. Assuming a body weight of 60 kg, this corresponds to an additional lead exposure of 0.07 to 457 µg/kg b.w. per day. In some cases, intake of traditional Asian herbal preparations, mainly of Ayurveda origin, could lead to exposures well above those from other known sources.

Cases of lead poisoning have been related to less common sources of exposure. Illicitly distilled alcohol made in stills composed of lead-soldered parts may contain high levels of lead. Detectable levels of lead with a maximum concentration of 5.3 mg/L were found in 7 of 12 samples of "moonshine" whiskey in Georgia, USA (Gerhardt et al., 1980). Of 115 suspected moonshine samples collected between 1995 and 2001, 33 samples contained lead concentrations above 3 mg/L. Exposure is also possible from, for example acidic drinks such as fruit juice, when kept in ceramics or crystalware containing lead. Indeed, ceramic tableware can serve as an important source of lead

(Centers for Disease Prevention & Epidemiology, 1998; BfR, 2004), and due to the large quantities of lead that can migrate from some glazes, lead intoxication may occur following such exposure. BfR has received 10 reports over a period of 10 years of lead intoxication from exposure due to consuming liquids (e.g. fruit juice, tea) stored in such containers, with reported B-Pb levels ranging from 76 to 500 µg/L. BfR has estimated that concentrations of up 4 mg/L were achieved by the migration of lead from ceramics. Drinking one litre per day of a liquid from such containers would lead to a lead intake of 66.7 µg/kg per day in an adult (assuming a b.w. of 60 kg) and, assuming a consumption of 0.5 litre per day, of 133 µg/kg per day in a 15-kg child.

It has also been shown that use of lead ammunition can result in exposure to lead from dust generated during gun or rifle discharge, at levels up to 1 mg/m$^3$ (U.S. EPA, 1985). Airborne lead dust from firearm discharge in indoor shooting ranges can result in increases in B-Pb concentrations by 1.5 to 2 fold (Greenberg and Hamilton, 1999; Gulson et al., 2002). Individuals who use recreational shooting ranges may be exposed to lead and soluble lead compounds in soil. Recreational shooting may also represent a threat from lead to groundwater and surface water (Murray et al., 1997). Lead pellets ingested by, or imbedded in, animals that are used as food sources may also contribute to human exposure, as described in 5.8.

Hair dyes and some cosmetics used to contain lead compounds (Cohen and Roe, 1991). Measured lead concentrations of 2.3 to 6 g of lead/kg of product have been reported in some hair dyes (Mielke et al., 1997). Lead acetate is soluble in water and easily transferred to hands and other surfaces during and following application of a hair dye product. However, the use of lead in cosmetic products is now banned in the EU (SCCNFP, 2004) and the recommendation is for the lowest technically achievable concentrations to be used in the manufacture of cosmetics. BfR recently concluded that the exposure of consumers to lead from cosmetic products manufactured in the EU is negligible (BfR, 2006). However, it is always possible that individuals will obtain lead-containing cosmetics or hair dyes in some other part of the world, where lead is permitted in their manufacture. In such cases, exposure to lead may be significant.

### 4.5. Transfer and bioaccumulation in the environment

Lead is present in the environment and can, for a number of different reasons, be mobilised in ecosystems to levels that could cause effects to terrestrial and aquatic species. In the atmosphere, lead compounds exist primarily in the particulate form (U.S. ATSDR, 2007). In the aquatic environment, lead can occur in ionic form, in organic complexes with dissolved humic materials, attached to colloidal particles such as iron oxide, or attached to solid particles of clay or dead remains of organisms (OECD, 1993). In general, lead decreases in concentration from rainwater (generally acidic (pH<5.5)) to fresh water (generally neutral (pH=7)) to seawater (alkaline (pH>8.2)) (OSPAR, 2009). In soil, lead is retained complexed to organic material or adsorbed to hydrous oxides near the soil surface (OECD, 1993).

Over time, elemental lead in the environment can be dissolved, e.g., as lead oxides, and therefore becomes available, but the extent and rate at which this transformation occurs are not known in detail (UNEP, 2008).

Many metals are converted to organic forms by microorganisms in soil. The biotransformation of inorganic lead to tetramethyl lead (TML) has been observed in aquatic systems, particularly in sediments. However, this is unlikely to have a significant effect on availability of either ionic lead or alkyl lead, since most of the lead in undisturbed sediments is in the form of lead sulphide which is not bioavailable (Schmidt and Huber, 1976). Organolead compounds, such as trialkyl-lead and tetraalkyl-lead compounds, are more toxic than inorganic forms of lead (UNEP, 2008).

Plants and animals may bioconcentrate lead, but lead is not biomagnified in the aquatic or terrestrial food chain (U.S. ATSDR, 2007). This is partly explained by the fact that in vertebrates, lead is stored

mainly in bone, which reduces the risk of lead transmission to other organisms in the food chain (Tukker et al., 2001).

The distribution of lead within animals is often associated with their calcium turn-over. In shellfish, lead concentrations are higher in the calcium-rich shell than in the soft tissue. Lead uptake by fish reaches equilibrium only after a number of weeks of exposure. Lead is accumulated mostly in gill, liver, kidney and bone (WHO/IPCS, 1989).

## 5. Occurrence in food

### 5.1. Previously reported lead occurrence results

Lead is commonly present in food and is regulated as a contaminant. Over the past decades, lead concentrations have decreased significantly due to the phase-out of leaded petrol, other actions, and the concomitant significant decrease in lead air pollution. As an example, the content of cadmium, lead, nickel, mercury and selenium in 83 foods was monitored from 1993 to 1997 in Denmark and compared with similar testing in 1988 to 1992. A general decrease in lead concentrations had occurred, whereas the contents of cadmium, nickel, mercury and selenium were stable or declined only slightly (Larsen et al., 2002).

A decrease in the concentration of lead was also noted in the United Kingdom (Food Standards Agency, 2009). The offal group had the highest mean lead concentration (0.065 mg/kg), while beverages (17 %), bread (16 %) and other vegetables (16 %) contributed the most to the population dietary exposure. Also in 2006, 310 food samples were analysed for lead in a study commissioned by the Food Standards Agency (Food Standards Agency, 2007). The highest level recorded was in game meat at 1.63 mg/kg.

In Poland, vegetables, cereal products, and meat and meat products contributed most to lead dietary exposure, representing close to 90 % of the total (Marzec and Schlegel-Zawadzka, 2004). In contrast, in Finland most lead exposure was from beverages and dairy products, 25 and 15 %, respectively (Tahvonen, 1997). Similarly, in the Netherlands, most lead in the diet came from beverages, followed by cereals and fresh fruits (Brussaard et al., 1996). In Greece (Tsoumbaris and Tsoukali-Papadopoulou, 1994) and Spain (Moreiras and Cuadrado, 1992) the main sources of lead in the diet were vegetables and fruit. In the 1st French total diet survey, in 998 composite samples of individual foods taken between 2000 and 2001, lead was found at an average level of 0.055 and 0.1 mg/kg in offal and shellfish, respectively (other food groups contained less than 0.04 mg/kg). The following food groups contributed most (5 to 11 %) to the exposure of populations: bread, rusk, soups, vegetables, fruits, drinking water, non-alcoholic beverages, alcoholic beverages and sugars and confectionery; other foods contributed less than 5 % of the total food exposure. So, six main categories (beverages; cereals and fine bakery wares; fruits and vegetables; salts, spices, soups and sauces; sweeteners, honeys and confectionery; meat and its products) contributed most to lead dietary exposure, contributing close to 83 % of the total (Leblanc et al., 2005).

In total diet surveys carried out in the U.S. between 1991 and 2005, 382 different product types were analysed for lead. The overall mean for the tested products was 0.003 mg/kg, varying between not detected and 0.033 mg/kg. High mean results were recorded for sweet cucumber pickles at 0.033 mg/kg, milk chocolate candy bar and beef liver 0.024 mg/kg, canned fruit cocktail and chocolate syrup 0.018 mg/kg, dry table wines 0.017 mg/kg, sweet canned potatoes 0.015 mg/kg and canned apricots 0.014 mg/kg. The maximum recorded was 0.210 mg/kg in a shrimp sample.

In a SCOOP study (SCOOP, 2004), food samples were analysed for lead in 10 European countries. In dairy products the lead concentration varied in the different countries between not detected to a mean of 0.058 mg/kg, for fats and oils between 0.005 and 0.049 mg/kg, for fruit between 0.008 and

0.049 mg/kg, for vegetables between 0.004 and 0.226 mg/kg, for confectionary between 0.004 and 0.067 mg/kg, for cereal and cereal products from not detected to 0.269 mg/kg, for meat and meat products from not detected to 0.114 mg/kg, for offal between 0.005 to 0.180 mg/kg, for fish and fish products from not detected to 0.550 mg/kg, for egg and egg products from not detected to 0.210 mg/kg, for soft drinks between 0.001 and 0.024 mg/kg and for alcoholic beverages between 0.001 and 0.156 mg/kg. There were some high maximum concentrations reported with several food groups having occasional products above 1 mg/kg. The highest concentration reported was for herbs and spices at 379 mg/kg, followed by game at 188 mg/kg, dietetic foods at 34.8 mg/kg, food supplements at 18.0 mg/kg and wine at 16.8 mg/kg.

## 5.2. Call for data on current lead occurrence in food

Since the last EU-wide data collection on lead was undertaken in 2002 (SCOOP, 2004), it was decided that there should be a new data collection, covering the years 2003 to 2008. The DATEX-2008-0002 call for data on lead covering this period was issued by EFSA in April 2008 with a closing date of July 2008. EFSA received a total of 139,423 results from food testing of which 97.9 % represented 14 Member States and Norway and 2.1 % represented three commercial operators. There were several partly overlapping Spanish submissions and thus 310 duplicate sample results from Spain were removed from further analysis.

### 5.2.1. Summary of data collected

The source of the 139,113 results remaining after the first review are reported in Figure 1 distributed over 14 EU Member States, Norway and three commercial organisations.

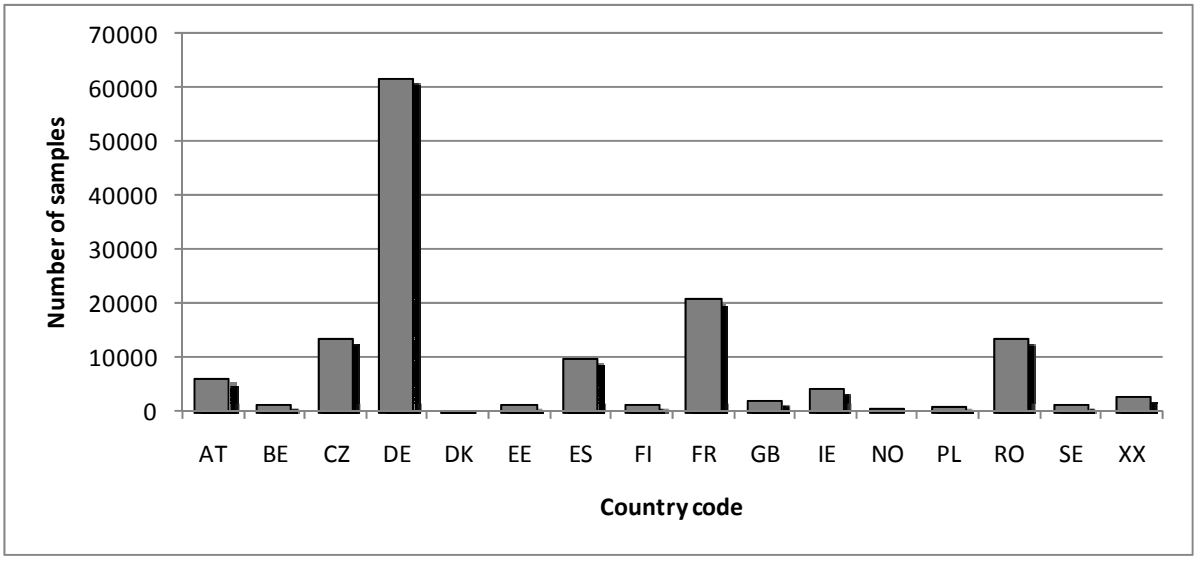



**Figure 1:** Distribution of samples across EU Member States, Norway and commercial organisations (referred to as "XX").

Germany was the major contributor providing 44 % of the data followed by France (15 %), the Czech Republic (9.7 %) and Romania (9.6 %).

The distribution of results over the years of sampling is illustrated in Figure 2.

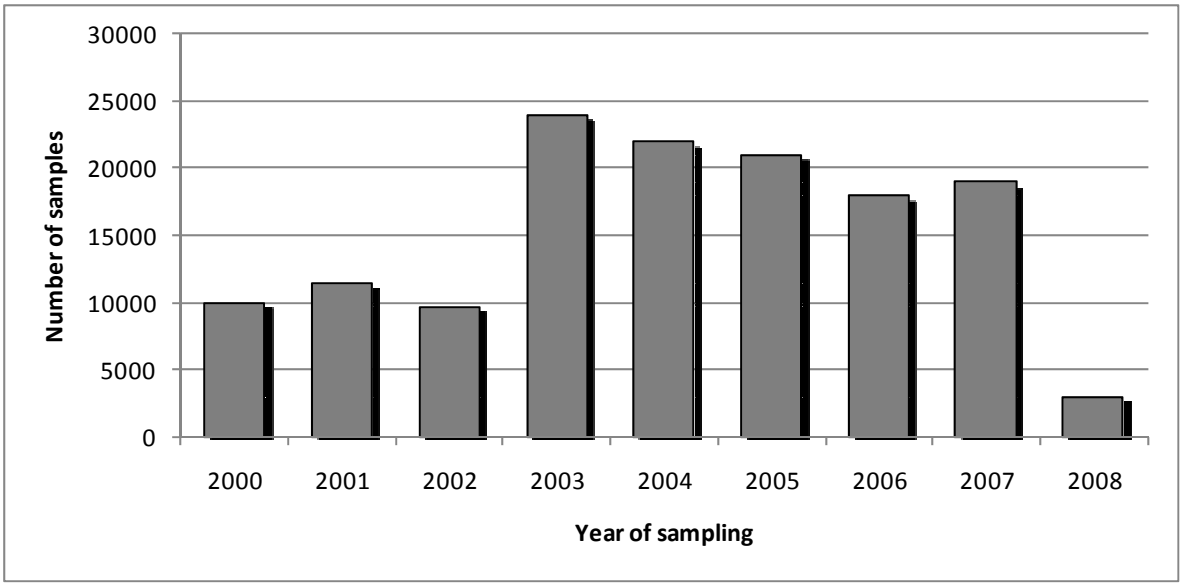



**Figure 2:** Distribution of samples over year of sampling (note that 2008 was not a complete year of sampling).

There were 122 results reported covering the period prior to year 2000 and 29,022 results covering the period of 2000 to 2002. Samples from the years prior to 2003 were excluded from further analysis, as were 15,843 samples identified during the data cleaning steps with incomplete or incorrect description of food type or value unit or insufficient sensitivity of the analytical method (an LOD of more than 0.1 mg/kg or an LOQ of more than 0.3 mg/kg). A total of 94,126 sample results were adequately described with sufficient detail to be included in the calculation of lead concentrations in the relevant food categories.

### 5.2.2. Distribution of samples across food categories

The food samples were classified using the aggregated food categories specified in the EFSA Concise European Food Consumption database (EFSA concise food categories). The distribution of samples across the aggregated food categories is shown in Figure 3.

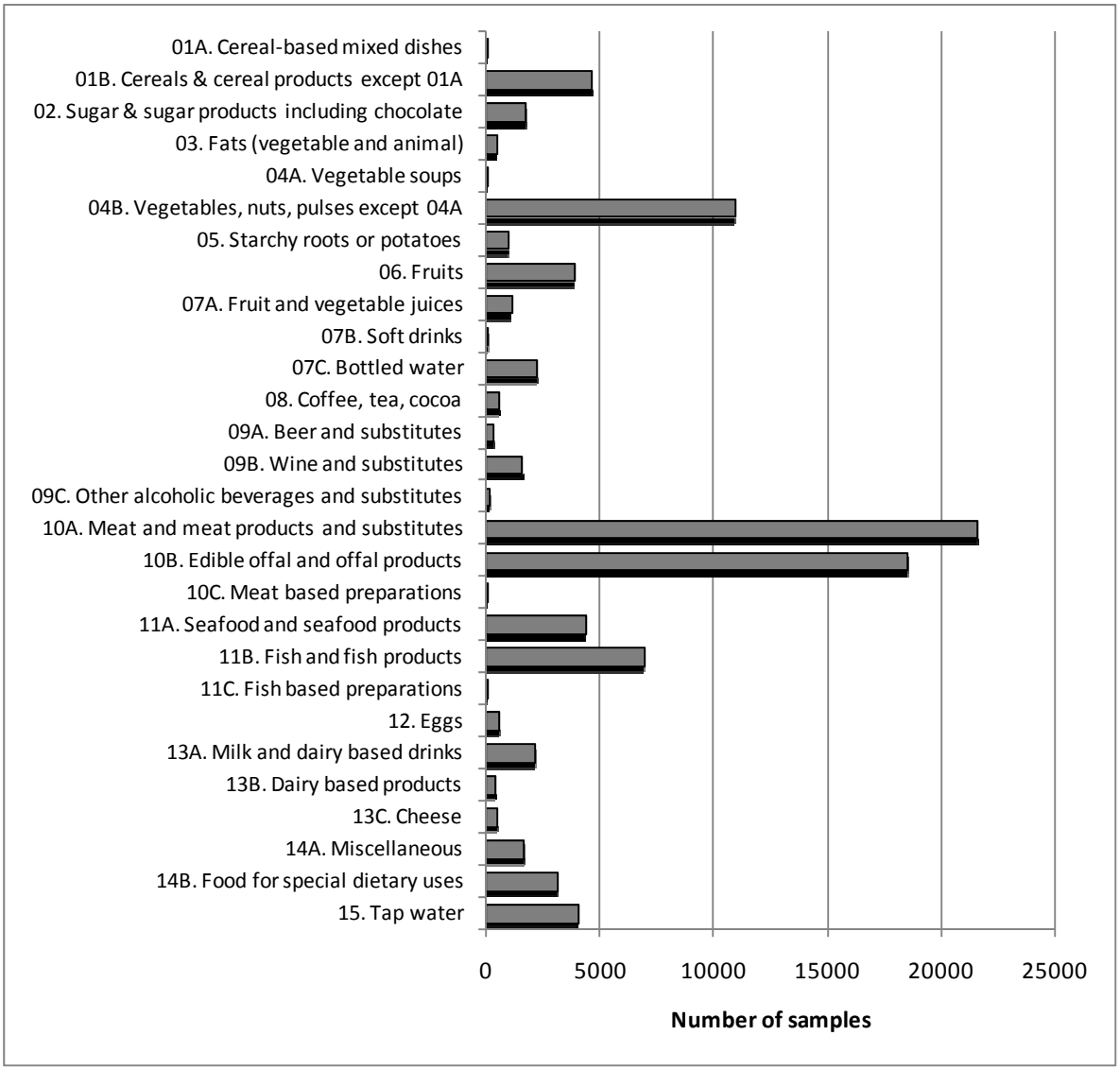



**Figure 3:** Distribution of samples in the EFSA concise food categories.

Meat and meat products and substitutes as well as edible offal and offal products dominated the food product coverage, representing 23 % and 20 % of the samples, respectively. They were followed by vegetables, nuts and pulses (12 %), fish and fish products (7 %) and cereals and cereal products (5 %). There were fewer than 200 samples submitted covering the food categories vegetable soups, cereal-based mixed dishes, fish-based preparations, meat-based preparations, other alcoholic beverages and substitutes and soft drinks. The lack of a representative number of samples in some food categories creates uncertainty for the overall result when calculating exposure.

### 5.3. Analytical methods used and limits of detection

The original results were reported in mg/kg (82.1 %), in µg/kg (15.2 %), in mg/L (1.7 %) and in µg/L (1.0 %). All the measures have been converted to mg/kg. For the measures expressed in relation to a volume unit, the approximate equivalence of 1 kg = 1 L has been used.

Several analytical methods were used to perform the analyses (Figure 4). The most commonly reported method was GFAAS, for 24.3 % of the samples, followed by ICP-MS for 9.8 %. However, it should be noted that for 49,450 (52.5 %) samples, no information was provided on the analytical method used, apart from detection and quantification limits. Since so many of the results lacked a description of the analytical method, it was not meaningful to cross-tabulate the food matrix results with the analytical method.

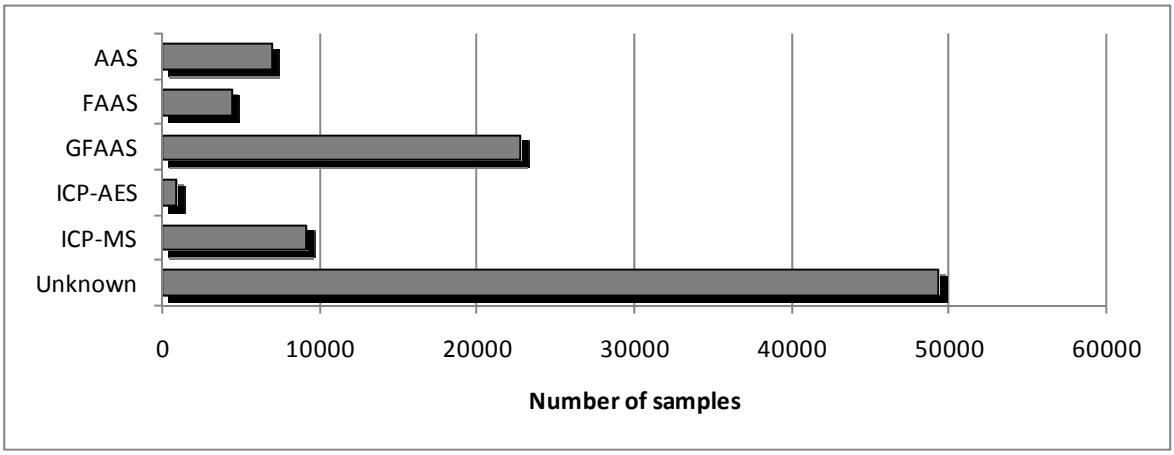



**Figure 4:** Distribution of analytical methods used. Abbreviations used are as follows: AAS - atomic absorption spectrometry; FAAS - furnace atomic absorption spectrometry; GFAAS - graphite furnace atomic absorption spectrometry; ICP-AES - inductively coupled plasma atomic emission spectrometry mass spectrometry; ICP-MS - inductively coupled plasma mass spectrometry; Unknown - method not reported.

The limits of detection (LOD) and quantification (LOQ) for the analyses varied with the analytical technique (Figure 5), the laboratory (not shown) and the food matrix (Figure 6).

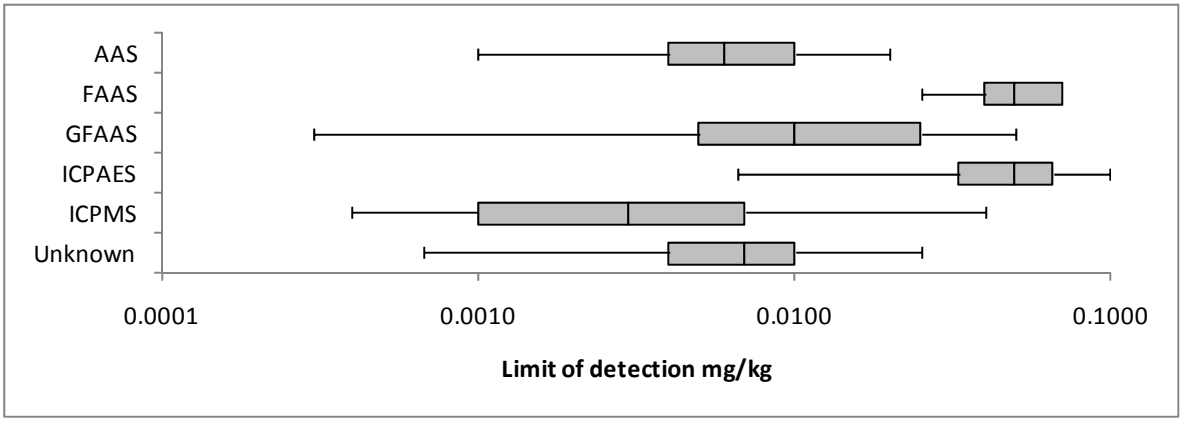



**Figure 5:** Box plot of the distribution of limit of reporting according to the analytical method used (box bounded by the 25$^{th}$ and 75$^{th}$ percentiles with median line and whiskers indicating 5$^{th}$ and 95$^{th}$ percentiles – note the logarithmic scale). For FAAS the 75$^{th}$ and the 95$^{th}$ percentiles were equal. Abbreviations used: AAS - atomic absorption spectrometry; FAAS - furnace atomic absorption spectrometry; GFAAS - graphite furnace atomic absorption spectrometry; ICP-AES - inductively coupled plasma atomic emission spectrometry mass spectrometry; ICP-MS - inductively coupled plasma mass spectrometry; Unknown - method not reported.

As expected, the highest sensitivity was achieved using ICP-MS, with a median LOD of 0.003 mg/kg followed by non-specified AAS and GFAAS with median LODs of 0.006 mg/kg and 0.01 mg/kg, respectively. The unknown group comprises a mix of the analytical methods available.

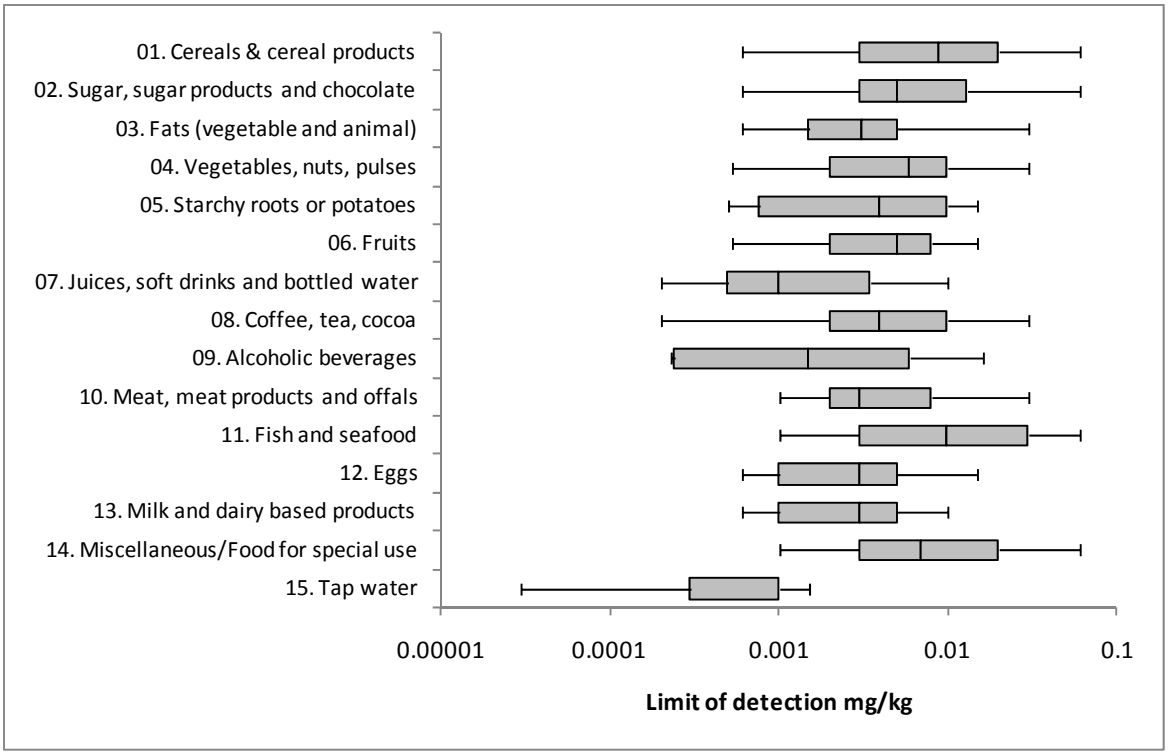



**Figure 6:** Box plot of the distribution of the limit of reporting according to the aggregated EFSA concise food categories (box bounded by the 25th and 75th percentiles with median line and whiskers indicating 5th and 95th percentiles – note the logarithmic scale). For tap water the 25th percentile and the median were equal.

Most of the aggregated food categories show a considerable spread in the limits reported. The highest reported sensitivity was for the liquid products (tap and bottled water, wine and other alcoholic beverages) with a median limit of reporting from 0.0003 to 0.0007 mg/kg compared to between 0.005 and 0.05 mg/kg for solid food. However, results reported are not only dependant on the food matrix but differences could also be due to the analytical method used and the testing laboratory. The sensitivity of the method is often set by the laboratory to fulfil legislative requirements and not fine-tuned to optimal sensitivity for reasons of cost and time. This is satisfactory for routine monitoring purposes, but may cause problems when results are used to calculate human exposure. This is particularly true for samples with levels below the limit of reporting when this is the LOQ and the LOQ is close to or even above the maximum levels specified in legislation. To reduce bias, a few such results were retained if they were associated with a number of results above the LOQ and the LOQ was not higher then 0.3 mg/kg. For submissions with a reported LOQ of more than 0.3 mg/kg, all associated results in the same food group were removed including those above the LOQ.

### 5.4. Occurrence data by food category

In total, the number of samples reported with quantified results was 37.4 %, varying from 11.4 % for eggs to 63.8 % for alcoholic beverages (Figure 7). When the number of samples with quantified results is below 40 %, the World Health Organisation Global Environment Monitoring System - Food Contamination Monitoring and Assessment Programme (GEMS/Food) recommends that the lower and

upper bounds be calculated, that is results below the detection or quantification limits as applied by the laboratories to be set at zero or the respective limits (WHO, 2003).

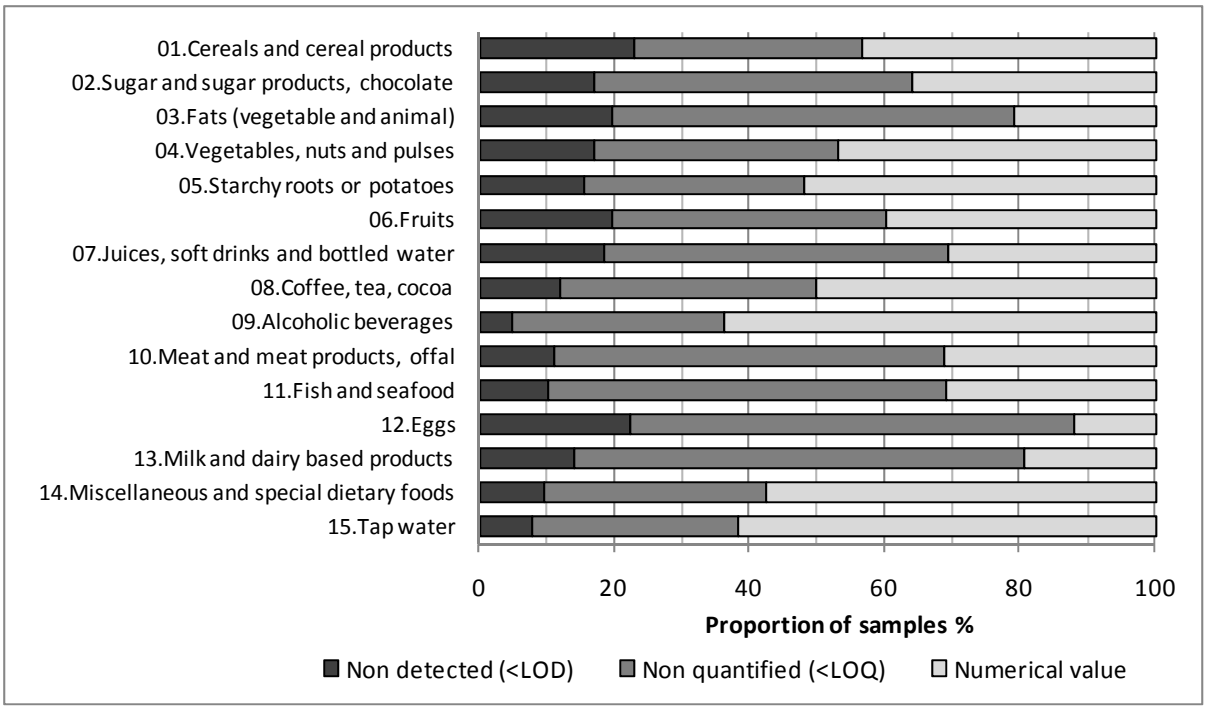



**Figure 7:** Proportion of samples with non-detected, non-quantifiable and quantified results in broad food categories as reported by the laboratories.

When scrutinising Figure 7, it should be noted that there is not always a clear distinction between the use of the LOD or the LOQ. Some laboratories always report anything other than quantifiable results as being less than LOQ even if they are below the LOD. There are also several options available for calculating the respective LOD or LOQ that can sometimes lead to overlapping results. This creates a problem when calculating exposure. However, the proportion of quantified results is clearly indicated in the figure.

Sampling adjustment factors (SAF) calculated from the German Nutrition Survey (Mensink et al., 2004) were applied when aggregating food sub-category averages to category averages in order to fit the information structure of the Concise European Food Consumption database (EFSA, 2008). The relative consumption of food sub-classes in the respective food sub-category was used to calculate a percentage for the respective SAF to correct for the unbalanced proportion of samples analysed in food subcategories in relation to their actual dietary contribution. In addition, a low arbitrary SAF was assigned to some rarely consumed food sub-categories not captured by the methodology used in the German survey.

The GEMS/Food Consumption Cluster Diet information (FAO/WHO, 2006) also provides SAFs. Those values were checked previously for some food groups against the calculated SAFs from the German survey and were found to be of a similar magnitude (EFSA, 2009). Nevertheless, the GEMS/Food database is based on the Codex Alimentarius standardised food classification system and therefore refers primarily to raw agricultural commodities. For this reason the data cannot be used to adjust means for all EFSA categories.

SAFs as reported in the respective occurrence tables (from Table 5 to Table 19), were applied at sub-class and sub-category level as described in detail in Table 4a and 4b, respectively.

An adjusted mean was calculated when a food sub-category comprised any sub-classes; in that case the SAF of each sub-class was corrected by the relative contribution of the subcategory to the overall food category (Table 4a). At food category level, SAFs, as reported in the occurrence tables (from Table 5 to Table 19), were applied to the adjusted or unadjusted means values of the food sub-categories to derive the overall adjusted mean of the food category to which they belonged.

**Table 4a:** An example of the use of sampling adjustment factors for deriving adjusted mean values (mg/kg) for food sub-categories.

| Food description | | N | Mean | SAF | Calculation | Adjusted Mean |
|---|---|---|---|---|---|---|
| **Sub-classes** | Fruit juices and nectar | 1,011 | 0.0250 | 14 % | (0.14/ 0.15) x 0.0250 | 0.0233+ |
| | Vegetable juices | 163 | 0.0177 | 1 % | (0.01/0.15) x 0.0177 | 0.0012+ |
| **Sub-category** | *Fruit & vegetable juices* | 1,174 | 0.0240 | 15 % | | 0.0245 |

N: number of samples; SAF: sampling adjustment factor.
Note that italics and the different grey colours refer to sub category/food category.

**Table 4b**: An example of the use of sampling adjustment factors for deriving adjusted mean values (mg/kg) for food categories.

| Food description | | N | Mean | SAF | Calculation | Adjusted Mean |
|---|---|---|---|---|---|---|
| **Sub-categories** | *Fruit & vegetable juices* | 1,174 | 0.0245[(a)] | 15 % | 0.15 x 0.0245 | 0.0037+ |
| | *Soft drinks* | 108 | 0.0403 | 15 % | 0.15 x 0.0403 | 0.0060+ |
| | *Bottled water* | 2,283 | 0.0018 | 70 % | 0.7 x 0.0018 | 0.0013= |
| **Food category** | *Total for Juices, soft drinks and bottled water* | 3,565 | | 100 % | | 0.0110 |

N: number of samples; SAF: sampling adjustment factor.
(a): Adjusted mean derived from food sub-group calculation (Table 4a).
Note that italics and the different grey colours refer to sub category/food category.

Tables 5 to 19 report the data for aggregated and detailed food categories (see Appendix A). Statistical descriptors include median, mean, and maximum concentrations as well as the 5$^{th}$ and 95$^{th}$ percentile concentrations (abbreviated as P5 and P95, respectively). N is the number of results reported and the column <LOD indicates the percentage of results below the LOD or the LOQ. The SAF was applied only when calculating adjusted aggregated category means in Table 20. The unadjusted means are shown in the respective tables with results for category totals.

Some occasional results at the very high end of the range, that were more than ten times higher than the next highest result, were considered as outliers and removed from the calculations as described below for each food category. An analysis of other high results did not show a uniform trend with any of the variables assessed. The results were spread across reporting countries and food groups. Since there can be occasional but genuine causes of high lead contamination, like lead pellets in game, lead equipment used in food manufacturing and lead containing dust in production facilities, these results were kept in the database. A specific analysis of these very high values follows after the general presentation.

The cereals and cereal products category comprises two major sub-categories, of which one is split into four sub-classes, with a total 4,774 analytical results reported (Table 5). Only a few results were reported covering the cereal-based mixed dishes category, typically including products like pizza and lasagne.

**Table 5:** Statistical description of concentrations of lead for food category "01. Cereal and cereal products" in mg/kg.

| Food category | N | <LOD | | P5 | Median | Mean | P95 | Max | SAF |
|---|---|---|---|---|---|---|---|---|---|
| *Cereal-based mixed dishes* | 70 | 67.1 % | LB | 0.0000 | 0.0000 | 0.0045 | 0.0179 | 0.0640 | *23 %* |
| | | | UB | 0.0052 | 0.0200 | 0.0283 | 0.1000 | 0.3000 | |
| Bran and germ | 292 | 64.0 % | LB | 0.0000 | 0.0000 | 0.0263 | 0.1400 | 0.8004 | 2 % |
| | | | UB | 0.0050 | 0.0370 | 0.0505 | 0.1400 | 0.8004 | |
| Cereal grains except rice | 2,336 | 54.1 % | LB | 0.0000 | 0.0000 | 0.0360 | 0.1100 | 7.120 | 22 % |
| | | | UB | 0.0050 | 0.0270 | 0.0510 | 0.1200 | 7.120 | |
| Cereal products | 1,464 | 53.2 % | LB | 0.0000 | 0.0000 | 0.0221 | 0.0870 | 0.8800 | 44 % |
| | | | UB | 0.0060 | 0.0250 | 0.0395 | 0.1038 | 0.8800 | |
| Rice | 612 | 69.6 % | LB | 0.0000 | 0.0000 | 0.0196 | 0.1040 | 0.5700 | 9 % |
| | | | UB | 0.0050 | 0.0250 | 0.0508 | 0.2000 | 0.5700 | |
| *Cereals/products excl. mixed dishes* | 4,704 | 67.1 % | LB | 0.0000 | 0.0000 | 0.0290 | 0.1041 | 7.120 | *77 %* |
| | | | UB | 0.0060 | 0.0270 | 0.0473 | 0.1250 | 7.120 | |
| *Total cereal and cereal products* | 4,774 | 56.6 % | LB | 0.0000 | 0.0000 | 0.0286 | 0.1033 | 7.120 | *100 %* |
| | | | UB | 0.0060 | 0.0267 | 0.0471 | 0.1250 | 7.120 | |

N: number of samples; LOD: limit of detection; P5: 5th percentile; P95: 95th percentile; Max: maximum level; SAF: sampling adjustment factor.

Lead levels in more than 50 % of samples in all sub-classes and sub-categories were below the LOD. The sub-classes 'bran and germ', 'cereal grains except rice' and 'rice' showed equally high mean upper bound lead levels. The highest lead level was recorded for a maize sample at 7.12 mg/kg.

The sugar and sugar products category comprises two sub-categories covering a total of 1,794 analytical results (Table 6).

**Table 6:** Statistical description of concentrations of lead for food category "02. Sugar and sugar products" in mg/kg.

| Food category | N | <LOD | | P5 | Median | Mean | P95 | Max | SAF |
|---|---|---|---|---|---|---|---|---|---|
| Chocolate and chocolate products | 315 | 25.1 % | LB | 0.0000 | 0.0530 | 0.0681 | 0.1730 | 1.240 | 33 % |
| | | | UB | 0.0110 | 0.0640 | 0.0828 | 0.2270 | 1.240 | |
| Other sugar and sugar products | 1,479 | 71.8 % | LB | 0.0000 | 0.0000 | 0.0266 | 0.1400 | 4.100 | 67 % |
| | | | UB | 0.0000 | 0.0270 | 0.0554 | 0.2000 | 4.100 | |
| *Total Sugar and sugar products* | 1,794 | 63.6 % | LB | 0.0000 | 0.0000 | 0.0339 | 0.1503 | 4.100 | 100 % |
| | | | UB | 0.0000 | 0.0370 | 0.0602 | 0.2000 | 4.100 | |

N: number of samples; LOD: limit of detection; P5: 5th percentile; P95: 95th percentile; Max: maximum level; SAF: sampling adjustment factor.

Only 25 % of the 'chocolate and chocolate product' results, but more than 70 % of 'other sugar and sugar product' results, had lead levels below the LOD. The highest result at 4.10 mg/kg was recorded for a banana-flavoured candy sample. One sample was excluded with a concentration of 54.1 mg/kg for a honey-flavoured liquorice sample because the value was more than ten times that of the next highest sample.

The fats category comprises three sub-categories covering a total of 518 analytical results (Table 7). Butter was separated from other animal fats and oils only to make clear that this sub-category should be reported under fats and not under dairy products, as requested for the Concise Database.

**Table 7:** Statistical description of concentrations of lead for food category "03. Fats (animal and vegetable)" in mg/kg.

| Food category | N | <LOD | | P5 | Median | Mean | P95 | Max | SAF |
|---|---|---|---|---|---|---|---|---|---|
| Animal fats and oils | 34 | 88.6 % | LB | - | 0.0000 | 0.0031 | - | 0.0720 | 23 % |
| | | | UB | - | 0.0125 | 0.0319 | - | 0.1000 | |
| Butter | 85 | 88.2 % | LB | 0.0000 | 0.0000 | 0.0102 | 0.0440 | 0.4910 | 22 % |
| | | | UB | 0.0088 | 0.0300 | 0.0372 | 0.0530 | 0.4910 | |
| Vegetable fats and oils | 399 | 73.2 % | LB | 0.0000 | 0.0000 | 0.0478 | 0.1600 | 7.300 | 55 % |
| | | | UB | 0.0060 | 0.0200 | 0.0718 | 0.1600 | 7.300 | |
| *Total Fats (animal and vegetable)* | 518 | 76.7 % | LB | 0.0000 | 0.0000 | 0.0387 | 0.1500 | 7.300 | 100 % |
| | | | UB | 0.0060 | 0.0200 | 0.0635 | 0.1500 | 7.300 | |

N: number of samples; LOD: limit of detection; P5: 5$^{th}$ percentile; P95: 95$^{th}$ percentile; Max: maximum level; SAF: sampling adjustment factor.

A majority of results were below the LOD for all sub-classes. Vegetable-based fats and oils had higher mean lead levels than the animal-based fats and oils. The highest recorded lead level was also reported in a sample of non-specified vegetable oil, with a concentration of 7.3 mg/kg. One sample, for fat tissue from a single game animal, was excluded as an outlier at 156 mg/kg since it might have included residue of lead shot.

The vegetable, nuts and pulses category was split into 2 major sub-categories and eleven sub-classes comprising a total of 11,011 analytical results (Table 8).

**Table 8:** Statistical description of concentrations of lead for food category "04. Vegetables, nuts and pulses" in mg/kg.

| Food category | N | <LOD | | P5 | Median | Mean | P95 | Max | SAF |
|---|---|---|---|---|---|---|---|---|---|
| *Vegetable soups* | 13 | 69.2 % | LB | - | 0.0000 | 0.0118 | - | 0.0500 | 1 % |
| | | | UB | - | 0.0200 | 0.0388 | - | 0.2000 | |
| Brassica vegetables | 1,084 | 58.3 % | LB | 0.0000 | 0.0000 | 0.0125 | 0.0500 | 0.7300 | 13 % |
| | | | UB | 0.0020 | 0.0150 | 0.0257 | 0.1000 | 0.7300 | |
| Dried vegetables | 37 | 27.0 % | LB | - | 0.1400 | 0.3797 | - | 6.400 | 2 % |
| | | | UB | - | 0.1400 | 0.3851 | - | 6.400 | |
| Fresh herbs | 461 | 32.1 % | LB | 0.0000 | 0.0300 | 0.1150 | 0.3700 | 8.500 | 1 % |
| | | | UB | 0.0020 | 0.0380 | 0.1249 | 0.3700 | 8.500 | |
| Fungi | 1,648 | 41.9 % | LB | 0.0000 | 0.0200 | 0.2703 | 1.2000 | 16.20 | 2 % |
| | | | UB | 0.0040 | 0.0440 | 0.3016 | 1.2000 | 16.20 | |
| Leafy vegetables | 2,303 | 44.4 % | LB | 0.0000 | 0.0100 | 0.0486 | 0.1150 | 11.30 | 21 % |
| | | | UB | 0.0050 | 0.0200 | 0.0631 | 0.1860 | 11.30 | |
| Nuts | 751 | 72.1 % | LB | 0.0000 | 0.0000 | 0.0330 | 0.1624 | 0.9390 | 1 % |
| | | | UB | 0.0001 | 0.0400 | 0.0686 | 0.2000 | 0.9390 | |
| Oilseeds | 276 | 51.3 % | LB | 0.0000 | 0.0000 | 0.0410 | 0.1645 | 0.5200 | 4 % |
| | | | UB | 0.0060 | 0.0500 | 0.0619 | 0.1915 | 0.5200 | |
| Other vegetables/products | 2,070 | 64.8 % | LB | 0.0000 | 0.0000 | 0.0237 | 0.0700 | 4.060 | 22 % |
| | | | UB | 0.0020 | 0.0120 | 0.0358 | 0.1000 | 4.060 | |
| Pulses (legumes) | 774 | 60.3 % | LB | 0.0000 | 0.0000 | 0.0162 | 0.0701 | 0.6200 | 13 % |
| | | | UB | 0.0054 | 0.0200 | 0.0422 | 0.2000 | 0.6200 | |
| Root vegetables | 1,111 | 41.1 % | LB | 0.0000 | 0.0100 | 0.0463 | 0.0670 | 10.10 | 16 % |
| | | | UB | 0.0040 | 0.0200 | 0.0572 | 0.1000 | 10.10 | |
| Stem vegetables | 483 | 60.9 % | LB | 0.0000 | 0.0000 | 0.0425 | 0.0910 | 4.250 | 4 % |
| | | | UB | 0.0050 | 0.0200 | 0.0703 | 0.3000 | 4.250 | |
| *Vegetable nuts and pulses* | 11,000 | 52.2 % | LB | 0.0000 | 0.0000 | 0.0734 | 0.1800 | 16.20 | 99 % |
| | | | UB | 0.0030 | 0.0200 | 0.0923 | 0.3000 | 16.20 | |
| *Total all vegetables, nuts and pulses* | 11,011 | 52.3 % | LB | 0.0000 | 0.0000 | 0.0733 | 0.1800 | 16.20 | 100 % |
| | | | UB | 0.0030 | 0.0200 | 0.0922 | 0.3000 | 16.20 | |

N: number of samples; LOD: limit of detection; P5: 5$^{th}$ percentile; P95: 95$^{th}$ percentile; Max: maximum level; SAF: sampling adjustment factor.

Very few results were reported for the vegetable soup category and for dried vegetables. The results for dried vegetables are expressed on a dry weight basis in Table 8, but were converted to a fresh weight basis by assuming a dilution factor of 9 before calculating exposure. The number of results reported with lead levels below the LOD varied considerably between the food sub-classes, from dried vegetables at 27 % to nuts at 72 %. Apart from dried vegetables that will be diluted before consumption, the fungus sub-class reported the highest mean upper bound lead level results. The highest lead levels were recorded for carrot at 10.1 mg/kg, unspecified wild mushroom at 16.2 mg/kg, two porcini samples at 11.2 mg/kg, a Lamb's lettuce sample at 11.3 mg/kg and one oregano sample at 8.5 mg/kg. The lead content in two samples exceeded any other sample in the respective sub-category by more than ten fold and they were excluded; an oilseed sample at 19.7 mg/kg and a pistachio nut sample at 11.7 mg/kg.

Although there are three sub-categories for the starchy roots and potatoes category, of the 1,059 analytical results, only 15 covered peeled potatoes and other starchy roots (Table 9). Separate reporting of peeled potatoes was requested because they are covered by a legislated maximum level.

**Table 9:** Statistical description of concentrations of lead for food category "05. Starchy roots and potatoes" in mg/kg.

| Food category | N | <LOD | | P5 | Median | Mean | P95 | Max | SAF |
|---|---|---|---|---|---|---|---|---|---|
| Other potatoes | 1,045 | 47.8 % | LB | 0.0000 | 0.0030 | 0.0217 | 0.0660 | 1.321 | 96 % |
| | | | UB | 0.0030 | 0.0200 | 0.0338 | 0.1000 | 1.321 | |
| Other starchy roots | 12 | 36.4 % | LB | - | 0.0179 | 0.0816 | - | 0.355 | 4 % |
| | | | UB | - | 0.0680 | 0.0999 | - | 0.355 | |
| Peeled potatoes | 3 | 100 % | LB | - | 0.0000 | 0.0000 | - | 0.000 | - |
| | | | UB | - | 0.0200 | 0.0200 | - | 0.020 | |
| *Total Starchy roots and potatoes* | 1,059 | 47.8 % | LB | 0.0000 | 0.0030 | 0.0223 | 0.0700 | 1.321 | 100 % |
| | | | UB | 0.0030 | 0.0200 | 0.0345 | 0.1000 | 1.321 | |

N: number of samples; LOD: limit of detection; P5: 5$^{th}$ percentile; P95: 95$^{th}$ percentile; Max: maximum level; SAF: sampling adjustment factor.

No peeled potato results exceeded the LOD but no conclusions can be drawn because of the very limited number of samples in this sub-class.

The fruit category was split into three sub-categories comprising a total of 3,915 samples (Table 10). The results for dried fruit are expressed on a dry weight basis in Table 10, but were converted to a fresh weight basis by assuming a dilution factor of 9 before calculating exposure.

**Table 10:** Statistical description of concentrations of lead for food category "06. Fruits" in mg/kg.

| Food category | N | <LOD | | P5 | Median | Mean | P95 | Max | SAF |
|---|---|---|---|---|---|---|---|---|---|
| Berries and small fruits | 1,435 | 56.2 % | LB | 0.0000 | 0.0000 | 0.0160 | 0.0500 | 3.700 | 26 % |
| | | | UB | 0.0030 | 0.0110 | 0.0262 | 0.0860 | 3.700 | |
| Dried fruits | 364 | 42.3 % | LB | 0.0000 | 0.0110 | 0.0350 | 0.1700 | 0.3900 | 4 % |
| | | | UB | 0.0000 | 0.0280 | 0.0451 | 0.1700 | 0.3900 | |
| Other fruits | 2,116 | 68.2 % | LB | 0.0000 | 0.0000 | 0.0084 | 0.0460 | 0.4680 | 70 % |
| | | | UB | 0.0010 | 0.0100 | 0.0215 | 0.0800 | 0.4680 | |
| *Total Fruits* | 3,915 | 61.4 % | LB | 0.0000 | 0.0000 | 0.0137 | 0.0580 | 3.700 | 100 % |
| | | | UB | 0.0010 | 0.0110 | 0.0254 | 0.0902 | 3.700 | |

N: number of samples; LOD: limit of detection; P5: 5$^{th}$ percentile; P95: 95$^{th}$ percentile; Max: maximum level; SAF: sampling adjustment factor.

Again the dried samples showed the highest mean upper bound lead levels. However, the highest concentration of 3.7 mg/kg was recorded for a strawberry sample in the 'berries and small fruit' sub-class.

There were a total of 3,565 samples analysed in the four sub-categories of the juices, soft drinks and bottled water category (Table 11).

**Table 11:** Statistical description of concentrations of lead for food category "07. Juices, soft drinks and bottled water" in mg/kg.

| Food category | N | <LOD | | P5 | Median | Mean | P95 | Max | SAF |
|---|---|---|---|---|---|---|---|---|---|
| Fruit juices and nectar | 1,011 | 68.6 % | LB | 0.0000 | 0.0000 | 0.0144 | 0.0700 | 0.6600 | 14 % |
| | | | UB | 0.0029 | 0.0200 | 0.0250 | 0.0700 | 0.6600 | |
| Vegetable juices | 163 | 82.2 % | LB | 0.0000 | 0.0000 | 0.0065 | 0.0210 | 0.6500 | 1 % |
| | | | UB | 0.0032 | 0.0100 | 0.0177 | 0.0218 | 0.6500 | |
| *Fruit and vegetable juices* | 1,174 | 70.5 % | LB | 0.0000 | 0.0000 | 0.0133 | 0.0570 | 0.6600 | 15 % |
| | | | UB | 0.0030 | 0.0200 | 0.0240 | 0.0570 | 0.6600 | |
| Soft drinks | 108 | 85.2 % | LB | 0.0000 | 0.0000 | 0.0040 | 0.0388 | 0.1100 | 15 % |
| | | | UB | 0.0003 | 0.0020 | 0.0403 | 0.2000 | 0.2000 | |
| Bottled water | 2,283 | 68.6 % | LB | 0.0000 | 0.0000 | 0.0004 | 0.0005 | 0.1220 | 70 % |
| | | | UB | 0.0005 | 0.0005 | 0.0018 | 0.0060 | 0.1220 | |
| *Total Juices, soft drinks, bottled water* | 3,565 | 69.7 % | LB | 0.0000 | 0.0000 | 0.0047 | 0.0220 | 0.6600 | 100 % |
| | | | UB | 0.0005 | 0.0010 | 0.0102 | 0.0327 | 0.6600 | |

N: number of samples; LOD: limit of detection; P5: 5th percentile; P95: 95th percentile; Max: maximum level; SAF: sampling adjustment factor.

Only about 30 % or less of the sample results in any of the sub-classes exceeded the LOD. The lead content of 9.25 mg/kg in one apple and peach juice sample exceeded any other sample in the sub-category by more than ten times and was excluded.

The coffee, tea and cocoa category is split into three sub-categories comprising a total of 655 samples (Table 12). The results are expressed on a dry weight basis in Table 12, but converted to liquid when presented in Table 20, by assuming dilution factors of 10 (20 g of cocoa powder in 180 ml of liquid), 18 (7 g of coffee powder in 120 ml of liquid) and 60 (2 g of tea leaves in 120 ml of liquid) for cocoa, coffee and tea, respectively. The dilutions for tea and coffee were used in a previous EFSA opinion (EFSA, 2008), while the cocoa dilution is estimated from manufacturers' recommendations. The dilutions give only an indication of average consumption, and because of large individual and country-to-country variations in consumption habits, the contribution of lead from the different types of diluting liquids used for this food category was not included here.

**Table 12:** Statistical description of concentrations of lead for food category "08. Coffee, tea and cocoa" in mg/kg.

| Food category | N | <LOD | | P5 | Median | Mean | P95 | Max | SAF |
|---|---|---|---|---|---|---|---|---|---|
| Cocoa (powder or cocoa beans) | 154 | 23.9 % | LB | 0.0000 | 0.0718 | 0.0983 | 0.3585 | 0.5720 | 14 % |
| | | | UB | 0.0220 | 0.0880 | 0.1202 | 0.3585 | 0.5720 | |
| Coffee (powder or coffee beans) | 105 | 81.9 % | LB | 0.0000 | 0.0000 | 0.0192 | 0.0900 | 0.8410 | 60 % |
| | | | UB | 0.0014 | 0.0200 | 0.0418 | 0.1000 | 0.8410 | |
| Tea and other infusions (leaves) | 396 | 51.5 % | LB | 0.0000 | 0.0000 | 0.3239 | 1.680 | 6.210 | 26 % |
| | | | UB | 0.0010 | 0.0200 | 0.3345 | 1.680 | 6.210 | |
| *Total Coffee, tea and cocoa* | 655 | 36.2 % | LB | 0.0000 | 0.0013 | 0.2220 | 1.100 | 6.210 | 100 % |
| | | | UB | 0.0010 | 0.0390 | 0.2372 | 1.100 | 6.210 | |

N: number of samples; LOD: limit of detection; P5: 5th percentile; P95: 95th percentile; Max: maximum level; SAF: sampling adjustment factor.

Although cocoa powder or cocoa beans had many more results above the LOD, tea leaves and other infusion concentrates showed much higher mean lead levels, based on a few high results. The highest concentration at 6.21 mg/kg was recorded in a sample of linden flower tea. The lead content in one

sample exceeded any other sample in the sub-category by more than ten times and was excluded; it was a cocoa sample at 14.1 mg/kg.

The alcoholic beverages category is split into three sub-categories comprising a total of 2,229 samples (Table 13).

**Table 13:** Statistical description of concentrations of lead for food category "09. Alcoholic beverages" in mg/kg.

| Food category | N | <LOD | | P5 | Median | Mean | P95 | Max | SAF |
|---|---|---|---|---|---|---|---|---|---|
| Beer and substitutes | 381 | 87.4 % | LB | 0.0000 | 0.0000 | 0.0132 | 0.0089 | 1.730 | 79 % |
| | | | UB | 0.0003 | 0.0100 | 0.0321 | 0.1000 | 1.730 | |
| Wine and substitutes | 1,656 | 23.4 % | LB | 0.0000 | 0.0130 | 0.0249 | 0.0620 | 5.800 | 20 % |
| | | | UB | 0.0020 | 0.0200 | 0.0340 | 0.1000 | 5.800 | |
| Other alcoholic beverages | 191 | 44.5 % | LB | 0.0000 | 0.0020 | 0.0093 | 0.0394 | 0.2180 | 1 % |
| | | | UB | 0.0010 | 0.0050 | 0.0137 | 0.0482 | 0.2180 | |
| *Total Alcoholic beverages* | 2,228 | 36.2 % | LB | 0.0000 | 0.0080 | 0.0215 | 0.0560 | 5.800 | 100 % |
| | | | UB | 0.0005 | 0.0010 | 0.0319 | 0.1000 | 5.800 | |

N: number of samples; LOD: limit of detection; P5: 5$^{th}$ percentile; P95: 95$^{th}$ percentile; Max: maximum level; SAF: sampling adjustment factor.

Wine and substitute sample results often showed low level lead contamination as reflected in the many results above the LOD. The highest concentration at 5.80 mg/kg was recorded in a red wine sample. The lead content in one sample exceeded any other sample in the sub-category by more than ten times and was excluded; it was a sample of the Spanish liquor Orujo at 19.58 mg/kg.

The meat, meat products and offal category comprises three sub-categories, of which two are sub-divided into a further seven and four sub-classes, respectively, with a total of 40,306 results reported (Table 14).

**Table 14:** Statistical description of concentrations of lead for food category "10. Meat, meat products and offal" in mg/kg.

| Food category | N | <LOD | | P5 | Median | Mean | P95 | Max | SAF |
|---|---|---|---|---|---|---|---|---|---|
| Bovine, sheep and goat meat | 7,229 | 77.1 % | LB | 0.0000 | 0.0000 | 0.0131 | 0.0700 | 0.8000 | 20 % |
| | | | UB | 0.0060 | 0.0200 | 0.0300 | 0.0780 | 0.8000 | |
| Game meat | 2,521 | 59.4 % | LB | 0.0000 | 0.0000 | 3.137 | 1.525 | 867.0 | 0.2 % |
| | | | UB | 0.0060 | 0.0200 | 3.153 | 1.525 | 867.0 | |
| Meat substitutes | 6 | 16.7 % | LB | - | 0.0120 | 0.0128 | - | 0.0320 | 0.1 % |
| | | | UB | - | 0.0130 | 0.0162 | - | 0.0320 | |
| Other meat | 1,419 | 82.2 % | LB | 0.0000 | 0.0000 | 0.0109 | 0.0700 | 0.4610 | 0.2 % |
| | | | UB | 0.0060 | 0.0200 | 0.0270 | 0.0700 | 0.4610 | |
| Pig meat | 5,244 | 82.5 % | LB | 0.0000 | 0.0000 | 0.0080 | 0.0500 | 1.443 | 41.9 % |
| | | | UB | 0.0030 | 0.0200 | 0.0272 | 0.0600 | 1.443 | |
| Poultry meat | 3,343 | 83.2 % | LB | 0.0000 | 0.0000 | 0.0070 | 0.0500 | 0.3120 | 12.3 % |
| | | | UB | 0.0060 | 0.0200 | 0.0276 | 0.0600 | 0.3120 | |
| Processed meat products | 1,843 | 74.3 % | LB | 0.0000 | 0.0000 | 0.0178 | 0.0610 | 7.320 | 16 % |
| | | | UB | 0.0050 | 0.0206 | 0.0416 | 0.1000 | 7.320 | |
| *Meat and meat products* | 21,605 | 77.4 % | LB | 0.0000 | 0.0000 | 0.3757 | 0.0760 | 867.0 | 90.7 % |
| | | | UB | 0.0050 | 0.0200 | 0.3941 | 0.0900 | 867.0 | |
| Kidney ruminants, pig, poultry, horse | 5,883 | 51.5 % | LB | 0.0000 | 0.0000 | 0.1085 | 0.1300 | 289.0 | 0.2 % |
| | | | UB | 0.0022 | 0.0310 | 0.1278 | 0.1310 | 289.0 | |
| Liver and kidney of game animals | 652 | 25.0 % | LB | 0.0000 | 0.0370 | 1.2514 | 0.2800 | 239.0 | 0.1 % |
| | | | UB | 0.0100 | 0.0410 | 1.2611 | 0.2814 | 239.0 | |
| Liver ruminants, pig, poultry, horse | 11,886 | 64.0 % | LB | 0.0000 | 0.0000 | 0.0535 | 0.1200 | 197.0 | 5 % |
| | | | UB | 0.0100 | 0.0390 | 0.0771 | 0.1340 | 197.0 | |
| Other offal products | 183 | 70.5 % | LB | 0.0000 | 0.0000 | 0.0119 | 0.0628 | 0.2800 | 2 % |
| | | | UB | 0.0050 | 0.0200 | 0.0528 | 0.2000 | 0.2800 | |
| *Edible offal and offal products* | 18,604 | 58.7 % | LB | 0.0000 | 0.0000 | 0.1125 | 0.1280 | 289.0 | 7.3 % |
| | | | UB | 0.0050 | 0.0380 | 0.1344 | 0.1400 | 289.0 | |
| *Meat-based mixed dishes* | 92 | 72.8 % | LB | 0.0000 | 0.0000 | 0.0159 | 0.0635 | 0.1860 | 2 % |
| | | | UB | 0.0080 | 0.0400 | 0.0467 | 0.1000 | 0.1860 | |
| *Total Meat, meat products, and offal* | 40,301 | 52.3 % | LB | 0.0000 | 0.0000 | 0.2534 | 0.1020 | 867.0 | 100 % |
| | | | UB | 0.0050 | 0.0200 | 0.2734 | 0.1100 | 867.0 | |

N: number of samples; LOD: limit of detection; P5: 5$^{th}$ percentile; P95: 95$^{th}$ percentile; Max: maximum level; SAF: sampling adjustment factor.

There was a range of high values for game meat, with a maximum of 867 mg/kg, as well as for liver and kidney from all animals, with maximum levels of 179 mg/kg and 289 mg/kg, respectively. One sample taken from a wild boar around the entry of a high velocity shot had a lead content of 3,090 mg/kg. This sample was excluded from the analysis. Four further samples exceeded any other sample in the sub-category by more than ten times and were excluded as they were considered outliers; they were fresh meat at 18 mg/kg, horse meat at 13 mg/kg and two samples at 89 mg/kg and 84 mg/kg in the processed meat sub-class.

The fish and seafood category comprises three sub-categories, of which one is sub-divided into a further four sub-classes, with a total of 11,453 results reported (Table 15).

**Table 15:** Statistical description of concentrations of lead for food category "11. Fish and seafood" in mg/kg.

| Food category | N | <LOD | | P5 | Median | Mean | P95 | Max | SAF |
|---|---|---|---|---|---|---|---|---|---|
| Bivalve molluscs | 2,231 | 35.0 % | LB | 0.0000 | 0.1100 | 0.2068 | 0.7578 | 4.060 | 0.1 % |
| | | | UB | 0.0200 | 0.2000 | 0.2676 | 0.7578 | 4.060 | |
| Cephalopods | 368 | 76.1 % | LB | 0.0000 | 0.0000 | 0.0224 | 0.1000 | 1.580 | 3 % |
| | | | UB | 0.0080 | 0.0400 | 0.0820 | 0.3000 | 1.580 | |
| Crustaceans | 1,580 | 79.7 % | LB | 0.0000 | 0.0000 | 0.0188 | 0.1000 | 0.9000 | 0.1 % |
| | | | UB | 0.0080 | 0.1700 | 0.1216 | 0.2000 | 0.9000 | |
| Other seafood and seafood products | 240 | 60.8 % | LB | 0.0000 | 0.0000 | 0.0786 | 0.3540 | 0.6700 | 0.8 % |
| | | | UB | 0.0100 | 0.2000 | 0.1843 | 0.3540 | 0.6700 | |
| *Seafood and seafood products* | 4,419 | 55.8 % | LB | 0.0000 | 0.0000 | 0.1173 | 0.5000 | 4.060 | 4 % |
| | | | UB | 0.0100 | 0.2000 | 0.1954 | 0.5000 | 4.060 | |
| *Fish and fish products* | 6,991 | 76.8 % | LB | 0.0000 | 0.0000 | 0.0146 | 0.0800 | 2.000 | 95 % |
| | | | UB | 0.0050 | 0.0200 | 0.0469 | 0.2000 | 2.000 | |
| *Fish-based mixed dishes* | 43 | 67.4 % | LB | - | 0.0000 | 0.0222 | - | 0.2900 | 1 % |
| | | | UB | - | 0.0250 | 0.0431 | - | 0.2900 | |
| *Total fish and seafood* | 11,453 | 68.7 % | LB | 0.0000 | 0.0000 | 0.0543 | 0.3000 | 4.060 | 100 % |
| | | | UB | 0.0060 | 0.0400 | 0.1042 | 0.3000 | 4.060 | |

N: number of samples; LOD: limit of detection; P5: 5th percentile; P95: 95th percentile; Max: maximum level; SAF: sampling adjustment factor.

Samples of bivalve molluscs had the highest incidence of lead contamination in this food category, with 65 % of the results above the LOD. Two samples of the wedge shell (*Donax trunculus*) showed the highest values with 4.06 and 3.88 mg/kg, respectively.

There were 615 results reported for the egg category.

**Table 16:** Statistical description of concentrations of lead for food category "12. Eggs" in mg/kg.

| Food category | N | <LOD | | P5 | Median | Mean | P95 | Max | SAF |
|---|---|---|---|---|---|---|---|---|---|
| *Total Eggs* | 615 | 88.6 % | LB | 0.0000 | 0.0000 | 0.0052 | 0.0420 | 0.2050 | 100 % |
| | | | UB | 0.0000 | 0.0200 | 0.0252 | 0.0500 | 0.2050 | |

N: number of samples; LOD: limit of detection; P5: 5th percentile; P95: 95th percentile; Max: maximum level; SAF: sampling adjustment factor.

Results for the egg category were mainly very low. The lead content in one sample exceeded any other sample in the sub-category by more than ten times and was excluded; it was a duck egg sample at 4.78 mg/kg.

The milk and dairy-based products category comprises three sub-categories, of which one is sub-divided into two sub-classes, with a total of 3,214 results submitted.

**Table 17:** Statistical description of concentrations of lead for food category "13. Milk and dairy-based products" in mg/kg.

| Food category | N | <LOD | | P5 | Median | Mean | P95 | Max | SAF |
|---|---|---|---|---|---|---|---|---|---|
| Milk and dairy drinks | 2,180 | 87.0 % | LB | 0.0000 | 0.0000 | 0.0050 | 0.0120 | 4.550 | 56.9 % |
| | | | UB | 0.0010 | 0.0100 | 0.0117 | 0.0200 | 4.550 | |
| Soya milk | 2 | 50.0 % | LB | - | 0.0040 | 0.0040 | - | 0.0080 | 0.1 % |
| | | | UB | - | 0.0075 | 0.0075 | - | 0.0080 | |
| *Milk and dairy-based drinks* | 2,182 | 86.9 % | LB | 0.0000 | 0.0000 | 0.0050 | 0.0120 | 4.550 | 57 % |
| | | | UB | 0.0010 | 0.0100 | 0.0117 | 0.0200 | 4.550 | |
| *Dairy-based products* | 493 | 72.7 % | LB | 0.0000 | 0.0000 | 0.0168 | 0.0909 | 0.7000 | 30 % |
| | | | UB | 0.0030 | 0.0200 | 0.0323 | 0.1000 | 0.7000 | |
| *Cheese* | 535 | 61.9 % | LB | 0.0000 | 0.0000 | 0.0176 | 0.0992 | 0.5700 | 13 % |
| | | | UB | 0.0060 | 0.0400 | 0.0436 | 0.1000 | 0.5700 | |
| *Total milk and dairy-based products* | 3,210 | 80.6 % | LB | 0.0000 | 0.0000 | 0.0089 | 0.0314 | 4.550 | 100 % |
| | | | UB | 0.0010 | 0.0100 | 0.0202 | 0.0690 | 4.550 | |

N: number of samples; LOD: limit of detection; P5: 5$^{th}$ percentile; P95: 95$^{th}$ percentile; Max: maximum level; SAF: sampling adjustment factor.

Overall, only 20 % of results in this category exceeded the LOD. The lead content in four samples exceeded the content in any other sample, except for the remaining highest milk sample of 4.55 mg/kg, which exceeded the level by more than ten fold and were excluded. They were two condensed milk concentrates (8 and 9 % water content, respectively) at about 14.6 mg/kg and two cream samples at about 12.6 mg/kg.

The miscellaneous food category comprises two sub-categories, each with five sub-classes, with a total of 4,923 results submitted. The infant and follow-on formula and the other infant food categories are used only to calculate the exposure of children and are not included in the overall summary for the category. The infant and follow-on formula category as shown in Table 18 contains a mixture of products to be diluted before use, and also ready-to-consume products. For Table 20, all products to be further prepared were transformed into ready-to-consume products by using a dilution factor of one part of dry powder to nine parts of water, including the lead content in both the powder and the water. Since the water category in the special children's exposure scenarios comprised a mix of tap water and bottled water, a proportion of 70 % tap water and 30 % bottled water consumption was assumed, although this varied from country to country (the average concentration of lead in water in these proportions, using UB estimates, would be 0.00523 mg/L; compared with 0.00327 mg/L with a 30 % tap water: 70 % bottled water combination). However, there is a maximum level for lead in tap water of 25 µg/L, and 111 of the 4,087 sample results exceeded this level (Table 19). Assuming that families with such water would use bottled water in place of tap water for their children, samples exceeding the maximum limit were excluded.

Equally for the other infant food categories, a few infant food supplements with high lead values were excluded in the adjusted mean column in Table 20 since the amount consumed would be much less than for jarred baby food. It was also decided, before transferring the results to the adjusted mean column in Table 20, to exclude 185 samples with results below the LOD with an LOD of 0.1 mg/kg since this was five times higher than the upper bound mean for the rest of the samples.

**Table 18:** Statistical description of concentrations of lead for food category "14. Miscellaneous products and products for special dietary use" in mg/kg.

| Food category | N | <LOD | | P5 | Median | Mean | P95 | Max | SAF |
|---|---|---|---|---|---|---|---|---|---|
| Algae as food | 60 | 30.0 % | LB | 0.0000 | 0.1526 | 0.3350 | 1.099 | 1.500 | 0.1 % |
| | | | UB | 0.0140 | 0.3000 | 0.3972 | 1.099 | 1.500 | |
| Dry herbs | 132 | 13.6 % | LB | 0.0000 | 0.5500 | 0.8809 | 3.542 | 10.50 | 3 % |
| | | | UB | 0.0200 | 0.5500 | 0.8896 | 3.542 | 10.50 | |
| Other miscellaneous products | 657 | 49.9 % | LB | 0.0000 | 0.0035 | 0.2179 | 0.9037 | 15.94 | 12 % |
| | | | UB | 0.0050 | 0.0310 | 0.2390 | 0.9037 | 15.94 | |
| Salt | 91 | 73.6 % | LB | 0.0000 | 0.0000 | 0.1122 | 0.6036 | 1.446 | 2 % |
| | | | UB | 0.0200 | 0.1000 | 0.1776 | 0.6036 | 1.446 | |
| Spices | 763 | 32.2 % | LB | 0.0000 | 0.1380 | 0.3313 | 0.9592 | 34.88 | 3 % |
| | | | UB | 0.0200 | 0.2000 | 0.3644 | 0.9592 | 34.88 | |
| *Miscellaneous products* | 1,703 | 39.8 % | LB | 0.0000 | 0.0000 | 0.3186 | 1.199 | 34.88 | 20.1 % |
| | | | UB | 0.0070 | 0.1000 | 0.3479 | 1.199 | 34.88 | |
| Algae based supplements | 204 | 13.1 % | LB | 0.0000 | 0.5700 | 1.0638 | 4.191 | 28.00 | 0.1 % |
| | | | UB | 0.0410 | 0.5700 | 1.0714 | 4.191 | 28.00 | |
| Infant and follow-on formulae | 423 | 55.3 % | LB | 0.0000 | 0.0000 | 0.0044 | 0.0197 | 0.0552 | - |
| | | | UB | 0.0020 | 0.0100 | 0.0143 | 0.0800 | 0.1000 | |
| Non-algae based supplements | 1,467 | 31.4 % | LB | 0.0000 | 0.0600 | 0.6229 | 1.700 | 155.0 | 0.4 % |
| | | | UB | 0.0100 | 0.1000 | 0.6410 | 1.700 | 155.0 | |
| Other food for special dietary use | 179 | 16.2 % | LB | 0.0000 | 0.0500 | 0.4889 | 3.360 | 14.08 | 79.4 % |
| | | | UB | 0.0130 | 0.0500 | 0.4961 | 3.360 | 14.08 | |
| Other infant food | 947 | 71.6 % | LB | 0.0000 | 0.0000 | 0.0370 | 0.0246 | 20.00 | - |
| | | | UB | 0.0040 | 0.0200 | 0.0701 | 0.1000 | 20.00 | |
| *Food for special dietary uses* | 3,220 | 44.4 % | LB | 0.0000 | 0.0000 | 0.3898 | 1.290 | 155.0 | 79.9 % |
| | | | UB | 0.0030 | 0.0500 | 0.4100 | 1.290 | 155.0 | |
| *Total miscellaneous/ special dietary uses* | 4,923 | 42.8 % | LB | 0.0000 | 0.0110 | 0.3652 | 1.240 | 155.0 | 100 % |
| | | | UB | 0.0040 | 0.0070 | 0.3885 | 1.240 | 155.0 | |

N: number of samples; LOD: limit of detection; P5: 5th percentile; P95: 95th percentile; Max: maximum level; SAF: sampling adjustment factor; LB: lower bound; UP: upper bound.

There are many high results in this food category. The highest result reported was for a sample of wakame at 155 mg/kg followed by a propolis sample at 59.6 mg/kg. The high of 20 mg/kg for a sample of other infant food was for a food supplement for children. All samples were retained since there were no large clear gaps in the continuum of results.

There were 4,087 results reported for the tap water category (Table 19).

**Table 19:** Statistical description of concentrations of lead for food category "15. Tap water" in mg/kg.

| Food category | N | <LOD | | P5 | Median | Mean | P95 | Max | SAF |
|---|---|---|---|---|---|---|---|---|---|
| *Total Tap water* | 4,087 | 38.2 % | LB | 0.0000 | 0.0005 | 0.0052 | 0.0120 | 1.950 | 100 % |
| | | | UB | 0.0003 | 0.0020 | 0.0067 | 0.0200 | 1.950 | |

N: number of samples; LOD: limit of detection; P5: 5th percentile; P95: 95th percentile; Max: maximum level; SAF: sampling adjustment factor.

Some lead, above the LOD, was recorded in about 60 % of the samples, mostly at low levels, because of the low LOD reported for water. The highest lead level recorded was 1.95 mg/kg.

### 5.5. Summary of occurrence

To normalise the unbalanced sampling frequency to better reflect products as consumed when aggregating the results into the concise food categories, adjustment factors based on detailed food consumption information, or in some cases on food production, as described in Section 5.6, were used (Table 20). Dried fruit and vegetables were converted to fresh weight and infant and follow-on formula, and coffee, tea and cocoa described as ready-to-consume product as explained earlier.

**Table 20:** Lower and upper bound original and adjusted lead mean concentration.

| EFSA Concise Food Category | Mean (mg/kg) | | Adjusted Mean (mg/kg) | |
|---|---|---|---|---|
| | LB | UB | LB | UB |
| 01. All cereals and cereal products | 0.0286 | 0.0471 | 0.0210 | 0.0407 |
| 01. A Cereal-based mixed dishes | 0.0045 | 0.0283 | 0.0045 | 0.0283 |
| 01. B Cereals and cereal products | 0.0290 | 0.0473 | 0.0259 | 0.0444 |
| 02. Sugar and sugar products including chocolate | 0.0339 | 0.0602 | 0.0403 | 0.0644 |
| 03. Fats (vegetable and animal) | 0.0387 | 0.0635 | 0.0292 | 0.0550 |
| 04. All vegetables, nuts and pulses | 0.0733 | 0.0922 | 0.0377 | 0.0536 |
| 04. A Vegetable soups | 0.0118 | 0.0388 | 0.0118 | 0.0388 |
| 04. B Vegetables, nuts and pulses | 0.0734 | 0.0923 | 0.0380 | 0.0538 |
| 05. Starchy roots or potatoes | 0.0223 | 0.0345 | 0.0241 | 0.0364 |
| 06. Fruits | 0.0137 | 0.0254 | 0.0102 | 0.0220 |
| 07. Juices, soft drinks and bottled water | 0.0047 | 0.0102 | 0.0030 | 0.0110 |
| 07. A Fruit and vegetable juices | 0.0133 | 0.0240 | 0.0139 | 0.0245 |
| 07. B Soft drinks | 0.0040 | 0.0403 | 0.0040 | 0.0403 |
| 07. C Bottled water | 0.0004 | 0.0018 | 0.0004 | 0.0018 |
| 08. Coffee, tea, cocoa (dry and liquid respectively, see legend) | 0.2220 | 0.2372 | 0.0034 | 0.0045 |
| 09. Alcoholic beverages | 0.0216 | 0.0319 | 0.0155 | 0.0323 |
| 09. A Beer and substitutes | 0.0132 | 0.0321 | 0.0132 | 0.0321 |
| 09. B Wine and substitutes | 0.0249 | 0.0340 | 0.0249 | 0.0340 |
| 09. C Other alcoholic beverages | 0.0093 | 0.0137 | 0.0093 | 0.0137 |
| 10. All meat and meat products and offal | 0.2534 | 0.2734 | 0.0207 | 0.0412 |
| 10. A Meat and meat products | 0.3757 | 0.3941 | 0.0176 | 0.0373 |
| 10. B Edible offal and offal products | 0.1125 | 0.1344 | 0.0600 | 0.0881 |
| 10. C Meat based preparations | 0.0159 | 0.0467 | 0.0159 | 0.0467 |
| 11. All fish and seafood | 0.0543 | 0.1042 | 0.0156 | 0.0493 |
| 11. A Seafood and seafood products | 0.1173 | 0.1954 | 0.0382 | 0.1081 |
| 11. B Fish and fish products | 0.0146 | 0.0469 | 0.0146 | 0.0469 |
| 11. C Fish-based preparations | 0.0222 | 0.0431 | 0.0222 | 0.0431 |
| 12. Eggs | 0.0052 | 0.0252 | 0.0052 | 0.0252 |
| 13. Milk and dairy based products | 0.0089 | 0.0202 | 0.0102 | 0.0220 |
| 13. A Milk and dairy-based drinks | 0.0050 | 0.0117 | 0.0050 | 0.0117 |
| 13. B Dairy-based products | 0.0168 | 0.0323 | 0.0168 | 0.0323 |
| 13. C Cheese | 0.0176 | 0.0436 | 0.0176 | 0.0436 |
| 14. Miscellaneous/special dietary products | 0.3652 | 0.3885 | 0.4568 | 0.4678 |
| 14. A Miscellaneous products | 0.3186 | 0.3479 | 0.3238 | 0.3495 |
| 14. B Food for special dietary uses | 0.3898 | 0.4100 | 0.4903 | 0.4975 |
| (Infant and follow-on formula - see Table 18 | 0.0044 | 0.0143 | 0.0020 | 0.0047) |
| (Other infant food – see Table 18 | 0.0370 | 0.0701 | 0.0082 | 0.0203) |
| 15. Tap water | 0.0052 | 0.0067 | 0.0052 | 0.0067 |

LB: lower bound; UB: upper bound.

The impact of the adjustment was particularly noticeable for the meat and meat products category where sampling bias favoured testing of game, although this category is only a small component of the average diet. The infant and follow-on formula adjusted mean was calculated by assuming that 90 % consisted of home-prepared ready-to-eat formula with lower and upper bound mean lead content, respectively of 0.0019 and 0.0046 µg/L, and 10 % purchased as liquid product with a mean lead content of 0.0029 and 0.0064 µg/L, respectively.

## 5.6. Lead content in samples with values above 1 mg/kg

The distribution across food groups of samples with a lead content of more than 1 mg/kg was reviewed in a separate exercise. There were 771 results with a lead content above 1 mg/kg. Of these, 17 samples characterised as outliers were excluded from further analyses. Those samples alone caused almost a doubling of exposure if left in the calculation. Of the remaining 754 samples, 14.1 % exceeded 10 mg/kg with a maximum of 867 mg/kg in muscle of wild pig. The histogram of lead content in the samples is shown in Figure 8.

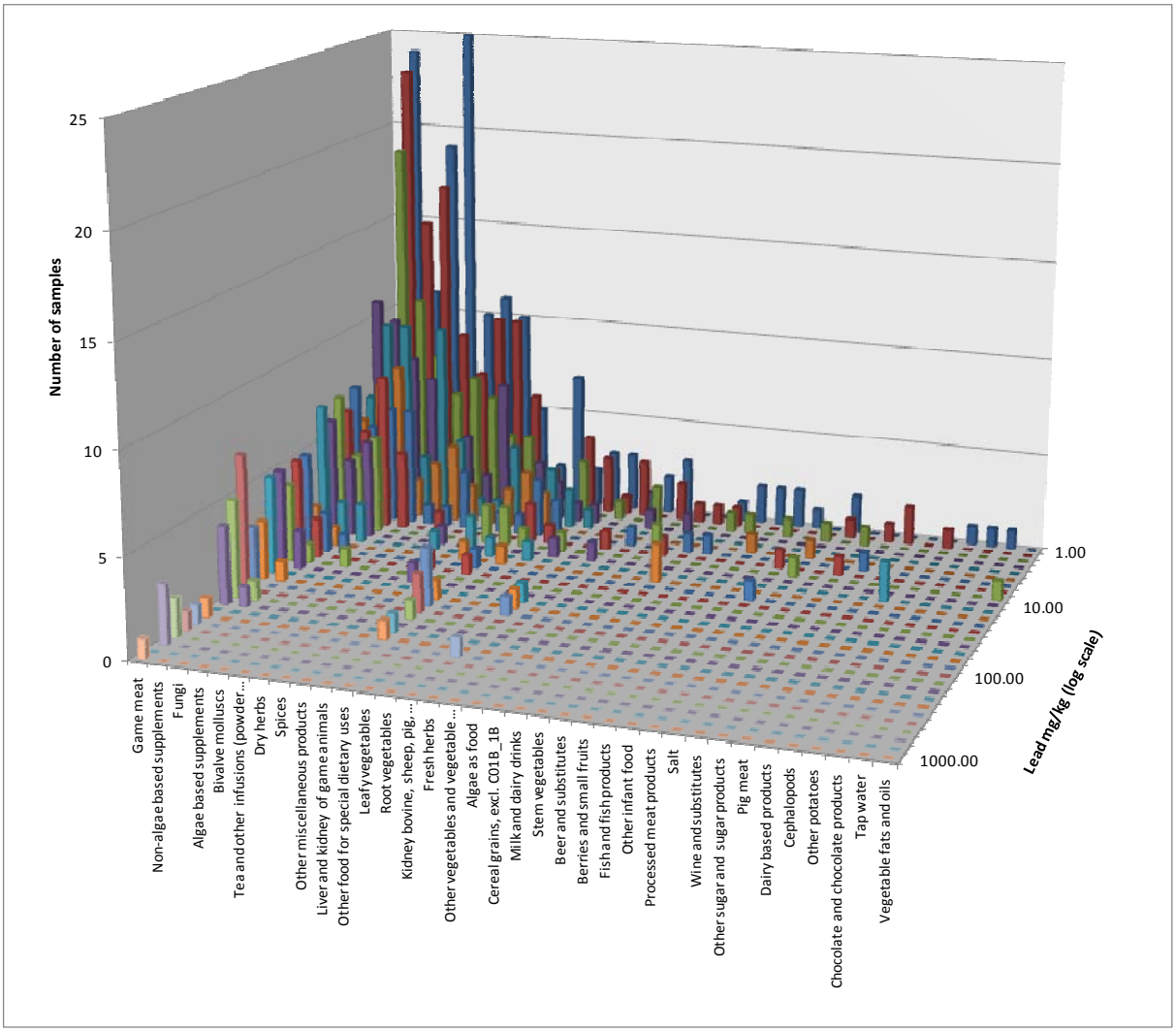



**Figure 8:** Lead content in samples above 1 mg/kg (note the logarithmic z-axis scale). Colour is used only for ease of identification of individual bars.

Game meat and offal dominate the group, followed by non-algae based food supplements and fungi and algae based food supplements. Some very high values are also recorded in the general offal category. Of the top ten groups, most are rarely consumed or consumed in small amounts (note that the value for tea is for dry product).

### 5.7. Occurrence data in breast milk and infant formulae

Babies and infants can be exposed to lead through breast milk or infant formula. During lactation, lead is mobilised from bone lead stores and partly excreted through human breast milk. Early studies of lead in human breast milk found concentrations ranging over two orders of magnitude from 0.0010 to 0.1000 mg/L (referenced in Gulson et al., 1998; Namihira et al., 1993). In Europe, the median lead concentration in breast milk from 41 volunteers in Sweden was 0.002 mg/L (Larsson et al., 1981) and the mean value for urban residents of Germany in 1983 was 0.0091 mg/L (Sternowsky and Wessolowski, 1985). A more recent study of Mexican women found lead content in breast milk ranging from 0.0002 to 0.0080 mg/L with an average of 0.0011 mg/L (Ettinger et al., 2004). An Austrian study estimated a mean lead content of 0.0016 mg/kg in breast milk (Gundacker et al., 2002). Since lead levels have been decreasing, and in the absence of any further information, this latter result was used to calculate exposure in nursing European babies.

Both formula concentrate and water can contribute to the lead content in ready-to-consume infant formula. Lower and upper bound lead content in ready-to-consume infant formula were calculated to be 0.0020 and 0.0047 mg/kg, respectively as indicated in Table 20. Thus, infant formula might contain up to three times the lead content of breast milk.

## 6. Food consumption

### 6.1. EFSA's Concise European Food Consumption Database

The EFSA Concise European Food Consumption Database was established to support exposure assessments in the EU with national data provided by 19 countries. To obtain comparable results, data were aggregated into 15 broad food groups and certain subgroups giving a total of 28 separate groups. Individual data on gender, age and body weight are included. A summary of the information is available on the EFSA website.

The concise database is intended as a screening tool for exposure assessment. It allows assessment of the overall exposure of population groups to a wide variety of substances. Limitations arise from the broad food categories defined and from the different methodologies for data collection applied in different countries. The use of this database may be sufficient when the exposure calculation, based on conservative assumptions for occurrence concentrations, is below the level of concern. If this is not the case, further refinements might be necessary, particularly in defining sub-categories of interest and adjusting occurrence means using the appropriate SAF. A guidance document for the use of the data has been published on the EFSA website (see Annex 3 to EFSA, 2008).

A modification from the guidance document was used for this assessment in that data at individual level from the EFSA Concise Food Consumption Database were used to calculate lead exposure. This provides a more accurate estimate in that individual body weights are used for the calculations. Only aggregated food consumption statistics for this database are presented on the EFSA web site.[22]

[22] Aggregated consumption figures for 19 Member States plus Norway can be found at http://www. efsa. europa. eu/en/datex/datexfooddb. htm.

For each single individual recorded in the database, the average daily consumption of each food group was linked with the corresponding average occurrence value as listed in Table 20 (adjusted mean columns). Individual exposure in µg/kg b.w. was then calculated by summing up the individual exposure from all different food groups divided by the specific body weight of the subject. Summary statistics of the individual exposure estimates were then calculated and reported at country level as in Table 22. Due to differences in methodologies, it is not appropriate to derive a mean of European exposure from these data[22].

## 6.2. Food consumption data for children

Estimating the potential lead exposure for infants from breast milk and infant formula requires information about the quantity of liquid consumed per day and the duration over which such consumption occurs (U.S. EPA 1997). According to the Institute of Medicine of the U.S. National Academies of Sciences (IOM), average breast milk consumption is about 750 to 800 g/day (range: 450 to1,200 g per day) for the first 4 to 5 months of life (IOM, 1991). Infant birth weight and nursing frequency have been shown to influence consumption (IOM, 1991). The World Health Organisation related breast milk consumption to body weight rather than age with an estimated 125 ml/kg or 763 ml for a 3 month old child weighing 6.1 kg (Onyango et al., 2002). According to the German DONALD study, mean consumption of infant formula for a three month old child weighing on average 6.1 kg, was 780 mL/day with a 95$^{th}$ percentile consumption of 1,060 mL/day (Kersting et al., 1998). A common mean of 800 mL/day will be used in this opinion for consumption of breast milk and infant formula when calculating exposure, with a high of 1,200 mL/day.

In a project funded by EFSA, long-term dietary exposure to lead was calculated for children living in eleven different European countries, Belgium, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Netherlands and Spain. Food consumption data for children aged 1 up to 14 years and collected in 13 different surveys were used. Not all participants had access to consumption information for all age groups; in particular only four of the surveys provided information for the ages 11 to 14 years. The statistical beta binomial-normal model (BBN model) in the Monte Carlo Risk Assessment software (MCRA) was used to estimate exposure as a function of age (De Boer and Van der Voet, 2007).

A special comparison between child and adult consumption patterns was also undertaken by the Istituto Superiore di Sanità using Italian food consumption information taken from the second national survey of 1,940 Italian subjects. It was carried out by Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione (Turrini et al., 2001; Turrini and Lombardi-Boccia, 2002). The database contains consumption data and other relevant information (e.g. body weight and age) expressed for each individual. The following low consumption entries could not be matched with the EFSA database: ready-to-eat dishes (first course and main course); preserved meat; dressing and sauces; fruit-based dishes; salt, vinegar and yeast; salted snacks, and sandwiches.

## 6.3. Food consumption for vegetarians

The CONTAM Panel selected data from the 65 individuals with a lacto-ovo-vegetarian food pattern in the EFSA Concise European Food Consumption database to use for this opinion to assess the impact of one type of vegetarian diet. In order to identify relevant individuals, data from only those countries that used a 7-day dietary record method were retained: they were the United Kingdom, Sweden, Italy, Ireland, France and Denmark. From an initial database of 37,599 consumers, 10,074 were retained for further analysis. Since the most common practice among vegetarians is to eat no meat or fish at all, but to include dairy products and eggs - a lacto-ovo-vegetarian diet - consumers who did not report any consumption in the meat category and fish category on any of the 7 days surveyed were selected for the final analysis. This included 65 subjects from five countries, with no subject with this dietary pattern identified in Denmark. The food consumption pattern is shown in Table 21.

**Table 21:** Food consumption pattern among 65 subjects identified as putative lacto-ovo-vegetarians in the EFSA Concise European Food Consumption Database.

| Category | Food consumption g per day | | | |
|---|---|---|---|---|
| | Mean | Std Dev | P95 | Maximum |
| 01. All cereals and cereal products | 283 | 128 | 510 | 749 |
| 01. A Cereal-based mixed dishes | 149 | 115 | 304 | 510 |
| 01. B Cereals and cereal products | 122 | 103 | 353 | 371 |
| 02. Sugar, products and chocolate | 25 | 25 | 66 | 131 |
| 03. Fats (vegetable and animal) | 24 | 25 | 67 | 144 |
| 04. All vegetables, nuts and pulses | 318 | 222 | 747 | 1,254 |
| 04. A Vegetable soups | 24 | 40 | 114 | 169 |
| 04. B Vegetables, nuts and pulses | 293 | 219 | 747 | 1,254 |
| 05. Starchy roots or potatoes | 94 | 93 | 296 | 487 |
| 06. Fruits | 144 | 166 | 494 | 912 |
| 07. Juices, soft drinks and bottled water | 322 | 422 | 888 | 2,750 |
| 07. A Fruit and vegetable juices | 57 | 88 | 226 | 414 |
| 07. B Soft drinks | 144 | 228 | 731 | 897 |
| 07. C Bottled water | 149 | 399 | 825 | 2,571 |
| 08. Coffee, tea, cocoa (dry and liquid respectively, see legend) | 483 | 395 | 1,117 | 1,728 |
| 09. Alcoholic beverages | 191 | 347 | 977 | 1,393 |
| 09. A Beer and substitutes | 158 | 342 | 925 | 1,371 |
| 09. B Wine and substitutes | 30 | 57 | 159 | 273 |
| 09. C Other alcoholic beverages | 3 | 9 | 21 | 51 |
| 12. Eggs | 17 | 27 | 84 | 114 |
| 13. Milk and dairy based products | 221 | 173 | 468 | 956 |
| 13. A Milk and dairy-based drinks | 171 | 163 | 449 | 854 |
| 13. B Dairy-based products | 32 | 41 | 112 | 176 |
| 13. C Cheese | 23 | 22 | 57 | 98 |
| 14. Miscellaneous/special dietary products | 14 | 30 | 52 | 220 |
| 14. A Miscellaneous products | 11 | 15 | 50 | 57 |
| 14. B Food for special dietary uses | 4 | 27 | 2 | 214 |
| 15. Tap water | 316 | 390 | 1143 | 1,671 |

Std dev: standard deviation; P95: 9$^{th}$ percentile.

## 7. Human exposure assessment

### 7.1. Previously reported exposure results

In a SCOOP report published in 2004, dietary exposure to lead in twelve participating EU Member States was estimated to be on average 42 µg/day in adults (SCOOP, 2004). This is equivalent to 0.7 µg/kg b.w. per day assuming a body weight of 60 kg. Exposure estimates for some individual Member States showed 60 to 70 % higher mean values for children aged 4 to 6 years than for adults on a body weight basis. Not all food groups were represented in the study in all participating countries and occurrence values below the limit of detection were handled differently, although half the LOD was frequently entered.

Several individual Member State or regional dietary lead surveys have also been published. Leblanc et al. (2005) estimated the dietary lead intake in France to be 13 µg/day for children aged 3 to 14 years and 18 µg/day for adults. In Germany, geometric mean weekly intake of lead for children aged 1 to

7 years was 5.3 µg/kg/week (about 15 µg/day for a child with a weight of 20 kg). Children living in the industrialized area with a substantial food consumption of own grown vegetables had no increased dietary intake of lead (Wilhelm et al., 2005). In Denmark, the mean estimated lead exposure decreased from 42 µg/day in 1983-87 to 18 µg/day in 1993-1997 (Larsen et al., 2002). An even more marked trend is apparent in the total diet studies of the United Kingdom, with lead exposure decreasing from an estimated 110 µg/day in 1976 to 6 µg/day in 2006, although the methodology was slightly changed in the last survey (FSA, 2009).

Lead exposure decreased appreciably over time in Catalonia, with successive studies reporting 115 µg/day (Schumacher et al., 1996), 49 µg/day (Llobet et al., 1998) and 28 µg/day (Llobet et al., 2003) for adults. To confirm the decrease, the group extended the latest survey to cover a more complete range of marine species commonly consumed in Catalonia, which resulted in an estimated adult exposure of 45 µg/day or 60 % higher than previously thought.

The U.S. ATSDR reviewed lead exposure in the U.S. calculated from total diet studies (U.S. ATSDR, 2007). A comparison of daily intakes of lead by age group (6 months, 2 years and adult) showed that lead intakes had declined by approximately 50 % in each group between 1980 and 1984 (Gunderson, 1988) and continued to decrease through 1990 for all age and sex groups (Bolger et al., 1991). In the more recent NHEXAS study, mean dietary lead exposure in the EPA Region V was estimated to be 17.5 µg/day for a 70-kg adult (Thomas et al., 1999). Daily dietary exposure to lead, estimated from the 1986–1988 Canadian Survey, was 24 µg/day for all ages including both male and female (Dabeka and McKenzie, 1995). From 1995-2003, daily estimated dietary lead intake for children up to 7 years in the USA amounted to between 1.95 to 2.26 µg/day (U.S. EPA, 2009).

### 7.2. Current mean and high dietary exposure to lead

Mean and the 95$^{th}$ percentile lead dietary exposure were calculated separately for each country recorded in EFSA Concise European Food Consumption Database for the whole and subgroups of the population, including infants, children and vegetarians, using a deterministic approach (Table 22). For this approach, the adjusted occurrence results, aggregated at the concise food category level (Table 20), were used with the individual consumption data for each country separately. The different statistical descriptors were then calculated with the SAS software version 7.1 (SAS Inc., USA). The Panel also performed a probabilistic exposure assessment using lower bound and upper bound values for the non-quantifiable samples and the Creme Monte Carlo computational system (Creme Ltd, Ireland). This approach resulted in similar exposure values for average consumers as the deterministic approach. To maintain consistency with its opinions on other heavy metals, the CONTAM Panel therefore decided to use the deterministic approach for its assessment of dietary exposure to lead.

Estimates of adult mean lead dietary exposure across European countries are in the range of 0.36 to 1.24 µg/kg b.w. per day. The adult 95$^{th}$ percentile lead dietary exposure is in the range 0.73 to 2.43 µg/kg b.w. per day. The variation in calculated exposure between countries is influenced only by differences in consumption pattern, since lead concentrations in food categories were calculated at the European level. The pooling of all submitted occurrence data provides a more complete coverage of foods available on the market across Europe, since individual Member States do not have the capability to cover the full range of foods with a sufficient number of samples. It is further justified by the extensive cross-border trade for many food commodities. However, it creates some uncertainty, because there is no assessment of potentially elevated local lead levels.

**Table 22:** Lower and upper bound dietary exposure to lead (µg/kg b.w. per day) for average (Mean) and 95th percentile exposure across a number of subjects in European countries using deterministic calculations.

| Countr | N | Mean LB | Mean UB | P95 LB | P95 UB |
|---|---|---|---|---|---|
| AT | 2,123 | 0.54 | 1.00 | 1.06 | 1.80 |
| BE | 1,723 | 0.46 | 0.96 | 0.73 | 1.63 |
| BG | 853 | 0.44 | 0.81 | 0.80 | 1.56 |
| CZ | 1,751 | 0.54 | 1.00 | 0.93 | 1.83 |
| DE | 3,550 | 0.74 | 1.24 | 1.74 | 2.43 |
| DK | 3,150 | 0.56 | 1.03 | 0.84 | 1.61 |
| EE | 2,010 | 0.37 | 0.63 | 0.73 | 1.26 |
| FI | 2,007 | 0.57 | 0.90 | 1.04 | 1.51 |
| FR | 1,195 | 0.51 | 0.99 | 0.79 | 1.47 |
| GB | 1,724 | 0.47 | 0.96 | 0.79 | 1.53 |
| HU | 927 | 0.50 | 0.87 | 0.74 | 1.33 |
| IE | 1,373 | 0.59 | 1.06 | 1.03 | 1.87 |
| IS | 1,075 | 0.54 | 1.04 | 1.11 | 1.93 |
| IT | 1,544 | 0.51 | 0.90 | 0.79 | 1.36 |
| NL | 4,285 | 0.53 | 0.97 | 0.80 | 1.51 |
| NO | 2,321 | 0.47 | 1.03 | 0.79 | 1.86 |
| PL | 2,692 | 0.67 | 1.14 | 1.19 | 2.01 |
| SE | 1,088 | 0.44 | 0.80 | 0.81 | 1.43 |
| SK | 2,208 | 0.36 | 0.74 | 0.74 | 1.71 |
| Minimum | | 0.36 | 0.63 | 0.73 | 1.26 |
| Median* | | 0.51 | 0.97 | 0.80 | 1.61 |
| Maximum | | 0.74 | 1.24 | 1.74 | 2.43 |

AT: Austria; BE: Belgium; BG: Bulgaria; CZ: Czechoslovakia; DE: Germany; DK: Denmark; EE: Estonia; FI: Finland; FR: France; GB: Great Britain; HU: Hungary; IE: Ireland; IS: Iceland; IT: Italy; NL: The Netherland; NO: Norway; PL: Poland; SE: Sweden; SK: Slovakia; N: number of subjects; LB: lower bound: UB: upper bound; P95: 95th percentile.
(*) This is the median of the means from the individual countries.

The results for adults are summarised in Table 29 and used for the risk characterisation.

### 7.3. Contributions of different food groups to lead exposure

The contribution of each broad food category to total lead exposure was calculated from the individual consumption figures for each country as summarised in Table 21, expressed in g per day multiplied by the corresponding lower bound and upper bound adjusted mean lead concentrations expressed in mg/kg from Table 20 and shown in Figure 9 and Table 23. Only the lower bound contribution is shown in detail as calculated per country.

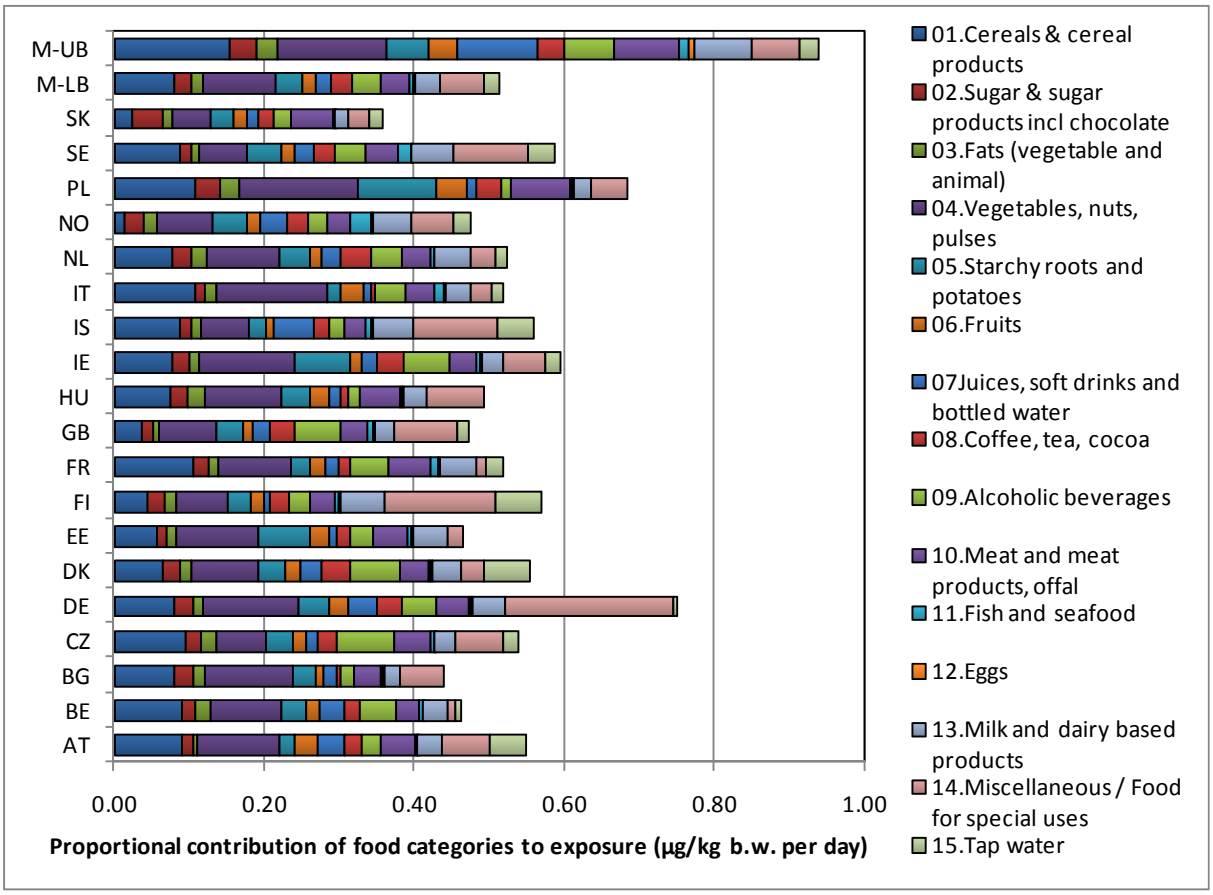



**Figure 9:** Relative contribution of each broad food category to overall lower bound mean lead exposure in each country and the median of the country mean for the lower bound (M-LB) as well as upper bound (M-UB) exposure.

There is considerable variation between countries in the contribution of different food categories to overall lead exposure in the respective population. An attempt was nevertheless made to calculate a European average. The largest contributors to the calculated overall lead exposure are vegetables, nuts and pulses with 19 % at the lower bound and 14 % at the upper bound and cereals and cereal products at 13 % and 14 %, respectively. This is followed for the lower bound by miscellaneous products and food for special uses at 12 %, starchy roots and potatoes and meat and meat products including offal both at 8 %, alcoholic beverages at 7 % and milk and dairy products at 6 %. For the upper bound the situation is slightly different with juices, soft drinks and bottled water at 11 %, alcoholic beverages and meat and meat products including offal, both at 9 %, milk and dairy products at 8 %, miscellaneous products and food for special uses at 7 % and starchy roots and potatoes at 6 %. Although the rank order is not dramatically different, the lower bound is considered to better reflect the actual food category contribution to overall lead exposure since it is not influenced by the high numbers of samples below the LOD in some food categories.

**Table 23:** Relative contribution of each broad food category to overall lower bound lead exposure in each country.

| Food Category | AT | BE | BG | CZ | DE | DK | EE | FI | FR | GB | HU | IE | IS | IT | NL | NO | PL | SE | SK | M-LB | M-UB |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 01.Cereals & cereal products | 9.03 | 9.05 | 7.94 | 9.33 | 8.02 | 6.33 | 5.58 | 4.31 | 10.57 | 3.54 | 7.47 | 7.55 | 8.59 | 10.70 | 7.79 | 1.28 | 10.65 | 8.57 | 2.37 | 7.94 | 15.38 |
| 02.Sugar & sugar products | 1.41 | 1.80 | 2.44 | 2.18 | 2.47 | 2.47 | 1.25 | 2.33 | 1.95 | 1.49 | 2.24 | 2.29 | 1.74 | 1.24 | 2.43 | 2.66 | 3.34 | 1.62 | 4.09 | 2.24 | 3.58 |
| 03.Fats (vegetable and animal) | 0.54 | 1.92 | 1.71 | 1.90 | 1.14 | 1.43 | 1.33 | 1.55 | 1.26 | 0.78 | 2.20 | 1.45 | 1.28 | 1.62 | 1.91 | 1.67 | 2.48 | 0.97 | 1.15 | 1.45 | 2.74 |
| 04.Vegetables, nuts, pulses | 11.02 | 9.47 | 11.66 | 6.84 | 12.93 | 8.79 | 11.10 | 6.93 | 9.80 | 7.61 | 10.29 | 12.65 | 6.16 | 14.68 | 9.71 | 7.51 | 15.89 | 6.36 | 5.06 | 9.71 | 14.57 |
| 05.Starchy roots and potatoes | 2.07 | 3.26 | 3.01 | 3.39 | 4.00 | 3.67 | 6.88 | 3.04 | 2.46 | 3.65 | 3.77 | 7.44 | 2.51 | 1.79 | 4.23 | 4.47 | 10.45 | 4.62 | 3.21 | 3.65 | 5.52 |
| 06.Fruits | 3.06 | 1.69 | 1.08 | 1.76 | 2.60 | 2.18 | 2.32 | 1.69 | 2.10 | 1.34 | 2.63 | 1.49 | 1.02 | 3.21 | 1.51 | 1.73 | 4.20 | 1.73 | 1.65 | 1.73 | 3.74 |
| 07Juices, soft drinks and bottled water | 3.59 | 3.41 | 1.63 | 1.64 | 3.88 | 2.58 | 1.14 | 0.85 | 1.74 | 2.15 | 1.59 | 2.19 | 5.29 | 0.82 | 2.40 | 3.59 | 1.07 | 2.78 | 1.52 | 2.15 | 10.80 |
| 08.Coffee, tea, cocoa | 2.26 | 2.10 | 0.61 | 2.64 | 3.16 | 3.93 | 1.67 | 2.64 | 1.49 | 3.39 | 0.87 | 3.38 | 1.94 | 0.66 | 4.14 | 2.88 | 3.40 | 2.75 | 2.24 | 2.64 | 3.49 |
| 09.Alcoholic beverages | 2.56 | 4.74 | 1.91 | 7.53 | 4.53 | 6.71 | 3.21 | 2.78 | 4.99 | 6.19 | 1.63 | 6.30 | 2.05 | 4.02 | 4.19 | 2.47 | 1.21 | 3.98 | 2.19 | 3.98 | 6.68 |
| 10.Meat and meat products, offal | 4.41 | 3.15 | 3.37 | 4.86 | 4.38 | 3.62 | 4.55 | 3.26 | 5.77 | 3.64 | 5.32 | 3.51 | 2.85 | 3.88 | 3.89 | 3.02 | 7.91 | 4.34 | 5.50 | 3.89 | 8.64 |
| 11.Fish and seafood | 0.40 | 0.48 | 0.46 | 0.40 | 0.39 | 0.46 | 0.51 | 0.56 | 1.04 | 0.78 | 0.18 | 0.51 | 0.84 | 1.35 | 0.33 | 3.01 | 0.37 | 1.77 | 0.26 | 0.48 | 1.47 |
| 12.Eggs | 0.04 | 0.07 | 0.16 | 0.14 | 0.16 | 0.12 | 0.20 | 0.12 | 0.15 | 0.13 | 0.20 | 0.14 | 0.08 | 0.14 | 0.11 | 0.15 | 0.23 | 0.10 | 0.09 | 0.14 | 0.67 |
| 13.Milk and dairy based products | 3.19 | 3.14 | 1.92 | 2.75 | 4.32 | 3.94 | 4.53 | 5.98 | 4.79 | 2.52 | 3.25 | 2.83 | 5.45 | 3.37 | 4.92 | 5.13 | 2.37 | 5.56 | 1.88 | 3.37 | 7.72 |
| 14.Miscellaneous/Food for special uses | 6.49 | 1.04 | 5.97 | 6.37 | 22.38 | 3.01 | 2.07 | 14.61 | 1.30 | 8.33 | 7.70 | 5.72 | 11.14 | 2.71 | 3.18 | 5.67 | 4.65 | 9.94 | 2.81 | 5.72 | 6.17 |
| 15.Tap water | 4.66 | 0.77 | N/A | 2.04 | 0.50 | 6.20 | N/A | 6.24 | 2.31 | 1.52 | 0.00 | 2.04 | 4.86 | 1.68 | 1.50 | 2.31 | N/A | 3.58 | 1.60 | 2.04 | 2.63 |

N/A, not available; AT: Austria; BE: Belgium; BG: Bulgaria; CZ: Czechoslovakia; DE: Germany; DK: Denmark; EE: Estonia; FI: Finland; FR: France; GB: Great Britain; HU: Hungary; IE: Ireland; IS: Iceland; IT: Italy; NL: The Netherland; NO: Norway; PL: Poland; SE: Sweden; SK: Slovakia; M-LB: country mean for the lower bound; M-UB: country mean for the upper bound.

Some of the above food categories contain a broad range of highly consumed products. Thus, a back calculation was performed dividing each broad food category with the previously applied SAFs (see Table 5 to 19) in order to split the broad categories into finer food groups (Figure 10). Only the lower bound lead levels were used to better reflect the high proportion of results below the LOD for some food categories. The exercise should be seen as producing a ranking order only with a large uncertainty associated with the calculation.

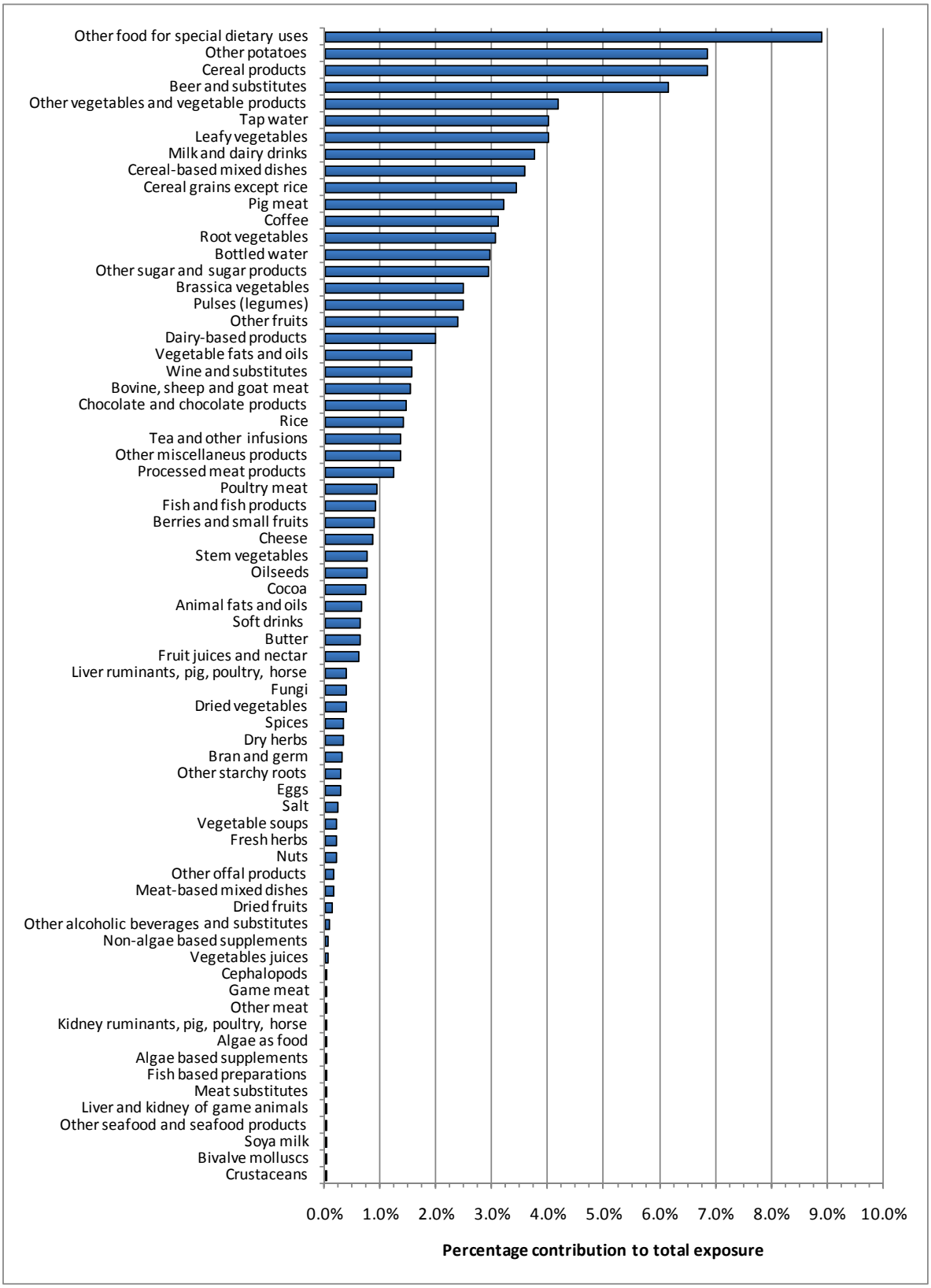



**Figure 10:** Lower bound estimated consumer exposure to lead from different food sub-categories and sub-classes calculated as the respective proportion of the overall lower bound median of the country mean from Figure 9 (M-LB).

For the disaggregated food groups, other food for special dietary uses contributed the most to lead exposure with 8.9 %, followed by potatoes with 6.9 %, cereal products with 6.8 %, beer and substitutes with 6.2 % and other vegetables and vegetable products with 4.2 %. High food consumption is the causative factor behind most of the high ranking results for disaggregated food groups. Variations will be large between and within countries dependent on individual consumption patterns as can partly be seen in Figure 9. The group "other foods for special dietary uses" comprises a disparate range of food products. Some products target special groups like diabetics or gluten intolerants, while others are "soothing", "stimulate intestinal activity" or "restore muscle strength". The range of 179 products tested is not considered representative for the indicated consumption of products in this group and exposure should be interpreted with care.

## 7.4. Specific sub-groups of the population

### 7.4.1. Nursing or formula-fed infants (0 to 0.5 year)

Breast- and formula-fed infants are exposed to exogenous lead from breast milk or infant formula consumption as well as possible endogenous release of lead accumulated during prenatal exposure and released through neonatal bone turnover (Gulson et al., 2001). In addition, lead is mobilised together with calcium from the maternal skeleton and readily transferred across the placenta. Although Ettinger et al. (2004) found that lead in breast milk accounted for only 12 % of the variance of the infant B-Pb level very early in life, Gulson et al. (1998) found that for the first 60 to 90 days postpartum, the contribution from breast milk to B-Pb in infants varied from 36 to 80 % and the contribution from formula to infant B-Pb levels varied from 24 to 68 % in formula-fed infants. This should be kept in mind when assessing dietary lead exposure in infants.

For the exposure assessment of infants below six months of age, a value of three months was selected, equivalent to a weight of about 6.1 kg, with an estimated average daily consumption of about 800 g, and a high consumption of 1,200 g, of breast milk and/or infant formula. Although lead levels in breast milk are highly variable, a mean of 0.0016 mg/L was taken from the available though limited data from Austria as discussed above for calculation of the breast milk exposure scenario. With these parameters, exposure is estimated to be 0.21 µg/kg b.w. per day on average, with a value of 0.32 µg/kg b.w. per day in high consumers. The mean lead level in ready-to-drink infant formula was estimated at between 0.0020 and 0.0047 mg/kg resulting in an estimated average exposure in the range of 0.27 to 0.63 µg/kg b.w. per day and an exposure of 0.40 to 0.94 µg/kg b.w. per day in high consumers, respectively, for the lower and upper bounds.

### 7.4.2. Children (1 to 14 years)

Food consumption data were collected in 13 different surveys for children aged 1-14 years living in ten different European countries and combined with the lead concentration data collected for this opinion. The aim was to estimate long-term dietary exposure as a function of age. The number of surveys covering the respective age group, and the resulting minimum, median and maximum lead exposures across the Member States, are shown in Table 24. For the estimation of long-term exposure, all daily consumption patterns were multiplied with the LB and UB mean lead concentration per food group, and aggregated over foods consumed per day per individual. The estimated exposures were adjusted for the individual's body weight. The distribution of daily exposures, calculated as described above using mean concentrations per food or food group, includes the variation both between individuals and between the days of one individual. However, to assess the long-term intake within a population only the former type of variation is of interest, since in the long run the variation between different days of one individual will average out. Therefore, to calculate a long-term dietary exposure distribution, the within-person (between days) variation should first be removed from the distribution of daily exposures using statistical models. The relatively new BBN model was used for this task (de

Boer and van der Voet, 2007; Slob, 2006). To remove the within-person variation from the daily exposures, the BBN model transforms the daily exposure distribution into a normal distribution using a logarithmic function. After removal of the within-person variation, the normal distribution is back-transformed and is then considered a long-term dietary exposure distribution.

**Table 24:** Table 24: Minimum, median and maximum estimates across four to thirteen surveys in up to ten Member States of 50th and 95th percentile lead exposure in children at different ages as calculated using the BBN model in the MCRA software.

| | **Age in years** (number of surveys in brackets) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** (6) | **2** (8) | **3** (9) | **4** (13) | **5** (13) | **6** (13) | **7** (9) | **8** (9) | **9** (9) | **10** (9) | **11** (4) | **12** (4) | **13** (4) | **14** (4) |
| **Lead exposure µg/kg b.w. per day for P50 lower bound** | | | | | | | | | | | | | | |
| Minimum | 1.27 | 1.19 | 1.10 | 0.93 | 0.87 | 0.81 | 0.80 | 0.75 | 0.71 | 0.66 | 0.46 | 0.43 | 0.40 | 0.35 |
| Median | 1.32 | 1.31 | 1.19 | 1.12 | 1.05 | 0.98 | 0.89 | 0.82 | 0.77 | 0.72 | 0.68 | 0.63 | 0.59 | 0.55 |
| Maximum | 1.60 | 1.72 | 1.58 | 1.44 | 1.32 | 1.26 | 0.99 | 0.92 | 0.86 | 0.80 | 0.72 | 0.68 | 0.64 | 0.60 |
| **Lead exposure µg/kg b.w. per day for P50 upper bound** | | | | | | | | | | | | | | |
| Minimum | 2.39 | 2.24 | 2.10 | 1.62 | 1.62 | 1.62 | 1.60 | 1.52 | 1.42 | 1.33 | 0.87 | 0.59 | 0.76 | 0.68 |
| Median | 2.54 | 2.58 | 2.37 | 2.19 | 2.04 | 1.91 | 1.72 | 1.59 | 1.46 | 1.37 | 1.24 | 0.82 | 1.04 | 0.96 |
| Maximum | 2.85 | 3.10 | 2.85 | 2.61 | 2.49 | 2.41 | 1.95 | 1.81 | 1.64 | 1.51 | 1.29 | 1.21 | 1.14 | 1.10 |
| **Lead exposure µg/kg b.w. per day for P95 lower bound** | | | | | | | | | | | | | | |
| Minimum | 2.08 | 1.83 | 1.71 | 1.50 | 1.39 | 1.30 | 1.39 | 1.28 | 1.18 | 1.09 | 0.76 | 0.72 | 0.67 | 0.58 |
| Median | 2.66 | 2.54 | 2.24 | 1.87 | 1.72 | 1.62 | 1.49 | 1.38 | 1.30 | 1.23 | 1.03 | 0.96 | 0.89 | 0.83 |
| Maximum | 3.09 | 2.93 | 2.80 | 2.66 | 2.56 | 2.43 | 1.81 | 1.68 | 1.55 | 1.44 | 1.16 | 1.09 | 1.03 | 0.97 |
| **Lead exposure µg/kg b.w. per day for P95 upper bound** | | | | | | | | | | | | | | |
| Minimum | 3.83 | 3.60 | 3.38 | 2.57 | 2.57 | 2.57 | 2.51 | 2.30 | 2.10 | 1.93 | 1.41 | 1.34 | 1.25 | 1.11 |
| Median | 5.07 | 4.67 | 4.33 | 3.54 | 3.32 | 3.12 | 2.95 | 2.75 | 2.57 | 2.39 | 1.79 | 1.68 | 1.59 | 1.53 |
| Maximum | 5.51 | 5.35 | 5.15 | 4.41 | 4.83 | 4.66 | 3.62 | 3.40 | 3.18 | 2.97 | 1.98 | 1.82 | 1.67 | 1.54 |

P50: 50th percentile; P95: 95th percentile; MCRA: Monte Carlo risk assessment; b.w.: body weight.

Ages included in the different national food consumption surveys differed. Since the dietary exposure of children is expected to be a function of age with higher exposure levels at lower ages (due to a higher amount of food consumed per kg body weight), exposure was calculated as a function of this co-variable using the BBN model. The long-term exposure is therefore reported per age using this approach and compared between countries at that level. The influence of age on lead exposure is illustrated in Figures 11 and 12, showing the 50th and 95th percentiles, respectively. For illustrative purposes, the medians of the means for the different countries and of the 95th percentile estimate for adult (i.e. >14 years) lead exposures are also shown (Table 22).

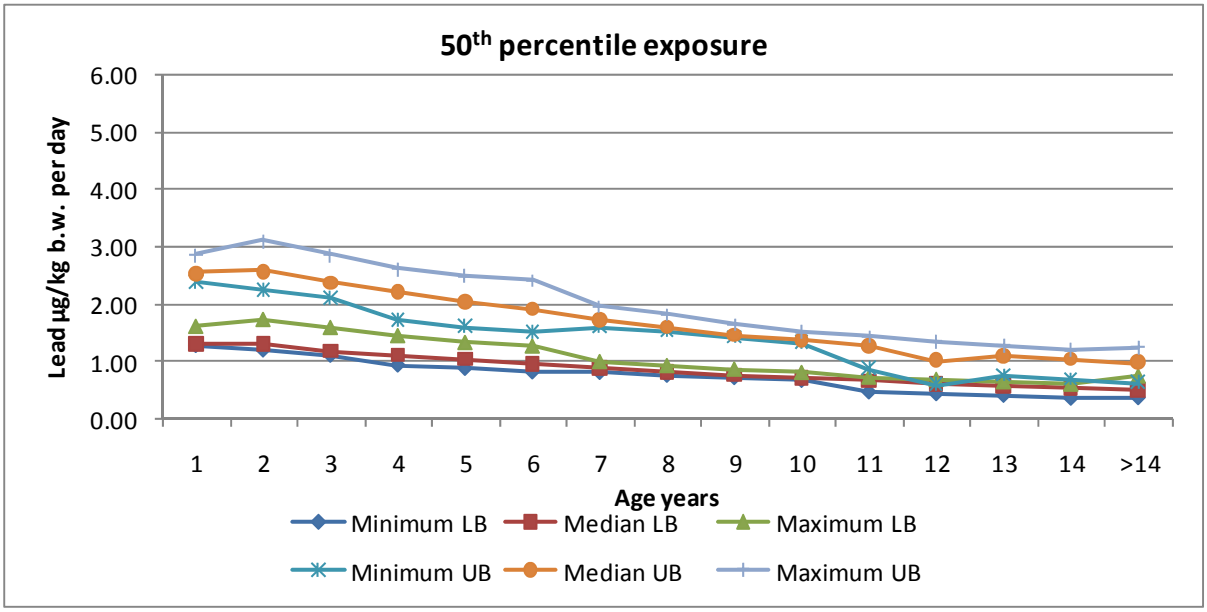



**Figure 11:** Lower and upper bound minimum, median and maximum estimates across four to thirteen surveys of the 50th percentile lead exposure in children at different ages as calculated using the BBN model in the MCRA software and previous (Table 22) mean results for adults (>14 years).

As expected, exposure was higher at younger age groups. At 14 years of age exposure was similar to the exposure for the overall adult population.

The results are sensitive to the matching classification of occurrence and consumption data, particularly when adult foods rarely consumed by children are included in calculating occurrence.

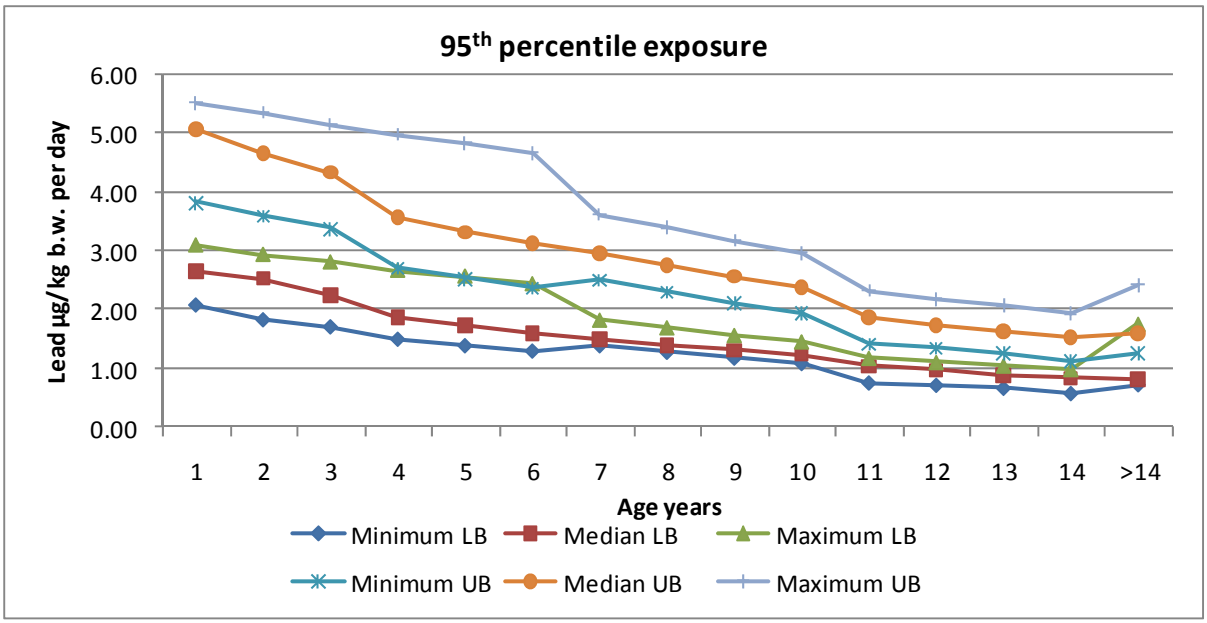



**Figure 12:** Lower and upper bound minimum, median and maximum estimates across four to thirteen surveys of the 95th percentile lead exposure in children at different ages as calculated using the BBN model in the MCRA software and previous (Table 22) mean results for adults (>14).

### 7.4.3. Comparison of exposure in Italian children vs. adults

A separate comparison of child and adult exposure was undertaken using Italian consumption information by the Istituto Superiore di Sanità. Four food consumption data sets were obtained according to the following age ranges (years): 0.5 to 3 (toddlers, breastfed not included; N = 52); 4 to 7 (children; N = 53); 8 to 12 (children; N = 88); 13 to 94 (adults; N = 1,747).

**Table 25:** Summary of exposure descriptors (µg/kg b.w. per day) for subjects of the Italian general population stratified by age ranges (95 % confidence intervals in brackets).

| Exposure descriptor | | 0.5 to 3 years | 4 to 7 years | 8 to 12 years | >13 years |
|---|---|---|---|---|---|
| **No of subjects** | | **52** | **53** | **88** | **1747** |
| Min | LB | 0.58 | 0.41 | 0.24 | 0.13 |
| | UB | 1.22 | 0.83 | 0.52 | 0.24 |
| Median | LB | 1.06 | 0.77 | 0.61 | 0.44 |
| | UB | 2.02 | 1.53 | 1.18 | 0.80 |
| Mean | LB | 1.11 (1.03-1.19) | 0.82 (0.74-0.89) | 0.61 (0.57-0.65) | 0.48 (0.47-0.50) |
| | UB | 2.12 (1.97-2.27) | 1.55 (1.43-1.67) | 1.16 (1.09-1.24) | 0.85 (0.84-0.87) |
| P95 | LB | *1.70** | *1.30** | *0.94** | 0.76 |
| | UB | *3.13** | *2.35** | *1.74** | 1.33 |
| Max | LB | 1.96 | 1.83 | 1.23 | 5.43 |
| | UB | 3.72 | 2.75 | 2.15 | 5.89 |

(*) Indicative value due to a limited number of data. P95: 95$^{th}$ percentile.

From the table above, the mean dietary lead exposure in Italian adults was 0.48 to 0.85 µg/kg b.w. per day (based on lower bound and upper bound lead occurrence means), whereas children's exposure can be up to 2.5 times greater than that of adults. The difference is particularly evident when considering 0.5 to 3 year old toddlers separately from other children, although the number of subjects is low in the toddler group.

There is a difference in the estimated median lead exposure for Italian children of 0.5 to 3 years of age with the combined study reported in Table 24. The latter shows11 % higher values than the standalone survey above. This difference is considered small since the two studies were based on different food consumption surveys and used different food classification systems when calculating exposure.

### 7.4.4. Vegetarian diet

Vegetarian exposure was calculated for a typical lacto-ovo-vegetarian diet using the EFSA Concise European Food Consumption Database. It should be noted that only 65 subjects were identified with such dietary habits and thus the estimates will be associated with considerable uncertainty in relation to both the mean and the high percentile exposure (Table 26).

Although the method was imprecise, to obtain an overall impression of lead exposure with a lacto-ovo-vegetarian diet, the estimated total for the 65 putative vegetarians was compared with the total for the 8,285 people with a conventional diet after weighting their contribution based on their proportional representation within each of the five countries. The difference in exposure between the diets varied by between -3 % and 9 %, with exposure via the vegetarian diet being slightly higher in most cases.

**Table 26:** Mean and high (extracted as the 95th percentile by the statistical program) lead exposure in putative lacto-ovo-vegetarians from the EFSA Concise European Food Consumption Database.

| Country | N | Lead exposure µg/kg b.w. per day | | | |
|---|---|---|---|---|---|
| | | Mean LB | Mean UB | High LB | High UB |
| GB | 46 | 0.46 | 0.87 | 0.80 | 1.36 |
| IE | 12 | 0.70 | 1.25 | 1.15 | 2.24 |
| IT | 4 | 0.66 | 1.07 | 0.83 | 1.30 |
| Estimated averages | 65* | 0.54 | 0.98 | 0.88 | 1.53 |
| Conventional diet | 8285 | 0.49 | 0.97 | 0.83 | 1.58 |

* One individual from France and two from Sweden (exposure 1.97, 0.64 and 1.58 µg/kg b.w. per day, respectively) are not shown separately but included in the estimated averages. N: number of subjects; LB: lower bound; UB: upper bound; b.w: body weight; GB: Great Britain; IE: Ireland; IT: Italy.

### 7.4.5. Specific diets

The influence of specific diets containing a high consumption of foods with elevated lead levels was tested assuming a weekly meal of 200 g of game meat, or bivalve molluscs, or 100 g of fungi, or game offal. The impact of a daily intake of 10 g of an algae food supplement was also tested. No change was assumed in median exposure via the base diet, of 0.36 and 1.24 µg/kg b.w. per day, respectively, for lower and upper bounds (from Table 22). Estimated lower and upper bound exposure for a 60 kg person with such a diet are shown in Table 27. It was not considered likely that a high consumer of the base diet would also regularly consume the specific products.

The impact of the specific diets on dietary exposure was modest in most cases with a 2.5 fold increase of exposure only for the game meat diet. The values shown in Table 27 do not exclude the possibility of higher consumption by an individual on one or more occasions.

**Table 27:** Contribution of special dietary components to a base diet to lead exposure.

| **Food item** | **Lead level mg/kg** | **Consumption g/day** | **Exposure µg/kg b.w. per day** | | | | |
|---|---|---|---|---|---|---|---|
| | | | **Added to diet** | **Base diet** | | **Total diet** | |
| | | | | **LB** | **UB** | **LB** | **UB** |
| Game meat | 3.15 | 28 | 1.47 | 0.36 | 1.24 | 1.98 | 2.44 |
| Bivalve molluscs | 0.268 | 28 | 0.12 | 0.36 | 1.24 | 0.63 | 1.09 |
| Fungi | 0.302 | 14 | 0.07 | 0.36 | 1.24 | 0.58 | 1.04 |
| Game offal | 1.26 | 14 | 0.30 | 0.36 | 1.24 | 0.81 | 1.27 |
| Algae food supplement | 1.07 | 10 | 0.18 | 0.36 | 1.24 | 0.69 | 1.15 |

b.w: body weight; LB: lower bound; UB: upper bound.

### 7.4.6. Women of child-bearing age

The fetus is at risk *in utero* through current exposure of the mother and through mobilisation of lead from bones subsequent to prior exposure to lead. As the CONTAM Panel had no access to direct dietary information for pregnant women, food consumption data for females between 20 and 40 years old were selected from the EFSA Concise European Food Consumption Database as a proxy for this category. Individual consumption data were used and combined with the adjusted mean lead occurrence to calculate exposure (Table 28).

**Table 28:** Total dietary exposure to lead (µg/kg b.w. per day) for average (Mean) and 95th percentile (P95) females aged between 20 and 40 years across a number of subjects in European countries using the lower (and upper bound lead concentrations.

| Country | N | Mean LB | Mean UB | P95 LB | P95 UB |
|---|---|---|---|---|---|
| AT | 725 | 0.58 | 1.05 | 1.03 | 1.77 |
| BE | 220 | 0.44 | 0.92 | 0.69 | 1.54 |
| BG | 190 | 0.42 | 0.76 | 0.77 | 1.48 |
| CZ | 313 | 0.50 | 0.90 | 0.82 | 1.51 |
| DE | 965 | 0.80 | 1.28 | 2.03 | 2.60 |
| DK | 742 | 0.56 | 1.07 | 0.86 | 1.65 |
| EE | 622 | 0.38 | 0.64 | 0.68 | 1.13 |
| FI | 411 | 0.60 | 0.93 | 1.11 | 1.53 |
| FR | 328 | 0.52 | 1.00 | 0.75 | 1.47 |
| GB | 459 | 0.44 | 0.92 | 0.72 | 1.49 |
| HU | 212 | 0.51 | 0.92 | 0.76 | 1.31 |
| IE | 368 | 0.59 | 1.07 | 1.06 | 1.87 |
| IS | 269 | 0.56 | 1.07 | 1.13 | 1.79 |
| IT | 420 | 0.54 | 0.95 | 0.84 | 1.39 |
| NL | 1,080 | 0.51 | 0.95 | 0.81 | 1.49 |
| NO | 593 | 0.48 | 1.07 | 0.78 | 1.90 |
| PL | 591 | 0.59 | 1.01 | 0.99 | 1.72 |
| SE | 259 | 0.46 | 0.82 | 0.86 | 1.43 |
| SK | 626 | 0.38 | 0.76 | 0.70 | 1.51 |
| Minimum | | 0.38 | 0.64 | 0.68 | 1.13 |
| Median | | 0.51 | 0.95 | 0.82 | 1.51 |
| Maximum | | 0.80 | 1.28 | 2.03 | 2.60 |

AT: Austria; BE: Belgium; BG: Bulgaria; CZ: Czechoslovakia; DE: Germany; DK: Denmark; EE: Estonia; FI: Finland; FR: France; GB: Great Britain; HU: Hungary; IE: Ireland; IS: Iceland; IT: Italy; NL: The Netherland; NO: Norway; PL: Poland; SE: Sweden; SK: Slovakia; N: number of subjects; LB: lower bound: UB: upper bound; P95: 95th percentile.

Lead exposure in 20 to 40 year old females is similar to exposure in the rest of the adult population. Figure 12 illustrates the small differences detected on a country basis for the mean and 95th percentile upper bound values. Exposure for the female proxy population selected to represent potentially pregnant women varied between 86 % and 108 % of exposure in the rest of the population for the mean upper bound, and between 79 % and 110 % for the 95th percentile upper bound calculations.

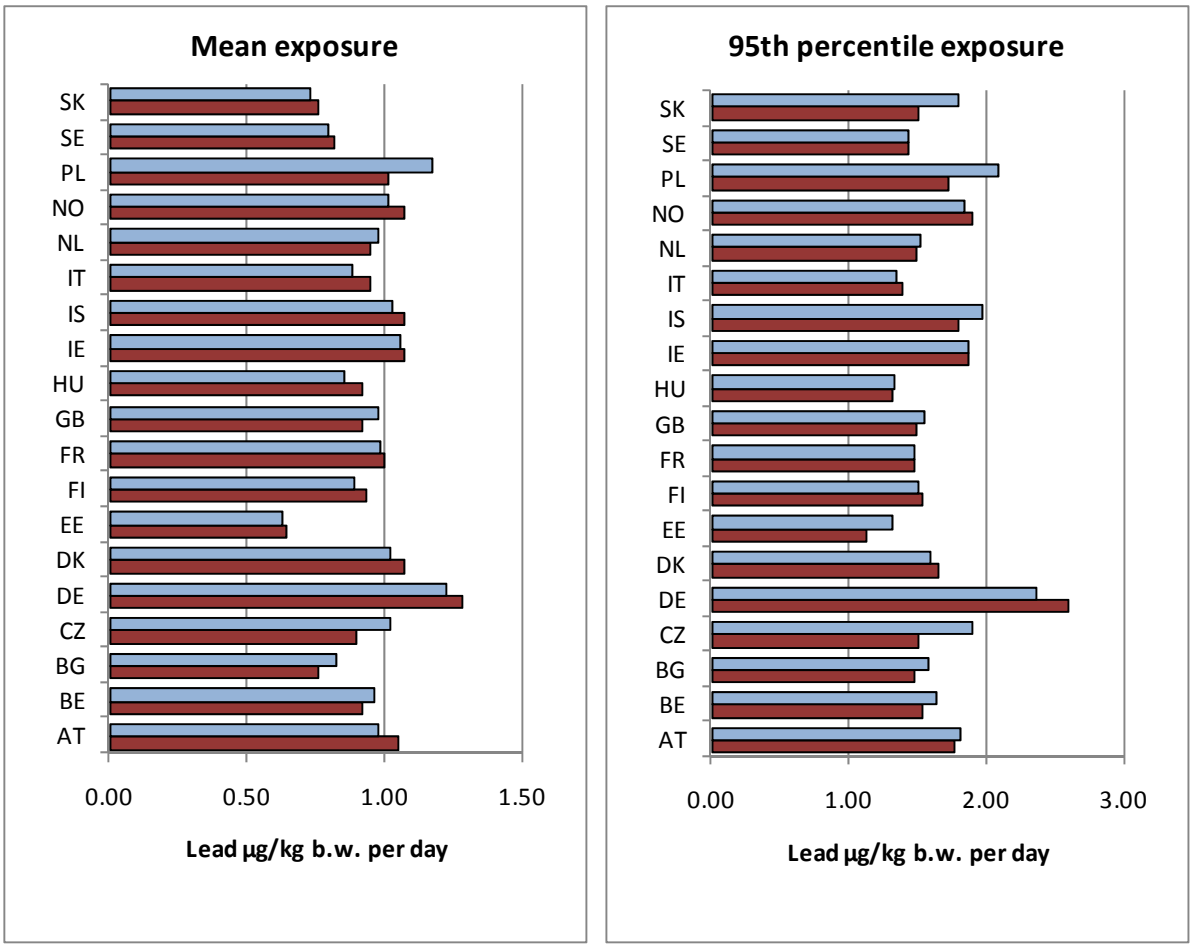



**Figure 13:** Mean and 95th percentile lead exposure in 20 to 40 year old females (in magenta) compared to the rest of the adult population, excluding 20 to 40 year old females (in blue).

### 7.5. Summary of different exposure pathways

A summary of different exposure sources of lead in different population groupings is presented in Tables 29. Dietary exposure is clearly the dominating source of overall lead exposure for adults as well as children although high soil intake can be a factor for children, particularly in contaminated areas. Inhalation from air or smoking contributes at most 10 % of overall lead exposure in adults. However, in a worst-case scenario, ETS could contribute up to 20 % of overall lead exposure in infants and soil ingestion 42 to 50 % to overall lead exposure in children depending on the age group. These estimates are based on maximum estimates for the exposure of concern (air, soil, etc) and minimum estimates for the other sources of exposure.

**Table 29:** Overview of estimated dietary lead exposure in average and high consumers for different parts of the population and of non-dietary exposure estimates. Note that due to data availability, the population areas covered are not the same for all of the calculated scenarios.

| Source | Pathway | Range of calculated or reported exposures µg/kg b.w. per day | |
|---|---|---|---|
| | | Average consumers | High consumers |
| **Dietary exposure** | | | |
| Adults[(a)] | Oral | 0.36-1.24 | 0.73-2.43 |
| Infants 3 months breast milk | Oral | 0.21 | 0.32 |
| Infants 3 months infant formula | Oral | 0.27-0.63 | 0.40-0.94 |
| Children 1-3 years | Oral | 1.10-3.10 | 1.71-5.51 |
| Children 4-7 years | Oral | 0.80-2.61 | 1.30-4.83 |
| Specific diets (game meat) | Oral | 1.98-2.44 | |
| Vegetarians | Oral | 0.46-1.25 | 0.80-2.24 |
| Women 20-40 years | Oral | 0.38-1.28 | 0.68-2.60 |
| Potential non-Dietary Exposure | | | |
| Soil and dust (children 2 years)[(b)] | Oral | 0.18-0.80 | |
| Outdoor air[(c)] | Inhalation | 0.001-0.003 | |
| Smoking (20 cigarettes)[(d)] | Inhalation | 0.003-0.018 | |
| Environmental Tobacco Smoke[(e)] | Inhalation | 0.009-0.037 (adults)<br>0.012-0.052 (children) | |

b.w.: body weight
(a) Age at which adulthood is assumed varies with country between 15 and 25 years in the different surveys. Details can be found at http://www.efsa.europa.eu/en/datex/datexfooddb.htm; (b) Exposure based on a mean and high lead content of 23 mg/kg and 100 mg/kg soil and dust, respectively, and ingestion of 100 mg soil and dust per day by a 12.5 kg child; (c) Exposure based on a mean and high lead content of 0.003 µg/m$^3$ and 0.010 µg/m$^3$, respectively, and 17 m$^3$ respired air and 60 kg b.w.; (d) Exposure based on 0.2 to 1.1 µg lead per person per day; (e) Exposure based on 0.03 to 0.13 µg/m$^3$ and 17 m$^3$ air respired by a 60 kg adult or 5 m$^3$ air respired by a 12.5 kg child.

## 8. Hazard identification and characterisation

### 8.1. Toxicokinetics

Absorption of lead from the gastrointestinal tract depends on host characteristics and on the physicochemical properties of the ingested material. Lead containing metallo-proteins and peptides are then transferred to soft tissues and bones, where lead accumulates with age. Lead is excreted primarily in urine and faeces, half-lives for lead in blood and bone are approximately 30 days and 10 to 30 years, respectively (Rabinowitz, 1991).

#### 8.1.1. Absorption

Oral Exposure

Gastrointestinal absorption of ingested lead is influenced by physiological factors (e.g., age, fasting, nutritional calcium and iron status, pregnancy) and physicochemical characteristics of particles (size, solubility and lead species). Details of the mechanism of absorption remain to be determined.

In radiotracer experiments in fasting subjects, the absorbed fraction was 37 to 70 % (average approximately 60 %) depending on the study (James et al., 1985; Rabinowitz et al., 1980). From studies of the uptakes of stable isotopes of lead in adults, an average absorption of 15 to 20 % may be estimated (Skerfving and Bergdahl, 2007).

Absorption of ingested soluble lead compounds appears to be higher in children than in adults (Alexander et al., 1974; Ziegler et al., 1978; Heard and Chamberlain, 1982; James et al., 1985; Rabinowitz et al., 1980), although kinetics of changes in stable isotope signatures of B-Pb in mothers and their children suggest that children aged 6 to 11 years and their mothers absorb a similar percentage of ingested lead (Gulson et al., 1997).

Studies in experimental animals provide additional evidence for an age-dependency of gastrointestinal absorption of lead. Absorption of lead, administered by oral gavage as lead acetate (6.37 mg lead/kg), was 38 % in juvenile Rhesus monkeys compared to 26 % in adult female monkeys (Pounds et al., 1978). Rat pups absorb approximately 40 to 50 times more lead via the diet than adult rats do (Aungst et al., 1981; Forbes and Reina, 1972; Kostial et al., 1978).

The presence of food decreases the absorption of water-soluble lead compounds (Blake and Mann, 1983; Blake et al., 1983; Heard and Chamberlain, 1982; James et al., 1985; Maddaloni et al., 1998; Rabinowitz et al., 1980), the reported absorption when taken with a meal varying from 3 to 21 % (average approximately 8 %). In adults, absorption of a tracer dose of lead acetate in water was approximately 63 % when ingested by fasting subjects, whereas it was only 3 % when ingested with a meal (James et al., 1985; Heard and Chamberlain, 1982). The arithmetic mean of reported estimates of absorption in fasting adults was 57 %, with reported fed/fasted ratios ranging from 0.04 to 0.2 (U.S. ATSDR, 2007, based on Blake et al., 1983; Heard and Chamberlain,1982; James et al., 1985; Rabinowitz et al., 1980).

Lead absorption in children is affected by nutritional iron status (Watson et al., 1986). A low iron intake (Cheng et al., 1998) and deficient iron status (Bárány et al., 2005) was associated with increased B-Pb. Evidence for the impact of iron deficiency on lead absorption has been provided by studies in rats, showing that iron deficiency increases lead absorption, possibly by enhancing its binding to iron binding carriers (Bannon et al., 2003; Barton et al., 1978; Morrison and Quaterman, 1987).

Dietary calcium intake appears to affect lead absorption. An inverse relationship has been observed between dietary calcium intake and B-Pb concentration in children, suggesting that children who are calcium-deficient may absorb more lead than calcium-replete children (Mahaffey et al., 1986; Ziegler et al., 1978). An effect of calcium on lead absorption is also evident in adults. In experimental studies of adults, absorption of a single dose of lead (100 to 300 □g lead chloride) was lower when the lead was ingested together with calcium carbonate (0.2 to 1 g calcium carbonate) than when the lead was ingested without additional calcium (Blake and Mann, 1983; Heard and Chamberlain, 1982). In experimental animal models, lead absorption was enhanced by dietary calcium depletion or administration of vitamin D (Mykkänen and Wasserman 1981, 1982). Although milk is a major source of calcium - and for more than a century milk has been recommended as a prophylactic for lead toxicity - it increases the uptake of lead (James et al., 1985). Lead salts and lead in milk appear to be absorbed by different mechanisms (Henning and Cooper, 1988). Lactose has a limited effect, whereas lactoferrin may cause an increase in absorption. Similar mechanisms may contribute to lead-iron and lead-calcium absorption interactions and to interactions between lead and other divalent cations such as cadmium, copper, magnesium and zinc.

Inhalation Exposure

Deposition and absorption of inhaled lead containing particles are influenced by their size and solubility. As compared to particles with lower density, lead fumes and lead-containing dusts tend to have a slightly different deposition, with a respirable fraction characterised by lower aerodynamic diameter. Particles larger than five micron are deposited on the lining fluid of trachea and bronchi, and from there they are transferred by the mucociliary transport into the pharynx and then swallowed, with possible absorption of lead from the gastrointestinal tract. Smaller particles can be deposited in the distal parts of the respiratory tract, from where they can be absorbed after extracellular dissolution or ingestion by alveolar macrophages.

The deposition and clearance from the respiratory tract have been measured in adult humans exposed to lead-bearing particles with aerodynamic diameter below 1 μm (Hursh and Mercer, 1970; Hursh et al., 1969; Morrow et al., 1980). Up to 95 % of deposited lead that is inhaled as submicron particles is absorbed (Hursh et al., 1969).

Dermal Exposure

Dermal absorption of lead compounds is generally considered to be much less than absorption by inhalation or oral routes of exposure. Dermal absorption has been estimated to be 0.06 % during normal use of lead-containing preparations (Moore et al., 1980), although few studies have provided quantitative estimates of dermal absorption of lead in humans.

### 8.1.2. Distribution

Several models have been proposed to characterise inter-compartmental lead exchange rates, retention of lead in various tissues, and relative rates of distribution amongst tissue groups, schematically presented in Figure 15.

Under steady-state conditions, lead in blood is found primarily in the red blood cells (96 to 99 %) (Bergdahl et al., 1997a, 1998, 1999; Hernandez-Avila et al., 1998; Manton et al., 2001; Schutz et al., 1996; Smith et al., 2002). At B-Pb concentrations <1.92 μM (400 μg/L), whole B-Pb levels increase linearly with serum levels. At higher B-Pb concentrations a non-linear relationship is apparent, and the serum to blood ratio increases dramatically as levels increase, due to saturation of binding in erythrocytes (WHO/IPCS, 1995). This kinetic relationship may be altered during pregnancy. From *in vitro* data (Ong and Lee, 1980), fetal haemoglobin appears to have a greater affinity for lead than adult haemoglobin.

Although the mechanisms by which lead crosses cell membranes have not been fully elucidated, results of studies in intact red blood cells and red blood cell ghosts indicate that the major pathway is likely to be an anion exchanger dependent upon $HCO_3^-$ (Simons, 1985, 1986a, 1986b, 1993). Lead and calcium may also share a permeability pathway, which might be a $Ca^{2+}$-channel (Calderon-Salinas et al., 1999). Lead is extruded from the erythrocyte by an active transport pathway, most likely a ($Ca^{2+}$, $Mg^{2+}$)-ATPase (Simons, 1988).

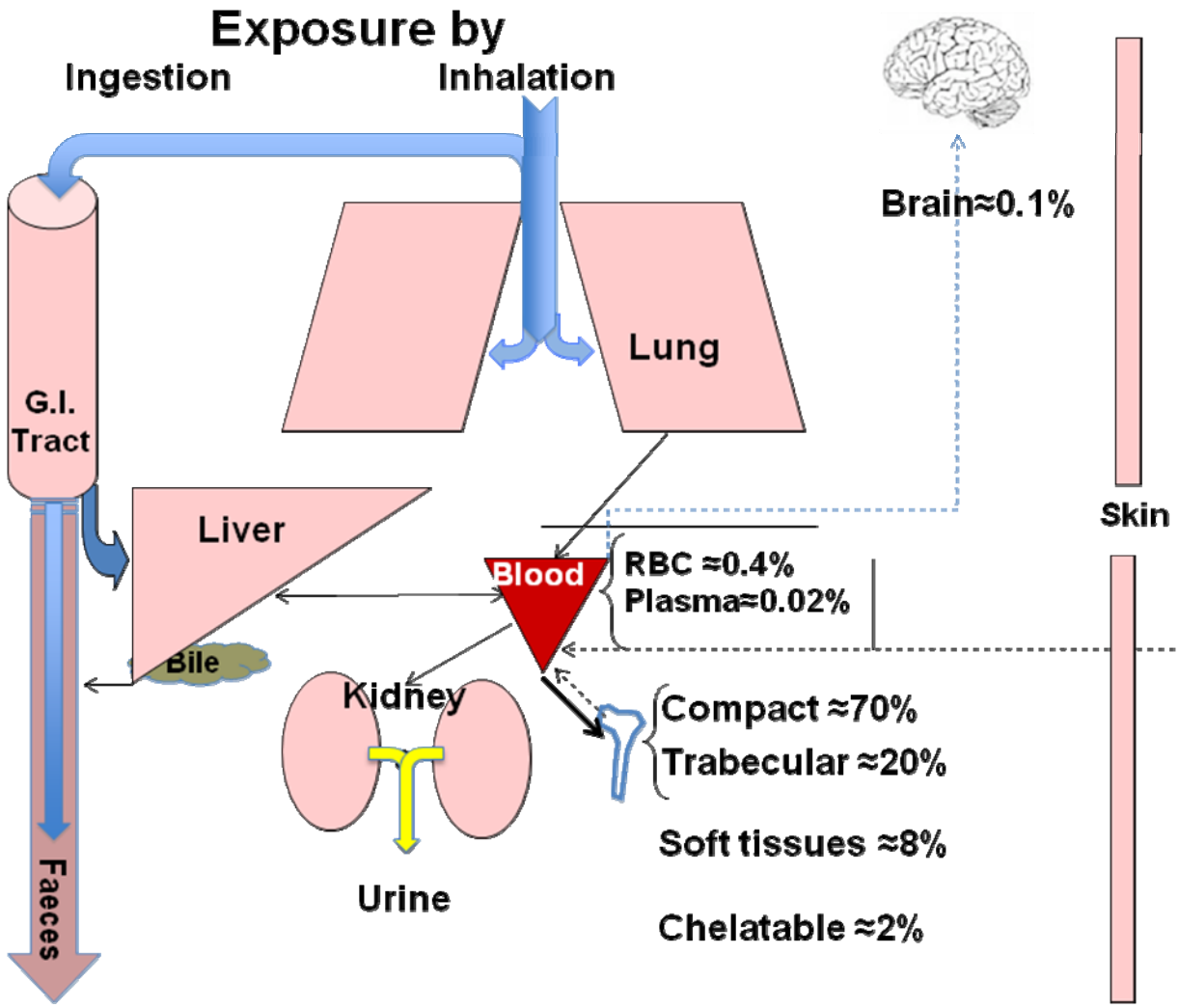



**Figure 14:** Schematic presentation of lead distribution in humans

Most of the lead found in erythrocytes is bound to proteins, the primary binding ligand being delta-aminolevulinic acid dehydratase (ALAD) (Bergdahl et al., 1997a, 1998; Sakai et al., 1982; Xie et al., 1998). Lead binding capacity of ALAD is approximately 8500 µg/L red blood cells (or approximately 400 µg/L whole blood) and the apparent dissociation constant is approximately 1.5 µg/L (Bergdahl et al., 1998). Two other lead-binding proteins have been identified in the red cell, a 45 kDa protein (Kd 5.5 µg/L) and a smaller protein(s) having a molecular weight <10 kDa (Bergdahl et al., 1996, 1997a, 1998). Of the three principal lead-binding proteins identified in red blood cells, ALAD has the strongest affinity for lead (Bergdahl et al., 1998) and appears to dominate the ligand distribution of lead (35 to 84 % of total erythrocyte lead) at B-Pb concentrations below 400 µg/L (Bergdahl et al., 1996, 1998; Sakai et al., 1982).

Lead binding inhibits the activity of ALAD (Gercken and Barnes, 1991; Sakai et al., 1982, 1983), thereby inducing its synthesis, which however might also be due to secondary accumulation of both ALA and protoporphyrin, the latter as a consequence of lead-induced inhibition of ferrochelatase (Fujita et al., 1982).

ALAD is a polymorphic enzyme with two alleles (ALAD 1 and ALAD 2) and three genotypes: ALAD 1,1, ALAD 1,2 and ALAD 2,2 (Battistuzzi et al., 1981). Higher B-Pb levels have been reported in

individuals with the ALAD 1,2 and ALAD 2,2 genotypes compared to similarly exposed individuals with the ALAD 1,1 genotype (Astrin et al., 1987; Hsieh et al., 2000, Schwartz et al., 2000; Wetmur et al., 1991). This observation has prompted the suggestion that the ALAD-2 allele may have a higher binding affinity for lead than the ALAD 1 allele (Bergdahl et al., 1997b), a difference that might alter dose-response relationships between B-Pb and lead-mediated outcomes. However, the overall impact of this polymorphism on the pharmacokinetics of lead is presently unclear.

Approximately 40 to 75 % of lead in the plasma is bound to proteins, mainly albumin, though lead also complexes to sulphydryl groups of cysteine, and other ligands, in other proteins (Al-Modhefer et al., 1991).

In human adults, approximately 90 % of the total body burden of lead is found in the bones. In contrast, bone lead accounts for only 70 % of the body burden in children but its concentration increases with age. The large pool of lead in adult bone maintains elevated B-Pb concentrations long after exogenous exposure has ended (Fleming et al., 1997; Inskip et al., 1996; Kehoe, 1987; O'Flaherty et al., 1982; Smith et al., 1996).

Lead accumulation occurs predominantly in trabecular bone during childhood, and in both cortical and trabecular bone in adulthood (Aufderheide and Wittmers, 1992). In the former, bone lead is essentially inert, having a half-life of several decades. Although a high bone formation rate in early childhood results in the rapid uptake of circulating lead into mineralizing bone, bone lead is also recycled to other tissue compartments or excreted in association with a high bone resorption rate (O'Flaherty, 1995).

In some bones (e.g. mid femur and pelvic bone), lead content decreases with aging (Drasch et al., 1987). This decrease is most pronounced in females and may be due to osteoporosis and release of lead from bone to blood (Gulson et al., 2002). During pregnancy, the mobilisation of bone lead also increases, apparently as the bone is catabolised to produce the fetal skeleton. This mobilisation of bone lead may contribute to the increase in lead concentration that has been observed during the later stages of pregnancy (Gulson et al., 1997; Lagerkvist et al., 1996; Schuhmacher et al., 1996).

Bone resorption during pregnancy can be reduced by ingestion of calcium supplements (Janakiraman et al., 2003). Additional evidence for increased mobilisation of bone lead into blood during pregnancy comes from studies in nonhuman primates and rats (Franklin et al., 1997; Maldonado-Vega et al., 1996). Kinetic changes in the stable isotope signatures of B-Pb in postpartum women indicated that the release of maternal bone lead to blood appears to accelerate during lactation (Gulson et al., 2003, 2004). Using a similar approach, increased release of bone lead to blood in women, in association with menopause has been shown (Gulson et al., 2002). These observations are consistent with epidemiological studies that have shown increases in B-Pb after menopause and in association with decreasing bone density in postmenopausal women (Berkowitz et al., 2004; Hernandez-Avila et al., 2000; Nash et al., 2004; Popovic et al., 2005).

The relative distribution of lead in soft tissues, in both males and females, expressed in terms of liver tissue concentration ratios, was: liver, 1.0 (approximately 1 µg/g wet weight); kidney cortex, 0.8; kidney medulla, 0.5; pancreas, 0.4; ovary, 0.4; spleen, 0.3; prostate, 0.2; adrenal gland, 0.2; brain, 0.1; fat, 0.1; testis, 0.08; heart, 0.07; and skeletal muscle, 0.05 (Barry, 1975; Gross et al., 1975). In contrast to lead in bone, which accumulates lead with continued exposure in adulthood, concentrations in soft tissues (e.g., liver and kidney) are relatively constant in adults (Barry, 1975; Treble and Thompson, 1997), reflecting faster turnover of lead in these tissues.

Maternal and cord B-Pb concentrations were measured at delivery in 888 mother-infant pairs; the cord/maternal ratio was relatively constant, 0.93, over a B-Pb range of approximately 30 to 400 µg/L (Graziano et al., 1990). A study of 159 mother-infant pairs also found a relatively constant cord/maternal ratio (0.84) over a maternal B-Pb range of approximately 10 to 120 µg/L (Carbone et

al., 1998). The fetal/maternal B-Pb concentration ratio is therefore, approximately 0.9 (Carbone et al., 1998; Goyer, 1990; Graziano et al., 1990). In a study of 159 mother-infant pairs, higher blood pressure and alcohol consumption late in pregnancy were associated with more lead in cord blood relative to maternal B-Pb. Higher haemoglobin levels and sickle cell trait were associated with reduced cord B-Pb relative to maternal B-Pb (Harville et al., 2005).

Maternal lead can be transferred to infants during breastfeeding. Although the breast milk/maternal blood concentration ratio is usually <0.1, values of up to 0.9 have been reported (Ettinger et al., 2006; Gulson et al., 1998). Lead in colostrum has also been found to be lower by at least one order of magnitude than in blood. Ettinger et al. (2006) found that B-Pb (mean, 80 to 90 µg/L; range 20 to 300) was a good predictor of breast milk lead (mean, 9 to 14 µg/L; range 2 to 80 µg/L), although Almeida et al. (2008) have reported that neither colostrum nor milk concentrations seem to correlate with B-Pb levels. Stable lead isotope dilution measurements in infant-mother pairs, measured as they came into equilibrium with a novel environmental lead isotope signature, suggested that lead in breast milk can contribute substantially to lead in infant blood (approximately 40 to 80 %; Gulson et al., 1998).

### 8.1.3. Excretion

The half-life of lead in blood is approximately 30 days in adult male humans, but varies with level of exposure, sex and age (Gulson, 2008).

Lead is excreted primarily in urine, most likely by passive diffusion, and faeces whilst sweat, saliva, hair and nails and breast milk are minor routes of excretion (Hursh and Suomela, 1968; Hursh et al., 1969; Kehoe, 1987; Rabinowitz et al., 1976; Stauber et al., 1994). Faecal excretion accounts for approximately one-third of total excretion of absorbed lead, and also of lead from inhalation of submicron lead particles (Hursh et al., 1969). Faecal lead includes both the unabsorbed fraction of ingested lead and the fraction of biliary excretion escaping any entero-hepatic re-circulation. The mechanisms for faecal excretion of absorbed lead have not been elucidated. However, there is evidence for lead excretion through bile (Ishihara and Matsushiro, 1986) and pancreatic juice (Ishihara et al., 1987). Possibly, the excretion in bile is in the form of a lead–glutathione complex (Alexander et al., 1986).

## 8.2. Biomarkers of exposure

### 8.2.1. Lead in blood (B-Pb)

Most of the information on human exposure to, and the health effects of, lead is based on B-Pb data. At steady-state, lead in blood is considered to be the most suitable indicator of the concentration of lead in soft tissues, and hence recent exposure. Lead in blood does not necessarily correlate with the total body burden of lead (Lauwerys and Hoet, 2001). A major advantage of this measure is the wealth of information that can be linked to B-Pb particularly the effects of low environmental exposure on central nervous system functions in children.

Lead in blood has two main pools. The short-half-life pool is in the blood and soft tissues ($t_{½}$ 20 to 40 days) and the long-half-life pool is mainly in the skeleton ($t_{½}$ in the range of 10 to 30 years). Thus, the B-Pb level reflects a combination of recent exposure and that which occurred several years previously.

B-Pb is usually determined from an analysis of venous blood. Most of the lead in blood is present in the cells. The relationship between lead uptake and B-Pb is curvilinear. At low lead uptake there is a steady linear increase in B-Pb with increasing uptake, while at high lead uptakes, the curve flattens out as binding sites in erythrocytes become saturated.

An increase of lead in ambient air by 1 µg/m$^3$ results in an increase in the steady state B-Pb level by 16.4 µg/L in adults and 19 µg/L in children (CDC, 1991). WHO/IPCS (1995) concluded that there was an increase of about 20 µg B-Pb/L per 1000 mg Pb/kg of soil or dust. It has been reported that an increase in lead intake by 1 µg/day through diet or water would result in an increase of B-Pb by 1.6 µg/L in children and 0.4 µg/L in adults (Carlisle and Wade, 1992).

There has been a significant worldwide decrease of B-Pb levels over the last twenty years, mainly due to the elimination of lead in petrol, together with other control measures. For example, in the U.S., the geometric mean B-Pb concentration in children, during subsequent NHANES II, III, IV and V phases in 1976-1980, 1988-1991, 1991-1994 and 2002 was 150 µg/L, 36 µg/L, 27 µg/L and 9 to 16 µg/L, respectively. The percentage of children with B-Pb levels higher than 100 µg/L decreased from 88.2 % to 4.4 % in less than two decades (U.S. ATSDR, 2007). In a sample of respondents in the NHANES III survey, comprising 4,394 women of child-bearing age, the geometric mean B-Pb level was 17.8 µg /L (cited in Rischitelli et al., 2006). In Sweden, the geometric mean B-Pb concentration in school children decreased from about 60 µg/L in 1978 to about 25 µg/L over a 15 year period (Gerhardsson et al., 1996).

In the German Environmental Survey on Children 2003-2006, 1,560 children aged 3 to 14 years were selected from the representative cross-sectional sample. The median B-Pb in these children was 16.3 µg/L (range from 14.6 µg/L in the 12 to 14 year old group, to 19.6 µg/L in the 3 to 5 year old group). The reference value (P95) was lowered from 50 to 35 µg/L (Schulz et al., 2009). In the Czech Republic, from 2001-2003, median B-Pb in 1,188 adults and 333 children were 33 µg/L and 31 µg/L, respectively (Batariova et al., 2006).

Recently, the time trend for lead levels was evaluated in Sweden. Concentrations in erythrocytes were determined in subgroups of the population-based MONICA surveys in 1990, 1994 and 1999 in a total of 600 men and women aged 25 to 74 years. Erythrocyte-Pb concentrations declined annually by 5 to 6 % (Wennberg et al., 2006).

Inhibition by lead of haem metabolism has been used for biomonitoring lead uptake and risk. Inhibition of ALAD activity in blood cells occurs at low lead uptake. In addition, the level of ALA in serum is a sensitive indicator of lead exposure. However, the sensitivity of ALA in serum or Zinc protoporphyrin in erythrocytes (with a threshold at about 300 µg/L B-Pb) is not sufficient to reflect the effects of current environmental levels of lead.

### 8.2.2. Lead in plasma or serum

The concentrations of lead in plasma and serum are very similar. Because of their low concentrations, determination of lead in plasma or serum has long been difficult and of doubtful accuracy, although the use of inductively coupled plasma mass spectrometry has made such analysis much more reliable. However, haemolysis in the blood sample, before the separation of plasma, will increase the apparent concentration of lead in plasma. Haemolysis of only 0.05 to 0.1 %, which may not be apparent, can increase the plasma lead concentration by 15 to 30 % (Bergdahl et al., 2006).

Although there are indications that the lead concentration in plasma or serum could be an alternative for biological monitoring, there are very few epidemiological studies in which plasma or serum lead levels have been used in exposure assessment. It is, therefore, not possible to determine whether plasma or serum lead is a better marker of exposure than B-Pb for use in risk assessment.

### 8.2.3. Lead in urine

Lead in urine reflects primarily the amount of lead recently absorbed. Since the analysis does not require blood sampling, urinary lead has been used in biological monitoring, but only to a limited

extent. Urinary lead excretion after administration of chelating agents has, however, been used as an index of risk and the body burden of lead.

There is an association between lead concentrations in urine and blood, but the variation is too large to allow prediction of an individual B-Pb concentration from a urinary lead concentration. Because of this poor association and the appreciable risk of external contamination during sampling, measuring lead in urine for the routine assessment of lead exposure is not recommended (Lauwerys and Hoet, 2001).

### 8.2.4. Bone lead

The lead concentration in bone reflects long-term uptake and the total body burden, since >90 % of the body burden of lead is in the skeleton. Lead concentration in bone can be determined *in vivo* by non-invasive methods based on X-ray fluorescence. Determinations are possible for the tibia, the calcaneus and the patella. Lead is incorporated into the teeth during their formation. Bone and teeth lead levels are attractive measures in epidemiological studies where assessments of retrospective exposures are required, even though the body of information is limited.

Shed deciduous teeth have been used to provide an index of exposure in early childhood. Interpretation of the analytical data is dependent on the type of tooth and the part of the tooth analysed (WHO, 1995).

### 8.2.5. Hair

Hair has sometimes been used for the biomonitoring of lead exposure. However, because of the potential for external contamination, it is not a useful index of uptake into the body (WHO, 2007; Lauwerys and Hoet, 2001).

## 8.3. Toxicity in experimental animals/models

The database for lead is unusual in that it contains a great deal of data concerning dose-effect relationships in humans. These data come from studies of occupationally exposed groups and the general population (U.S. ASTDR, 2007). The human database includes data on neurodevelopmental effects, mutagenicity, carcinogenicity, cardiovascular effects, renal effects, effects on the endocrine system, gastrointestinal effects, haematological effects, musculoskeletal effects, reproductive effects and developmental effects. Most of these endpoints have also been studied in experimental animals, and these studies may provide support for the human data regarding effect or mode of action. The developing nervous system has been identified as the critical target organ for lead. Studies on genotoxicity in cell systems, animals and humans indicate that lead is an indirect mutagen. In an evaluation performed by IARC it was concluded that "there is sufficient evidence in experimental animals for the carcinogenicity of lead compounds" and that "there is limited evidence in humans for the carcinogenicity of lead compounds" (IARC 2006). This chapter focuses on the neurodevelopmental, renal and cardiovascular effects, genotoxicity and carcinogenicity of lead, as these are the endpoints of greatest potential concern, either because of the nature or the sensitivity of the effect.

### 8.3.1. Acute toxicity

Lead has been described as a classic chronic poison. Health effects are generally not observed after a single exposure, and oral $LD_{50}$ values for lead salts have been reported to be greater than 2,000 mg/kg b.w. The lowest observed lethal doses in animals after multiple short-term oral exposure to lead

acetate, lead chlorate, lead nitrate, lead oleate, lead oxide or lead sulphate range from 300 to 4,000 mg/kg b.w. (JECFA, 2000).

### 8.3.2. Repeat dose and chronic toxicity

#### 8.3.2.1. Neurotoxicity and neurodevelopmental toxicity

Experimental studies, primarily with rodent and non-human primate models, have provided evidence that chronic low-level exposure to lead affects behaviour such as learning abilities, in particular in the developing animal. The magnitude of these effects appears to be strongly dependent on the developmental period in which exposure takes place (IARC, 2006).

Studies in rats and nonhuman primates have demonstrated deficits in learning associated with B-Pb concentrations between 100 and 150 µg/L, a range that is comparable to those reported in epidemiological studies, in which learning deficits were found in children (Cory-Slechta, 2003).

Deficits in reversal or repeated learning have been a consistent finding with lead exposure. These learning impairments appear to be generalised, having been reported across species and stimulus dimensions. Prenatal exposure of 8 pregnant (5 to 8.5 weeks of gestation) squirrel monkeys to lead, resulting in a maternal blood concentration between 210 µg/L and 790 µg/L showed that at blood concentrations above 400 µg/L, behavioural alterations, such as learning ability of the offspring, were affected at an age of 5 to 6 years. At lower maternal B-Pb concentrations there were still some effects on learning ability (Newland et al., 1994). In monkeys given lead at doses resulting in a blood concentration of 320 to 360 µg/L from birth, the spatial discrimination reversal task was impaired at age 7 to 8 years (Rice, 1990). Whether this reflects different critical exposure period(s) for different types of learning paradigms is not known. Studies on the impact of lead on various behavioural domains have not been carried out systematically across developmental periods of exposure, and thus the ability to define critical periods for any behavioural deficit is not possible (Cory-Slechta, 2003).

Rats were exposed to 775 mg lead/kg feed at different stages of development, and tested with respect to active-avoidance learning and hippocampal long-term potentiation. When exposure comprised the prenatal and the early postnatal period and was continued into adulthood, both processes were impaired. However, when exposure started 16 days after birth, neither learning nor hippocampal potentiation was affected. These results reflect the higher vulnerability of the developing hippocampus to lead-induced functional deficits compared with the mature hippocampus (Altmann et al., 1993).

Numerous human studies ascribe lead-related cognitive dysfunction to attention deficits. In experimental studies, discrimination reversal deficits in lead-exposed non-human primates have been interpreted as increased distractibility to irrelevant stimuli (Rice, 1985).

Clinical diagnosis of attention deficit hyperactivity disorder (ADHD) relies on three behavioural domains: inattention, hyperactivity and impulsivity (Goldman et al., 1998). Collective findings across two recent studies support the possibility, based on the clinical diagnosis domains, that impulsivity is more strongly affected by lead than sustained attention, and that impulsivity as a behavioural dysfunction could ultimately lead to cognitive impairments (Cory-Slechta, 2003). With respect to hyperactivity, reports of hyperkinetic behaviour have been inconsistent in animal models of lead exposure, with increases, decreases and no change all reported (Bornschein et al., 1980).

Two groups of 7-week old rats were given 50 mg/L sodium acetate and 50 mg/L lead acetate, respectively, in the drinking-water for 3 months. Ocular motor function was tested by rotating the animals on a platform at an increasing angular velocity and measuring ocular nystagmus when the rotation is abruptly stopped. The lead-exposed animals showed a reduction in post-rotatory nystagmus that was statistically significantly correlated with B-Pb and brain lead concentrations, while no such

alterations were observed in animals treated with sodium acetate. The results show that low concentrations of lead may impair both sensory and motor functions, and indicate that such measurements provide a screening tool for neurotoxic effects of lead even in the absence of clinical signs of lead intoxication (Mameli et al., 2001).

Five cats at an age of 12 to 28 months were stimulated with a precisely controlled electrical current via electrodes inserted into the lateral hypothalamus. The response measure was the predatory attack threshold, i.e. the current required to elicit an attack response in 50 % of the trials. Lead was mixed (as lead acetate) into cat food at doses of 50 to 150 mg/kg b.w. lead per day for 4 to 5 weeks. B-Pb concentrations were <10, 210 to 770 and <200 µg/L before, during and after lead exposure, respectively. The predatory attack threshold decreased significantly during lead exposure in three of the five cats and increased after cessation of exposure in four of the five cats ($p < 0.01$). There was a significant ($p < 0.002$) negative association between threshold current and B-Pb concentration. These data show that lead exposure enhances predatory aggression in cats (Li et al., 2003).

Results of behavioural tests performed primarily in rats and monkeys exposed to lead have suggested that the impaired performance is the result, at least in part, of a combination of distractibility, inability to inhibit inappropriate responding, and perseverance of behaviours that are no longer appropriate (U.S. ATSDR, 2007).

#### 8.3.2.2. Cardiovascular effects

A number of animal experiments have suggested a biphasic response of blood pressure to lead exposure (Victery et al., 1982; Victery, 1988). Evis et al. (1985) reported that prolonged (3 or 12 months) low-level exposure of spontaneously hypertensive rats to lead (25 mg/L as lead acetate in the drinking-water) enhanced the susceptibility of the heart to ischaemia-induced arrhythmias at 3 but not at 12 months, in the absence of any effect on blood pressure. In contrast, subchronic (3 months) high-level exposure of these rats to lead (250 or 1,000 mg/L in the drinking-water) resulted in only slightly enhanced susceptibility of the heart to arrhythmias induced by myocardial ischaemia. Both doses of lead accelerated the development of high blood pressure and in normotensive rats the higher dose also resulted in an elevated blood pressure (Evis et al., 1987). A consistent and significantly higher systolic blood pressure (SBP) was seen in all dosed groups of 15 to 18 female weanling Long-Evans rats fed a diet low in lead and exposed to lead at 0.1, 1.0 or 5.0 mg/L in the drinking water for 3 months, 6 months or 1 year compared to control (Perry et al., 1988). This was confirmed in a study where groups of ten albino rats were given lead at 25, 50 or 1,000 mg/L in the drinking water. After 90 days the blood pressure was measured in five rats per group and the remaining five were terminated for histopathological and histochemical studies on heart tissue. The increase in arterial blood pressure was statistically significant only in the two highest dose groups. In this study lead increased calcium influx in atrial trabeculae and papillary muscles. It was suggested that the mechanism is related to the ability of lead to alter calcium transport processes (Lai et al., 1991).

#### 8.3.2.3. Nephrotoxicity

Chronic intoxication with lead is associated with the presence of characteristic intranuclear inclusions in proximal tubular epithelial cells of the kidney. Lead-induced formation of nuclear inclusion bodies has been observed in kidneys of rabbits, rats (Six and Goyer, 1970; Choie and Richter, 1972a, b), monkeys (Allen et al., 1974) and dogs (Stowe et al., 1973).

Experimental models of lead nephropathy were developed in male Sprague-Dawley rats fed a low calcium diet (Khalil-Manesh et al, 1992, 1993). Lead acetate was used in concentrations of 0.5 % (high dose) and 0.01 % (low dose) in drinking water for periods from 1 to 12 months and lead-exposed animals were compared to pair-fed control rats. Animals treated with 0.5 % reached a maximum B-Pb of 1,254 ± 101 µg/L after 6 months, when lead acetate was reduced from 0.5 to 0.1 % (Khalil-Manesh

et al, 1992). B-Pb in these animals at 12 months averaged 550 µg/L. In lead-treated rats, Glomerular Filtration Rate (GFR) was increased at 3 months (1.00 ± 0.14 vs.0.83 ± 0.26 ml/min/ 100 g b.w., P=0.05), then declined after 6 months (0.78 ±0.16 ml/min/100 g b.w. vs.0.96 ± 0.08). At 6 months, focal tubular atrophy and interstitial fibrosis appeared, increasing in extent up to 12 months. Glomeruli at 12 months showed focal and segmental sclerosis.

In the second study (Khalil-Manesh et al, 1993) the course of events was examined over 12 months in continuous low level lead-exposed animals. Maximum B-Pb levels in these animals were reached at three months, averaging 294 ± 41 µg/L. GFR was statistically significantly increased above that in pair-fed controls at 1 and 3 months, but was normal at other time points. There were no other pathological alterations in the kidneys up to 12 months, when mild tubular atrophy and interstitial fibrosis were seen. It should be noted that both low dose lead-treated and high dose lead-treated animals showed a "hyperfiltration" effect during the first 3 months of lead exposure.

#### 8.3.2.4. Genotoxicity

Many tests *in vivo* as well as *in vitro* have been used to investigate the genotoxicity of lead. In bacterial systems only lead chromate and lead bromide are mutagenic but the active moiety in these tests is probably chromate or bromide, respectively. In cell-free systems an increase in DNA strand breaks was observed in plasmid DNA. It was suggested that this was due to a Fenton-like reaction, involving reduction of $Pb^{2+}$ to $Pb^{1+}$ or lead oxygen or lead-peroxide complexes (IARC, 2006).

Several tests have been performed in mammalian cells with lead chromate. In these studies it cannot be excluded that the observed effects were caused by chromate. With respect to the induction of chromosomal aberrations by lead acetate, treatment of human leukocytes showed clearly elevated frequencies of achromatic lesions, chromatid breaks and isochromatid breaks in 72-h cultures but not 48-h cultures (Beek and Obe, 1974). Other studies with lead nitrate and lead glutamate were mostly negative. Concerning the induction of micronuclei, a dose-dependent increase starting at concentrations of 1.1 µM lead chloride or 0.05 µM lead acetate has been reported (Elias et al., 1989; Sidhu et al., 1991). Both positive and negative results have been reported for the induction of sister chromatid exchange (IARC, 2006).

The induction of DNA damage in mammalian cells by lead acetate and lead nitrate has been investigated in a number of studies, yielding negative or (mostly weakly) positive results for DNA strand breaks; one study found no 8-hydroxy-2′-deoxyguanosine (oxo8dG) in nuclear DNA, and one suggested the induction of DNA–protein crosslinks (IARC, 2006).

*In vivo,* DNA damage was assessed using the Comet assay in cells isolated from the kidneys of male rats. The Comet tail length and the number of sister chromatid exchanges were increased in rats dosed with lead (IARC, 2006). When mice were exposed for three generations to lead acetate in the drinking-water, Comet tail length increased in blood cells in the F1 and F2 generations, but not in the dams (Yuan and Tang, 2001). Increased frequencies of chromosomal aberrations (gaps and fragments) and enhanced aneuploidy were seen in lymphocytes of monkeys given lead acetate orally or by intubation in one study (Deknudt et al., 1977) but not in another (Jacquet and Tachon, 1981). In a single *in-vivo* mutagenesis study, lead chloride in the drinking-water had no effect in the dominant lethal assay in mice (Kristensen et al., 1993).

In conclusion, the data on genotoxicity indicate that lead may be a weak indirect genotoxin.

#### 8.3.2.5. Carcinogenicity

Oral exposure to lead acetate has been shown to be carcinogenic in the rat kidney in several studies, producing adenomas and adenocarcinomas after chronic exposure in males and females. The doses in

these studies were high compared to human intake. In three of these studies the lowest dose was 3 or 5 mg lead/kg feed and in the remaining studies the doses was either 500 or 1,000 ppm in the feed equivalent to about 20 or 40 mg/kg b.w. per day for adult rats. In the two studies suitable for the purpose, i.e. there were more than two dose groups, a dose-response relationship was demonstrable. In a study where rats were given lead acetate in the feed to achieve a daily dose of 3 mg per animal for two months followed by a daily dose of 4 mg lead per animal for 16 months, tumours were found in several organs, with a statistically significant increase in the incidence of tumours of the adrenal gland, testes and prostate in males and adrenal gland in females (IARC, 2006). In another study, of a mixed population of male and female rats, oral exposure to 3 mg lead acetate/rat per day was associated with tumours of the lung, pituitary, prostate, mammary gland and adrenal gland (IARC, 2006). In a study where primigravid mice (20 in each group) were given lead acetate in doses of 0, 500, 750 and 1,000 mg/L in the drinking water during gestation and lactation, renal tumours were observed in the offspring. The kidney tissue adjacent to the tumours appeared to be normal in most cases, or had only a mild degree of aging nephropathy similar to that of control mice (Waalkes et al., 1995). A study in metallothionein double knockout mice showed that these mice are more susceptible to lead induced kidney carcinogenesis than wild type mice. Renal lead-containing nuclear inclusion bodies were frequently observed in wild type mice but did not form in metallothionein-null mice. Metallothionein was often found associated with the outer portion of these inclusion bodies. Thus, the metallothionein-null mice cannot form renal inclusion bodies, even after protracted lead exposure, and this increases the carcinogenic potential of lead. Poor production of metallothionein may predispose some individuals to lead carcinogenicity (Waalkes et al., 2004). Brain gliomas were observed after oral exposure to lead acetate in rats in two separate studies (dose 3 mg lead/rat per day). Also, lead subacetate induced renal cancer in rats and mice after oral administration in doses of 0.1 % in the feed. In one study, hamsters exposed orally to lead subacetate did not develop tumours. In four separate studies, injection of lead phosphate subcutaneously, or combined subcutaneously and intraperitoneally, was shown to produce renal cancers in rats. Three experiments showed that oral exposure to lead subacetate enhanced N-ethyl-N-hydroxyethylnitrosamine-induced renal carcinogenesis in male rats. Oral exposure to lead nitrate increased the incidence of N-nitrosodimethylamine-induced renal tumours in male rats while intraperitoneal injections of lead subacetate enhanced N-nitrosodimethylamine-induced lung tumour multiplicity in mice (IARC, 2006).

Overall, extensive experimental evidence shows that various water-soluble and –insoluble lead compounds in high doses can induce tumours at different sites in rodents. In addition, one study showed that renal tumours may occur with minimal lead-induced nephropathy (Waalkes et al., 1995). It is also noteworthy that brain gliomas, which are rarely spontaneous, were induced after oral exposure to lead in rats (IARC, 2006). Lead proved to be a renal tumour carcinogen/promoter in rats and mice exposed to various organic renal carcinogens. As lead is not a direct acting genotoxin and the doses used to induce tumours in the rodent experiments are very high compared to human intake the CONTAM Panel considered human exposure to lead through food unlikely to represent a significant cancer risk.

## 8.4. Mechanisms of action

### 8.4.1. Introduction

Epidemiological studies suggest that the system most sensitive to inorganic lead toxicity in the intact organism is the developing nervous system. Accordingly, most attention will be devoted to the possible mechanisms by which the nervous system may be affected. However, other systems are also susceptible to damage by inorganic lead and this damage may impact indirectly on the nervous system. Effects on other systems will, therefore, be reviewed briefly. At the molecular level, many toxic effects of lead can be attributed to the affinity of lead for thiol groups (-SH) (Vallee and Ulmer, 1972)

and other organic ligands in proteins. Further, the ability of lead to substitute for calcium (and perhaps zinc (Bressler and Goldstein, 1991)) is a factor common to many of its toxic actions.

### 8.4.2. Neurotoxic effects

The following summary of mechanisms related to neurotoxicity in humans has been taken from a number of recent expert reviews (Bouton and Pevsner, 2000; Bressler et al., 1999; Cory-Slechta, 1995, 2003; Gilbert and Lasley, 2002; Lasley and Gilbert, 2000; Nihei and Guilarte, 2002; Suszkiw, 2004; Toscano and Guilarte, 2005; Verina et al., 2007; Zawia et al., 2000; 2009).

Among the most important ways that inorganic lead can affect the nervous system are those involving interference with calcium-dependent reactions and (or) disruption of calcium homeostasis. One process that has been studied in detail is the activation of protein kinase C (PKC). Picomolar concentrations of lead activate preparations of PKC *in vitro* (Markovac and Goldstein, 1988). The PKC family is made up of 12 isozymes, each with different enzymatic cofactor requirements, tissue expression, and cellular distributions. The gamma-isoform is one of several calcium ion dependent forms of PKC that is a likely target for lead neurotoxicity; it is neurone-specific and is thought to be involved in spatial learning, and memory processes. It has also been suggested that lead-induced increase in neuronal calcium levels may cause an excessive calcium influx into mitochondria, resulting in the production of free radicals and in the opening of the membrane transition pores (Sidhu and Nehru, 2003), thus damaging the neurones.

The particular vulnerability of the fetus and infant to the neurotoxicity of lead may be due in part to immaturity of the blood-brain barrier and to the lack of the high-affinity lead binding protein in astroglia that enables them to trap divalent lead ions in adults (Lindahl et al., 1999). In addition, other membrane changes may affect the blood-brain barrier and brain cells. Lead has been found to accumulate in brain myelin of rats and myelin membrane fluidity was higher in such rats than in controls (Dabrowska-Bouta et al., 1999). Myelin from lead intoxicated animals showed gross morphological alterations, and altered composition (higher phosphatidylethanolamine content and altered glycoproteins) when compared to the controls (Dabrowska-Bouta et al., 1999, 2008). It seems likely that similar changes may occur in human brain. Further, Adonaylo and Oteiza (1999) have shown from studies on liposomes that lead may cause lipid rearrangement in the lateral phase of the phospholipid bilayer of the cell membrane. Exposure to lead causes lipid clustering in liposomes and increases the rate of iron-initiated lipid oxidation and consequent membrane damage.

The dopaminergic system has a role in aspects of cognitive function since lesions of dopaminergic neurones impair performance of various learning and cognitive tasks. There is evidence that suggests that lead may affect regulation of dopamine synthesis and release, indicating a presynaptic site of action (Cory-Slechta, 1995).

The cholinergic system plays a role in learning and memory processes. In general, it is clear that lead blocks the evoked release of acetylcholine and diminishes cholinergic function (Cooper et al., 1984; Silbergeld, 1977; Shih and Hanin, 1978; Suszkiw et al., 1984). This has been demonstrated in central and peripheral synapses. Studies with the neuromuscular junction have shown that lead reduces acetylcholine release by blocking calcium entry into the terminal.

Chronic exposure of rats to lead resulted in decreased muscarinic-receptor expression in the hippocampus. Whether lead exposure during development alters muscarinic receptor sensitivity is unclear as there are reports with conflicting results. The preponderance of the binding data suggests that lead does not directly affect muscarinic receptors except in the visual cortex, where lead may have a direct inhibitory effect on muscarinic receptors in the retina (Costa and Fox, 1983).

Reports on lead-exposed workers and experimental animals have shown that lead also causes changes in the hypothalamic-pituitary axis which in turn has been found to affect thyroid, adrenal and gonadal

function (reviewed by Doumouchtsis et al., 2009). Lead-induced changes in both the response to neurotransmitters and hormone production and response have been observed, with the most consistent observations reported being elevated circulating levels of prolactin and decreased circulating levels of growth hormone releasing hormone, growth hormone and insulin-like growth factor-1 (Doumouchtsis et al., 2009). These neuroendocrine changes have been linked to lead-induced suppression of growth, fertility and skeletal disorders.

Bolin et al. (2006) have reported that developmental exposure to lead increases levels of the Alzheimer's disease (AD) related β-amyloid peptide (Aβ), known to generate reactive oxygen species (ROS) in the ageing brain. This study measured the lifetime cerebral oxo8dG levels and the activity of the DNA repair enzyme 8-oxoguanine DNA glycosylase (Ogg1) in rats developmentally exposed to lead. Oxo8dG was transiently modulated early in life (Postnatal day 5), but was later elevated 20 months after exposure to lead had ceased, while Ogg1 activity was not altered. An age-dependent loss in the inverse correlation between Ogg1 activity and oxo8dG accumulation was also observed. There was no effect of lead on oxo8dG levels when animals were exposed to lead in old age. Wu et al., (2008a,b) reported that the expression of AD-related genes [APP, BACE1 (beta-site APP cleaving enzyme 1)] as well as their transcriptional regulator (Sp1) were elevated in aged (23-year-old) monkeys exposed to lead as infants. Developmental exposure to lead also altered the levels, characteristics, and intracellular distribution of amyloid beta staining and amyloid plaques in the frontal association cortex.

In relation to AD, a target for lead is the 78-kDa molecular chaperone glucose-regulated protein (GRP78) (White et al., 2007). GRP78 chaperones the secretion of the cytokine interleukin-6 (IL-6) by astrocytes. *In vitro* evidence shows that lead strongly binds to GRP78, induces GRP78 aggregation, and blocks IL-6 secretion by astroglial cells. These findings provide evidence for a significant chaperone deficiency in lead-exposed astrocytes in culture. Chaperone deficiency could contribute to protein conformational diseases such as AD.

### 8.4.3. Cardiovascular effects

Increased blood pressure is observed with chronic exposure to lead (Carmignani et al., 2000; Ni et al., 2004; Vaziri and Sica 2004). Lead exerts direct constrictive effects on vascular smooth muscle. These effects may be mediated by inhibition or Na-K-ATPase activity and associated elevation of intracellular $Ca^{2+}$ levels, possibly with activation of protein kinase C (Hwang et al., 2001; Kramer et al., 1986; Piccinini et al., 1977; Watts et al., 1995). Lead-induced hypertension is accompanied by depletion of nitric oxide (NO), which plays an important role in regulating blood pressure, through peripheral (i.e., vasodilation, natriuresis) and central (anti-sympathetic) mechanisms (Gonick et al., 1997; Vaziri et al., 1997; Vaziri, 2008).

### 8.4.4. Renal effects

A characteristic histological feature of lead nephrotoxicity is the formation of intranuclear inclusion bodies in the renal proximal tubule (Goyer et al., 1970a, b). Inclusion bodies contain lead complexed with protein (Moore et al., 1973). The toxicological consequences of the formation of inclusion bodies are not clear, but it may reflect a disposition mechanism, involving metallothionein. Cytosolic proteins can serve as carriers of lead or intermediary ligands for uptake of lead into the nucleus. These proteins can also participate in ligand exchange reactions with other cytosolic binding sites, including δ-aminolevulinic dehydratase, which binds to lead and is inhibited by it (Goering and Fowler, 1985). Other high-affinity lead-binding proteins (affinity constant (Kd) approximately 14 nM) have been isolated from human kidney (Smith et al., 1998).

Characteristic of lead-induced nephropathy is the occurrence of structural abnormalities of mitochondria of the renal proximal tubule cells (Fowler et al., 1980; Oskarsson and Fowler, 1985).

Mitochondria isolated from lead intoxicated rats contain lead, principally associated with the intramembrane space or bound to the inner and outer membranes. Such mitochondria also show abnormal respiratory function, including decreased respiratory control ratio during pyruvate/malate- or succinate-mediated respiration (Fowler et al., 1980; Oskarsson and Fowler, 1985). The mitochondrial effects may be the result of membrane changes similar to those described in the section on neurotoxic effects.

### 8.4.5. Haematological effects

The haematological effects of lead can result in increased urinary levels of porphyrins, coproporphyrins, ALA, erythrocyte protoporphyrin (EP), free erythrocyte protoporphyrin (FEP) and zinc protoporphyrin (ZPP) (U.S. EPA, 1986). The most serious haematological effect is anaemia. Lead interferes with haem biosynthesis by altering the activity of three enzymes: δ-aminolevulinic acid synthetase (ALAS), δ-aminolaevulinic acid dehydratase (ALAD) and ferrochelatase. Lead indirectly stimulates the mitochondrial enzyme ALAS, which catalyses the condensation of glycine and succinyl-coenzyme A to form ALA. The activity of ALAS is the rate-limiting step in haem biosynthesis; increase of ALAS activity occurs through feedback derepression.
Lead inhibits noncompetitively the zinc-containing cytosolic enzyme ALAD, which catalyzes the condensation of two units of ALA to form porphobilinogen. Lead also inhibits noncompetitively the activity of the zinc-containing mitochondrial enzyme ferrochelatase, which catalyzes the insertion of iron(II) into the protoporphyrin ring to form haem (U.S. EPA, 1986; Goering, 1993). It has been postulated that metals bind to thiol groups of allosteric sites and, according to their structure, provoke allosteric transitions to the active or inactive form of the enzymes (Bernard and Lauwerys, 1987). As a result of the inhibition of ALAD and ferrochelatase, there is increased production and excretion of the precursors ALA and coproporphyrin (COPRO) with increased circulatory protoporphyrin (PROTO) usually bound to zinc. Diminished synthesis of haem-containing monooxygenases (cytochromes P450) may reduce oxidation of xenobiotics.

Inherited dysfunctions in haem biosynthetic enzymes give rise to a number of conditions that are referred to as the porphyrias (Warren et al., 1998). Although most of these conditions are inherited in an autosomal dominant fashion and display only partial penetrance, hereditary dysfunctions in ALAD are recessive and are extremely rare (Kappas et al., 1995). ALAD porphyria (or Doss porphyria) is characterised by excessive accumulation of ALA and acute attacks of severe pain (Doss et al., 1979). Individuals who inherit this condition, as well as their carrier parents and other heterozygotes for ALAD deficiency, show greater sensitivity to the porphyriogenic effects of environmental and dietary lead.

Lead interference with haem synthesis causes a reduction in haemoglobin concentration in blood. Decreased haemoglobin production, coupled with an increase in erythrocyte destruction, results in a hypochromic, normocytic anaemia with associated reticulocytosis.

### 8.4.6. Genotoxicity and carcinogenicity

The main genotoxic mechanisms of lead at non-cytotoxic concentrations demonstrated to date are: (1) those involving ROS; and (2) interference with DNA repair processes. It has been shown in many systems that exposure to lead results in altered levels of a number of types of ROS. The mechanisms by which this can occur include inhibition of antioxidant defence systems, catalysis of Fenton-type reactions and via accumulation of ALA. Nucleotide excision repair has been shown to be blocked by exposure to lead. Such inhibition would be expected to enhance the mutagenicity of agents such as polycyclic aromatic hydrocarbons, UV and other agents causing bulky lesions in DNA. The co-mutagenicity of lead with UV and with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) is consistent with the hypothesis that both nucleotide excision repair and base excision repair are affected by lead.

Another mechanism that may be relevant for the carcinogenesis of lead is its ability to alter gene expression. One pathway by which this could occur is via activation of PKC, which occurs at low concentrations of lead. PKC activation starts a signalling pathway that leads to upregulation of 'immediate early response' genes, which ultimately results in a proliferative response (IARC, 2006). The chemical similarity between lead and zinc may explain several effects related to its carcinogenic mechanism. Zinc-binding transcription-regulating proteins act by binding to specific recognition elements in DNA. The replacement of zinc by lead in these proteins may result in altered transcription of e.g. oncogenes or tumour suppressor genes. It has also been suggested that displacement of zinc in the tumour suppressor protein p53 may result in a structurally altered form of the protein with functional consequences similar to those caused by mutation or deletion of the p53 gene (Silbergeld et al., 2000).

## 8.5. Observations in humans

Human lead toxicity has been reviewed in recent risk assessments (CDC, 2005; U.S. ATSDR, 2007) and the present evaluation will therefore focus on the main target organs and recent scientific evidence. EFSA is aware that lead will be evaluated by JECFA in 2010. In accordance with the IARC (2006) classification of lead as a class 2A carcinogen (insufficient evidence of human carcinogenicity), cancer epidemiology will not be considered here.

### 8.5.1. Acute effects

Colic is a typical early symptom of lead poisoning, especially in occupational exposure or other high-level intakes of lead. The most prominent symptoms are abdominal pain, constipation, nausea, vomiting and anorexia (U.S. ATSDR, 2007). Children are particularly prone to develop toxic encephalopathy at high-level acute exposures. However, due to the long elimination half-life of lead in the body, chronic toxicity is a much greater risk.

### 8.5.2. Chronic effects

Early studies of occupationally exposed groups suggested increased mortality due to renal disease and cardiovascular disease (U.S. ATSDR, 2007). Although these outcomes are less relevant at lower occupational exposure levels, recent studies have also reported excess mortality due to cardiovascular disease at current background exposure levels. In addition, there is increasing concern about non-fatal effects, in particular neurotoxicity and cardiovascular effects.

#### 8.5.2.1. Neurotoxicity

The nervous system is the main target organ for lead toxicity. Emphasis in occupational medicine initially was on encephalopathy, seizures, paraesthesias and other serious signs and symptoms that revealed clinical lead poisoning. Following pioneering studies in Finland by Hänninen (1988), the existence of subclinical neurotoxicity was documented. This syndrome consisted of vague and non-specific symptoms (such as irritability, fatigue and headache), decreased peripheral nerve conduction velocity and deficits on neuropsychological tests of attention, memory, motor function and other functions (U.S. ASTDR, 2007). A substantial number of studies have been carried out using a variety of methods and study designs in which exposure assessment has often relied on the concurrent[23] B-Pb concentration.

[23] Unless specified otherwise, concurrent B-Pb refers to the B-Pb at the time when the clinical tests were performed, i.e. IQ, BP, CKF.

Among recent studies that have identified lead neurotoxicity at lower exposure levels, Schwartz et al. (2005) conducted a longitudinal study of lead-exposed workers using lead concentrations both in blood and in the tibia. The results showed consistent associations with current neurobehavioural test scores and also with declines in test scores over time, especially for executive abilities, manual dexterity and peripheral vibration threshold.

Meyer-Baron and Seeber (2000) conducted a meta-analysis of studies involving lead exposures corresponding to B-Pb concentrations below 700 µg/L. Among 22 studies that met the minimum requirements for inclusion, twelve provided data suitable for joint analysis of 13 test outcomes. The strongest associations were seen with central information processing, especially for visuospatial organisation and short-term verbal memory, and for manual dexterity. The decreased performance associated with lead exposure in the adult workers was comparable to effects caused by aging by up to 20 years.

While the main source of evidence on neurotoxicity is from adults exposed to lead at work, some recent studies have examined subjects from the general population in the post-leaded-petrol era, where lead exposures have declined. Using the NHANES III data on adults of varying ages, Krieg et al. (2005) failed to detect any association between the concurrent B-Pb concentration (geometric mean, 25.1 µg/L; range 7 to 418 µg/L) and the performance on three computer-assisted neurobehavioural tests, i.e. simple reaction time, symbol-digit substitution, and serial-digit learning, after adjusting for covariates.

In a study in women above 65 years, lead-associated cognitive deficits in the absence of occupational lead exposure were investigated by Moldoon et al. (1996) who showed that, after adjustment for covariates, a B-Pb concentration above 70 µg/L was associated with a deficit in attention, psychomotor speed, manual dexterity and mental flexibility. However, these findings were statistically significant only in the larger group of rural women (N = 325), but not in the city residents (N = 205).

In agreement with this study, a study of 141 male participants in the Normative Aging Study showed a decline in similar cognitive functions associated with increasing B-Pb, and also with increases in lead concentrations in tibia bone (Payton et al., 1998). More recent studies of larger numbers of participants from the same study cohort reported that patellar lead was significantly associated with psychiatric symptoms, such as anxiety, depression and phobic anxiety (Rhodes et al., 2003), and with poor test performance (Weisskopf et al., 2004). The mean B-Pb concentration was 45 µg/L.

These findings are in agreement with another study from the US (Shih et al., 2006), which reported that tibia lead levels were negatively associated with performance on cognitive tests after adjustment for confounders, although no association was apparent for the concurrent B-Pb concentration. The mean tibia lead concentration in this study was 18.7 µg/g, and the B-Pb concentration averaged 35 µg/L. These authors suggested that a proportion of what is commonly termed normal age-related decrements in cognitive function could be attributable to neurotoxicants such as lead. In agreement with this notion, Weisskopf et al. (2007) found an association between lead exposure and the extent of functional decline with age, although there was little evidence of associations on a cross-sectional basis. In a review of 21 studies (Shih et al. 2007) that compared associations of recent (in blood) and cumulative (in bone) lead levels with neurobehavioural outcomes, it was found that associations with biomarkers of cumulative exposure (mainly lead in tibia) were stronger and more consistent than associations with B-Pb levels. However, in currently exposed workers, associations were generally more apparent with B-Pb levels.

***Neurotoxicity in adults.*** The CONTAM Panel examined available information on effects of lead on the developed brain in adults. For this, the recent overview of Murata et al. (2009) was identified as the most informative source. Aimed on clarification of "whether the critical level of B-Pb actually differs between adults and children" these authors provided the results of a BMD analysis of neuromotor function and neurophysiological endpoints including postural stability and maximal motor

nerve conduction velocity (MCV), which were considered by the CONTAM Panel as relevant endpoints for assessing the effects of lead in adults. An association between increased concurrent B-Pb concentrations and changes in neuromotor function and MCV has been reported in adults by Iwata et al. (2005) and by Araki and Honma (1976) and Seppäläinen et al. (1979), respectively. Murata et al. (2009) derived from increased postural sagittal and transversal sway in 121 lead exposed workers (with B-Pb levels ranging between 60 and 890 µg/L) $BMDL_{05}$ values ranging between 121 and 169 µg/L calculated from six sway parameters, with a sample number weighted mean $BMDL_{05}$ of 143 µg/L. These authors used for their BMD analysis the so-called hybrid approach, in which continuous data are transformed to quantal data on the basis of a cut-off value, a procedure not recommended by the Scientific Committee of EFSA (EFSA, 2009). The cut-off values in that analysis, defined through an estimated 95$^{th}$ percentile of the unexposed persons were determined from the data available from the studies for which the BMD analysis was applied. Using the same procedure, Murata and colleagues derived $BMDL_{05}$ values for two MCV parameters, median MCV (two studies) and posterior tibial MCV (one study), which ranged between 75 and 84 µg/L, with a sample number weighted mean $BMDL_{05}$ of 80 µg/L, based on a total of 150 occupationally exposed adults (n=37-38 from Araki and Honma (1976) and n=112 from Seppäläinen et al. (1979)). The B-Pb levels in these studies on MCV ranged between 20 and 730 µg/L. Notwithstanding methodological concerns on the use of the hybrid approach, the CONTAM Panel did not use these results for risk assessment since the exposure in adults was about twice that observed in the neurodevelopmental studies in children. Likewise, the BMDLs for effects on kidney and SBP were lower, and hence, these were considered the critical effects in adults (see below).

***Neurodevelopmental effects.*** The developing brain seems to be more vulnerable to lead exposure than the mature brain. This finding is in accordance with other evidence on neurotoxicant exposure and the susceptibility of brain developmental processes (Grandjean and Landrigan, 2006). The characteristics of developmental lead toxicity have been well documented. Encephalopathy with seizures may occur in children at B-Pb concentrations much below those that will induce similar effects in adults. Further, decreased nerve conduction velocity and cognitive deficits have been shown at lower B-Pb concentrations in children than in adults (U.S. ATSDR, 2007).

A wide range of neurobehavioural tests has been applied to assess the influence of lead exposure on CNS functions. However, the most consistently used end-point of cognitive ability assessed in such studies is that of general intelligence (IQ). Intelligence tests employ a variety of tasks probing cognitive abilities including memory, verbal and spatial reasoning, planning, learning, and the comprehension and use of language. The child's raw IQ score is mathematically transformed to provide a rank of that score in the IQ standardisation sample (based on a Gaussian distribution). The IQ distribution is normalised to have a mean of 100 and a standard deviation of (typically) 15. As scores on IQ tests have been increasing by about 3 to 5 points per decade (the so-called Flynn effect), IQ tests are routinely re-normalised.

For example, the Wechsler Intelligence Scale for Children (WISC) was re-normalised in 1974 (WISC-R), 1991 (WISC-III) and 2003 (WISC-IV) – at each re-normalisation, the actual tests used undergo revised administration and scoring, the materials updated, and new tests introduced in place of older tests.

IQ tests can be influenced by anxiety, unfamiliarity with materials and testing procedure, the value placed by the "culture" on academic abilities, and the rapport established with the tester. In general, IQ tests are reasonably reliable and the high correlations between various IQ tests (range: 0.70 to 0.80) is logical given they were designed to provide broadly similar scores by virtue of the ranking process. Nevertheless, differences in the skills required by different IQ tests (e.g. WISC-R vs. WISC-IV) render some sub-tests more sensitive (or resistant) to impairment than others. It should be noted that this is especially true when impairments of function are suspected because the impaired function may be under or over represented in the IQ test and therefore in the IQ score obtained. This makes it difficult to compare studies using different IQ instruments because the instruments do not attempt to

equate to each other on the details of the tests – the emphasis is on ranking the individual within a population.

It is highly unlikely that childhood lead exposure "impairs" intelligence. Rather, any impairment measured by IQ scores most likely arises due to the particular constellation of cognitive functions the individual tests required (e.g., use of language, attention, or speed of performance). Given this, and the fact that a child's IQ score will reflect a combination of spared and impaired abilities, an overall IQ score may underestimate (by being non-specific) the child's difficulties.

Negative associations between B-Pb and psychometric performance have been reported in several prospective and cross-sectional studies of children. Based on evidence from cross-sectional and prospective studies available in the mid-1990s, the size of apparent IQ effect (at ages 4 and above) was a deficit of between 0 and 5 points (on a scale of 100 with a standard deviation of 15) for each 100 µg/L increment in the B-Pb concentration, with a likely apparent effect size of between 1 to 3 points (WHO/IPCS, 1995).

**Table 30:** Characteristics of 1,333 children in seven cohort studies of environmental lead exposure and IQ of the pooled analysis of Lanphear et al. (2005). Geometric means ($5^{th}$ - $95^{th}$ percentiles) of B-Pb (in µg/L) are reported. The last two lines report the number of children (% relative to the sample size of the respective cohort) with B-Pb levels lower than 100 µg/L and lower than 75 µg/L, respectively.

| Characteristic | Boston (n=116) | Cincinnati (n=221) | Cleveland (n=160) | Mexico (n=99) | Port Pirie (n=324) | Rochester (n=182) | Yugoslavia (n=231) |
|---|---|---|---|---|---|---|---|
| IQ test | WISC-R | WISC-R | WPPSI | WISC-S | WISC-R | WPPSI | WISC-III |
| IQ score | 116.0 ± 14.2 | 87.0 ± 11.4 | 86.7 ± 16.2 | 107.8 ± 11.0 | 106.0 ± 13.7 | 84.9 ± 14.4 | 74.2 ± 13.3 |
| Concurrent B-Pb | 54 (8-127) | 75 (35-200) | 142 (70-285) | 70 (30-165) | 130 (60-240) | 40 (15-120) | 159 (47-478) |
| Peak B-Pb | 120 (54-270) | 179 (90-380) | 180 (90-340) | 150 (60-400) | 270 (150-460) | 90 (35-233) | 238 (76-615) |
| Early children B-Pb | 81 (33-180) | 120 (66-266) | 134 (79-248) | 114 (43-268) | 205 (110-333) | 58 (24-131) | 141 (56-493) |
| Lifetime mean B-Pb | 76 (36-152) | 117 (58-249) | 145 (81-253) | 106 (45-213) | 186 (108-302) | 55 (24-128) | 158 (56-493) |
| Peak B-Pb <100 µg/L | 41 (35.3) | 23 (10.4) | 11 (6.9) | 20 (20.2) | 0 (0.0) | 103 (56.6) | 46 (19.9) |
| Peak B-Pb <75 µg/L | 13 (11.2) | 1 (0.4) | 1 (0.6) | 8 (8.1) | 0 (0.0) | 69 (37.9) | 11 (4.8) |

IQ: intelligence quotient; B-Pb: Blood lead; WISC: Wechsler Intelligence Scale for Children; WISC-R: Wechsler Intelligence Scale for Children Re-normalised.

In Europe, Walkowiak et al. (1998) concluded that non-IQ measures, such as measures of sustained attention, were negatively affected in children, of whom 95 % had a B-Pb below 90 µg/L. The data were such that it was not possible to identify an effect threshold. A US study by Canfield et al. (2003) showed that B-Pb concentrations, even those below 100 µg/L, were inversely associated with children's IQ scores at three and five years of age, and associated relative declines in IQ were greater at these concentrations than at higher exposure levels. Using a linear model, each increase of 100 µg/L in the lifetime average B-Pb concentration was associated with a 4.6 point decrease in IQ (P=0.004). In a subsample of 101 children, whose maximal B-Pb concentration remained below 100 µg/L, the change in IQ associated with a given change in lead concentrations was even greater. When the complete data set was analysed using a nonlinear model, IQ declined by 7.4 points as lifetime average B-Pb concentrations increased from 10 to 100 µg/L. Other recently published results also suggest possible impairment of neuropsychological functions (Canfield et al., 2004), including lead-related deficits in colour vision (Canfield et al., 2003a) as a result of low-level lead exposure of children.

Lanphear et al. reported in 2005 a pooled analysis of the results of seven of the studies initiated prior to 1995 (Table 30). Although the results of the individual studies differ in some respects, perhaps because of the influence of co-variates, individual susceptibility and test methods, the overall evidence strongly supports an association between biomarkers of early-life chronic exposure to lead and lowered IQ and similar neuropsychological measures in children at school age, after adjustment for possible confounders. However, two studies failed to reveal any negative effect of lead exposure, and reasons for the discrepancies are unclear. The three largest studies offer the strongest support for an association between lead exposure and developmental neurotoxicity, i.e. the Treatment of Lead-exposed Children Trial, the Australian Port Pirie study, and the Kosovo study. In an international pooled analysis (Lanphear et al., 2005), the full-scale IQ score was used as the primary outcome measure. The geometric mean B-Pb concentration peaked at 178 µg/L and declined to 94 µg/L by 5 to 7 years of age. A total of 244 of the children had a maximal B-Pb concentration below 100 µg/L, with 103 having a maximum below 75 µg/L. Using a log-linear model, the estimated IQ point decrements associated with an increase in B-Pb from 24 to 100 µg/L, 100 to 200 µg/L and 200 to 300 µg/L were 3.9 (95 % CI, 2.4 to 5.3), 1.9 (95 % CI, 1.2 to 2.6) and 1.1 (95 % CI, 0.7 to 1.5), respectively. For a given increase in B-Pb, the intellectual decrement for children with a maximal B-Pb concentration <75 µg/L was significantly greater than that observed for those with a maximal B-Pb concentration ≥ 75 µg/L (p = 0.015). This analysis also showed that the lead-associated deficits at lower exposures have become apparent only in recent studies that included children born in the post-leaded-petrol era. No threshold for these effects has been identified, and the evidence suggests that the response at B-Pb concentrations below 100 µg/L is steeper than at higher exposure levels.

Studies published since then seem to support these conclusions concerning neurobehavioural outcomes, as shown for example by Kordas et al. (2006) and Téllez-Rojo et al. (2006) (Table 31). Miranda et al. (2007) evaluated the relationship between B-Pb concentrations in early childhood and educational achievement in early elementary school, as measured by performance on end-of-grade (EOG) testing. A discernible impact of B-Pb on EOG testing was found for early childhood blood levels as low as 20 µg/L. A B-Pb of 50 µg/L was associated with a decline in EOG reading and mathematics scores roughly equal to 15 % of the interquartile range.

Jusko et al. (2008) examined the association between B-Pb assessed throughout early childhood from 6 months and IQ at 6 years of age. At 6 years of age, intelligence was assessed in 194 children using the Wechsler Preschool and Primary Scale of Intelligence-Revised. After adjustment for maternal IQ, Home Observation for Measurement of the Environmental (HOME) Inventory Score, and other potential confounders, lifetime average B-Pb (mean 72 µg/L; median 62 µg/L) was inversely associated with Full-Scale IQ and Performance IQ Scores. Compared with children who had lifetime average B-Pb concentrations <50 µg/L, children with lifetime average concentrations between 50 and 99 µg/L scored 4.9 points lower on Full-Scale IQ (p=0.03). Nonlinear modelling of the peak B-Pb concentration revealed an inverse association (p=0.003) with Full-Scale IQ down to a B-Pb of 21 µg/L.

The adverse effects of lead may also include delinquent behaviour resulting in criminal arrests, although educational underachievement may also play a role in this regard (Ferguson et al., 2008; Wright et al., 2008). A possible anatomical basis for the developmental neurotoxicity of lead has been demonstrated by magnetic resonance scanning (Cecil et al., 2008). Following increased lead exposure during childhood, reductions were documented in adult gray matter volume, especially of the prefrontal cortex that is responsible for executive functions, mood regulation and decision-making.

A recent prospective study found adverse effects at B-Pb concentrations of 50 µg/L and above in children with diverse backgrounds that included maternal illicit drug exposure during pregnancy (Min et al., 2009). The B-Pb concentration at age 4 years was negatively associated with IQ at age 4 through 11 years, while reading, mathematics and verbal scores were affected only at age 11 years, possibly because these functions were not testable at earlier developmental stages. This study therefore emphasises the importance of age at testing, and that the full spectrum of developmental neurotoxicity may not be apparent at preschool ages. Likewise, the time of exposure assessment is important. Hornung et al. (2009) examined the data from two prospective studies and concluded that the B-Pb concentration obtained at age 6 years provided a better prediction of school age lead-associated cognitive deficits than did earlier exposure measurements. Although age 6 is unlikely to represent the most vulnerable age, it is possible that lead concentrations at this age are more stable and therefore more predictive.

**Table 31:** Representative papers published since 2005 on the neurodevelopmental effects of lead.

| Country of study | Details of cohort | Design of study | Main Findings | Reference |
|---|---|---|---|---|
| **Effect on IQ and cognitive functions** | | | | |
| USA | 506 children (age: 7 years) of African American origin from Detroit, MI, region | Cross-sectional | B-Pb-dependent decrease in cognitive functions with no safe threshold. | Chiodo et al., 2007 |
| USA | 112 children (mean age: 4) from Oklahoma | Cross-sectional | B-Pb >25 µg/L was associated lower MSCA[(a)] perceptual scores. | Hubbs-Tait et al., 2009 |
| USA | 278 inner city children of African American origin from Ohio followed from age 4 to age 11 | Cross-sectional | B-Pb ≥50 µg/L had detrimental effect on verbal and non-verbal reasoning. | Min et al., 2009 |
| USA | 534 children from Boston, MA, and Farmington, Maine (age: 6 to 10) | Cross-sectional | Children with B-Pb 50-100 µg/L had 5.0 ± 2.3 IQ points lower than children with B-Pb 10-20 µg/L. Verbal IQ was more affected than performance IQ. | Surkan et al., 2007 |
| USA | 194 children from Rochester, NY, (age: 6 years) | Longitudinal | Children with B-Pb 50-99 µg/L had 4.9 IQ points lower compared to children with life-time B-Pb <50 µg/L. Performance IQ was more affected than verbal IQ. | Jusko et al., 2008 |
| USA | 36,000 children from North Carolina | Longitudinal | Negative impact on End-of-Grade Test results from B-Pb ≥20. | Miranda et al., 2007 |
| Egypt | 100 children from different regions (age: 6 to 12 years) and B-Pb 30-280 µg/L | Cross-sectional | Children with cognitive dysfunction had significantly higher B-Pb than children without | Mostafa et al., 2009 |
| Philippines | 877 children from central regions (age 0.5 to 6 years) | Cross-sectional | A B-Pb increase of 10 µg/L was associated with a 2.5-3.3 point decline in cognitive function. | Solon et al., 2008 |
| Mexico | 586 children from Torreón (age: 7 years) | Cross-sectional | Significant decrease in cognitive function at B-Pb <100 µg/L | Kordas et al., 2006 |
| Mexico | 175 children from Mexico City followed until age 10 | Longitudinal | IQ at age 6 to 10 years decreased with increasing natural-log third trimester maternal B-Pb. Lead exposure around 28 weeks gestation appears critical for negative effects on neurodevelopment. | Schnaas et al., 2006 |
| Mexico | 146 children from Mexico City | Longitudinal | Comparison between maternal blood, cord blood and blood in offsprings at 1 and 2 years showed that 1st trimester maternal B-Pb was associated with a 3.5 point reduction the Mental Development Index score. | Hu et al., 2006 |
| Ecuador | 166 children from villages with local ceramic glazing industry (age: 6 to 16 years) | Cross-sectional | Significant inverse correlation between B-Pb and the Digit Span scale score. | Counter et al., 2008. |
| Canada | 104 Inuit children from artic Quebec assessed at age 5 and 11 | Cross-sectional | Prenatal Pb exposure was associated with delayed cognitive electrophysiological measurements (event-related potential P3b) | Boucher et al., 2009 |
| Belgium | 120 girls and 80 boys (mean age: 17.4 years) | Cross-sectional | Slowing of symbol-digit substitution was seen in boys but not girls with increasing B-Pb (range 8 to 321 µg/L). | Vermeir et al., 2005 |
| Poland LO | 457 children from Krakow (age: 3 years) | Longitudinal | Prenatal lead exposure based on cord B-Pb was inversely and significantly associated with cognitive function in boys but not girls. | Jedrychowski et al., 2009 |
| Korea CS | 261 children aged 8 to 11 years recruited from 4 different geographical areas | Cross-sectional | A cross-sectional examination of B-Pb and B-Mn[(a)] showed that at high B-Mn there was an association between decrease in full-scale and verbal IQ and B-Pb. This association was lost with low B-Mn. | Kim et al., 2009 |

**Table 31:** continued

| | | | | |
|---|---|---|---|---|
| **Behavioural effects of lead** | | | | |
| USA | 780 urban children with B-Pb 200-440 µg/L at age 1-2.5 years were followed from ages 2 to 7 years | Longitudinal | Concurrent B-Pb was associated with externalizing and school problems at age 7 years. | Chen et al., 2007 |
| USA | 560,000 children from NHANES 2001-2004 aged 8 to 15 years | Cross-sectional | Increased B-Pb ($4^{th}$ vs $1^{st}$ quartile was associated with a 8.6-fold increased odds of meeting the criteria for conduct disorder. | Braun et al., 2008 |
| USA | 150 children from the State of Michigan of which 53 were control subjects and 97 were suffering from ADHD. Highest B-Pb was 34 µg/L (age: 8 to 17 years) | Cross-sectional | B-Pb was statistically higher in ADHD-combined type than in non-ADHD control children, and was associated with hyperactivity-impulsivity. | Nigg et al., 2008 |
| Canada | 169 Inuit children from artic Quebec assessed at age 1 with 46 µg/L and 59 µg/L for cord and maternal B-Pb, respectively | Cross-sectional | Cord B-Pb correlated with behavioural function (infant attention). | Plusquellec et al., 2007 |
| India | 756 children from Chennai with mean B-Pb 114 $\pm$ 5.3 µg/L (age: 3 to 7 years). | Cross-sectional | B-Pb was associated with higher anxiety, social problems and ADHD. | Roy et al., 2009 |
| India | 176 neonates from Nagpur (average age: 45 weeks) with cord B-Pb determinations | Cross-sectional | Increased B-Pb was associated with abnormal walking reflex. | Patel et al., 2006 |
| Korea | 61 children with mean B-Pb 29 µg/L from Seoul (age: 7 to 16 years) | Cross-sectional | Small but significant association between B-Pb and reaction time and digit span, reflecting attention and short-term memory. | Min et al., 2007 |
| Mexico | 294 children with B-Pb <100 µg/L at 1 and 2 years of age from Mexico City | Longitudinal | B-Pb was significantly associated with a depressed Psychomotor Development Index at age 2 years. | Tellez-Rojo et al., 2006 |
| China | 630 control and 630 ADHD children matched by age, sex and socioeconomic status (age: 4 to 12 years) | Cross-sectional | B-Pb was statistically higher in ADHD- than in non-ADHD control children. The authors conclude that ADHD is a deleterious outcome to lead exposure, even down to <10 µg/L. | Wang et al., 2008 |

(a) Abbreviations used: MSCA, McCarthy Scales of Children's abilities; B-Mn, blood manganese; ADHD, Attention Deficit Hyperactivity Disorder

In conclusion, developmental lead neurotoxicity has been reported at exposures that correspond to a B-Pb of as low as 20 µg/L. The dose-effect relationship seems to be nonlinear, reflecting a greater relative impact at lower lead concentrations. It is unclear whether this association is due to toxicokinetic properties of lead at these dose levels (see section 8.2.1). It is also possible that the outcome scales are not linearly linked to functional levels. Although the prospective studies provide better evidence, the use of lifetime average or peak exposure levels fail to take into account the age at greatest vulnerability. In addition, some important brain functions that are vulnerable to lead toxicity may not be testable in preschool children. The global tests, such as IQ scales, may not capture deficits that are restricted to certain types of brain function. However, the overall evidence clearly indicates developmental lead neurotoxicity. Lead-related developmental neurotoxicity appears to persist to at least the late teenage years; more severe forms of lead poisoning are known to have effects that are apparent in adulthood.

#### 8.5.2.2. Cardiovascular Effects

Studies conducted in animal models support the plausibility of an effect of lead on blood pressure in humans. Moreover, the association between B-Pb and elevated blood pressure has been identified not only in cross-sectional, but also in prospective studies, showing that new cases of hypertension and within-person elevations in blood pressure levels over follow-up were related to B-Pb at first presentation (Glenn et al., 2003; Møller and Kristensen, 1992). Glenn at al. (2003) related lead measured in blood and in tibia bone in 1997 to the change in systolic and diastolic blood pressure measured longitudinally from 1994 to 1998 three to four times in current and former employees of a US chemical manufactory. Changes in SBP were statistically significantly associated in 496 evaluable workers (mean age 55.8 years) with B-Pb, year 3 tibia lead and peak past tibia lead, the latter two based on back-calculations using a clearance half-life of lead in tibia of 27 years following a first-order clearance. Some of these authors (Glenn at el., 2006) studied the same associations also in a younger cohort of 435 men and 140 women (mean age 41 years) occupationally exposed to lead in South Korea for whom at least three measurements were on average available in a three year period. Change in SBP during the study was statistically significantly associated with concurrent B-Pb. Some studies have demonstrated a dose–related trend between lead exposure and blood pressure (Pocock et al., 1984; Schwartz, 1988). However, the shape of the dose–response relationship is not well characterised, particularly at low levels of lead exposure. The lowest level of lead exposure associated with no effect on blood pressure is unknown, and available studies provide little evidence for a threshold (Hertz-Picciotto and Croft, 1993).

The precise mechanisms for the hypertensive effect of low chronic exposure to environmental lead are unknown. An inverse association between estimated glomerular filtration rate and B-Pb has been observed at B-Pb levels <50 µg/L in the general population (Ekong et al., 2006; Muntner et al., 2005), indicating that Pb-induced reductions in renal function could play a major role in hypertension. Other potential mechanisms include enhanced oxidative stress (Stohs and Bagchi, 1995), stimulation of the renin-angiotensin system (Carmignani et al., 1999), and down-regulation of nitric oxide (Dursun et al., 2005) and soluble guanylate cyclase (Farmand et al., 2005). Effects on Na/K ATPase and on $Ca^{2+}$ levels could also contribute to the effect on blood pressure. These mechanisms could result in increased vascular tone and peripheral vascular resistance.

Few cohort studies have evaluated prospectively the association of B-Pb with clinical cardiovascular outcomes in the general population. The findings of the NHANES II and NHANES III Mortality Follow-up studies, although cross-sectional, are notable. Despite a marked decline in B-Pb levels in U.S. adults, both surveys showed statistically significant increases in cardiovascular mortality with increasing B-Pb (Schober et al., 2006). In addition, a cross-sectional analysis of NHANES 1999–2002 data identified an association between B-Pb and the prevalence of peripheral arterial disease (Muntner et al., 2005). The British Regional Heart Study (Pocock et al., 1988) and two other small cohort studies (Kromhout, 1988) showed positive but not statistically significant associations between

coronary heart disease or stroke incidence and B-Pb levels. The confidence intervals from these studies were wide but included the point estimates of the NHANES studies.

The associations between B-Pb and clinical cardiovascular end-points in the NHANES studies were moderately strong, with a clear dose–response gradient. An unresolved issue is the impact of uncontrolled confounding and measurement error on the relative risk estimates in studies of B-Pb and clinical cardiovascular end-points. NHANES studies adjusted for race, education, income and urban versus rural location, which reduces potential confounding by socioeconomic status. Studies with more detailed information on the determinants of lead exposure may contribute to a better understanding of this issue. Similarly, evaluating lead effects using a single B-Pb measure may result in measurement error with substantial over or underestimation of the magnitude of the association. This is particularly problematic when there are marked temporal trends in lead levels.

The validity of occupational studies of lead and cardiovascular mortality is limited by several methodological problems. The comparison of exposed workers with the general population is particularly inappropriate for cardiovascular mortality because workers are healthier and their lifestyles and cardiovascular risk factors are likely to differ from those of the general population. In addition, cardiovascular diseases are associated with prolonged disability and changes in employment status. Even in studies based on comparisons with unexposed workers, the selection of healthier individuals at time of hire or for specific jobs within an industry may have resulted in biased estimates of the association. Correcting the bias introduced by the healthy worker survivor effect is extremely challenging, and stratifying by duration of employment or time since hire is unlikely to account completely for this source of bias (Arrighi and Hertz-Picciotto, 1994). Additional limitations include the assignment of lead exposure based on job titles and of cardiovascular deaths based on death certificates. Misclassification of exposure and outcome may have resulted in underestimation of the association of lead and cardiovascular end-points. Finally, the lack of determination of established cardiovascular risk factors and of other occupational exposures may have contributed to uncontrolled confounding. As a result of these methodological limitations, and despite many occupational cohort studies published in the literature, available information on occupational lead exposure and cardiovascular mortality is inadequate to infer the presence or absence of a causal relationship.

At low exposure levels, it is also possible that observed changes reflect a phenomenon known as reverse causality, i.e. hemodynamic changes associated with aging, diabetes, renal diseases, etc. could result in altered lead disposition leading eventually to increased B-Pb levels.

As a whole, meta-analyses of the epidemiological findings have found a persistent trend in the data that supports a relatively weak, but significant association between B-Pb levels and SBP. Quantitatively, this association amounts to an increase in SBP of approximately 1 mmHg with each doubling of B-Pb (Nawrot et al., 2002; Schwartz et al., 2000; Staessen et al., 1994), without any clearly identifiable threshold.

Nash et al. (2003) performed a cross-sectional study in 2,165 women ages 40 to 59 years drawn from the NHANES III data base (1988-1994) for associations between B-Pb and blood pressure and hypertension. In multivariable analyses statistically significant associations were observed for SPB and DPB as well as for the risk of (systolic and diastolic) hypertension, strongest in postmenopausal women.

Vupputuri et al. (2003) sampled for a cross-sectional study from the same NHANES III data base (1988-1994) 7,464 (5,360 white and 2,104 black) men and 8,488 women (5,188 white and 2,300 black). In a multivariable analysis adjusted for age, education, BMI, alcohol consumption, physical activity potassium and total calories a statistically significant association between B-Pb and SBP was observed in black men (regression parameter associated with one standard deviation of B-Pb with 95 % CI: 0.82; 0.19 to 1.44) as well as black women (1.55; 0.47 to 2.64), similar but weaker between B-Pb and DPB (men: 0.64; 0.08 to 1.20, women: 1.07; 0.37 to 1.77).

8.5.2.3. Renal effects

Most epidemiological studies on the renal effects of lead have involved occupationally exposed workers; however, environmental and/or mixed exposures are represented and a few surveys of children are also included in available studies (Bernard et al., 1995; Fels et al., 1998; Verberk et al., 1996; De Burbure, 2006). Usually, such studies have a cross-sectional design, in which a temporal relationship between exposure and the measured health effect cannot be established. In occupational settings, a selection bias is also known to occur, the so-called "healthy worker effect". As a result, caution must be exercised in the interpretation of the results from such surveys, in which the estimates provided may be biased in either direction.

***Effects on biomarkers of renal tubular toxicity.*** Functional deficits that have been associated with lead exposure include low- and high-molecular weight proteinuria and impaired transport of organic anions and glucose. Enzymuria (mainly the activity of urinary N-acetyl-D-glucosaminidase - NAG) has also been measured as a marker of tubular cell shedding. A detailed discussion and a comprehensive summary table of biomarkers for the renal effects of lead have been published recently (U.S. ATSDR, 2007). The same biomarkers have been used in experimental studies in rats (reviewed in 8.3.2.3), which provided experimental evidence supporting the plausibility of lead effects on both glomerular and tubular function in humans. As a whole, biomarkers of tubular function are not very sensitive in detecting lead-induced renal changes, probably because lead accumulation occurs in the distal part (S3) of the proximal tubule.

***Effects on GFR.*** In humans, reduced GFR, as indicated by decreases in creatinine clearance or increases in serum creatinine concentration, has been observed in association with exposures resulting in average B-Pb levels <200 µg/L, taking into account age and other co-variates that might contribute to glomerular disease. Given the evidence for an association between lead exposure and hypertension, and that decrements in GFR can be a contributor to hypertension, it is possible that the reported hypertension-adjusted regression coefficients may underestimate the actual slope of the B-Pb concentration relationship with serum creatinine concentration or creatinine clearance. In the Kim et al. (1996) and Muntner et al. (2003) studies, a significant relationship between serum creatinine and B-Pb was evident in subjects with B-Pb below 100 µg/L (serum creatinine increased 0.14 mg/dL per 10-fold increase in B-Pb). Estimating the change in GFR from the incremental changes in serum creatinine concentration is far less certain because of the large functional reserve of the kidneys. Indeed, up to 50 % decrement in GFR can occur without a measurable change in serum creatinine excretion (Brady and O'Leary, 1998).

Another important complication in the assessment of associations between lead exposure and adverse effects on kidney function is that not only a decrease, but also an increase in GFR may be a forerunner of late chronic renal disease. Indeed, lead exposure has been associated with increases in GFR (Hsiao et al., 2001; Hu et al., 1991; Roels et al., 1994). This may represent a benign outcome or a potentially adverse hyperfiltration, which may contribute to subsequent adverse renal effects. Increases in GFR have been observed in the early phases of development of chronic renal injury in rats (Khalil-Manesh et al., 1992). The above observations suggest that significant decrements in GFR may occur in association with B-Pb below 200 µg/L and, possibly, below 100 µg/L (Kim et al., 1996; Muntner et al., 2003).

The NHANES III collected data on serum creatinine concentrations and B-Pb on approximately 20,000 U.S. residents during the period 1988–1994. Muntner et al. (2003) analyzed data on 15,211 of these subjects, aged 20 years or older, stratified into normotensive (n=10,398) or hypertensive (n=4,813; ≥140 mmHg systolic pressure or ≥90 mmHg diastolic pressure) categories. Elevated serum creatinine was defined as greater than or equal to 99$^{th}$ percentile of each race-sex specific distribution for healthy young adults and chronic kidney disease (CKD) as a glomerular filtration rate (GFR) below 60 mL/min per 1.73 m$^2$ estimated using the Modification of Diet in Renal Disease formula (Muntner et al., 2003, 2005). Mean B-Pb was 33.0 µg/L in the normotensive group and 42.1 µg/L in the hypertensive group. Associations between B-Pb and risk of elevated serum creatinine

concentrations or chronic renal disease (i.e., depressed GFR) were explored using multivariate regression. GFR was estimated from serum creatinine concentration. Chronic renal disease was defined as GFR <60 mL/minute per 1.73 $m^2$ of body surface area. Co-variate-adjusted odds ratios (ORs) were estimated for B-Pb quartiles 2 (25 to 38 µg/L), 3 (39 to 59 µg/L) and 4 (60 to 560 µg/L), relative to the 1st quartile (7 to 24 µg/L). The ORs for elevated serum creatinine concentration showed a significant upward trend with B-Pb. Co-variate-adjusted ORs for chronic renal disease were: 2nd quartile, 1.44 (95 % CI, 1.00 to 2.09); 3rd quartile, 1.85 (95 % CI, 1.32 to 2.59); and 4th quartile, 2.60 (95 % CI, 1.52 to 4.45). A 2-fold increase in B-Pb was associated with an OR of 1.43 (95 % CI, 1.20 to 1.72) for elevated serum creatinine concentration or 1.38 (95 % CI, 1.15 to 1.66) for chronic renal disease. Co-variates included in the models were age, gender and body mass index; SBP; cardiovascular disease and diabetes mellitus; alcohol consumption and cigarette smoking; and household income, marital status and health insurance. A stronger association between B-Pb and depressed GFR was also found in hypertensive compared to normotensive people (Tsaih et al., 2004).

An analysis of relationships between B-Pb and renal creatinine clearance was conducted as part of the Belgian Cadmibel Study (Staessen et al., 1992). The Cadmibel study was cross-sectional, originally intended to assess health outcomes from cadmium exposure. B-Pb and creatinine clearance measurements were obtained for 965 males (mean age, 48 years) and 1,016 females (mean age, 48 years). Mean B-Pb was 114 µg/L (range: 23 to 725) in males and 74 µg/L (range: 17 to 60) in females. Based on multivariate linear regression (with log-transformed B-Pb), co-variate-adjusted creatinine clearance was significantly associated with B-Pb. A 10-fold increase in B-Pb was associated with a decrease in creatinine clearance of 13 mL/minute in males and 30 mL/minute in females. This would represent a decrease in creatinine clearance of approximately 13 % from the group mean of 99 mL/minute in males, or 38 % from the group mean of 80 mL/minute in females. Co-variates included in the regression model were age and body mass index; urinary γ-glutamyltransferase activity; and diuretic therapy. A logistic regression model was applied to the data to examine the relationship between risk of impaired renal function, defined as less than the 5th percentile value for creatinine clearance in subjects who were not taking analgesics or diuretics (<52 mL/minute in males or 48 mL/minute in females). A 10-fold increase in B-Pb was associated with a co-variate-adjusted risk for impaired renal function of 3.76 (95 % CI, 1.37 to 10.4; p=0.01). Co-variates included in the logistic model were age and body mass index; urinary γ-glutamyltransferase activity; diabetes mellitus; and analgesic or diuretic therapy.

***Longitudinal Studies –General Population.*** In a longitudinal study of a random population (459 subjects, observed between 1991 and 1994) conducted as part of the Normative Aging Study in the United States, co-variate-adjusted serum creatinine (mg/dL) was significantly associated with B-Pb (Kim et al., 1996). A 10-fold increase in B-Pb was associated with an increase of 0.08 mg/dL in co-variate-adjusted serum creatinine (95 % CI, 0.02 to 0.13). In subjects with B-Pb ≤100 µg/L, serum creatinine was predicted to increase 0.14 mg/dL per 10-fold increase in B-Pb. Co-variates included in the models were age and body mass index; hypertension; alcohol consumption and tobacco smoking and education.

Another prospective study, conducted as part of the Normative Aging Study, included 707 subjects examined in 1991–1995 (baseline), and a subset (n=448) in whom follow-up serum creatinine measurements were made 4 to 8 years later (Tsaih et al., 2004). Mean B-Pb was 65 µg/L at baseline and 45 µg/L at follow-up. Baseline bone lead concentrations were: tibia, 21.5 µg/g and patella, 32.4 µg/g and were essentially the same at follow-up. Associations between co-variate-adjusted serum creatinine and lead measures were significant (p<0.05) only for B-Pb and follow-up serum creatinine. When stratified by diabetes and hypertension status, significant associations between serum creatinine concentration and lead measures (in blood or bone) were found in the diabetic (n=26) and hypertensive groups (n=115), suggesting the possibility of interactions between lead exposure, glomerular function, diabetes, or hypertension.

A systematic review of the literature was carried out by Ekong et al. (2006), who reviewed 17 studies, concluding that lead contributes to nephrotoxicity, even at B-Pb levels below 50 µg/L, particularly in subjects with hypertension, diabetes and CKD.

Muntner et al. (2005) examined the declining trend of B-Pb in the American population (NHANES III (1988-1994) and NHANES (1999-2002)) and showed a clear dose-response relationship between the quartiles of B-Pb and the prevalence of both hypertension and CKD. The latter was defined as GFR below 60 ml/min/1.73 $m^2$, calculated on the basis of serum creatinine relying on a simplified version of Levey's formula.

Navas-Acien at al. (2009) evaluated the associations between joint exposure to low-level cadmium and lead in 14,778 adults aged at least 20 years sampled from the more recent NHANES (1999-2006) survey. The LOD for B-Pb was 3 µg/L in NHANES (1999-2004) and 2.5 µg/L in NHANES (2005-2006) with 30 % of the values lower than the respective LODs. The geometric mean of P-Pb was 15.8 µg/L. After adjustment for survey year, sociodemographic factors, CDK risk factors and blood cadmium the odds ratio for reduced estimated GFR comparing the second (11 to 16 µg/L), third (16 to 24µg/L) and highest quartile (>24µg/L) with the lowest quartile (<11µg/L) were (with 95 %CI) 1.10 (0.80 to 1.51), 1.36 (0.99 to 1.85) and 1.56 (1.17 to 2.08), respectively, exhibiting a statistically significant trend ($p<0.001$), see also Table 35. The authors conclude that their data support the consideration of cadmium and lead as joint risk factors for CDK. That study was chosen as the key study for the assessment of the effect of lead on GFR and on the prevalence of CDK.

Fadrowsky and a number of authors of the aforementioned study investigated in 769 adolescents aged 12 to 20 years sampled from the NHANES (1988-1994) survey the association between B-Pb and GFR determined by cystatin C-based and creatinine-based estimating equations very recently (Fadrowsky et al., 2010) following the approach used by Navas-Acien et al. (2009). Median whole B-Pb was 15 µg/L. When adjusting for age, sex, race/ethnicity, urban versus rural residence, tobacco smoke exposure, obesity, income, education of family reference person GFR was reduced in the second (10 to 15µg/L), third (16 to 29µg/L) and fourth quartile (>29 µg/L), compared to the first (<10 µg/L) by 1.4, 2.6 and 6.6 (mL per minute per 1.73 square meter estimated GFR) when based on cystatin C and by 0,5, 1.7 and 1.9 (mililiter per minute per 1.73 square meter estimated GFR) when based on creatinine. The trend was significant for cystatin based GFR ($p=0.009$) but not for creatinine based GFR. The authors of that study estimated the mean reduction in estimated GFR per doubling of B-Pb as 2.9 (95 % CI: 0.7 to 5.0) mililiter per minute per 1.73 square meter when based on cystatin C; 1.0 (-0.9 to 2.8) when based on creatinine. The Panel had no access to the original data. In addition, no further details were reported on the prevalence of CDK, the information on the distribution of the 769 persons in the four quartiles was not available, and cystatin C-based GFR values appeared to depend on log(B-Pb) non-linear. Consequently, these results were not amenable for a dose-response analysis in this opinion.

#### 8.5.2.4. Other effects

Lead has effects on reproduction, the immune system and other organs, besides those on blood pressure, kidneys and the nervous system but these effects are apparent only at higher lead exposures and will therefore not be further discussed (U.S. ATSDR, 2007).

## 8.6. Establishment of a health based guidance value

### 8.6.1. Dose response modelling

As a result of the analysis of the toxicity of lead in previous sections, the dose-response modelling was based on chronic effects in humans and a detailed dose-response assessment focussed on neurotoxicity, cardiovascular effects and renal toxicity.

The CONTAM Panel identified the developing brain as the most vulnerable organ for lead exposure and analyzed therefore available neurodevelopmental data for their appropriateness for a dose-response analysis. Data were available from studies in children at ages between 4 and 10 to 12 years on average in whom the Full Scale IQ was the primary endpoint for neurodevelopmental effects. Dose-response analysis of cardiovascular effects concentrated on blood pressure as the most sensitive endpoint and SBP was the preferred critical endpoint. Presence or absence of CDK was analyzed as a quantal response. Four dose metrics available in published studies were as follows.

- blood lead (B-Pb) concentration measured in units of µg lead per litre (L) blood;
- concurrent B-Pb determined at the time of the examination for the presence and extent of health effects;
- B-Pb measured in early childhood and
- B-Pb determined as the average or as the peak concentration over the duration of the study.

When available, the Panel used also tibia bone concentration measured concurrently or in the past in units of µg lead/g bone mass.

The CONTAM Panel used the BMD approach to derive references points for risk characterisation, where the BMD is defined as that B-Pb or tibia bone Pb concentration, respectively, which is associated with a pre-specified change in the outcome (i.e. loss in IQ, increase in blood pressure, or increase in the incidence of CKD), denoted the benchmark response (BMR). The lower one-sided 95 % confidence bound of the BMD, denoted BMDL, is then taken as the reference point. The CONTAM Panel chose for the two continuous responses, IQ and SBP, a BMR of 1 %, i.e. BMR = 1 IQ point (see sections 8.6.1.1 and 8.6.1.4 for explanation of this choice) and BMR= 1.2 mmHg (see sections 8.6.1.2 and 8.6.1.4 for explanation of this choice), respectively, resulting in the calculation of a $BMD_{01}$ and a $BMDL_{01}$. For the quantal endpoint of the prevalence of CKD, a BMR of 10 % was chosen (see sections 8.6.1.3 and 8.6.1.4 for explanation of this choice), resulting in the calculation of a $BMD_{10}$ and $BMDL_{10}$. It should be noted that the term BMD(L) as used in this Opinion refers to the concentrations in blood (or tibia bone) associated with a specified response. Whilst strictly speaking this is a concentration, it does not represent a BMC(L) in the conventional sense and hence this term has been avoided. For a given BMDL it is possible to calculate a corresponding dietary intake (as described in section 8.6.2). This has been referred to as the BMDL intake value throughout this Opinion.

#### 8.6.1.1. Neurodevelopmental effects

The CONTAM Panel identified the decrease of (full scale) IQ as intellectual deficit in children at ages 4 and higher as the critical endpoint for neurodevelopmental effects. Data on IQ effects were available from a series of longitudinal prospective studies initiated before 1995, where repeated IQ measurements had been correlated with individual exposure measurements.

The pooled analysis performed by Lanphear et al. (2005) on the quantitative relationship between IQ test outcomes taken from children of ages between 4 years and 10 months and 7 years in six cohorts and taken at ages between 5 years and 10 years in one cohort was selected as the database for the dose-response modelling. B-Pb concentration had been measured as concurrent B-Pb (i.e. closest to IQ testing), peak B-Pb level measured at any time before the IQ test, average lifetime lead level measured from 6 months to concurrent B-Pb test, and early childhood B-Pb concentration determined as the mean of all available measurements from 2 months to 2 years. Lanphear at al. (2005) determined the association between IQ scores and B-Pb in 1,333 children taken from seven studies published between 1989 and 2003 which had recruited a total of 1,581 children. The sample reduced to 1,333 (84 %) when only files with complete data on four co-factors (HOME inventory; child's birth weight; maternal education and maternal IQ) were included in the analysis. Cohort sizes, IQ scores and the four B-Pb metrics in units of µg/L are displayed in Table 30 based on Table 2 of Lanphear et al. (2005).

There were differences between the seven cohorts with respect to the sample size (varying between n=116 and n=324) as well as in the children's characteristics. These authors also investigated the association in two subsets of children at the lower exposure range: a low dose (LD) set of 244 children with peak B-Pb less than 100 µg/L, and a subset of 103 very low dose children with a peak B-Pb less than 75 µg/L. Notably, two cohorts contributed most to the LD set: the Rochester cohort, where n=103 (56.6 %) and the Boston cohort, where n=41 (35.3 %) of the sample had peak lead levels lower than 100µg/L. The VLD set was dominated by n=69 children out of the 103 LD children of the Rochester cohort. IQ was measured as full scale IQ, four different Wechsler Intelligence Scales being used in the different studies (three times WISC-R (Boston, Cincinnati, Port Pirie), two times WIPPSI (Cleveland, Rochester), and once each WISC-S and WISC-III). Using B-Pb measurements at ages 6, 12 (or 15), 36, 48 and 60 months, exposure was quantified as concurrent B-Pb concentration with the overall median of 97.5 µg/L (95 % CI: 25.0 to 332.5 µg/L), peak B-Pb concentration (median:180.5 µg/L; 95 %CI: 62.0 to 470.5 µg/L), life-time B-Pb concentration (median: 124.5 µg/L; 95 % CI 41.0 to 348.5 µg/L) and childhood B-Pb concentration (median: 127.5 µg/L; 95 % CI 40.0 to 345.5 µg/L). Note that the IQ score is based on a scale of dimensionless values standardised such that the mean is equal to 100 in each population and for each test system. Therefore the measure is automatically age standardised, so that different age groups can be compared with each other. The objective of the study of Lanphear et al. (2005) had been to examine the association between IQ and B-Pb and to estimate their quantitative relationship, especially for children who had B-Pb concentrations lower than 100 µg/L. Therefore, using a multi-step process, the authors developed a multivariable regression model relating each of the four B-Pb exposure measures to the IQ while controlling for site (study) and available confounders. The authors selected finally a log-linear model of the form

$$IQ = \alpha + ß \cdot \log (B\text{-}Pb) + \gamma \cdot confounders + error$$

to describe the dependency of IQ on concurrent B-Pb, adjusting for four significant confounders, namely maternal IQ, HOME inventory, birth weight and maternal education. Six further co-variates (child's sex, birth order, maternal age and marital status, maternal prenatal smoking and alcohol use) did not fulfil the stated criteria for inclusion as confounders. The concurrent B-Pb concentration exhibited the strongest relationship with IQ and was therefore used as the primary lead exposure index by these authors in all their subsequent analyses. The concurrent B-Pb values ranged between 8 µg/L and 478 µg/L with 5$^{th}$ percentile equal to 25 µg/L and 95$^{th}$ percentile equal to 332 µg/L (see Table 1 of Lanphear et al., 2005). The dose-response curve for concurrent B-Pb over this range was clearly nonlinear, see Figure 15 which combines Figures 1 and 3 taken from Lanphear et al. (2005) and Figure 6 taken from Jusko et al. (2008) describing a subset of n=169 children of the Rochester cohort. In order to account for this nonlinear behaviour in the dose-response relationship, the log-linear model above was chosen by these authors as the preferred model for analyzing the quantitative relationship between IQ score and concurrent B-Pb. This was of the form

$$IQ = \alpha - 2.7 \log (\text{concurrent B-Pb}) + \gamma \text{ confounders},$$

The parameter ß= 2.7, as the regression coefficient of the log-linear model, describes the decrease in IQ depending on concurrent B-Pb in units of µg/dL as used by these authors, with a 95 % CI from 1.66 to 3.74 (see Budtz-Jørgensen (2010) for details on the log-linear and piece-wise linear modelling based on the results reported in Lanphear et al., 2005, as subcontracted by EFSA for this opinion). The term 'confounders' represents HOME inventory, child's birth weight, maternal education and maternal IQ. Notably, the regression coefficients of the four confounders (HOME score: 4.23, 95 % CI: 3.15 to 5.31; birth weight (g): 1.53, 95 % CI 0.83 to 2.23; maternal IQ: 4.7, 95 % CI 3.63 to 5.91 and maternal education grade at delivery: 1.12, 95 % CI 0.20 to 2.04) were of a similar magnitude to the coefficient of the log(B-Pb) concentration when B-Pb was analyzed in units of µg/dL by these authors.

The magnitude of the slope of the fitted non-linear (log linear) curve depends on the section of the curve examined. It increases with decreasing concurrent B-Pb values (at the extreme, namely where concurrent B-Pb is zero, the slope is infinite, i.e. parallel to the IQ ordinate). Therefore, one can derive from such a log-linear model only an approximate estimate of the linear decline in IQ per concurrent

B-Pb concentration unit. Some authors, e.g. Carlisle et al. (2009), have used such approximations to derive $BMD_{01}$ values for the relationship between IQ score and B-Pb concentrations from the published results of Lanphear et al. (2005). However, using such approaches, only approximate $BMD_{01}$ or $BMDL_{01}$ values can be derived.

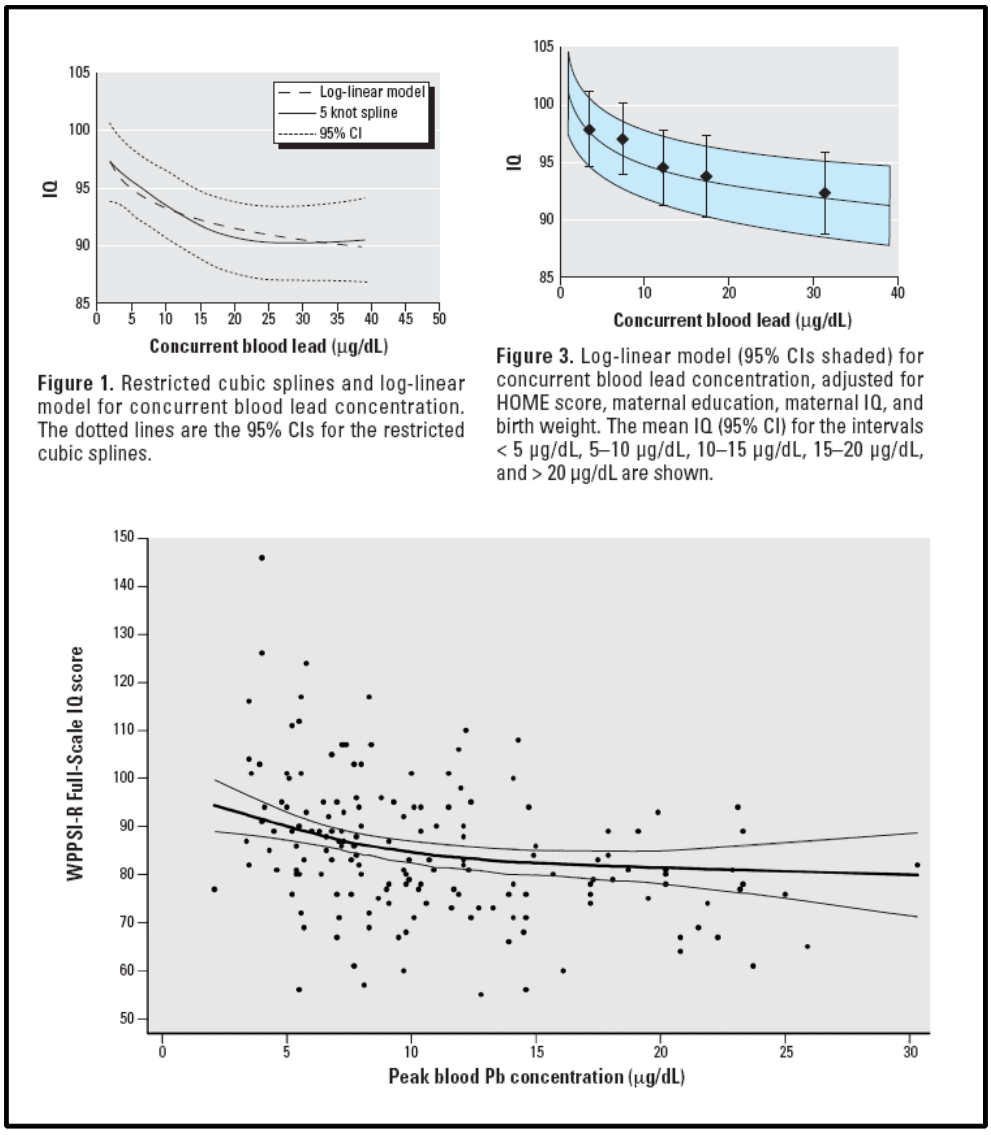



**Figure 1.** Restricted cubic splines and log-linear model for concurrent blood lead concentration. The dotted lines are the 95% CIs for the restricted cubic splines.

**Figure 3.** Log-linear model (95% CIs shaded) for concurrent blood lead concentration, adjusted for HOME score, maternal education, maternal IQ, and birth weight. The mean IQ (95% CI) for the intervals < 5 µg/dL, 5–10 µg/dL, 10–15 µg/dL, 15–20 µg/dL, and > 20 µg/dL are shown.

**Figure 15:** The figures were taken from Lanphear et al. (2005) Figures 1 and 3 (top) and Figure 6 from Jusko et al. (2008) (bottom). Note the units of B-Pb used were µg/dL.

Consequently, EFSA asked the University of Copenhagen to carry out a refined BMD analysis on the same complete individual data from the n=1,333 children that had been used for the international pooled analysis performed by Lanphear at al. (2005) (Budtz-Jørgensen, 2010). The Benchmark calculations were based on standard multiple regression models. As the dependent variable the full IQ score was used. Covariates included study site, birth weight, HOME score, maternal education and maternal IQ, as these variables were found to be statistically significant in the original analysis. $BMD_{01}$ and $BMDL_{01}$ were calculated using each of the four B-Pb exposure variables available: concurrent lead, peak lead, life time average lead and early childhood lead. The $BMD_{01}$ was determined based on the estimated dose-response relationship after confounder adjustment, using a model of the generic form

$$IQ = a + ß\ f(d) + \gamma\ confounder + \varepsilon$$

where d is the B-Pb concentration, and f is the pre-determined dose-response function satisfying f(0) = 0. If ß <0, then an increase in exposure will lead to a lower IQ. The expected IQ loss at concentration d is given by −ßf(d). A logarithmic dose-response model of the form f(d) = log(d + 1), a linear model of the form f(d) = d and a piecewise linear function with breakpoint at 100 µg/L were considered. Details of the calculations are provided in a separate document (Budtz-Jørgensen, 2010) and entitled "An international pooled analysis for obtaining a benchmark dose for environmental lead exposure in children". Table 32 shows the results of that analysis. The same trends are seen for all four exposure metrics. The logarithmic model yields the lowest BMDs and BMDLs, while the linear model (shown in Budtz-Jørgensen, 2010) gives the highest estimates. The linear model also has the poorest fit to the data in accordance with the analysis of Lanphear et al. (2005). The linear model failed a 'goodness of fit test' for concurrent lead. The superiority of the piecewise linear model compared to the linear model was confirmed when these models was compared using statistical methods for assessing goodness-of-fit. Although the logarithmic model generally had a better fit than the piece-wise linear model, the differences were small.

For the preferred dose metric of concurrent B-Pb, the BMD analysis based on the individual data resulted in $BMD_{01}$ values of 3.5 and 18.0 µg/L for the logarithmic and the piece-wise linear model with a cut-off at 100 µg/L, respectively and $BMDL_{01}$ values of 2.6 and 12.0 µg/L at comparable goodness-of-fit. The $BMD/L_{01}$ values of 3.5/2.6 obtained using the logarithmic model were very similar to the values of $BMD_{01}$ = 7.1 and $BMDL_{01}$= 2.1 µg/L obtained when the data set was restricted to n=583 children with concurrent B-Pb concentration below 100 µg/L where there was an overlap amongst the seven studies.

**Table 32:** Benchmark dose calculation initiated by EFSA using the complete individual data of n=1,333 children of the Lanphear et al. (2005) study.

| B-Pb concentration levels | logarithmic model | | piecewise linear model | |
|---|---|---|---|---|
| | $BMD_{01}$ µg/L | $BMDL_{01}$ µg/L | $BMD_{01}$ µg/L | $BMLD_{01}$ µg/L |
| Concurrent | 3.5 | 2.6 | 18.0 | 12.0 |
| Peak | 3.9 | 2.7 | 10.3 | 6.9 |
| Life time | 3.6 | 2.5 | 14.8 | 9.7 |
| Early childhood | 5.6 | 3.4 | 37.7 | 16.1 |
| Subsample of n=583 children with B-Pb concentration <100µg/L from the overlap region of the seven studies | 7.1 | 2.1 | - | - |

B-Pb: blood lead; BMD: benchmark dose; BMDL: benchmark dose lower confidence limit.

Based on the dose-response analysis of the individual data from the comprehensive study on seven populations of Lanphear et al. (2005), the CONTAM Panel chose a $BMDL_{01}$ of 12 µg/L B-Pb as reference point for the risk characterisation of lead for assessing the risk of intellectual deficits in children as measured by the Full Scale IQ (see section 8.6.1.4). The $BMDL_{01}$, associated with a BMR=1 %, i.e. a decrease of cognitive ability by 1 IQ point, was chosen to account for the fact that a shift of the distribution of the IQ by 1 IQ point to lower values would have an impact on the socioeconomic status of the population and its productivity (see also section 8.6.1.4). Schwartz (1994) related a 1 point reduction in IQ to a 4.5 % increase in the risk of failure to graduate from high school. Grosse et al. (2002) studied economic benefits from projected improvements in worker productivity from the reduction in children's exposure to lead in the US and estimated that each IQ point raises worker productivity by 1.76 to 2.38 % using a causal model of cognitive ability and economic productivity and they estimated from there an economic benefit. Therefore, a decrease of 1 IQ in children can be associated with a decrease of later productivity of about 2 %.

A number of uncertainties must be considered in the determination of the dose-response relationship between lead exposure and developmental neurotoxicity. Firstly, because of the blood-brain barrier, the B-Pb concentration at a single point in time may not be an accurate reflection of the amount of lead that has reached the brain. Secondly, lead absorption and therefore, the lead concentration in blood changes with time. For instance, the fractional lead absorption is much higher in infants than in adults. Thirdly, changes in behaviour such as the adoption of hand-to-mouth behaviour by toddlers may contribute to additional exposure from dust and lead from toys, along with food and drinking water. Fourthly, IQ tests were not developed for the purpose of neurotoxicity assessment and more subtle and specific effects on the CNS may be missed. Finally, caution is needed when including covariates which may control at the same time for an effect of, rather than a confounder of the lead effect.

#### 8.6.1.2. 8.6.1.2 Cardiovascular effects

Exposure to lead has been associated with a variety of adverse effects on the cardiovascular system in animals and humans. The most studied dose-response relationship is that on the effect of lead exposure on blood pressure; more frequently reported for systolic (SBP) than for diastolic (DBP) blood pressure. Increased concurrent B-Pb and tibia bone lead concentration (TB-Pb) have both been associated with an increase of SBP. The CONTAM Panel selected SBP as the most relevant endpoint for cardiovascular effects and based its dose-response analysis on available data relating SBP levels in adult humans to concurrent B-Pb or concurrent tibia bone Pb concentrations for deriving a reference point. The CONTAM Panel identified five studies for the quantification of a dose-response relationship between SBP and B-Pb and tibia bone Pb concentration, respectively:

**Cheng et al. (2001)** performed a cross-sectional and a longitudinal study on the relationship between tibia bone-Pb concentration and SBP in participants of the US Veterans Affairs Normative Aging Study (NAS) from greater Boston, MA. They reported a significant positive association based on the cross-sectional study in 519 participants, with an increase of SBP by 0.1 mmHg (95 % CI: 0.0015 to 0.20 mmHg) per µg Pb/g tibia bone.

**Glenn et al. (2003)** studied B-Pb and TB-Pb concentrations in 496 chemical workers in New Jersey (USA). The average annual increase in SBP was 0.025 mm Hg per µg Pb/L (95 % CI: 0.0054 to 0.044) when relating to concurrent B-Pb and 0.078 mmHg per µg-Pb/g tibia bone (95 % CI: 0.024 to 0.13), respectively.

**Vupputuri et al. (2003)** analyzed 4,404 Afro-Americans from the US NHANES III survey cross-sectional study (n=14,952). After adjusting for age, education, body mass index (BMI), alcohol consumption, physical activity, dietary sodium and potassium intake and total calorie intake the average increase in SBP in women (n=2,300) was 1.55 mmHg per µg Pb/L (95 % CI: 0.47 to 2.64) and in men (n=2,104), 0.82 mmHg per µg Pb/L (95 % CI: 0.19 to 1.44).

**Nash et al. (2003)** examined the relationship between SBP and B-Pb in 2,165 women aged 40 to 59 years from the NHANES III (1988-1994) cross-sectional survey. Subdividing the sample into four exposure levels (see Table 33) they observed a statistically significant positive trend between SBP and concurrent B-Pb concentration ($p<0.001$). Multivariable regression in n=1,786 women not treated for hypertension yielded a slope estimate of 0.032 mmHg per µg Pb/L with a standard error (s.e.) of 0.016 mmHg per µg Pb/L. The approximate 95 % CI therefore ranged from 0.0006 to 0.0634 mmHg per µg Pb/L (see Appendix B). For DBP, the slope estimate was 0.025 mmHg per µg Pb/L with s.e. = 0.009, i.e. with an approximate 95 % CI: 0.007 to 0.037 mmHg per µg Pb/L. The authors reported that age, race, ethnicity, alcohol use, cigarette smoking, BMI and kidney function were included as covariates in the final regression and that other potential confounding variables such as education, poverty income ratio, alcohol use and cigarette smoking, were included if found significantly associated with blood pressure in any one of the models before inclusion of B-Pb. The effect of B-Pb on SBP was greater in postmenopausal women than in premenopausal women (slope estimate 0.042 versus 0.014 mmHg per µg Pb/L) contrary to that of B-Pb on DBP (slope estimate 0.013 versus 0.038 mmHg per µg Pb/L).

**Table 33:** Dose-response relationship between concurrent B-Pb and SBP as well as with hypertension, as reported by Nash et al. (2003) for n=2,165 women. Exposure was reported in quartiles and hypertension was defined as SBP ≥ 140mmHg or DPB ≥ 90 or self-reported prescription of antihypertensive treatment.

| Concurrent B-Pb quartile range µg/L | quartile mean (n) µg/L | SBP mean (s.e) mmHg | | Hypertension % | n | OR |
|---|---|---|---|---|---|---|
| 5-16 | 10 (568) | 117.2 | (0.95) | 19.4 % | 110 | 1 |
| 17-25 | 21 (498) | 117.7 | (0.83) | 20.6 % | 103 | 1.0 (0.63-1.6) |
| 26-39 | 32 (556) | 119.3 | (1.10) | 25.5 % | 142 | 1.3 (0.87-2.0) |
| 40-311 | 64(534) | 121.2 | (0.92) | 28.3 % | 154 | 1.4 (0.92-2.0) |

B-Pb: blood lead; SBP: systolic blood pressure; n: number of subjects; OR: odds ratio

**Glenn at al. (2006)** reported the results of a longitudinal study over more than three years (October 1997 to June 2001) in 575 workers from lead-using facilities in South Korea. Using four different models, all based on generalised estimating equations, they evaluated blood pressure changes between study visits in relation to TB-Pb at each prior visit and in relation to changes in concurrent B-Pb. Mean B-Pb level was 314 µg/L (s.d.= 142 µg/L) and mean TB-Pb was 38.4 µg/g tibia bone mineral (s.d.= 42.9µg/g tibia bone mineral) at first presentation. The average annual increase of SBP was 0.009 (95 % CI: 0.001 to 0.016) mmHg per µg Pb/L. Modelling was adjusted for visit, age, alcohol consumption, BMI, gender and use of blood pressure lowering medication. The average annual increase of SBP with TB-Pb was not quantified in this study; in contrast TB-Pb levels were used for adjustment of the B-Pb slope estimate in some models.

An average 1 % annual increase in SBP in the whole population under consideration was regarded as a health concern (see also section 8.6.1.4). Using the results reported by Selmer at al. (2000) who studied the effects of reduced salt intake, an increase of SBP by 1 % (i.e. 1.2 mmHg) would increase the percentage of the population treated for hypertension by 3.1 % and the expected annual mortality from cerebral stroke or myocardial infarction would be by 2.6 % or 2.4 %, respectively (Selmer et al., 2000). Assuming an average SBP of 120 mmHg and critical BMR of 1 %, an increase in SBP by 1.2 mmHg then corresponds to the response at the $BMDL_{01}$. Table 34 summarises the results of the calculation of $BMD_{01}$ and $BMDL_{01}$ based on the slope estimates derived from the five selected studies in the unit of mm Hg/µg Pb/L for B-Pb concentration and in the unit of µg/g tibia bone mineral. Available longitudinal data allowed calculation of the $BMD_{01}$ and $BMDL_{01}$ for an average annual increase of SBP by 1 % in an individual, whereas the cross-sectional data allowed calculation of the $BMD/L_{01}$ for a population-based increase of the mean SBP by 1 %. That distinction is indicated as study type in the first column of Table 34; the second column summarises the slope estimates of the five selected studies from which the BMD/Ls were derived. Details of the statistical calculations are given in Appendix B.

An average $BMDL_{01}$ of 36 µg/L for B-Pb and 8.1 µg/g for TB-Pb were computed from the data in Table 34 and used for risk characterisation of the cardiovascular effects of lead in adults (see section 8.6.1.4 for explanation of the approach used).

**Table 34:** $BMD_{01}$ and $BMDL_{01}$ values obtained from the relationship between blood lead (B-Pb) or tibia bone mineral lead (TB-Pb), respectively, and systolic blood pressure (SBP) in the five studies selected for cardiovascular risk characterisation of lead. Slopes estimates with their 95 % CIs (given in brackets) are displayed. The $BMD_{01}$ and $BMDL_{01}$ values were derived from these slopes and the upper confidence limits, relating to an increase of SBP by 1.2 mmHg.

| Studies Selected | Slope of SBP per change | $BMD_{01}$ | $BMDL_{01}$ |
|---|---|---|---|

| | in lead level | | |
|---|---|---|---|
| **B-Pb** | **mmHg/µg/L (range)** | **µg/L** | **µg/L** |
| Glenn et al. (2003) (longitudinal data)[(a)] | 0.025 (0.005-0.044) | 48 | 29 |
| Vupputuri et al. (2003) (cross-sectional data)[(b)] | 0.047 (0.014 - 0.08) | 26 | 16 |
| Nash et al. (2003) (cross-sectional data) | 0.032 (0.001- 0.0634) | 38 | 21 |
| Glenn et al. (2006) (longitudinal data) | 0.009 (0.001 to 0.016) | 133 | 78 |
| **TB-Pb** | **mmHg/µg/g TB (range)** | | |
| Cheng et al. (2001) (cross-sectional data) | 0.10 (0.0015-0.20 | 12 | 6.5 |
| Glenn et al. (2003) (longitudinal data) | 0.078 (0.024-0.13) | 13 | 9.7 |

(a) For longitudinal studies, the annual increase of SBP was related to concurrent B-Pb or TB-Pb levels, respectively; (b) For cross-sectional studies the observed increase in SBP in the population was related to increases of B-Pb or TB-Pb, respectively; B-PB: blood lead; SBP: systolic blood pressure; BMD: benchmark dose; BMDL: benchmark dose lower confidence limit.

#### 8.6.1.3. Renal effects

Exposure to lead has been associated with functional renal deficits e.g., changes in proteinuria, GFR or creatinine levels and clearance. The CONTAM Panel chose as critical endpoint for the characterisation of renal toxicity the prevalence of CKD, defined as a GFR below 60mL/1.73 $m^2$ body surface/min (Levey et al., 2009).

Data from recent updates (Muntner et al. (2005), Navas-Acien et al. (2009), Fadrowski et al., 2010) of previous investigations of Muntner et al. (2003) and Vupputuri et al. (2003), based on the NHANES III survey were chosen as the key data sets for the derivation of a reference point

Muntner et al. (2005) had studied two populations, n=16,609 persons from the NHANES II (1988-1994) and n=9,961 from the NHANES III (1999-2002). The geometric mean of the measured B-Pb concentration was 16.4 µg/L in the NHANES III (1999-2002) population of 9,961 individuals; amongst those were 655 persons (6.6 %) with levels >50 µg/L and 113 persons with B-Pb >100 µg/L. The OR, adjusted for confounding through age, race/ethnicity, sex, diabetes mellitus, BMI, current cigarette smoking, alcohol consumption, school education and having health insurance, exhibited a statistically significant increase with increasing B-Pb concentration. Two follow-ups were recently published by Navas-Acien et al. (2009) and Fadrowski et al. (2010). Navas-Acien et al. (2009) report associations between blood cadmium (B-Cd) and B-Pb and the prevalence of CKD as defined above, separately for B-Cd and B-Pb as well as jointly where B-Pb was adjusted for the effect of B-Cd and where B-Cd was adjusted for B-Pb, respectively. They examined therefore data of a subset of 14,778 participants, from the total of 18,986 adults who participated in the National Health and Nutrition Examination Survey NHANES (1999–2006). Limits of detection of B-Pb were 3 µg/L in NHANES (1999-2004) and 2.5 µg/L in NHANES (2003-2006) with 0.4 and 0.2 % of the study participants with B-Pb values below the detection limit. The geometric mean B-Pb was 15.8 µg/L.

The prevalence of CKD (based on reduced GFR), as shown in Table 35 increased significantly compared to the reference group of participants with concurrent B-Pb concentrations lower than 11 µg/L in those in the higher quartiles. The odds ratios adjusted for survey year, age, sex, race/ethnicity and BMI, and in addition for education, smoking, alcohol intake, hypertension, diabetes mellitus, menopausal status (not displayed in the table) and further adjusted for blood cadmium level (log-10 µg/L) showed a significant trend ($p<0.001$) in all three modelling approaches, differing by the extent of adjustment.

The CONTAM Panel decided to define renal toxicity of lead by using the CKD and B-Pb data of the most recent cross-sectional study (NHANES (1999-2006)) and fitted the quantal dose-response

models recommended by EFSA to the incidence data as shown in columns 1-3 of Table 35. When fitting these data, separately from cadmium, using a BMR of 10 % as recommended by the Scientific Committee of EFSA (EFSA, 2009) and an acceptability criterion of $p \geq 0.01$ for the model fit, a $BMDL_{10}$ of 15 µg/L was obtained (see Appendix C).

**Table 35:** Dose-response relationship between B-Pb and CKD as reported by Navas-Acien et al. (2009) when exposure was categorised by quartiles of concurrent B-Pb. The odds ratios were adjusted for survey year, age, sex, race/ethnicity and BMI (4th column) and in addition, for education, smoking, alcohol intake, hypertension, diabetes mellitus, menopausal status and blood cadmium level (log-10 µg/L) (5th column).

| B-Pb Quartiles µg/L | Median µg/L(n) | CKD Prevalence number of cases ( %) | Odds ratio (95 % CI non-adjusted for cadmium | Adjusted for cadmium |
|---|---|---|---|---|
| ≤11 | 8 (3,242) | 147 (4.5) | 1 (reference) | 1(reference) |
| 11-16 | 13 (3,167) | 274 (8.7) | 1.08 (0.79-1.47) | 1.10 (0.80-1.51) |
| 16-24 | 19 (3,734) | 468 (12.5) | 1.25 (0.92-1.69) | 1.36 (0.99-1.85) |
| >24 | 32 (4,635) | 779 (16.8) | 1.41(1.07-1.86) | 1.56 (1.17- 2.08) |

B-PB: blood lead; CKD: chronic kidney disease; CI: confidence interval.

8.6.1.4. Selection of critical reference point

The CONTAM Panel identified a number of potential adverse effects of lead for which there was both experimental and epidemiological evidence. The most sensitive of these were developmental neurotoxicity in young children (up to 7 years of age), and effects on SBP and on the kidney in adults.

The CONTAM Panel concludes that the current database, although substantial, also has deficiencies that limit the assessment of dose-response relationships between these endpoints of potential concern and low levels of lead exposure.

However, it was possible to conduct dose-response modelling and to obtain acceptable models for the endpoints of concern. This yielded estimates for the BMDLs, albeit there are many caveats in regard to their interpretation. The CONTAM Panel noted the uncertainty associated with the derivation of the BMDL values. The epidemiological data provided little or no evidence for the existence of thresholds for the critical endpoints. The CONTAM Panel therefore concluded that it would not be appropriate to derive health based guidance values for lead (e.g. tolerable weekly intake), but rather calculated approximate margins of exposure.

The choice of benchmark responses was based on the principles recommended by EFSA in its opinion on the benchmark dose (EFSA, 2005).

*Developmental neurotoxicity*

The benchmark response selected for this endpoint was a 1 % change in full scale IQ score, i.e. a decrease in IQ by 1 point on the full scale IQ score. This BMR was selected because such a change was within the range of observable values and could have significant consequences for human health on a population basis (Grosse et al., 2002). The CONTAM Panel considered several metrics for B-Pb for deriving the $BMDL_{01}$, but concluded that concurrent B-Pb was the most reliable for assessing effects on developmental neurotoxicity, based on published information suggesting that this measure best reflects steady state concentrations of lead in the body of children (Lanphear et al., 2005). The dose-response relationship for change in IQ and B-Pb was nonlinear, with a greater relative change in IQ at low levels of B-Pb than at higher levels. The CONTAM Panel considered several possible approaches to model this relationship. The logarithmic and piecewise linear models gave acceptable and similar fits. The mathematical properties of the logarithmic model, with infinite slope and zero B-Pb and the marked uncertainty associated with the relationship at B-Pb levels below around 80 to

100 µg/L were such that the CONTAM Panel concluded the piecewise linear model, using the segment fit to the lower B-Pb levels, provided less uncertain estimates of the $BMDL_{01}$.

The $BMDL_{01}$ for developmental neurotoxicity = 12 µg/L (B-Pb)

*Cardiovascular effects*

The benchmark response selected for this endpoint was a 1 % change in SBP, corresponding to an increase of 1.2mmHg from the baseline value of 120 mmHg in a normotensive adult. This value was selected for the BMR as such a change was within the range of observable values and could have significant consequences for human health on a population basis (Selmer et al., 2000) The CONTAM Panel based its estimates of $BMDL_{01}$ on the dose-response data published by the authors. This was because the CONTAM Panel did not have access to either the raw data or a suitable aggregated form. The authors of the respective papers all used a linear dose response model. This was considered acceptable, as it is unclear whether there is a threshold for an adverse change in SBP (Nawrot et al., 2002; Schwartz, 1995; Staessen et al., 1994). Four estimates of the $BMDL_{01}$ were based on B-Pb and two estimates were based on TB-Pb. The CONTAM Panel evaluated the studies on which these independent estimates were based and was unable to determine whether one or more estimates were more reliable than the others, as there were no obvious deficiencies in any of the studies. The CONTAM Panel therefore decided to use the arithmetic average of the $BMDL_{01}$ estimates based on B-Pb and TB-Pb respectively for the derivation of reference points for this endpoint.

Effects on SBP in adults: $BMDL_{01}$ = 36 µg/L (B-Pb);

Effects on SBP in adults: $BMDL_{01}$ = 8.1 µg/g (TB-Pb)

*Renal effects*

The benchmark response selected for this endpoint was a 10 % change in the prevalence of CKD, defined by the study authors as a GFR below 60mL/1.73 $m^2$ body surface/min. A 10 % response was selected for the BMR as such a change was within the range of observable values and could have significant consequences for human health on a population basis (EFSA, 2009). The CONTAM Panel fit the quantal models recommended by EFSA (2009) to the data. None of the models was acceptable at $P \geq 0.10$. However, as the precision in the incidence rates using the cross-sectional data from NHANES (1999-2006) was high, due to the large sample size, the CONTAM Panel reduced the model acceptance criterion to $P \geq 0.01$. This resulted in the unrestricted log-probit and multistage models being acceptable. The estimates of the $BMDL_{10}$ using these two models were the same, rounded to two significant figures.

Effects on kidney in adults: $BMDL_{10}$ = 15 µg/L (B-Pb)

**Model-based estimation of the relationship between B-Pb and dietary lead intake**

The three main toxicokinetic models currently being used in lead risk assessment are the O'Flaherty Model (a physiologically based toxicokinetic (PBTK) model for children and adults), the Integrated Exposure Uptake Biokinetic (IEUBK) Model for Lead in children and the Carlisle and Wade model.

The IEUBK is the most widely validated exposure assessment model and is a classic multi-compartmental model linked to an exposure and probabilistic model of B-Pb distributions in populations of children 0 to 7 years. The biokinetic component of the IEUBK model is a mathematic

expression of the movement of absorbed lead throughout the body over time by physiologic or biochemical processes. The biokinetic component converts the total lead uptake rate from the uptake component into an input to the central plasma-extracellular fluid compartment. Transfer coefficients are used to model movement of lead between internal compartments and to the excretion pathways. These quantities are combined with the total lead uptake rate to continuously recalculate the lead masses in each body compartment and especially the changing concentration of lead in blood. The main limitation of the IEUBK model is that its applicability is restricted to children.

Exposure is determined by estimating the concentration of lead in the environmental media with which the child is in contact, multiplied by a term to describe the amount of contact the child has with the medium (usually in g per day, $m^3$ per day, or litre per day), and the term for the duration of that contact (usually days). The media addressed by the IEUBK model include soil, house dust, drinking water, air and food. In this Opinion, lead exposure in children and infants was converted into B-Pb using IEUBKwin version 1.1[24].

The Carlisle and Wade model, used by the California EPA, considers lead from dietary, drinking water, soil and dust and an empirically determined pathway-specific factor that reflects the ratio between lead intake and B-Pb. The model estimates B-Pb using exposure from dietary and non-dietary sources, their corresponding medium-specific contact rates and an empirically determined ratio between intake and blood level (Carlisle and Wade, 1992). The Carlisle and Wade model has successfully been applied to adults but is less suitable for children (Lakind, 1998).

Because of their robustness and suitability, the CONTAM Panel derived its estimation of B-Pb from dietary and non-dietary exposure on the IEUBK model and Carlisle and Wade model for children (0 to 7 years of age) and adults, respectively (Table 36). B-Pb in infants and children were computed using IEUBKwin version 1.1 whereas for adults, the following equation from Carlisle and Wade (1992) was applied: [food exposure (µg/kg b.w. per day)*b.w. *0.4]+[soil and dust lead level (mg/kg)*0.025*0.18]+[air lead level (µg/$m^3$)*16.4] = B-Pb (µg/L).

[24] Available from http://www. epa. gov/superfund/lead/products. htm

**Table 36:** Calculated B-Pb levels resulting from the combined food, soil and dust, air and smoking exposure.

| Source | Range of calculated B-Pb µg/L | |
|---|---|---|
| | **Average consumer** | **High consumer** |
| **Dietary exposure** | | |
| Adults[a,b] | 9-30 | 18-58 |
| Infants 3 months breast milk[c] | 3 | 5 |
| Infants 3 months infant formulae[c] | 4-9 | 6-14 |
| Children 1 to 3 years[c] | 18-48 | 28-77 |
| Children 4 to 7 years[c] | 15-46 | 24-77 |
| Specific diets (game meat)[b] | 48-59 | |
| Vegetarians[b] | 11-30 | 19-54 |
| Women 20 to 40 years[b] | 9-31 | 16-62 |
| **Potential non-Dietary Exposure** | | |
| Soil and dust (children 2 years)[c] | 4 | 15 |
| Outdoor air[b] | 0.1-0.2 | |
| Smoking (20 cigarettes)[b] | 0.2 – 1 | |
| ETS | 0.5-2 (adults)[b]<br>0-1 (children 2 years)[c] | |

(a) Age at which adulthood is assumed varies with country between 15 and 25 years in the different surveys. Details can be found at http://www.efsa.europa.eu/en/datex/datexfooddb.htm; (b) B-Pb calculated using the Carlisle and Wade (1992) equation based on 60 kg b.w.; (c) B-Pb calculated using IEUBKwin version 1.1 and the exposure estimates reported in Table 29.

### 8.6.2. Conversion of BMDLs expressed as B-Pb levels into dietary exposure values

For renal effects in adults, using the equation of Carlisle and Wade (1992), the $BMDL_{10}$ of 15 µg/L based on B-Pb levels corresponds to a dietary lead exposure of 0.63 µg/kg b.w. per day (Table 37). For cardiovascular effects in adults, using the equation of Carlisle and Wade (1992), the $BMDL_{01}$ of 36 µg/L based on B-Pb levels corresponds to a dietary lead exposure of 1.50 µg/kg b.w. per day (Table 37). In both cases, negligible exposure from air and soil were assumed. Using the IEUBK model, the $BMDL_{01}$ of 12 µg/L for neurodevelopmental effects, based on B-Pb levels, corresponds to a dietary lead exposure in infants and children of 0.5 µg lead/kg b.w. per day (Table 37). This is assuming a negligible exposure from air and from soil and dust.

**Table 37:** Conversion of B-Pb levels to dietary exposures at the BMDLs.

| Endpoint | Population | BMDL B-Pb (µg/L) | Corresponding dietary Pb exposure µg/kg b.w. per day | µg/person per day |
|---|---|---|---|---|
| Developmental neurotoxicity | Children | 12 | 0.50 | 10[a] |
| Nephrotoxicity | Adults | 15 | 0.63 | 37.5[b] |
| Cardiovascular effects | Adults | 36 | 1.50 | 90[b] |

(a) Based on a 20 kg child; (b) Based on a 60 kg adult; B-Pb: blood lead; BMDL: benchmark dose lower confidence limit; b.w.: body weight.

## 9. Risk characterisation

The CONTAM Panel evaluated the risk to human health related to the presence of lead in foodstuffs by applying the Margin of Exposure (MOE)[25] approach as there was no evidence for a threshold for the critical endpoints, systolic blood pressure, chronic kidney disease and IQ scores. The interpretation of the MOE is dependent on the magnitude and nature of the BMR, the dose metric on which the BMD is based and the relevance of the population in whom the BMDL was determined. Interpretation of the MOE also depends on where the exposure actually lies within the range of intake estimates based on lower and upper bounds assumptions for samples at the limit of reporting. A summary of the estimated MOEs for the different endpoints for the different populations is given in Table 38.

**Table 38:** Estimated MOEs for different endpoints by type of population

| Population/Diet | Endpoint | MOE | |
|---|---|---|---|
| | | Average consumer | High consumer |
| Adult | Cardiovascular effects[(a)] | 1.2 - 4.2 | 0.62 - 2.1 |
| | Nephrotoxicity[(b)] | 0.51 - 1.8 | 0.26 - 0.86 |
| Vegetarians | Cardiovascular effects | 1.2 - 3.3 | 0.67- 1.9 |
| | Nephrotoxicity[)] | 0.50 - 1.4 | 0.28 - 0.79 |
| Specific diet (game meat) | Cardiovascular effects | 0.76 | 0.61 |
| | Nephrotoxicity | 0.32 | 0.26 |
| Infants 3 months breast milk | Developmental | 2.4 | 1.6 |
| Infants 3 months infant | Developmental neurotoxicity | 0.79 - 1.9 | 0.53 - 1.3 |
| Children 1-3 years | Developmental neurotoxicity | 0.16 - 0.45 | 0.09 - 0.29 |
| Children 4-7 years | Developmental neurotoxicity | 0.19 - 0.63 | 0.10 - 0.38 |
| *In utero* exposure(a) | Developmental neurotoxicity | 0.39 - 1.3 | 0.19 - 0.74 |

(a) For cardiovascular effects the MOE was calculated by dividing the $BMDL_{01}$ intake value of 1.50 µg/kg b.w. per day by the respective dietary exposure estimates taken from Table 29; (b) For nephrotoxicity the MOE was calculated by dividing the $BMDL_{10}$ intake value of 0.63 µg/kg b.w. per day by the respective dietary exposure estimates taken from Table 29; (c) For neurodevelopmental toxicity, the MOE was calculated by dividing the $BMDL_{01}$ intake value of 0.50 µg/kg b.w. per day by the respective dietary exposure estimates taken from Table 29. The MOE range for *in utero* exposure was derived from the 20 to 40 year old female consumer data; MOE: margin of exposure

### 9.1. Adult consumers

#### 9.1.1. 9.1.1 Cardiovascular effects

For the cardiovascular effects, a $BMDL_{01}$ intake value of 1.50 µg/kg b.w. per day was derived from the B-Pb levels (see Chapter 8.6.3.). For adult average consumers across European countries, estimates of dietary exposure to lead ranged from 0.36 to 1.24 µg/kg b.w. per day (lower bound for country with lowest average exposure – upper bound for country with highest average exposure) (Table 29) and are below the $BMDL_{01}$ intake value. The endpoint used, SBP, is not in itself adverse but a physiological parameter known to be linearly correlated with the incidence of stroke and myocardial infarction. The populations in whom the $BMDL_{01}$ values were derived were relatively large, up to 4,400 subjects, and were representative of the general population. The use of concurrent B-Pb as the dose metric on which

[25] MOEs are calculated by dividing the BMDL values derived from human data for the different endpoints by the estimates of dietary exposure.

to base the $BMDL_{01}$ was such that it accounted for a substantial proportion of inter-individual variation in toxicokinetics. The estimated MOEs are small (Table 38). The CONTAM Panel concluded that a margin of exposure of 10 or greater would be sufficient to ensure that there was no appreciable risk of a clinically significant effect on SBP. Indeed, even at MOEs of greater than 1.0 the risk would be very low.

For adult high consumers across European countries, estimates of dietary exposure to lead ranged from 0.73 to 2.43 µg/kg b.w. per day and are below the $BMDL_{01}$ intake values using lower bound assumptions and slightly above the $BMDL_{01}$ intake values using upper bound assumptions. Given the magnitude of the MOEs, if the exposure is closer to the lower end of the range of estimates, the risk is very low to negligible, however if closer to the upper end of the range, the possibility of an effect of lead on SBP in some high consumers cannot be excluded.

The limited available evidence does not indicate a different dietary exposure to lead or risk for vegetarians from that in the general population (Table 29 and 38).

In consumers with specific diets, which included a specific foodstuff with a high lead concentration (see Table 27), estimated dietary exposure to lead is in the range of 0.58 to 1.98 µg/kg b.w. per day (LB assumptions) and 1.04 to 2.44 µg/kg b.w. per day (UB assumptions). The MOE using the $BMDL_{01}$ intake values for the effect of lead on SBP is small (Table 38). Other than game meat, estimated dietary exposure to lead in consumers of these specific diets, using UB assumptions, was less than the $BMDL_{01}$ intake value. Hence, such subjects do not appear to be at any greater risk of an effect of lead on SBP than adult consumers of a typical diet. However, the possibility of an effect in some consumers of a diet rich in game meat cannot be excluded.

### 9.1.2. Nephrotoxicity

For effects on the kidney, a $BMDL_{10}$ intake value of 0.63 µg/kg b.w. per day was derived from the B-Pb levels (see Chapter 8.6.3.). For the adult average consumer across European countries, estimates of dietary exposure to lead (0.36 to 1.24 µg/kg b.w. per day) ranged from below to above the $BMDL_{10}$ intake value for effects on the kidney. The endpoint used was CKD, defined as a 50 % reduction in GFR, to below 60mL/1.73 $m^2$ body surface/min. This is clearly an adverse effect. The magnitude of the BMR, a 10 % change in the prevalence of CKD, is such that the response would be of potential concern. The populations in whom the $BMDL_{10}$ values were derived, a large number of individuals from NHANES, almost 15,000 were representative of the US general population, and the use of concurrent B-Pb as the dose metric on which to base the $BMD_{10}$ was such that it accounted for a substantial proportion of inter-individual variation in toxicokinetics. The prevalence of kidney disease was compared with concurrent B-Pb, whereas this effect would depend on lead exposure over a prolonged interval of time, during which such exposure was declining appreciably. Hence, the $BMDL_{10}$ intake value for this endpoint is likely to be numerically lower than necessary to protect against lead-induced CKD. The estimated MOEs are small (Table 38). The CONTAM Panel concluded that a margin of exposure of 10 or greater would be sufficient to ensure that there was no appreciable risk of a clinically significant change in the prevalence of CKD. Indeed, overall, the risk at MOEs of greater than 1.0 would be very low.

In average adult consumers, estimated dietary exposure to lead, based on LB assumptions, was less than the $BMDL_{10}$ intake value for the effects of lead on the prevalence of CKD, in 17 countries, but was just above the $BMDL_{10}$ intake value in two countries. Estimated dietary exposure to lead, based on UB assumptions, was greater than the $BMDL_{10}$ intake value in all but one country. Hence, if exposure is closer to that based on LB assumptions, the risk of an effect of lead on the prevalence of CKD is considered to be low, in light of the above considerations. If exposure is closer to that based on UB assumptions, the possibility of an effect of lead on the prevalence of CKD in some consumers cannot be excluded.

For adult high consumers, estimated dietary exposure is above the $BMDL_{10}$ intake value using even lower bound assumptions (see Table 22), the MOE ranged from 0.36 to 0.86. Using upper bound assumptions, the MOE ranged from 0.26 to 0.50, across the European countries in which data were available. Hence, the possibility of an effect of lead on the prevalence of CKD in some high consumers, regardless of assumptions about concentrations at the level of reporting, cannot be excluded.

The limited available evidence does not indicate a different dietary exposure to lead or risk of an effect of lead on the prevalence of CKD for vegetarians from than in the general population (Table 29 and 38).

Consumers with specific diets, which included a specific foodstuff with a high lead concentration (see Table 27), estimated dietary exposure to lead is in the range of 0.58 to 1.98 µg/kg b.w. per day (LB assumptions) and 1.04 to 2.44 µg/kg b.w. per day (UB assumptions). The MOEs are small (Table 38) and the estimated dietary exposure to lead ranged from greater than the $BMDL_{10}$ intake value for effects on the prevalence of kidney disease (UB assumptions) in those consuming game meat to slightly less than the $BMDL_{10}$ intake value (LB assumptions) in those consuming fungi. Hence, those consuming a diet rich in fungi or bivalve molluscs do not appear to be at any greater risk of an effect of lead on the prevalence of CKD than adult consumers of a typical diet. In contrast, for those consuming diets rich in the other foodstuffs, i.e. game meat, game offal and algae food supplements, regardless of where exposure lies within the range of estimates, the possibility of an effect in some consumers cannot be excluded.

## 9.2. Infants and children

The estimated dietary exposures to lead are in the ranges of 0.27 to 0.63 µg/kg b.w. per day for average infant consumers of formula feed (3 months of age, mid-point age for 0 to 0.5 years old group), 1.10 to 3.10 µg/kg b.w. per day for child consumers of 1 to 3 years of age and 0.80 to 2.61 µg/kg b.w. per day for child consumers of 4 to 7 years of age, across nineteen European countries (lower bound for age group with lowest average exposure – upper bound for age group with highest average exposure) (see Tables 24 and 29). The age groups infants, 1 to 3 years and 4 to 7 years were selected as they encompass the range of ages at which children will be most sensitive to the neurodevelopmental effects of lead. In high infant consumers of formula feed (3 months of age), exposure estimates ranged from 0.40 to 0.94 µg/kg b.w. per day, in high child consumers of 1 to 3 years of age, estimates ranged from 1.71 to 5.51 µg/kg b.w. per day and in high child consumers of 4 to 7 years of age, estimates ranged from 1.30 to 4.83 µg/kg b.w. per day (lower bound for age group with lowest $95^{th}$ percentile exposure – upper bound for age group with highest $95^{th}$ percentile exposure). In breast milk fed infants of 3 months of age the mean exposure to lead was 0.21 µg/kg b.w. per day in average consumers and 0.32 µg/kg b.w. per day in high consumers. It should be noted that these estimates do not include non-dietary sources of lead.

For changes in full scale IQ score a $BMDL_{01}$ value of 12 µg/L was derived from the B-Pb levels in 6 year old children. Using the IEUBK model, this corresponds to an exposure of 0.50 µg/kg b.w. per day (see Chapter 8.6.3.). Amongst dose metrics available, concurrent B-Pb levels in 6 year old children showed the strongest association with changes in IQ, most likely because it takes some years for B-Pb levels to achieve a steady state, best reflected in measurements at 6 years of age (see Section 8.6.1.2). In addition, it is not possible to test certain functional behavioural domains in younger children. Hence, the CONTAM Panel concluded that the $BMDL_{01}$ determined in 6 year old children is applicable to infants and children of all ages.

The endpoint used, full scale IQ score, is not in itself adverse but a surrogate for neurobehavioural impairment, and most likely reflects effects on one or more cognitive functions. The magnitude of the BMR, a change in full scale IQ score of 1 point, is a small effect in an individual. The populations in whom the $BMDL_{01}$ values were derived were of reasonable size, 1,333 children, and were

representative of the susceptible population. The use of concurrent B-Pb as the dose metric on which to base the $BMDL_{01}$ was such that it accounted for a substantial proportion of inter-individual variation in toxicokinetics. No threshold could be identified for an effect of lead on IQ score and the magnitude of the effect was proportionately greater at lower B-Pb levels. The CONTAM Panel therefore concluded that a margin of exposure of 10 or greater 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 is likely to be low, but not such that it could be dismissed as of no potential concern.

For 3 month old infants (mid-point between 0 and 0.5 years) consuming average or high amounts of breast milk, estimated dietary exposure to lead is less than the $BMDL_{01}$ for effects on IQ score. Hence, the risk from effects of lead is likely to be low in breast-fed infants.

In those consuming average amounts of infant formula, estimated dietary exposure to lead based on LB assumptions is less than the $BMDL_{01}$ intake value but greater than the $BMDL_{01}$ intake value when based on UB assumptions. The situation is similar, in those consuming high amounts of infant formula. In infants fed formula feed, the magnitude of the risk will depend upon whether exposure is closer to that estimated using the LB or the UB for samples below the reporting limit. If closer to the estimates based on LB assumptions, even in high consumers, the risk of any effect of lead on IQ score is likely to be low. However, if exposure is closer to that based on UB assumptions, the possibility of an effect in some consumers, even with average exposure, cannot be excluded.

In average and high child consumers from 1 to 3 years of age, estimated dietary exposure to lead, based on either LB or UB assumptions, was greater than the $BMDL_{01}$ for effects on full scale IQ score. Hence, the possibility of an effect of lead on full scale IQ score in some child consumers in the age range 1 to 3 years cannot be excluded. It is not possible to provide estimates of the potential numbers of children who might be affected, as even in average consumers the MOE was <1.

In average and high child consumers from 4 to 7 years of age, estimated dietary exposure to lead, based on either LB or UB assumptions, was greater than the $BMDL_{01}$ intake value for effects on full scale IQ score. Hence, as in younger children, the possibility of an effect of lead on full scale IQ score in some child consumers in the age range 4 to 7 years cannot be excluded. It is not possible to provide estimates of the potential numbers of children who might be affected, as even in average consumers the MOE was <1.

### 9.3. Developing fetus

*In utero* exposure of the developing fetus to lead is of potential concern. In such cases, maternal exposure to lead would be a critical determinant of risk. Women of 20 to 40 years of age were used as a proxy for pregnant women (Table 28), for whom specific consumption data were not available. Dietary exposure to lead in this group is in the range of 0.38 to 1.28 µg/kg b.w. per day for average consumers and 0.68 to 2.60 µg/kg b.w. per day for high consumers (lower bound for country with lowest average exposure – upper bound for country with highest average exposure). Exposure is therefore no greater than in the adult population overall. 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. Given that the fetal/maternal cord B-Pb concentration ratio is approximately 0.9, the maternal B-Pb level corresponding to the $BMDL_{01}$ for effects on neurodevelopment (12 µg/L) is 13 µg/L, which is equivalent to a dietary exposure of 0.54 µg/kg b.w. per day.

In average 20 to 40 year old female consumers, estimated dietary exposure to lead, based on LB assumptions, was greater than the $BMDL_{01}$ intake value for effects on IQ score in children in approx 50 % of the countries. Based on UB assumptions, estimated dietary exposure was greater than the $BMDL_{01}$ intake value in all 19 European countries in which data were available. In high 20 to 40 year old female consumers, estimated dietary exposure to lead, based on either LB or UB assumptions, was

greater than the $BMDL_{01}$ intake value in all 19 countries. Hence, the possibility of an effect of lead on the IQ scores of the offspring of some female consumers cannot be excluded.

It is not possible to provide estimates of the potential numbers of offspring who might be affected by prenatal exposure to lead, as even in average consumers the MOE was <1 in approx 50 % of countries.

### 9.4. Conclusions

After due consideration to both limitations of epidemiological data and health significance of observed changes associated with blood lead levels, the CONTAM Panel concluded that the risk of clinically important effects on either the cardiovascular system or kidneys of adult consumers, at current levels of lead exposure is low to negligible. In infants, children and pregnant women, there is potential concern at current levels of exposure to lead for effects on neurodevelopment. Protection of children and women of child-bearing age against the potential risk of neurodevelopmental effects would be protective for all other adverse effects of lead, in all populations.

## 10. Uncertainty analysis

The evaluation of the inherent uncertainties in the assessment of exposure to lead was performed following the guidance of the Opinion of the Scientific Committee related to Uncertainties in Dietary Exposure Assessment (EFSA, 2006). In addition, the report on "Characterizing and Communicating Uncertainty in Exposure Assessment" published by WHO/IPCS (WHO-IPCS, 2008) was considered in this evaluation. According to the guidance provided by the EFSA opinion (EFSA, 2006), the following sources of uncertainties have been considered: assessment objectives, exposure scenario, exposure model and model input (parameters).

### 10.1. Assessment objectives

The objectives of the assessment were clearly specified in the terms of reference, and the CONTAM Panel assessed the new occurrence data that were collected by EFSA, and evaluated the food commodities in the different Member States that contribute most to lead exposure. The uncertainty in the assessment objectives is considered to be negligible. The CONTAM Panel has sufficient indications that the basis of approaches and instruments used were sufficient to prepare the opinion. The methods applied for the exposure assessment are widely used and well accepted.

Risk characterisation was based on reference points derived using the benchmark dose (BMD) approach for the analysis of the dose-response relationship observed for critical human health effects of lead on the central nervous system, the cardiovascular system and the renal system following the principles of the Scientific Opinion of the EFSA (EFSA, 2009)

### 10.2. Exposure scenario

Food intake and all relevant foods having lead as an important contaminant are the focus of this opinion. However, lead exposure may also arise from environmental sources, in particular house dust, and this may represent an important source of exposure for children. Direct contact with consumer products, e.g. toys, may also contribute to exposure to an unknown extent, particularly in children. Therefore, considering only food as a source of lead may lead to an underestimation of the risk from lead exposure.

The dietary sources of lead exposure are described as completely as possible, and it is not expected that important sources are missing. The information about sources stems from measurements of the lead content of different foods, from monitoring programmes, but also from targeted market control initiatives. Although the measurements of lead in foods are driven by targeting, they are considered to cover almost all of the sources.

The concise food consumption database providing food consumption data aggregates some food items which brings some uncertainties, due to generic stratification coefficients which in reality will vary with country and subpopulations.

The oral path is considered as the most important route of exposure to lead in the general population. It is assumed that all lead present in the food will be available for absorption in the gastrointestinal tract which may result in overestimation of exposure.

### 10.3. Exposure model

The generally accepted model for estimation of food exposure was used. The model considers the specific aggregation of data in EFSA's concise food consumption database and also occurrence data reported from 19 Member States of the EU plus Norway.

A standard algorithm for calculating the exposure has been applied.

### 10.4. Dose-response parameters

For the food consumption figures, the average amounts of food have been used according to the food groups of the EFSA concise food consumption data base. The database provides data from 19 Member States of the EU plus Norway. The primary purpose of these food consumption surveys is to study nutrient intake. Aggregation of inhomogeneous food groups, e.g. vegetables, nuts and pulses, due to differences in consumption and contamination may lead to uncertainty which can lead to over- or underestimation of exposure. Therefore, for risk assessment purposes, the data are sometimes needed at a higher degree of disaggregation.

Lead concentrations in the different food classes have been measured in foods primarily for market control from different EU member states in order to identify foods having lead levels that exceed the regulatory limits. For this reason, the samples may not be representative for each country and hence the EU. For instance, data from Germany are overrepresented. In addition, foods that are frequently controlled for high concentrations are often eaten less frequently. Vice-versa, frequently eaten foods do not always represent the most frequently measured samples. Combining high consumption figures with non-representative and incomplete data may therefore result in uncertainty. Also, if the majority of these data are below the LOD then high LODs reported from laboratories might lead to an overestimation rather then an underestimation of exposure.

There are no prescribed fixed official methods for the analysis of lead, and there are no requirements for laboratories to reach certain LODs and LOQs. This may have added to the uncertainty in the analytical results.

The CONTAM Panel considered that the impact of the uncertainties on the risk assessment of exposure to lead is limited and concluded that its assessment of the risk is likely to be conservative, i.e. more likely to overestimate than to underestimate the risk.

In determining dose-response relationships for lead exposure and developmental neurotoxicity, the following sources of uncertainties must be considered.

- The B-Pb concentration at a single point in time is not an accurate reflection of the amount of lead that has entered the body and reached the brain, because lead does not distribute evenly in the body and only slowly passes the blood-brain barrier. Therefore, the lead concentration in a blood sample taken at a certain age of a child may not be representative for the long-term exposure of the child. However, the evidence suggests that concurrent B-Pb levels at 6 years of age are the most reliable dose metric amongst those available.

- Lead concentration in blood changes with time, thereby creating different time profiles of B-Pb concentrations.

- The fractional lead absorption is much higher in infants than in adults. Some studies suggest that B-Pb concentrations are the highest at around 2 years of age. However, this conclusion may, in part, be due to the hand-to-mouth behaviour at that age, where lead in dust and lead from toys, etc. may contribute to the exposure, along with food and drinking water.

- In considering neurodevelopment, the velocity of development is a critical factor when a cohort of children is investigated and those children do not necessarily follow the same trajectory. This means that when assessing brain functions at one age tests that correspond to brain development at that age must be applied. Deficits will then be apparent only in regard to the age-appropriate tests.

- Many studies have applied IQ tests, since they are commonly available and have been standardised in many languages. However, the IQ tests were not developed for the purpose of neurotoxicity assessment and more subtle and more specific deficits may be missed. IQ tests may be less sensitive to specific types of neurotoxicity than domain-related tests that better reflect specific functions.

- IQ testing can be affected by the environment in which the test is applied and by the attitude of the subject to the tester.

- Measurement errors of clinical endpoints such as blood pressure and creatinine determination and differences in protocols between centres may contribute to uncertainty.

### 10.5. Modelling

- Differences in the strength of the design of human studies (e.g. lower evidence level of observational and cross-sectional studies which partly or totally have to rely on retrospective data collection compared to prospective designed studies) and in detail of reporting (e.g. of the sampling confounder information and the ascertainment of the health effect endpoints) contribute to the uncertainty of reference points (BMDs/BMDLs). Causation is inferred from these studies but is rarely established. Indeed, it is possible that associations are not causal, or may even occur by chance, adding uncertainty to the assessment.

- There were limitations in the assessment of individual exposure, e.g., when using aggregate dose information. Dose categories were pre-specified by the investigators by design or by convenience for data analysis. Note that a median dose used as a measure of exposure in the highest dose interval might not reflect high exposure appropriately.

- Model uncertainty due to the selection of dose-response models for the BMD analysis cannot be excluded but in general it was a minor source of uncertainty, and the range of BMDLs of the set of acceptable models was very narrow.

- Confounding is usually an important factor in human studies which may turn an effect towards a nil effect and hence towards a less stringent dose-response relationship. However the designs of the studies chosen for this risk characterisation were in the upper quality range of human studies and they usually had adjusted for effects of relevant confounders, such as age, sex, BMI, socioeconomic status, smoking, alcohol consumption and other lifestyle factors including even exposure to concurrent agents e.g. cadmium in the case of nephrotoxicity.

- A weakness in particular of cross-sectional studies when using B-Pb concentration for the characterisation of nephrotoxicity is the possible distortion of the dose-response relationship

by effects caused by previous higher B-Pb values than those measured e.g. in NHANES (1999-2006) survey. That could indicate an underestimation of the dose and thereby an overestimation of the risk. Note also that kidney dysfunction may result in decreased excretion of lead which may lead to higher B-Pb levels and thereby also to reverse causation, in the sense that lower renal function could have increased B-Pb.

- There is a large heterogeneity and some inconsistency in study outcomes on the relationship between lead exposure and hypertension (Nawrot et al, 2002). This variability could be due to population differences or due to differences in study design and conduct of measurement of exposure as well as measurement of the SBP endpoint. SBP measurement depends on clinical measurement protocols, e.g., single versus repeated measurement, measurement on one or both arms, position of the person. It is also possible that some associations occurred due to chance, or were not causal.

- Using the log linear model could possibly lead to a substantial overestimation of the risk in the low dose range where only few human observations were available. Therefore, the CONTAM Panel chose to use the piecewise linear model for the risk assessment of neurodevelopmental effects, and this is less likely to be overly conservative.

### 10.6. Uncertainty evaluation

In Table 39, a summary of the uncertainty evaluation is presented that highlights the main sources of uncertainty and indicates an estimate of whether the respective source of uncertainty may have led to an over- or underestimation of the exposure or the resulting risk.

**Table 39:** Summary of qualitative evaluation of the impact of uncertainties on the risk assessment of the dietary exposure to lead.

| Sources of uncertainty | Direction and Magnitude |
|---|---|
| Extrapolation of data from a limited number of Member States to whole Europe | +/-[a)] |
| Measurement uncertainty of analytical results | +/- |
| Some analytical data were from targeted sampling | + |
| Influence of non-detects on exposure estimate | + |
| • Using the highest UB | + |
| • Using the lowest LB | - |
| Use of broad food categories / Aggregation of food categories | +/- |
| Estimation of exposure in consumers of specific diets | + |
| Estimation of exposure from other pathways | +/- |
| Absorption for different exposure routes | +/- |
| Reliability of identification and characterisation of critical effects | +/- |
| Dietary exposure estimates from B-Pb | + |
| Modelling assumptions for B-Pb levels | + |

[a)] +, = uncertainty with potential to cause over-estimation of risk; -, = uncertainty with potential to cause under-estimation of risk

The CONTAM Panel concluded that the impact of the uncertainties on the risk assessment of exposure to lead could be appreciable and that its assessment of the risk is likely to be conservative, i.e. more likely to overestimate than to underestimate the risk.

## CONCLUSIONS

### *General*

- Lead is a metal that occurs naturally but whose presence in the environment has greatly increased as a result of anthropogenic activities such as mining and smelting and battery manufacturing. Although lead occurs in organic and inorganic forms, it is the inorganic forms that predominate in the environment.

- Control measures taken to regulate lead in paint, petrol, food cans and pipes in Europe since the 1970s have led to a substantial decrease in exposure.

### *Methods of analysis*

- The primary techniques for analysing lead in food samples are based on atomic absorption spectrometry, atomic emission spectrometry and mass spectrometry after digestion of organic material with concentrated acids.

### *Occurrence and exposure*

- Following a call for data, 14 Member States and Norway submitted approximately 140,000 results of lead concentrations in various food commodities and tap water.

- A total of 94,126 results covered the period from 2003 to 2009 and were suitable for calculating lead concentrations in the various food categories. The lead level in approximately two thirds of the samples was below the limit of detection or limit of quantification.

- Mean lead dietary exposure estimates for adults across European countries ranged from 0.36 to 1.24 µg/kg b.w. per day and from 0.73 to 2.43 µg/kg b.w. per day for high consumers, based on lower bound and upper bound assumptions for the level of reporting, respectively.

- Overall, cereals, vegetables and tap water were the most important contributors to lead exposure in the general European population. More specifically, the following food groups were identified as the major contributors to lead exposure: cereal products, followed by potatoes, cereal grains (except rice), cereal-based mixed dishes and leafy vegetables and tap water. Considerable variation between and within countries in the contribution of different food categories/groups exists.

- The available evidence for women of child-bearing age and vegetarians does not indicate a dietary exposure that is different from that of the general adult population.

- Based on limited data, exposure of breast-fed infants was estimated to be 0.21 µg/kg b.w. per day on average or 0.32 µg/kg b.w. per day for high consumers. For infants fed ready-to-consume infant formula, the average exposure estimates range from 0.27 to 0.63 µg/kg b.w. per day, based on lower bound and upper bound assumptions, respectively; for high consumers, lead exposure estimates range from 0.40 to 0.94 µg/kg b.w. per day, respectively.

- For children aged 1 to 3 years mean lead dietary exposure estimates range from 1.10 to 3.10 µg/kg b.w. per day based on lower bound and upper bound assumptions, respectively; for high consumers, lead exposure estimates range from 1.71 to 5.51 µg/kg b.w. per day, respectively.

- For children aged 4 to 7 years mean lead dietary exposure estimates range from 0.80 to 2.61 µg/kg b.w. per day based on lower bound and upper bound assumptions, respectively; for high consumers, lead exposure estimates range from 1.30 to 4.83 µg/kg b.w. per day.

- For adults, non-dietary exposure to lead is likely to be of minor importance for the general population in the EU. House dust and soil can be an important source of exposure to lead for children.

- Lead in blood is considered to be the biomarker of choice for the concentration of lead in soft tissues, and hence recent exposure, although in part it also reflects long term exposure. Bone lead in vivo reflects the long-term uptake and body burden.

***Hazard identification and characterisation***

- Absorption of lead appears to be highly variable and tends to be higher in children than in adults. It is lower in the presence of food. Absorbed lead is transferred to soft tissues, including liver and kidneys, and to bone tissue, where it accumulates with age.

- Half-lives for lead in blood and bone are approximately 30 days and 10 to 30 years, respectively, and excretion primarily is in urine and faeces.

- The CONTAM Panel identified the following potential adverse effects of lead, the developmental neurotoxicity in young children, cardiovascular effects and nephrotoxicity in adults as the basis for the risk assessment.

- A decrease in Full Scale IQ score was considered to reflect a change in cognitive function in children at ages 4 and higher as it is the most consistently used end-point of cognitive ability assessed in such studies and was used as the critical endpoint for neurodevelopmental effects. An increase in SBP and an increase in the prevalence of CKD as assessed by a decrease in glomerular filtration rate were used as endpoints for adults.

- The computed BMDL were as follows:

  Developmental neurotoxicity: $BMDL_{01}$ = 12 µg/L (B-Pb)

  Effects on SBP in adults: $BMDL_{01}$ = 36 µg/L (B-Pb); 8.1 µg/g (TB-Pb)

  Effects on kidney in adults: $BMDL_{10}$ = 15 µg/L (B-Pb)

- Using the equation of Carlisle and Wade (1992), dietary lead intake values in adults, in whom there is negligible exposure from air and from soil and dust (<1 µg B-Pb/L), corresponding to the respective BMDL dietary intake values were as follows.

  Effects on SBP– B-Pb 36 µg/L ~ 90.0 µg/60kg = 1.50 µg/kg per day

  Effects on kidney – B-Pb 15 µg/L ~ 37.5 µg/60kg = 0.63 µg/kg b.w. per day.

- Using the IEUBK model, a B-Pb level of 12 µg/L, the $BMDL_{01}$ dietary intake value for developmental neurotoxicity in 6 year old children, corresponds to a dietary lead intake value of 0.50 µg/kg b.w. per day.

- The CONTAM Panel concluded that the present PTWI of 25 µg/kg b.w. is no longer appropriate and noted that there was no evidence for a threshold for a number of critical endpoints including developmental neurotoxicity and renal effects in adults. Therefore, a margin of exposure approach was applied to risk characterisation.

***Risk characterisation***

- Dietary exposures to lead based on LB and UB assumptions for average adult consumers in Europe are lower than the BMDL intake value for effects on SBP (1.50 µg/kg b.w. per day),

but vary from above to below the BMDL intake value for effects on the prevalence of CKD, (0.63 µg/kg b.w. per day). The respective MOEs range from 1.2 to 4.2 and from 0.51 to 1.8, respectively. Hence, if exposure were closer to the upper bound estimates, the possibility of effects in some consumers cannot be excluded.

- The limited available evidence does not indicate a different average dietary exposure or risk for vegetarians from the adult population, Consumer groups with higher lead exposure levels include high consumers of game meat (1.98 to 2.44 µg/kg b.w. per day) and high consumers of game offal (0.81 to 1.27 µg/kg b.w. per day). The estimated dietary exposures of these groups are also within, or at the higher end of the range of the respective BMDL intake values.

- Estimated exposure in children up to age seven exceeds the $BMDL_{01}$ intake level of 0.50 µg/kg b.w. per day for neurodevelopmental effects. The MOE in average 1 to 3 year old child consumers ranged from 0.16 to 0.45, and was only slightly higher in 4 to 7 year old children. Therefore, the possibility of effects in some children cannot be excluded. It was not possible to estimate the potential numbers of children who might be affected, as even in average consumers the MOE was <1.

- Breast-fed 3-month old infants are predicted to have a lead exposure that is below the $BMDL_{01}$ intake value of 0.50 µg/kg b.w. per day. Lead exposure based on lower bound assumptions in both average and high 3-month old infant consumers of infant formula is below the $BMDL_{01}$ intake value, but may exceed this level, based on upper bound estimates. Therefore, the possibility of an effect in some infants cannot be excluded.

- Women of 20 to 40 years of age were used as a surrogate for pregnant women to calculate the risk of lead exposure in utero on neurodevelopment in the offspring. Estimates of exposure were at or above the BMDL for neurodevelopmental effects, and the CONTAM Panel concluded that it was not possible to exclude a risk to the developing fetus through exposure of some pregnant female consumers.

## RECOMMENDATIONS

- Further efforts should be made to increase the understanding of the lead dose-response relationship.

- At the same time, work should continue to reduce exposure to lead, from both dietary and non-dietary sources.

- When results are reported as below LOD, LOQ or as non-detected, the respective numerical values should be reported.

- An additional recommendation is that the EFSA food category database should be expanded and refined.

## REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists), 2004. Lead. Threshold limit values for chemical substances and physical agents and biological exposure indices. Cincinnati, OH.

Adonaylo VN and Oteiza PI, 1999. Lead intoxication: antioxidant defenses and oxidative damage in rat brain. Toxicology, 135, 77-85.

Alexander FW, Clayton BE and Delves HT, 1974. Mineral and trace-metal balances in children receiving normal and synthetic diets. Quarterly Journal of Medicine, 43, 89-111.

Alexander J, Aaseth J and Mikalsen A, 1986. Excretion of lead in rat bile--the role of glutathione. Acta Pharmacologica et Toxicologica, 59 Suppl 7, 486-489.

Allen JR, McWey PJ and Suomi SJ, 1974. Pathobiological and behavioral effects of lead intoxication in the infant rhesus monkey. Environmental Health Perspectives, 7, 239-246.

Almeida AA, Lopes CM, Silva AM and Barrado E, 2008. Trace elements in human milk: correlation with blood levels, inter-element correlations and changes in concentration during the first month of lactation. Journal of Trace Elements in Medicine and Biology, 22, 196-205.

Al-Modhefer AJA, Bradbury MWB and Simmons TJB, 1991. Observations on the chemical nature of lead in human blood serum. Clinical Science, 81, 823-829.

Altmann L, Weinsberg F, Sveinsson K, Lilienthal H, Wiegand H and Winneke G, 1993. Impairment of long-term potentiation and learning following chronic lead exposure. Toxicology Letters, 66, 105-112.

Araki S and Honma T, 1976. Relationships between lead absorption and peripheral nerve conduction velocities in lead workers. Scandinavian Journal of Work, Environment & Health, 2, 225-231.

Arrighi HM and Hertz-Picciotto I, 1994. The evolving concept of the healthy worker survivor effect. Epidemiology, 5, 189-196.

Astrin KH, Bishop DF, Wetmur JG, Kaul B, Davidow B and Desnick RJ, 1987. Delta-aminolevulinic-acid dehydratase isozymes and lead toxicity. Annals of the New York Academy of Sciences, 514, 23-29.

Aufderheide AC and Wittmers LE, 1992. Selected aspects of the spatial-distribution of lead in bone. Neurotoxicology, 13, 809-819.

Aungst BJ, Dolce JA and Fung HL, 1981. The effect of dose on the disposition of lead in rats after intravenous and oral-administration. Toxicology and Applied Pharmacology, 61, 48-57.

Bacon JR and Dinev NS, 2005. Isotopic characterisation of lead in contaminated soils from the vicinity of a non-ferrous metal smelter near Plovdiv, Bulgaria. Environmental Pollution, 134, 247-255.

Bannon DI, Abounader R, Lees PSJ and Bressler JP, 2003. Effect of DMT1 knockdown on iron, cadmium, and lead uptake in Caco-2 cells. American Journal of Physiology-Cell Physiology, 284, C44-C50.

Bárány E, Bergdahl IA, Bratteby LE, Lundh T, Samuelson G, Skerfving S and Oskarsson A, 2005. Iron status influences trace element levels in human. Environmental Research, 98, 215-223.

Barry PSI, 1975. Comparison of concentrations of lead in human tissues. British Journal of Industrial Medicine, 32, 119-139.

Barton JC, Conrad ME, Nuby S and Harrison L, 1978. Effects of iron on the absorption and retention of lead. The Journal of Laboratory and Clinical Medicine, 92, 536-547.

Batariova A, Spevackova V, Benes B, Cejchanova M, Smid J and Cerna M, 2006. Blood and urine levels of Pb, Cd and Hg in the general population of the Czech Republic and proposed reference values. International Journal of Hygiene and Environmental Health, 209, 359-366.

Battistuzzi G, Petrucci R, Silvagni L, Urbani FR and Caiola S, 1981. Delta-aminolevulinate dehydrase - a new genetic-polymorphism in man. Annals Of Human Genetics, 45, 223-229.

Beek B and Obe G, 1974. Effect of lead acetate on human leukocyte chromosomes in vitro. Experientia, 30, 1006-1007.

Bergdahl IA, Gerhardsson L, Liljelind IE, Nilsson L and Skerfving S, 2006. Plasma-lead concentration: investigations into its usefulness for biological monitoring of occupational lead exposure. American Journal of Industrial Medicine, 49, 93-101.

Bergdahl IA, Gerhardsson L, Schutz A, Desnick RJ, Wetmur JG and Skerfving S, 1997b. Delta-aminolevulinic acid dehydratase polymorphism: Influence on lead levels and kidney function in humans. Archives of Environmental Health, 52, 91-96.

Bergdahl IA, Grubb A, Schutz A, Desnick RJ, Wetmur JG, Sassa S and Skerfving S, 1997a. Lead binding to delta-aminolevulinic acid dehydratase (ALAD) in human erythrocytes. Pharmacology & Toxicology, 81, 153-158.

Bergdahl IA, Schutz A and Grubb A, 1996. Application of liquid chromatography inductively coupled plasma mass spectrometry to the study of protein-bound lead in human erythrocytes. Journal of Analytical Atomic Spectrometry, 11, 735-738.

Bergdahl IA, Sheveleva M, Schutz A, Artamonova VG and Skerfving S, 1998. Plasma and blood lead in humans: Capacity-limited binding to delta-aminolevulinic acid dehydratase and other lead-binding components. Toxicological Sciences, 46, 247-253.

Bergdahl IA, Vahter M, Counter SA, Schutz A, Buchanan LH, Ortega F, Laurell G and Skerfving S, 1999. Lead in plasma and whole blood from lead-exposed children. Environmental Research, 80, 25-33.

Berkowitz GS, Wolff MS, Lapinski RH and Todd AC, 2004. Prospective study of blood and tibia lead in women undergoing surgical menopause. Environmental Health Perspectives, 112, 1673-1678.

Bernard A and Lauwerys R, 1987. Metal-induced alterations of delta-aminolevulinic acid dehydratase. Annals of the New York Academy of Sciences, 514, 41-47.

Bernard AM, Vyskocil A, Roels H, Kriz J, Kodl M and Lauwerys R, 1995. Renal effects in children living in the vicinity of a lead smelter. Environmental Research, 68, 91-95.

BfR (Bundesinstitut für Risikobewertung), 2004. Lead and Cadmium from Ceramics. Updated Expert Opinion* No. 023/2005 of BfR from 26 March 2004. 12 pp.

BfR (Bundesinstitut für Risikobewertung), 2006. Kosmetische Mittel: BfR empfiehlt Schwermetallgehalte über Reinheitsanforderungen der Ausgangsstoffe zu regeln Stellungnahme Nr. 025/2006 des BfR vom 05. April 2006. 4 pp.

BfR (Bundesinstitut für Risikobewertung), 2009. Blei und Cadmium gehören nicht in Spielzeug. Stellungnahme Nr. 048/2009 des BfR vom 1. Juni 2009.

Blake KCH, Barbezat GO and Mann M, 1983. Effect of dietary constituents on the gastrointestinal absorption of Pb-203 in man. Environmental Research, 30, 182-187.

Blake KCH and Mann M, 1983. Effect of calcium and phosphorus on the gastrointestinal absorption of Pb-203 in man. Environmental Research, 30, 188-194.

Bolger PM, Carrington CD, Capar SG and Adams MA, 1991. Reductions in dietary lead exposure in the United States. Chem Speciat Bioavail, 3, 31-36.

Bolin CM, Basha R, Cox D, Zawia NH, Maloney B, Lahiri DK and Cardozo-Pelaez F, 2006. Exposure to lead and the developmental origin of oxidative DNA damage in the aging brain. FASEB Journal, 20, 788-790.

Bonanno LJ, Freeman NCG, Greenberg M and Lioy PJ, 2001. Multivariate analysis on levels of selected metals, particulate matter, VOC, and household characteristics and activities from the Midwestern states NHEXAS. Applied Occupational & Environmental Hygiene, 16, 859-874.

Bornschein R, Pearson D and Reiter L, 1980. Behavioral effects of moderate lead exposure in children and animal models: part 2, animal studies. Critical Reviews in Toxicology, 8, 101-152.

Boucher O, Muckle G, Saint-Amour D, Dewailly E, Ayotte P, Jacobson SW, Jacobson JL and Bastien CH, 2009. The relation of lead neurotoxicity to the event-related potential P3b component in Inuit children from arctic Quebec. Neurotoxicology, 30, 1070-1077.

Bouton CM and Pevsner J, 2000. Effects of lead on gene expression. Neurotoxicology, 21, 1045-1055.

Brady J and O'Leary N, 1998. Interference due to haemolysis in routine photometric analysis--a survey. Annals of Clinical Biochemistry, 35 ( Pt 1), 128-134.

Braun JM, Froehlich TE, Daniels JL, Dietrich KN, Hornung R, Auinger P and Lanphear BP, 2008. Association of environmental toxicants and conduct disorder in U.S. children: NHANES 2001-2004. Environmental Health Perspectives, 116, 956-962.

Bressler J, Kim KA, Chakraborti T and Goldstein G, 1999. Molecular mechanisms of lead neurotoxicity. Neurochemical Research, 24, 595-600.

Bressler JP and Goldstein GW, 1991. Mechanisms of lead neurotoxicity. Biochemical Pharmacology, 41, 479-484.

Brussaard JH, vanDokkum W, vanderPaauw CG, deVos RH, deKort W and Lowik MRH, 1996. Dietary intake of food contaminants in the Netherlands (Dutch Nutrition Surveillance System). Food Additives and Contaminants, 13, 561-573.

Budtz-Jørgensen E, 2010. An international pooled analysis for obtaining a benchmark dose for 2 environmental lead exposure in children. SCIENTIFIC / TECHNICAL REPORT submitted to EFSA. Available from www.efsa.europa.eu

Calderon-Salinas JV, Quintanar-Escorcia MA, Gonzalez-Martinez MT and Hernandez-Luna CE, 1999. Lead and calcium transport in human erythrocyte. Human & Experimental Toxicology, 18, 327-332.

Canfield RL, Gendle MH and Cory-Slechta DA, 2004. Impaired neuropsychological functioning in lead-exposed children. Developmental Neuropsychology, 26, 513-540.

Canfield RL, Henderson CR, Jr., Cory-Slechta DA, Cox C, Jusko TA and Lanphear BP, 2003. Intellectual impairment in children with blood lead concentrations below 10 microg per deciliter. New England Journal of Medicine, 348, 1517-1526.

Canfield RL, Kreher DA, Cornwell C and Henderson CR, Jr., 2003a. Low-level lead exposure, executive functioning, and learning in early childhood. Child Neuropsychology, 9, 35-53.

Carbone R, Laforgia N, Crollo E, Mautone A and Iolascon A, 1998. Maternal and neonatal lead exposure in southern Italy. Biology of the Neonate, 73, 362-366.

Carlisle JC, Dowling KC, Siegel DM and Alexeeff GV, 2009. A blood lead benchmark for assessing risks from childhood lead exposure. J Environ Sci Health A Tox Hazard Subst Environ Eng, 44, 1200-1208.

Carlisle JC and Wade MJ, 1992. Predicting blood lead concentrations from environmental concentrations. Regulatory Toxicology and Pharmacology, 16, 280-289.

Carmignani M, Boscolo P, Poma A and Volpe AR, 1999. Kininergic system and arterial hypertension following chronic exposure to inorganic lead. Immunopharmacology, 44, 105-110.

Carmignani M, Volpe AR, Boscolo P, Qiao N, Di Gioacchino M, Grilli A and Felaco M, 2000. Catecholamine and nitric oxide systems as targets of chronic lead exposure in inducing selective functional impairment. Life Sciences, 68, 401-415.

CDC (Centers for Disease Control and Prevention), 1991. Preventing Lead Poisoning in Young Children. Atlanta, GA. Available from http://www.cdc.gov/nceh/lead/publications/books/plpyc/contents.htm

CDC (Centers for Disease Control and Prevention), 2004. Lead poisoning associated with Ayurvedic medications – five states, 2000–2003. MMWR Morb Mortal Weekly Reports. 53, 582-584.

CDC (Centers for Disease Control and Prevention), 2005. Preventing Lead Poisoning in Young Children. Atlanta, GA.

CDPE (Centers for Disease Prevention and Epidemiology), 1998. Ceramic and pottery cookware: A potential source of Pb. Oregon Health Division. http://www.oregon.gov/DHS/ph/cdsummary/1998/ohd4721.pdf

Cecil KM, Brubaker CJ, Adler CM, Dietrich KN, Altaye M, Egelhoff JC, Wessel S, Elangovan I, Hornung R, Jarvis K and Lanphear BP, 2008. Decreased brain volume in adults with childhood lead exposure. PLoS Med, 5, e112.

Celik M, Ekerbicer HC, Ergun UG and Kilinc M, 2007. Effects of environmental lead pollution, smoking, and smokeless tobacco (Maras powder) use on blood lead level. Biological Trace Element Research, 120, 121-126.

Chen A, Cai B, Dietrich KN, Radcliffe J and Rogan WJ, 2007. Lead exposure, IQ, and behavior in urban 5- to 7-year-olds: does lead affect behavior only by lowering IQ? Pediatrics, 119, e650-658.

Cheng Y, Schwartz J, Sparrow D, Aro A, Weiss ST and Hu H, 2001. Bone lead and blood lead levels in relation to baseline blood pressure and the prospective development of hypertension: the Normative Aging Study. American Journal of Epidemiology, 153, 164-171.

Cheng Y, Willet WC, Schwartz J, Sparrow D, Weiss S and Hu H, 1998. Relation of nutrition to bone lead and blood lead levels in middle-aged to elderly men. The Normative Aging Study. American Journal of Epidemiology, 147, 1147-1174.

Chiodo LM, Covington C, Sokol RJ, Hannigan JH, Jannise J, Ager J, Greenwald M and Delaney-Black V, 2007. Blood lead levels and specific attention effects in young children. Neurotoxicology and Teratology, 29, 538-546.

Choie DD and Richter GW, 1972a. Cell proliferation in rat kidney induced by lead acetate and effects of uninephrectomy on the proliferation. American Journal of Pathology, 66, 265-275.

Choie DD and Richter GW, 1972b. Cell proliferation in rat kidneys after prolonged treatment with lead. American Journal of Pathology, 68, 359-370.

Cohen AJ and Roe FJC, 1991. Review of lead toxicology relevant to the safety assessment of lead acetate as a hair coloring. Food and Chemical Toxicology, 29, 485-507.

Cooper GP, Suszkiw JB and Manalis RS, 1984. Presynaptic effects of heavy metals. In: Cellular and molecular neurotoxicology. T Narahashi. Raven Press, New York, 1-21.

Cory-Slechta DA, 1995. Relationships between lead-induced learning impairments and changes in dopaminergic, cholinergic, and glutamatergic neurotransmitter system functions. Annual Review of Pharmacology and Toxicology, 35, 391-415.

Cory-Slechta DA, 2003. Lead-induced impairments in complex cognitive function: offerings from experimental studies. Child Neuropsychology, 9, 54-75.

Costa LG and Fox DA, 1983. A selective decrease of cholinergic muscarinic receptors in the visual cortex of adult rats following developmental lead exposure. Brain Research, 276, 259-266.

COT (Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment), 2008. COT statement on the 2006 UK Total Diet Study of Metals and other Elements.

Counter SA, Buchanan LH and Ortega F, 2008. Zinc protoporphyrin levels, blood lead levels and neurocognitive deficits in Andean children with chronic lead exposure. Clinical Biochemistry, 41, 41-47.

Dabeka RW and McKenzie AD, 1995. Survey of lead, cadmium, fluoride, nickel, and cobalt in food composites and estimation of dietary intakes of these elements by Canadians in 1986-1988. Journal of AOAC International, 78, 897-909.

Dabrowska-Bouta B, Struzynska L, Walski M and Rafalowska U, 2008. Myelin glycoproteins targeted by lead in the rodent model of prolonged exposure. Food and Chemical Toxicology, 46, 961-966.

Dabrowska-Bouta B, Sulkowski G, Bartosz G, Walski M and Rafalowska U, 1999. Chronic lead intoxication affects the myelin membrane status in the central nervous system of adult rats. Journal of Molecular Neuroscience, 13, 127-139.

De Boer WJ and Van der Voet H, 2007. MCRA, Release 6. A web-based programme for Monte Carlo Risk Assessment. Bilthoven, Wageningen: Biometris, Wageningen UR, RIKILT-Institute of Food Safety, Wageningen UR and National Institute for Public Health and the Environment (RIVM). Available from http://mcra.rikilt.wur.nl/mcra/Manual.pdf.

de Burbure C, Buchet JP, Leroyer A, Nisse C, Haguenoer JM, Mutti A, Smerhovsky Z, Cikrt M, Trzcinka-Ochocka M, Razniewska G, Jakubowski M and Bernard A, 2006. Renal and neurologic effects of cadmium, lead, mercury, and arsenic in children: evidence of early effects and multiple interactions at environmental exposure levels. Environmental Health Perspectives, 114, 584-590.

Deknudt G, Colle A and Gerber GB, 1977. Chromosomal abnormalities in lymphocytes from monkeys poisoned with lead. Mutation Research, 45, 77-83.

Doss M, von Tiepermann R, Schneider J and Schmid H, 1979. New type of hepatic porphyria with porphobilinogen synthase defect and intermittent acute clinical manifestation. Klinische Wochenschrift, 57, 1123-1127.

Doumouchtsis KK, Doumouchtsis SK, Doumouchtsis EK and Perrea DN, 2009. The effect of lead intoxication on endocrine functions. Journal of Endocrinological Investigation, 32, 175-183.

Drasch GA, Bohm J and Baur C, 1987. Lead in human bones. Investigations on an occupationally non-exposed population in southern Bavaria (F.R.G.). I. Adults. The Science of The Total Environment, 64, 303-315.

Dursun N, Arifoglu C, Süer C and Keskinol L, 2005. Blood pressure relationship to nitric oxide, lipid peroxidation, renal function, and renal blood flow in rats exposed to low lead levels. Biological Trace Element Research, 104, 141-149.

EFSA (European Food Safety Authority), 2004. Opinion of the Scientific Panel on Contaminants in the Food Chain (CONTAM) related to leas as undesirable substance in animal feed. EFSA Journal. 71, 1-20.

EFSA (European Food Safety Authority), 2005. Guidance of the Scientific Committee on a request from EFSA related to a harmonised approach for risk assessment of substances which are both genotoxic and carcinogenic. The EFSA Journal. 282, 1-31.

EFSA (European Food Safety Authority), 2006. Guidance of the Scientific Committee on a request from EFSA related to Uncertainties in Dietary Exposure Assessment. The EFSA Journal. 438, 1-54.

EFSA (European Food Safety Authority), 2008. Guidance Document for the use of the Concise European Food Consumption Database in Exposure Assessment. 1-11. Available from http://www.efsa.europa.eu/cs/BlobServer/General/Coincise_database_guidance_document_and_annexes.pdf?ssbinary=true.

EFSA (European Food Safety Authority), 2009. Guidance of the Scientific Committee on Use of the benchmark dose approach in risk assessment. The EFSA Journal. 1150, 1-72.

Ekong EB, Jaar BG and Weaver VM, 2006. Lead-related nephrotoxicity: a review of the epidemiologic evidence. Kidney International, 70, 2074-2084.

Elias Z, Poirot O, Pezerat H, Suquet H, Schneider O, Daniere MC, Terzetti F, Baruthio F, Fournier M and Cavelier C, 1989. Cytotoxic and neoplastic transforming effects of industrial hexavalent chromium pigments in Syrian hamster embryo cells. Carcinogenesis, 10, 2043-2052.

EMEP (European Monitoring and Evaluation Programme), 2001. Cyromazine summary report (2).Committee for veterinary medicinal products. European Agency for the Evaluation of

medicinal products, veterinary medicines and inspections (EMEA). EMEA/MRL/770/00-FINAL, January 2001. Available from http://www.emea.europa.eu/pdfs/vet/mrls/077000en.pdf.

EMEP/CCC (European Monitoring and Evaluation Programme-Chemical Co-ordinating Centre), 2006. Heavy metals and POP measurements, 2004. Available from http://tarantula.nilu.no/projects/ccc/reports/cccr7-2006.pdf.

Ernst E, 2002. Heavy metals in traditional Indian remedies. European Journal of Clinical Pharmacology, 57, 891-896.

Ettinger AS, Tellez-Rojo MM, Amarasiriwardena C, Gonzalez-Cossio T, Peterson KE, Aro A, Hu H and Hernandez-Avila M, 2004. Levels of lead in breast milk and their relation to maternal blood and bone lead levels at one month postpartum. Environmental Health Perspectives, 112, 926-931.

Ettinger AS, Tellez-Rojo MM, Amarasiriwardena C, Peterson KE, Schwartz J, Aro A, Hu H and Hernandez-Avila M, 2006. Influence of maternal bone lead burden and calcium intake on levels of lead in breast milk over the course of lactation. American Journal of Epidemiology, 163, 48-56.

Evis MJ, Dhaliwal K, Kane KA, Moore MR and Parratt JR, 1987. The effects of chronic lead treatment and hypertension on the severity of cardiac arrhythmias induced by coronary artery occlusion or by noradrenaline in anaesthetised rats. Archives of Toxicology, 59, 336-340.

Evis MJ, Kane KA, Moore MR and Parratt JR, 1985. The effects of chronic low lead treatment and hypertension on the severity of cardiac arrhythmias induced by coronary artery ligation in anesthetized rats. Toxicology and Applied Pharmacology, 80, 235-242.

Fadrowski JJ, Navas-Acien A, Tellez-Plaza M, Guallar E, Weaver VM and Furth SL, 2010. Blood lead level and kidney function in US adolescents: The Third National Health and Nutrition Examination Survey. Archives of Internal Medicine, 170, 75-82.

FAO/WHO (Food and Agriculture Organization/ World Health Organization), 2006. GEMS/Food Consumption Cluster Diets. Available from http://www.who.int/foodsafety/chem/gems/en/index1.html.

Farmand F, Ehdaie A, Roberts CK and Sindhu RK, 2005. Lead-induced dysregulation of superoxide dismutases, catalase, glutathione peroxidase, and guanylate cyclase. Environmental Research, 98, 33-39.

Federal Clean Air Act, 1970.42 United States Code (U.S. C.), § 7401,

Fels LM, Wunsch M, Baranowski J, Norska-Borowka I, Price RG, Taylor SA, Patel S, De Broe M, Elsevier MM, Lauwerys R, Roels H, Bernard A, Mutti A, Gelpi E, Rosello J and Stolte H, 1998. Adverse effects of chronic low level lead exposure on kidney function--a risk group study in children. Nephrology, Dialysis, Transplantation, 13, 2248-2256.

Fergusson DM, Boden JM and Horwood LJ, 2008. Dentine lead levels in childhood and criminal behaviour in late adolescence and early adulthood. Journal of Epidemiology and Community Health, 62, 1045-1050.

Fleming DE, Boulay D, Richard NS, Robin JP, Gordon CL, Webber CE and Chettle DR, 1997. Accumulated body burden and endogenous release of lead in employees of a lead smelter. Environmental Health Perspectives, 105, 224-233.

Forbes GB and Reina JC, 1972. Effect of age on gastrointestinal absorption (Fe, Sr, Pb) in the rat. Journal of Nutrition, 102, 647-652.

Fowler BA, Kimmel CA, Woods JS, McConnell EE and Grant LD, 1980. Chronic low-level lead toxicity in the rat. III. An integrated assessment of long-term toxicity with special reference to the kidney. Toxicology and Applied Pharmacology, 56, 59-77.

Franklin CA, Inskip MJ, Baccanale CL, Edwards CM, Manton WI, Edwards E and O'Flaherty E J, 1997. Use of sequentially administered stable lead isotopes to investigate changes in blood lead

during pregnancy in a nonhuman primate (Macaca fascicularis). Fundamental and Applied Toxicology, 39, 109-119.

FSA (Food Standards Agency), 2007. Survey of metals in a variety of foods. FSIS 01/07.

FSA (Food Standards Agency), 2009. Survey on measurement of the concentrations of metals and other elements from the 2006 UK total diet study. Available from http://www.food.gov.uk/multimedia/pdfs/fsis0909metals.pdf.

Fujita H, Sato K and Sano S, 1982. Increase in the amount of erythrocyte delta-aminolevulinic acid dehydratase in workers with moderate lead exposure. International Archives of Occupational and Environmental Health, 50, 287-297.

Gemenetzis P, Moussas P, Arditsoglou A and Samara C, 2006. Mass concentration and elemental composition of indoor PM2.5 and PM10 in university rooms in Thessaloniki, northern Greece. Atmospheric Environment, 40, 3195-3206.

Gercken B and Barnes RM, 1991. Determination of lead and other trace-element species in blood by size exclusion chromatography and inductively coupled plasma mass-spectrometry. Analytical Chemistry, 63, 283-287.

Gerhardsson L, Kazantzis G and Schutz A, 1996. Evaluation of selected publications on reference values for lead in blood. Scandinavian Journal of Work, Environment & Health, 22, 325-331.

Gerhardt RE, Crecelius EA and Hudson JB, 1980. Trace-element content of moonshine. Archives of Environmental Health, 35, 332-334.

Gilbert ME and Lasley SM, 2002. Long-term consequences of developmental exposure to lead or polychlorinated biphenyls: Synaptic transmission and plasticity in the rodent CNS. Environmental Toxicology and Pharmacology, 12, 105-117.

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

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

Goering PL, 1993. Lead-protein interactions as a basis for lead toxicity. Neurotoxicology, 14, 45-60.

Goering PL and Fowler BA, 1985. Mechanism of renal lead-binding protein reversal of delta-aminolevulinic acid dehydratase inhibition by lead. Journal of Pharmacology and Experimental Therapeutics, 234, 365-371.

Goldman LS, Genel M, Bezman RJ and Slanetz PJ, 1998. Diagnosis and treatment of attention-deficit/hyperactivity disorder in children and adolescents. Council on Scientific Affairs, American Medical Association. JAMA : the Journal of the American Medical Association, 279, 1100-1107.

Gonick HC, Ding Y, Bondy SC, Ni Z and Vaziri ND, 1997. Lead-induced hypertension: interplay of nitric oxide and reactive oxygen species. Hypertension, 30, 1487-1492.

Goyer RA, 1990. Transplacental transport of lead. Environmental Health Perspectives, 89, 101-105.

Goyer RA, Leonard DL, Moore JF, Rhyne B and Krigman MR, 1970a. Lead dosage and the role of the intranuclear inclusion body. An experimental study. Archives of Environment Health, 20, 705-711.

Goyer RA, May P, Cates MM and Krigman MR, 1970b. Lead and protein content of isolated intranuclear inclusion bodies from kidneys of lead-poisoned rats. Laboratory Investigation, 22, 245-251.

Grandjean P and Landrigan PJ, 2006. Developmental neurotoxicity of industrial chemicals. Lancet, 368, 2167-2178.

Graziano JH, Popovac D, Factor-Litvak P, Shrout P, Kline J, Murphy MJ, Zhao YH, Mehmeti A, Ahmedi X, Rajovic B, Zvicer Z, Nenezic DU, Lolacono NJ and Stein Z, 1990. Determinants of elevated blood lead during pregnancy in a population surrounding a lead smelter in Kosovo, Yugoslavia. Environmental Health Perspectives, 89, 95-100.

Greenberg M and Hamilton R, 1999. Lack of blood lead elevations in police officers following small arms qualification on an indoor range (Abstract). Journal of Toxicology - Clinical Toxicology, 37, 627.

Gross SB, Pfitzer EA, Yeager DW and Kehoe RA, 1975. Lead in human tissues. Toxicology and Applied Pharmacology, 32, 638-651.

Grosse SD, Matte TD, Schwartz J and Jackson RJ, 2002. Economic gains resulting from the reduction in children's exposure to lead in the United States. Environmental Health Perspectives, 110, 563-569.

Gulson B, 2008. Stable lead isotopes in environmental health with emphasis on human investigations. Sci Total Environ, 400, 75-92.

Gulson BL, Jameson CW, Mahaffey KR, Mizon KJ, Patison N, Law AJ, Korsch MJ and Salter MA, 1998. Relationships of lead in breast milk to lead in blood, urine, and diet of the infant and mother. Environmental Health Perspectives, 106, 667-674.

Gulson BL, Mahaffey KR, Vidal M, Jameson CW, Law AJ, Mizon KJ, Smith AJ and Korsch MJ, 1997. Dietary lead intakes for mother/child pairs and relevance to pharmacokinetic models. Environmental Health Perspectives, 105, 1334-1342.

Gulson BL, Mizon KJ, Korsch MJ, Mahaffey KR and Taylor AJ, 2001. Dietary intakes of selected elements from longitudinal 6-day duplicate diets for pregnant and nonpregnant subjects and elemental concentrations of breast milk and infant formula. Environmental Research, 87, 160-174.

Gulson BL, Mizon KJ, Korsch MJ, Palmer JM and Donnelly JB, 2003. Mobilization of lead from human bone tissue during pregnancy and lactation--a summary of long-term research. The Science of The Total Environment, 303, 79-104.

Gulson BL, Mizon KJ, Palmer JM, Korsch MJ, Taylor AJ and Mahaffey KR, 2004. Blood lead changes during pregnancy and postpartum with calcium supplementation. Environmental Health Perspectives, 112, 1499-1507.

Gulson BL, Palmer JM and Bryce A, 2002. Changes in blood lead of a recreational shooter. The Science of The Total Environment, 293, 143-150.

Gundacker C, Pietschnig B, Wittmann KJ, Lischka A, Salzer H, Hohenauer L and Schuster E, 2002. Lead and mercury in breast milk. Pediatrics, 110, 873-878.

Gunderson EL, 1988. FDA Total Diet Study, April 1982-April 1984, dietary intakes of pesticides, selected elements, and other chemicals. Journal of the Association of Official Analytical Chemists, 71, 1200-1209.

Gzyl J, 1995. Ecological impact and remediation of contaminated sites around lead smelters in Poland. Journal of Geochemical Exploration, 52, 251-258.

Hänninen H, 1988. The psychological performance profile in occupational intoxications. Neurotoxicology and Teratology, 10, 485-488.

Harville EW, Hertz-Picciotto I, Schramm M, Watt-Morse M, Chantala K, Osterloh J, Parsons PJ and Rogan W, 2005. Factors influencing the difference between maternal and cord blood lead. Occupational and Environmental Medicine, 62, 263-269.

Heard MJ and Chamberlain AC, 1982. Effect of minerals and food on uptake of lead from the gastrointestinal tract in humans. Human Toxicology, 1, 411-415.

HELCOM (Helsinki Commission), 2007. Baltic Marine Environment Protection Commission. HELCOM Indicator Fact Sheets for 2007.

Henning SJ and Cooper LC, 1988. Intestinal accumulation of lead salts and milk lead by suckling rats. Proceedings of The Society for Experimental Biology and Medicine, 187, 110-116.

Hernandez-Avila M, Smith D, Meneses F, Sanin LH and Hu H, 1998. The influence of bone and blood lead on plasma lead levels in environmentally exposed adults. Environmental Health Perspectives, 106, 473-477.

Hernandez-Avila M, Villalpando CG, Palazuelos E, Hu H, Villalpando ME and Martinez DR, 2000. Determinants of blood lead levels across the menopausal transition. Archives of Environment Health, 55, 355-360.

Hertz-Picciotto I and Croft J, 1993. Review of the relation between blood lead and blood pressure. Epidemiologic Reviews, 15, 352-373.

Hill SJ, 2005. Speciation of Lead. In: Handbook of Elemental Speciation II. R Cornelis, J Caruso, H Crews, K Heumann (eds.). John Wiley & Sons, Chichester, 239-246.

Hornung RW, Lanphear BP and Dietrich KN, 2009. Age of greatest susceptibility to childhood lead exposure: a new statistical approach. Environmental Health Perspectives, 117, 1309-1312.

Hsiao CY, Wu HD, Lai JS and Kuo HW, 2001. A longitudinal study of the effects of long-term exposure to lead among lead battery factory workers in Taiwan (1989-1999). Sci Total Environ, 279, 151-158.

Hsieh LL, Liou SH, Chen YH, Tsai LC, Yang T and Wu TN, 2000. Association between aminolevulinate dehydrogenase genotype and blood lead levels in Taiwan. Journal of Occupational and Environmental Medicine, 42, 151-155.

Hu H, Pepper L and Goldman R, 1991. Effect of repeated occupational exposure to lead, cessation of exposure, and chelation on levels of lead in bone. American Journal of Industrial Medicine, 20, 723-735.

Hu H, Tellez-Rojo MM, Bellinger D, Smith D, Ettinger AS, Lamadrid-Figueroa H, Schwartz J, Schnaas L, Mercado-Garcia A and Hernandez-Avila M, 2006. Fetal lead exposure at each stage of pregnancy as a predictor of infant mental development. Environmental Health Perspectives, 114, 1730-1735.

Hubbs-Tait L, Mulugeta A, Bogale A, Kennedy TS, Baker ER and Stoecker BJ, 2009. Main and interaction effects of iron, zinc, lead, and parenting on children's cognitive outcomes. Developmental Neuropsychology, 34, 175-195.

Hursh JB and Mercer TT, 1970. Measurement of Pb-212 loss rate from human lungs. Journal of Applied Physiology, 28, 268-274.

Hursh JB, Schraub A, Sattler EL and Hofmann HP, 1969. Fate of 212Pb inhaled by human subjects. Health Physics, 16, 257-267.

Hursh JB and Suomela J, 1968. Absorption of 212Pb from the gastrointestinal tract of man. Acta Radiologica: Therapy, Physics, Biology, 7, 108-120.

Hwang KY, Schwartz BS, Lee BK, Strickland PT, Todd AC and Bressler JP, 2001. Associations of lead exposure and dose measures with erythrocyte protein kinase C activity in 212 current Korean lead workers. Toxicological Sciences, 62, 280-288.

IARC 2006. Inorganic and Organic Lead Compounds. IARC Monographs on the Evaluation of Carcinogenic Risks to Human. 87, 519.

Inskip MJ, Franklin CA, Baccanale CL, Manton WI, O'Flaherty EJ, Edwards CM, Blenkinsop JB and Edwards EB, 1996. Measurement of the flux of lead from bone to blood in a nonhuman primate

(Macaca fascicularis) by sequential administration of stable lead isotopes. Fundamental and Applied Toxicology, 33, 235-245.

IOM (Institute of Medicine), 1991. Nutrition during Lactation. Washington, DC: National Academies Press.

Ishihara N and Matsushiro T, 1986. Biliary and urinary-excretion of metals in humans. Archives of Environmental Health, 41, 324-330.

Ishihara N, Matsushiro T, Chen ZP and Zhou WS, 1997. Metals in gallstones in Japan and in the Peoples' Republic of China. Trace Elements and Electrolytes, 14, 187-190.

Iwata T, Yano E, Karita K, Dakeishi M and Murata K, 2005. Critical dose of lead affecting postural balance in workers. American Journal of Industrial Medicine, 48, 319-325.

Jacquet P and Tachon P, 1981. Effects of long-term lead exposure on monkey leukocyte chromosomes. Toxicology Letters, 8, 165-169.

James HM, Hilburn ME and Blair JA, 1985. Effects of meals and meal times on uptake of lead from the gastrointestinal tract in humans. Human Toxicology, 4, 401-407.

Janakiraman V, Ettinger A, Mercado-Garcia A, Hu H and Hernandez-Avila M, 2003. Calcium supplements and bone resorption in pregnancy: a randomized crossover trial. American Journal of Preventive Medicine, 24, 260-264.

JECFA (Joint FAO/WHO Expert Committee on Food Additives), 2000. Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives.

Jedrychowski W, Perera F, Jankowski J, Mrozek-Budzyn D, Mroz E, Flak E, Edwards S, Skarupa A and Lisowska-Miszczyk I, 2009. Gender specific differences in neurodevelopmental effects of prenatal exposure to very low-lead levels: the prospective cohort study in three-year olds. Early Human Development, 85, 503-510.

Jusko TA, Henderson CR, Lanphear BP, Cory-Slechta DA, Parsons PJ and Canfield RL, 2008. Blood lead concentrations < 10 microg/dL and child intelligence at 6 years of age. Environmental Health Perspectives, 116, 243-248.

Kabata-Pendias A and Mukherjeee A, 2007. Trace elements from soil to human. Editor. Springer, 550 pp.

Kabata-Pendias A and Pendias H, 1999. Biogeochemistry of trace elements. 2nd edition. Editor. Wyd. Nauk. PWN, Warszawa (in Polish),

Kappas A, Sassa S, Galbraith RA and Nordmann Y, 1995. The porphyrias In: The metabolic basis of inherited diseases. 7th ed. CR Scriver, AL Beaudet, WS Sly, D Valle. McGraw-Hill, New York, 2103-2159.

Kazi TG, Jalbani N, Arain MB, Jamali MK, Afridi HI and Shah AQ, 2009. Determination of toxic elements in different brands of cigarette by atomic absorption spectrometry using ultrasonic assisted acid digestion. Environmental Monitoring and Assessment, 154, 155-167.

Kehoe RA, 1987. Studies of lead administration and elimination in adult volunteers under natural and experimentally induced conditions over extended periods of time. Food and Chemical Toxicology, 25, i-iv, 425-453.

Kersting M, Alexy U, Sichert-Hellert W, Manz F and Schoch G, 1998. Measured consumption of commercial infant food products in German infants: Results from the DONALD study. Journal of Pediatric Gastroenterology and Nutrition, 27, 547-552.

Khalil-Manesh F, Gonick HC and Cohen AH, 1993. Experimental model of lead nephropathy. III. Continuous low-level lead administration. Archives of Environmental Health, 48, 271-278.

Khalil-Manesh F, Gonick HC, Cohen AH, Alinovi R, Bergamaschi E, Mutti A and Rosen VJ, 1992. Experimental model of lead nephropathy. I. Continuous high-dose lead administration. Kidney International, 41, 1192-1203.

Kim R, Rotnitsky A, Sparrow D, Weiss S, Wager C and Hu H, 1996. A longitudinal study of low-level lead exposure and impairment of renal function. The Normative Aging Study. JAMA, 275, 1177-1181.

Kim Y, Kim BN, Hong YC, Shin MS, Yoo HJ, Kim JW, Bhang SY and Cho SC, 2009. Co-exposure to environmental lead and manganese affects the intelligence of school-aged children. Neurotoxicology, 30, 564-571.

Kordas K, Canfield RL, Lopez P, Rosado JL, Vargas GG, Cebrian ME, Rico JA, Ronquillo D and Stoltzfus RJ, 2006. Deficits in cognitive function and achievement in Mexican first-graders with low blood lead concentrations. Environmental Research, 100, 371-386.

Korn MG, de Andrade JB, de Jesus DS, Lemos VA, Bandeira ML, Dos Santos WN, Bezerra MA, Amorim FA, Souza AS and Ferreira SL, 2006. Separation and preconcentration procedures for the determination of lead using spectrometric techniques: A review. Talanta, 69, 16-24.

Kostial K, Kello D, Jugo S, Rabar I and Maljkovic T, 1978. Influence of age on metal metabolism and toxicity. Environmental Health Perspectives, 25, 81-86.

Kramer HJ, Gonick HC and Lu E, 1986. In vitro inhibition of Na-K-ATPase by trace metals: relation to renal and cardiovascular damage. Nephron, 44, 329-336.

Krieg EF, Jr., Chrislip DW, Crespo CJ, Brightwell WS, Ehrenberg RL and Otto DA, 2005. The relationship between blood lead levels and neurobehavioral test performance in NHANES III and related occupational studies. Public Health Reports, 120, 240-251.

Kristensen P, Eilertsen E, Einarsdottir E, Ovrebo S and Haugen A, 1993. Effect modification by inorganic lead in the dominant lethal assay. Mutation Research, 302, 33-38.

Kromhout D, 1988. Blood lead and coronary heart disease risk among elderly men in Zutphen, The Netherlands. Environmental Health Perspectives, 78, 43-46.

Kulka E and Gzyl J, 2008. Assessment of lead and cadmium soil contamination in the vicinity of a nonferrous metal smelter. Archives of Environmental Protection, 34, 105-115.

Lagerkvist BJ, Ekesrydh S, Englyst V, Nordberg GF, Soderberg HA and Wiklund DE, 1996. Increased blood lead and decreased calcium levels during pregnancy: a prospective study of Swedish women living near a smelter. American Journal of Public Health, 86, 1247-1252.

Lai B, Murthy RC, Anand M, Chandra SV, Kumar R, Tripathi O and Srimal RC, 1991. Cardiotoxicity and hypertension in rats after oral lead exposure. Drug and Chemical Toxicology, 14, 305-318.

Lakind JS, 1998. Comparison of three models for predicting blood lead levels in children: episodic exposures to lead. Journal of Exposure Analysis and Environmental Epidemiology, 8, 399-406.

Landrigan P, Nordberg M, Lucchini R, Nordberg G, Grandjean P, Iregren A and Alessio L, 2006. The Declaration of Brescia on Prevention of the Neurotoxicity of Metals Brescia, Italia 17-18 June 2006. Medicina del Lavoro, 97, 811-814.

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, 2005. Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. Environmental Health Perspectives, 113, 894-899.

Larsen EH, Andersen NL, Moller A, Petersen A, Mortensen GK and Petersen J, 2002. Monitoring the content and intake of trace elements from food in Denmark. Food Additives & Contaminants, 19, 33-46.

Larsson B, Slorach SA, Hagman U and Hofvander Y, 1981. WHO collaborative breast-feeding study. 2. Levels of lead and cadmium in Swedish human-milk, 1978-1979. Acta Paediatrica Scandinavica, 70, 281-284.

Lasley SM and Gilbert ME, 2000. Glutamatergic components underlying lead-induced impairments in hippocampal synaptic plasticity. Neurotoxicology, 21, 1057-1068.

Lauwerys RR and Hoet P, 2001. Industrial chemical exposure. Guidelines for biological monitoring. Editor. Lewis Publishers, CRC Press, Inc., Boca Raton, London, NY, Washington DC, 638 pp.

Leblanc JC, Tard A, Volatier JL and Verger P, 2005. Estimated dietary exposure to principal food mycotoxins from the first French Total Diet Study. Food Additives & Contaminants, 22, 652-672.

Levey AS, Stevens LA, Schmid CH, Zhang YP, Castro AF, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T and Coresh J, 2009. A New Equation to Estimate Glomerular Filtration Rate. Annals of Internal Medicine, 150, 604-607.

Li G, Jiao S, Yin X, Deng Y, Pang X and Wang Y, 2009. The risk of melamine-induced nephrolithiasis in young children starts at a lower intake level than recommended by the WHO. Pediatric Nephrology, 25, 135-141.

Li W, Han S, Gregg TR, Kemp FW, Davidow AL, Louria DB, Siegel A and Bogden JD, 2003. Lead exposure potentiates predatory attack behavior in the cat. Environmental Research, 92, 197-206.

Lindahl LS, Bird L, Legare ME, Mikeska G, Bratton GR and Tiffany-Castiglioni E, 1999. Differential ability of astroglia and neuronal cells to accumulate lead: dependence on cell type and on degree of differentiation. Toxicological Sciences, 50, 236-243.

Llobet JM, Falco G, Casas C, Teixido A and Domingo JL, 2003. Concentrations of arsenic, cadmium, mercury, and lead in common foods and estimated daily intake by children, adolescents, adults, and seniors of Catalonia, Spain. Journal of Agricultural and Food Chemistry, 51, 838-842.

Llobet JM, Granero S, Torres A, Schuhmacher M and Domingo JL, 1998. Biological monitoring of environmental pollution and human exposure to metals in Tarragona, Spain. IV. Estimation of the dietary intake. Trace Elements and Electrolytes, 25, 136-141.

Maddaloni M, Lolacono N, Manton W, Blum C, Drexler J and Graziano J, 1998. Bioavailability of soilborne lead in adults, by stable isotope dilution. Environmental Health Perspectives, 106 Suppl 6, 1589-1594.

Mahaffey KR and Annest JL, 1986. Association of erythrocyte protoporphyrin with blood lead level and iron status in the second National Health and Nutrition Examination Survey, 1976-1980. Environmental Research, 41, 327-338.

Maldonado-Vega M, Cerbon-Solorzano J, Albores-Medina A, Hernandez-Luna C and Calderon-Salinas JV, 1996. Lead: intestinal absorption and bone mobilization during lactation. Human & Experimental Toxicology, 15, 872-877.

Mameli O, Caria MA, Melis F, Solinas A, Tavera C, Ibba A, Tocco M, Flore C and Sanna Randaccio F, 2001. Neurotoxic effect of lead at low concentrations. Brain Research Bulletin, 55, 269-275.

Mannino DM, Albalak RA, Grosse S and Repace J, 2003. Second-hand smoke exposure and blood lead levels in US children. Epidemiology, 14, 719-727.

Manton WI, Rothenberg SJ and Manalo M, 2001. The lead content of blood serum. Environmental Research, 86, 263-273.

Markovac J and Goldstein GW, 1988. Picomolar concentrations of lead stimulate brain protein kinase C. Nature, 334, 71-73.

Martena MJ, Van Der Wielen JCA, Rietjens I, Klerx WNM, De Groot HN and Konings EJM, 2010. Monitoring of mercury, arsenic, and lead in traditional Asian herbal preparations on the Dutch

market and estimation of associated risks. Food Additives and Contaminants Part a-Chemistry Analysis Control Exposure & Risk Assessment, 27, 190-205.

Marzec Z and Schlegel-Zawadzka M, 2004. Exposure to cadmium, lead and mercury in the adult population from Eastern Poland, 1990-2002. Food Additives and Contaminants, 21, 963-970.

Mensink GBM and Beitz R, 2004. Food and nutrient intake in East and West Germany, 8 years after the reunification - The German Nutrition Survey 1998. European Journal of Clinical Nutrition, 58, 1000-1010.

Meyer-Baron M and Seeber A, 2000. A meta-analysis for neurobehavioural results due to occupational lead exposure with blood lead concentrations <70 microg/100 ml. Archives of Toxicology, 73, 510-518.

Mielke HW, Dugas D, Mielke PW, Jr., Smith KS and Gonzales CR, 1997. Associations between soil lead and childhood blood lead in urban New Orleans and rural Lafourche Parish of Louisiana. Environmental Health Perspectives, 105, 950-954.

Millot R, Allegre CJ, Gaillardet J and Roy S, 2004. Lead isotopic systematics of major river sediments: a new estimate of the Pb isotopic composition of the Upper Continental Crust. Chemical Geology, 203, 75-90.

Min JY, Min KB, Cho SI, Kim R, Sakong J and Paek D, 2007. Neurobehavioral function in children with low blood lead concentrations. Neurotoxicology, 28, 421-425.

Min MO, Singer LT, Kirchner HL, Minnes S, Short E, Hussain Z and Nelson S, 2009. Cognitive development and low-level lead exposure in poly-drug exposed children. Neurotoxicology and Teratology, 31, 225-231.

Miranda ML, Kim D, Galeano MA, Paul CJ, Hull AP and Morgan SP, 2007. The relationship between early childhood blood lead levels and performance on end-of-grade tests. Environmental Health Perspectives, 115, 1242-1247.

Mishra VK, Kim KH, Kang CH and Choi KC, 2004. Wintertime sources and distribution of airborne lead in Korea. Atmospheric Environment, 38, 2653-2664.

Moldoon SB, Cauley JA, JKuller LH, Morrow LA, Needleman HL, Scott J and F.J. H, 1996. Effects of blood lead levels on cognitive function of older women. Neuroepidemiology, 15, 62-72.

Møller L and Kristensen TS, 1992. Blood lead as a cardiovascular risk factor. American Journal of Epidemiology, 136, 1091-1100.

Moore JF, Goyer RA and Wilson M, 1973. Lead-induced inclusion bodies. Solubility, amino acid content, and relationship to residual acidic nuclear proteins. Laboratory Investigation, 29, 488-494.

Moore MR, Meredith PA, Watson WS, Sumner DJ, Taylor MK and Goldberg A, 1980. The percutaneous absorption of lead-203 in humans from cosmetic preparations containing lead acetate, as assessed by whole-body counting and other techniques. Food and Cosmetics Toxicology, 18, 399-405.

Moreiras O and Cuadrado C, 1992. Theoretical-study of the intake of trace-elements (nutrients and contaminants) via total diet in some geographical areas of Spain. Biological Trace Element Research, 32, 93-103.

Morrison JN and Quarterman J, 1987. The relationship between iron status and lead absorption in rats. Biological Trace Element Research, 14, 115-126.

Morrow PE, Beiter H, Amato F and Gibb FR, 1980. Pulmonary retention of lead: an experimental study in man. Environmental Research, 21, 373-384.

Morton MJ and Laffoon SW, 2008. Cigarette smoke chemistry market maps under Massachusetts Department of Public Health smoking conditions. Regulatory Toxicology and Pharmacology, 51, 1-30.

Mostafa GA, El-Shahawi HH and Mokhtar A, 2009. Blood lead levels in Egyptian children from high and low lead-polluted areas: impact on cognitive function. Acta Neurologica Scandinavica, 120, 30-37.

Muntner P, He J, Vupputuri S, Coresh J and Batuman V, 2003. Blood lead and chronic kidney disease in the general United States population: results from NHANES III. Kidney International, 63, 1044-1050.

Muntner P, Menke A, DeSalvo KB, Rabito FA and Batuman V, 2005. Continued decline in blood lead levels among adults in the United States: the National Health and Nutrition Examination Surveys. Archives of Internal Medicine, 165, 2155-2161.

Murata K, Iwata T, Dakeishi M and Karita K, 2009. Lead toxicity: does the critical level of lead resulting in adverse effects differ between adults and children? Journal of Occupational Health, 51, 1-12.

Murray K, Bazzi A, Carter C, Ehlert A, Harris A, Kopec M, Richardson J and Sokol H, 1997. Distribution and mobility of lead in soils at an outdoor shooting range. Journal of Soil Contamination, 6, 79-93.

Mykkänen HM and Wasserman RH, 1981. Gastrointestinal absorption of lead (203Pb) in chicks: influence of lead, calcium, and age. Journal of Nutrition, 111, 1757-1765.

Mykkänen HM and Wasserman RH, 1982. Effect of vitamin D on the intestinal absorption of 203Pb and 47Ca in chicks. Journal of Nutrition, 112, 520-527.

Namihira D, Saldivar L, Pustilnik N, Carreon GJ and Salinas ME, 1993. Lead in human blood and milk from nursing women living near a smelter in Mexico City. Journal of Toxicology and Environment Health, 38, 225-232.

Nash D, Magder L, Lustberg M, Sherwin RW, Rubin RJ, Kaufmann RB and Silbergeld EK, 2003. Blood lead, blood pressure, and hypertension in perimenopausal and postmenopausal women. JAMA, 289, 1523-1532.

Nash D, Magder LS, Sherwin R, Rubin RJ and Silbergeld EK, 2004. Bone density-related predictors of blood lead level among peri- and postmenopausal women in the United States: The Third National Health and Nutrition Examination Survey, 1988-1994. American Journal of Epidemiology, 160, 901-911.

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

Nawrot TS, Thijs L, Den Hond EM, Roels HA and Staessen JA, 2002. An epidemiological re-appraisal of the association between blood pressure and blood lead: a meta-analysis. Journal of Human Hypertension, 16, 123-131.

Newland MC, Yezhou S, Logdberg B and Berlin M, 1994. Prolonged behavioral effects of in utero exposure to lead or methyl mercury: reduced sensitivity to changes in reinforcement contingencies during behavioral transitions and in steady state. Toxicology and Applied Pharmacology, 126, 6-15.

Ni Z, Hou S, Barton CH and Vaziri ND, 2004. Lead exposure raises superoxide and hydrogen peroxide in human endothelial and vascular smooth muscle cells. Kidney International, 66, 2329-2336.

Nigg JT, Knottnerus GM, Martel MM, Nikolas M, Cavanagh K, Karmaus W and Rappley MD, 2008. Low blood lead levels associated with clinically diagnosed attention-deficit/hyperactivity disorder and mediated by weak cognitive control. Biological Psychiatry, 63, 325-331.

Nihei MK and Guilarte TR, 2002. Molecular mechanisms of low-level Pb2+ neurotoxicity. In: Handbook of neurotoxicology. EJ Massaro. Humana Press, Totowa, NJ, 107-133.

NTP (National Toxicology Programme), 2004. Lead (CAS No 7439-92-1) and lead compounds. In: Report on carcinogens. 11th ed. Research Triangle Park: US Department of Health and Human Services. Available from http://ntp.niehs.nih.gov/ntp/roc/eleventh/profiles/s101lead.pdf.

OECD (Organization for Economic Cooperation and Development), 1993. Risk Reduction Monograph No. 1 Lead Background And National Experience With Reducing Risk. Paris. 1-295.

O'Flaherty EJ, 1995. Physiologically-based models for bone-seeking elements. 5. Lead absorption and disposition in childhood. Toxicology and Applied Pharmacology, 131, 297-308.

O'Flaherty EJ, Hammond PB and Lerner SI, 1982. Dependence of apparent blood lead half-life on the length of previous lead exposure in humans. Fundamental and Applied Toxicology, 2, 49-54.

Ong CN and Lee WR, 1980. High affinity of lead for fetal haemoglobin. British Journal of Industrial Medicine, 37, 292-298.

Onyango AW, Receveur O and Esrey SA, 2002. The contribution of breast milk to toddler diets in western Kenya. Bulletin of The World Health Organization, 80, Available from http://www.who.int/bulletin/archives/80(84)292.pdf.

Oskarsson A and Fowler BA, 1985. Effects of lead inclusion bodies on subcellular distribution of lead in rat kidney: the relationship to mitochondrial function. Experimental and Molecular Pathology, 43, 397-408.

OSPAR (The Convention for the Protection of the Marine Environment of the North-East Atlantic), 2009. Background document on lead. Hazardous Substances Series. 28 pp.

Pacyna JM and Pacyna EG, 2001. An assessment of global and regional emissions of trace metals to the atmosphere from anthropogenic sources worldwide. Environmental Reviews, 9, 269-298.

Pacyna JM, Pacyna EG and Aas W, 2009. Changes of emissions and atmospheric deposition of mercury, lead, and cadmium. Atmospheric Environment, 43, 117-127.

Patel A, Mamtani MR, Thakre TP and Kulkarni H, 2006. Association of umbilical cord lead with neonatal behaviour at varying levels of exposure. Behavioral and Brain Functions, 2, 1-12.

Payton M, Riggs KM, Spiro A, 3rd, Weiss ST and Hu H, 1998. Relations of bone and blood lead to cognitive function: the VA Normative Aging Study. Neurotoxicology and Teratology, 20, 19-27.

Perry HM, Jr., Erlanger MW and Perry EF, 1988. Increase in the blood pressure of rats chronically fed low levels of lead. Environmental Health Perspectives, 78, 107-111.

Piccinini F, Favalli L and Chiari MC, 1977. Experimental investigations on the contraction induced by lead in arterial smooth muscle. Toxicology, 8, 43-51.

Plusquellec P, Muckle G, Dewailly E, Ayotte P, Jacobson SW and Jacobson JL, 2007. The relation of low-level prenatal lead exposure to behavioral indicators of attention in Inuit infants in Arctic Quebec. Neurotoxicology and Teratology, 29, 527-537.

Pocock SJ, Shaper AG, Ashby D, Delves HT and Clayton BE, 1988. The relationship between blood lead, blood pressure, stroke, and heart attacks in middle-aged British men. Environmental Health Perspectives, 78, 23-30.

Pocock SJ, Shaper AG, Ashby D, Delves T and Whitehead TP, 1984. Blood lead concentration, blood pressure, and renal function. British Medical Journal (Clinical Research Ed.), 289, 872-874.

Popovic M, McNeill FE, Chettle DR, Webber CE, Lee CV and Kaye WE, 2005. Impact of occupational exposure on lead levels in women. Environmental Health Perspectives, 113, 478-484.

Pounds JG, Marlar RJ and Allen JR, 1978. Metabolism of lead-210 in juvenile and adult rhesus monkeys (Macaca mulatta). Bulletin of Environment Contamination and Toxicology, 19, 684-691.

Pruvot C, Douay F, Herve F and Waterlot C, 2006. Heavy metals in soil, crops and grass as a source of human exposure in the former mining areas. Journal of Soils and Sediments, 6, 215-220.

Rabinowitz MB, 1991. Toxicokinetics of bone lead. Environmental Health Perspectives, 91, 33-37.

Rabinowitz MB, Kopple JD and Wetherill GW, 1980. Effect of food intake and fasting on gastrointestinal lead absorption in humans. American Journal of Clinical Nutrition, 33, 1784-1788.

Rabinowitz MB, Wetherill GW and Kopple JD, 1976. Kinetic-analysis of lead metabolism in healthy humans. Journal of Clinical Investigation, 58, 260-270.

Rhodes D, Spiro A, 3rd, Aro A and Hu H, 2003. Relationship of bone and blood lead levels to psychiatric symptoms: the normative aging study. Journal of Occupational and Environmental Medicine, 45, 1144-1151.

Rice DC, 1985. Chronic low-lead exposure from birth produces deficits in discrimination reversal in monkeys. Toxicology and Applied Pharmacology, 77, 201-210.

Rice DC, 1990. Lead-induced behavioral impairment on a spatial discrimination reversal task in monkeys exposed during different periods of development. Toxicology and Applied Pharmacology, 106, 327-333.

Richter PA, Bishop EE, Wang JT and Swahn MH, 2009. Tobacco Smoke Exposure and Levels of Urinary Metals in the US Youth and Adult Population: The National Health and Nutrition Examination Survey (NHANES) 1999-2004. International Journal of Environmental Research and Public Health, 6, 1930-1946.

Rickert WS and Kaiserman MJ, 1994. Levels of lead, cadmium, and mercury in Canadian cigarette tobacco as indicators of environmental-change - results from a 21-year study (1968-1988). Environmental Science & Technology, 28, 924-927.

Rischitelli G, Nygren P, Bougatsos C, Freeman M and Helfand M, 2006. Screening for elevated lead levels in childhood and pregnancy: an updated summary of evidence for the US Preventive Services Task Force. Pediatrics, 118, e1867-1895.

Rodríguez-Ortíz JC, Valdez-Cepeda RD, Lara-Mireles JL, Rodrígues-Fuentes H, Vázquez-Alvarado RE, Magallanes-Quintanar R and García-Hernández JL, 2006. Soil Nitrogen Fertilization Effects on Phytoextraction of Cadmium and Lead by Tobacco (*Nicotiana tabacum* L.). Bioremediation Journal, 10, 105-114.

Roels H, Lauwerys R, Konings J, Buchet JP, Bernard A, Green S, Bradley D, Morgan W and Chettle D, 1994. Renal function and hyperfiltration capacity in lead smelter workers with high bone lead. Occupational and Environmental Medicine, 51, 505-512.

Roy A, Bellinger D, Hu H, Schwartz J, Ettinger AS, Wright RO, Bouchard M, Palaniappan K and Balakrishnan K, 2009. Lead exposure and behavior among young children in Chennai, India. Environmental Health Perspectives, 117, 1607-1611.

Sakai T, Yanagihara S, Kunugi Y and Ushio K, 1982. Relationships between distribution of lead in erythrocytes in vivo and in vitro and inhibition of ALA-D. British Journal of Industrial Medicine, 39, 382-387.

Sakai T, Yanagihara S, Kunugi Y and Ushio K, 1983. Mechanisms of ALA-D inhibition by lead and of its restoration by zinc and dithiothreitol. British Journal of Industrial Medicine, 40, 61-66.

Sand S, von Rosen D, Victorin K and Filipsson AF, 2006. Identification of a critical dose level for risk assessment: developments in benchmark dose analysis of continuous endpoints. Toxicological Sciences, 90, 241-251.

SCCNFP (The Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers), 2004. Opinion concerning lead acetate. SCCNFP/0832/04. 2 pp. Available from http://ec.europa.eu/health/ph_risk/committees/sccp/documents/out286_en.pdf.

SCF (Scientific Committee on Food), 1992. Opinion of 19 June 1992, Thirty second series, 1994, p. 7. Available from http://ec.europa.eu/food/fs/sc/scf/reports/scf_reports_32.pdf.

Schmidt U and Huber F, 1976. Methylation of organolead and lead(II) compounds to (CH3)4Pb by microorganisms. Nature, 259, 157-158.

Schnaas L, Rothenberg SJ, Flores MF, Martinez S, Hernandez C, Osorio E, Velasco SR and Perroni E, 2006. Reduced intellectual development in children with prenatal lead exposure. Environmental Health Perspectives, 114, 791-797.

Schober SE, Mirel LB, Graubard BI, Brody DJ and Flegal KM, 2006. Blood lead levels and death from all causes, cardiovascular disease, and cancer: results from the NHANES III mortality study. Environmental Health Perspectives, 114, 1538-1541.

Schuhmacher M, Hernandez M, Domingo JL, FernandezBallart JD, Llobet JM and Corbella J, 1996. A longitudinal study of lead mobilization during pregnancy: Concentrations in maternal and umbilical cord blood. Trace Elements and Electrolytes, 13, 177-181.

Schulz C, Angerer J, Ewers U, Heudorf U and Wilhelm M, 2009. Revised and new reference values for environmental pollutants in urine or blood of children in Germany derived from the German environmental survey on children 2003-2006 (GerES IV). International Journal of Hygiene and Environmental Health, 212, 637-647.

Schutz A, Bergdahl IA, Ekholm A and Skerfving S, 1996. Measurement by ICP-MS of lead in plasma and whole blood of lead workers and controls. Occupational and Environmental Medicine, 53, 736-740.

Schwartz BS, Lee BK, Bandeen-Roche K, Stewart W, Bolla K, Links J, Weaver V and Todd A, 2005. Occupational lead exposure and longitudinal decline in neurobehavioral test scores. Epidemiology, 16, 106-113.

Schwartz BS, Stewart WF, Bolla KI, Simon PD, Bandeen-Roche K, Gordon PB, Links JM and Todd AC, 2000. Past adult lead exposure is associated with longitudinal decline in cognitive function. Neurology, 55, 1144-1150.

Schwartz J, 1988. The relationship between blood lead and blood pressure in the NHANES II survey. Environmental Health Perspectives, 78, 15-22.

Schwartz J, 1994. Societal benefits of reducing lead exposure. Environmental Research, 66, 105-124.

Schwartz J, 1995. Lead, blood pressure, and cardiovascular disease in men. Archives of Environment Health, 50, 31-37.

SCOOP (Scientific Cooperation), 2004. SCOOP Report of experts participating in Task 3.2.11. March 2004. Assessment of the dietary exposure to arsenic, cadmium, lead and mercury of the population of the EU Member States. 125 pp.

Selmer RM, Kristiansen IS, Haglerod A, Graff-Iversen S, Larsen HK, Meyer HE, Bonaa KH and Thelle DS, 2000. Cost and health consequences of reducing the population intake of salt. Journal of Epidemiology and Community Health, 54, 697-702.

Seppäläinen AM, Hernberg S and Kock B, 1979. Relationship between blood lead levels and nerve-conduction velocities. Neurotoxicology, 1, 313-332.

Shih RA, Glass TA, Bandeen-Roche K, Carlson MC, Bolla KI, Todd AC and Schwartz BS, 2006. Environmental lead exposure and cognitive function in community-dwelling older adults. Neurology, 67, 1556-1562.

Shih RA, Hu H, Weisskopf MG and Schwartz BS, 2007. Cumulative lead dose and cognitive function in adults: a review of studies that measured both blood lead and bone lead. Environmental Health Perspectives, 115, 483-492.

Shih TM and Hanin I, 1978. Effects of chronic lead exposure on levels of acetylcholine and choline and on acetylcholine turnover rate in rat brain areas in vivo. Psychopharmacology (Berl), 58, 263-269.

Sidhu MK, Fernandez C, Khan MY and Kumar S, 1991. Induction of morphological transformation, anchorage-independent growth and plasminogen activators in non-tumorigenic human osteosarcoma cells by lead chromate. Anticancer Research, 11, 1045-1053.

Sidhu P and Nehru B, 2003. Relationship between lead-induced biochemical and behavioral changes with trace element concentrations in rat brain. Biological Trace Element Research, 92, 245-256.

Silbergeld EK, 1977. Interactions of lead and calcium on the synaptosomal uptake of dopamine and choline. Life Sciences, 20, 309-318.

Silbergeld EK, Waalkes M and Rice JM, 2000. Lead as a carcinogen: experimental evidence and mechanisms of action. American Journal of Industrial Medicine, 38, 316-323.

Simons TJ, 1985. Influence of lead ions on cation permeability in human red cell ghosts. Journal of Membrane Biology, 84, 61-71.

Simons TJ, 1986a. The role of anion transport in the passive movement of lead across the human red cell membrane. The Journal of Physiology, 378, 287-312.

Simons TJ, 1986b. Passive transport and binding of lead by human red blood cells. The Journal of Physiology, 378, 267-286.

Simons TJ, 1988. Active transport of lead by the calcium pump in human red cell ghosts. The Journal of Physiology, 405, 105-113.

Simons TJ, 1993. Lead transport and binding by human erythrocytes in vitro. Pflugers Archiv. European Journal of Physiology, 423, 307-313.

Six KM and Goyer RA, 1970. Experimental enhancement of lead toxicity by low dietary calcium. The Journal of Laboratory and Clinical Medicine, 76, 933-942.

Skerfving S and Bergdahl IA, 2007. Lead. In: Handbook on the toxicology of metals, 3rd edition. GF Nordberg, BA Fowler, M Nordberg, LT Friberg (eds.). Elsevier, 599-643.

Skjelkvale BL and Traaen TS, 2003. Heavy metals in surface waters; results from ICP Waters. In: ICP waters Report, 73/2003. Convention on Long-range Transboundary Air Pollution. International Cooperative Programme on Assessment and Monitoring of Acidification of Rivers and Lakes. The 15-year report: Assessment and monitoring of surface waters in Europe and North America; acidification and recovery, dynamic modelling and heavy metals. Norwegian Institute for Water Research NIVA.

Slezakova K, Pereira MC and Alvim-Ferraz MC, 2009. Influence of tobacco smoke on the elemental composition of indoor particles of different sizes. Atmospheric Environment, 43, 486-493.

Slob W, 2006. Probabilistic dietary exposure assessment taking into account variability in both amount and frequency of consumption. Food and Chemical Toxicology, 44, 933-951.

Smith D, Hernandez-Avila M, Tellez-Rojo MM, Mercado A and Hu H, 2002. The relationship between lead in plasma and whole blood in women. Environmental Health Perspectives, 110, 263-268.

Smith DR, Kahng MW, Quintanilla-Vega B and Fowler BA, 1998. High-affinity renal lead-binding proteins in environmentally-exposed humans. Chemico-Biological Interactions, 115, 39-52.

Smith DR, Osterloh JD and Flegal AR, 1996. Use of endogenous, stable lead isotopes to determine release of lead from the skeleton. Environmental Health Perspectives, 104, 60-66.

Solon O, Riddell J, Quimbo SA, Butrick E, Aylward GP, Bacate ML and Peabody JW, 2008. Associations between congnitive function, blood lead concentration, and nutrition among children in the Central Philippines. The Journal of Pediatrics, 152, 237-243.

Staessen JA, Bulpitt CJ, Fagard R, Lauwerys RR, Roels H, Thijs L and Amery A, 1994. Hypertension caused by low-level lead exposure: myth or fact? Journal of Cardiovascular Risk, 1, 87-97.

Staessen JA, Lauwerys RR, Buchet JP, Bulpitt CJ, Rondia D, Vanrenterghem Y and Amery A, 1992. Impairment of renal function with increasing blood lead concentrations in the general population. The Cadmibel Study Group. The New England Journal of Medicine, 327, 151-156.

Stauber JL, Florence TM, Gulson BL and Dale LS, 1994. Percutaneous absorption of inorganic lead compounds. Science of the Total Environment, 145, 55-70.

Sternowsky HJ and Wessolowski R, 1985. Lead and cadmium in breast-milk - higher levels in urban vs rural mothers during the 1st 3 months of lactation. Archives of Toxicology, 57, 41-45.

Stohs SJ and Bagchi D, 1995. Oxidative mechanisms in the toxicity of metal ions. Free Radical Biology & Medicine, 18, 321-336.

Stowe HD, Goyer RA, Krigman M, Wilson M and Cates M, 1973. Experimental oral lead toxicity in young dogs. Clinical and morphologic effects. Archives of Pathology, 95, 106-116.

Surkan PJ, Zhang A, Trachtenberg F, Daniel DB, McKinlay S and Bellinger DC, 2007. Neuropsychological function in children with blood lead levels <10 microg/dL. Neurotoxicology, 28, 1170-1177.

Suszkiw J, Toth G, Murawsky M and Cooper GP, 1984. Effects of Pb2+ and Cd2+ on acetylcholine release and Ca2+ movements in synaptosomes and subcellular fractions from rat brain and Torpedo electric organ. Brain Research, 323, 31-46.

Suszkiw JB, 2004. Presynaptic disruption of transmitter release by lead. Neurotoxicology, 25, 599-604.

Tahvonen R, 1997. Contents of lead in foods and diet in Finland in the early 1990s. Food Reviews International, 13, 77-90.

Tait PA, Vora A, James S, Fitzgerald DJ and Pester BA, 2002. Severe congenital lead poisoning in a preterm infant due to a herbal remedy. Medical Journal of Australia, 177, 193-195.

Tellez-Rojo MM, Bellinger DC, Arroyo-Quiroz C, Lamadrid-Figueroa H, Mercado-Garcia A, Schnaas-Arrieta L, Wright RO, Hernandez-Avila M and Hu H, 2006. Longitudinal associations between blood lead concentrations lower than 10 microg/dL and neurobehavioral development in environmentally exposed children in Mexico City. Pediatrics, 118, e323-330.

Thomas KW, Pellizzari ED and Berry MR, 1999. Population-based dietary intakes and tap water concentrations for selected elements in the EPA Region V National Human Exposure Assessment Survey (NHEXAS). Journal of Exposure Analysis and Environmental Epidemiology, 9, 402-413.

Toscano CD and Guilarte TR, 2005. Lead neurotoxicity: from exposure to molecular effects. Brain Research. Brain Research Reviews, 49, 529-554.

Treble RG and Thompson TS, 1997. Preliminary results of a survey of lead levels in human liver tissue. Bulletin of Environment Contamination and Toxicology, 59, 688-695.

Tsaih SW, Korrick S, Schwartz J, Amarasiriwardena C, Aro A, Sparrow D and Hu H, 2004. Lead, diabetes, hypertension, and renal function: the normative aging study. Environmental Health Perspectives, 112, 1178-1182.

Tsoumbaris P and Tsoukali-Papadopoulou H, 1994. Heavy-metals in common foodstuff - quantitative-analysis. Bulletin of Environmental Contamination and Toxicology, 53, 61-66.

Tukker A, Buijst H, van Oers L and van der Voet E, 2001. Risks to health and the environment related to the use of lead in products. TNO Report STB-01-39 for the European Commission. Brussels, Directorate General Enterprise.

Turrini A and Lombardi-Boccia G, 2002. The formulation of the market basket of the Italian total diet 1994-96. Nutrition Research, 22, 1151-1162.

Turrini A, Saba A, Perrone D, Cialfa E and D'Amicis A, 2001. INN-CA (Nationwide Nutritional Survey of Food Behaviour). Food consumption patterns in Italy: the INN-CA Study 1994-1996. European Journal of Clinical Nutrition, 55, 571-588.

UNEP (United Nations Environment Programme), 2008. Interim review of scientific information on lead. Version of March 2008.

US ATSDR (United States Agency for Toxic Substances and Disease Registry), 2007. Toxicological Profile for lead. U.S. Department of Health and Human Services. 1-582.

US EPA (U.S. Environmental Protection Agency), 1973. EPA Requires Phase-Out of Lead in All Grades of Gasoline [EPA press release - November 28, 1973].

US EPA (U.S. Environmental Protection Agency), 1977. Control techniques for Lead Air Emissions. Office of Air Quality Planning and Standards. EPA-450/2-77-012. Research Triangle Park, N.C.

US EPA (U.S. Environmental Protection Agency), 1985. Lead exposures in the human environment. Research Triangle Park, NC: U.S. Environmental Protection Agency, Environmental Criteria and Assessment Office. EPA600D86185, PB86241007.

US EPA (U.S. Environmental Protection Agency), 1986. Air quality criteria for lead. Office of Research and Development, Office of Health and Environmental Assessment,Environmental Criteria and Assessment Office. EPA600883028F,

US EPA (U.S. Environmental Protection Agency), 1996. EPA Takes Final Step in Phaseout of Leaded Gasoline, [EPA press release-January 29, 1996]. Available from http://www.epa.gov/history/topics/lead/02.htm.

US EPA (U.S. Environmental Protection Agency), 1997. Exposure Factors Handbook. Vol 2: PB98-124233—Food Ingestion Factors. Chapter 14: Breast Milk Intake. EPA/600/P-95/002Fa:1–8. Washington, DC:U.S. Environmental Protection Agency.

US EPA (U.S. Environmental Protection Agency), 2000. Lead compounds. Available from http://www.epa.gov/ttn/atw/hlthef/lead.html.

US EPA (U.S. Environmental Protection Agency), 2004. IRIS Integrated Risk Information System, Lead and compounds (inorganic) (CASRN 7439-92-1). Available from http://www.epa.gov/ncea/iris/subst/0277.htm#noncar.

US EPA (U.S. Environmental Protection Agency), 2009. Guidance Manual for the Integrated Exposure Uptake Biokinetic Model for Children. Publication Number 9285.7-215-1, EPA 540-R-93-081, PB93-963510. February 1994. Version 1.1.

US FDA (U.S. Federal and Drug Administration), 1993. US FDA CSFAN Federal Register, Vol. 58, p. 33860, June 21, 1993.

Vallee BL and Ulmer DD, 1972. Biochemical effects of mercury, cadmium, and lead. Annual Review of Biochemistry, 41, 91-128.

Vaziri ND, 2008. Mechanisms of lead-induced hypertension and cardiovascular disease. American Journal of Physiology Heart and Circulatory Physiology, 295, H454-465.

Vaziri ND, Ding Y, Ni Z and Gonick HC, 1997. Altered nitric oxide metabolism and increased oxygen free radical activity in lead-induced hypertension: effect of lazaroid therapy. Kidney International, 52, 1042-1046.

Vaziri ND and Sica DA, 2004. Lead-induced hypertension: role of oxidative stress. Current Hypertension Reports, 6, 314-320.

Verberk MM, Willems TE, Verplanke AJ and De Wolff FA, 1996. Environmental lead and renal effects in children. Archives of Environment Health, 51, 83-87.

Verina T, Rohde CA and Guilarte TR, 2007. Environmental lead exposure during early life alters granule cell neurogenesis and morphology in the hippocampus of young adult rats. Neuroscience, 145, 1037-1047.

Vermeir G, Viaene M, Staessen J, Hond ED and Roels H, 2005. Neurobehavioural investigations in adolescents exposed to environmental pollutants. Environmental Toxicology and Pharmacology, 19, 707-713.

Victery W, 1988. Evidence for effects of chronic lead exposure on blood pressure in experimental animals: an overview. Environmental Health Perspectives, 78, 71-76.

Victery W, Vander AJ, Markel H, Katzman L, Shulak JM and Germain C, 1982. Lead exposure, begun in utero, decreases renin and angiotensin II in adult rats. Proceedings of The Society for Experimental Biology and Medicine, 170, 63-67.

von Storch H, Costa-Cabral M, Hagner C, Feser F, Pacyna J, Pacyna E and Kolb S, 2003. Four decades of gasoline lead emissions and control policies in Europe: a retrospective assessment. Science of the Total Environment, 311, 151-176.

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

Waalkes MP, Diwan BA, Ward JM, Devor DE and Goyer RA, 1995. Renal tubular tumors and atypical hyperplasias in B6C3F1 mice exposed to lead acetate during gestation and lactation occur with minimal chronic nephropathy. Cancer Research, 55, 5265-5271.

Waalkes MP, Liu J, Goyer RA and Diwan BA, 2004. Metallothionein-I/II double knockout mice are hypersensitive to lead-induced kidney carcinogenesis: role of inclusion body formation. Cancer Research, 64, 7766-7772.

Wadge A and Hutton M, 1987. The leachability and chemical speciation of selected trace elements in fly ash from coal combustion and refuse incineration. Environmental Pollution, 48, 85-99.

Walkowiak J, Altmann L, Kramer U, Sveinsson K, Turfeld M, Weishoff-Houben M and Winneke G, 1998. Cognitive and sensorimotor functions in 6-year-old children in relation to lead and mercury levels: adjustment for intelligence and contrast sensitivity in computerized testing. Neurotoxicology and Teratology, 20, 511-521.

Wang HL, Chen XT, Yang B, Ma FL, Wang S, Tang ML, Hao MG and Ruan DY, 2008. Case-control study of blood lead levels and attention deficit hyperactivity disorder in Chinese children. Environmental Health Perspectives, 116, 1401-1406.

Warren MJ, Cooper JB, Wood SP and Shoolingin-Jordan PM, 1998. Lead poisoning, haem synthesis and 5-aminolaevulinic acid dehydratase. Trends in Biochemical Sciences, 23, 217-221.

Watson WS, Morrison J, Bethel MI, Baldwin NM, Lyon DT, Dobson H, Moore MR and Hume R, 1986. Food iron and lead absorption in humans. American Journal of Clinical Nutrition, 44, 248-256.

Watts SW, Chai S and Webb RC, 1995. Lead acetate-induced contraction in rabbit mesenteric artery: interaction with calcium and protein kinase C. Toxicology, 99, 55-65.

Weisskopf MG, Proctor SP, Wright RO, Schwartz J, Spiro A, 3rd, Sparrow D, Nie H and Hu H, 2007. Cumulative lead exposure and cognitive performance among elderly men. Epidemiology, 18, 59-66.

Weisskopf MG, Wright RO, Schwartz J, Spiro A, 3rd, Sparrow D, Aro A and Hu H, 2004. Cumulative lead exposure and prospective change in cognition among elderly men: the VA Normative Aging Study. American Journal of Epidemiology, 160, 1184-1193.

Wennberg M, Lundh T, Bergdahl IA, Hallmans G, Jansson JH, Stegmayr B, Custodio HM and Skerfving S, 2006. Time trends in burdens of cadmium, lead, and mercury in the population of northern Sweden. Environmental Research, 100, 330-338.

Wetmur JG, Lehnert G and Desnick RJ, 1991. The delta-aminolevulinate dehydratase polymorphism: higher blood lead levels in lead workers and environmentally exposed children with the 1-2 and 2-2 isozymes. Environmental Research, 56, 109-119.

White LD, Cory-Slechta DA, Gilbert ME, Tiffany-Castiglioni E, Zawia NH, Virgolini M, Rossi-George A, Lasley SM, Qian YC and Basha MR, 2007. New and evolving concepts in the neurotoxicology of lead. Toxicology and Applied Pharmacology, 225, 1-27.

WHO (World Health Organization), 1972. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series 4: Lead. Available from http://www.inchem.org/documents/jecfa/jecmono/v004je03.htm.

WHO (World Health Organization), 1978. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series 13: Lead. Available from http://www.inchem.org/documents/jecfa/jecmono/v13je13.htm.

WHO (World Health Organization), 1986. WHO 1986. Lead (evaluation of health risk to infants and children). Available from http://www.inchem.org/documents/jecfa/jecmono/v21je16.htm.

WHO (World Health Organization), 1993. Evaluation of certain food additives and contaminants. Forty-first report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, No. 837, Geneva.

WHO (World Health Organization), 1995. Environmental health criteria 165. Inorganic Lead. Lead. Available from http://www.inchem.org/documents/ehc/ehc/ehc165.htm.

WHO (World Health Organization), 2000a. Air Quality Guidelines for Europe. Second Edition. WHO Regional Publications, European Series, No 91. Copenhagen.

WHO (World Health Organization), 2000b. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series 44: Lead. Available from http://www.inchem.org/documents/jecfa/jecmono/v44jec12.htm.

WHO (World Health Organization), 2003. Instructions for electronic submission of data on chemical contaminants in food and the diet. Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food). Available from http://www.who.int/foodsafety/publications/chem/en/gemsmanual.pdf. 160 pp.

WHO (World Health Organization), 2007. Health risks of heavy metals from long-range transboundary air pollution. Joint WHO/Convention Task Force on the Health Aspects of Air Pollution, Copenhagen.

WHO (World Health Organization), 2009. Toxicological and Health aspects of melamine and cyanuric acid. Report of a WHO expert meeting in collaboration with FAO. Supported by Health Canada.

WHO/IPCS (World Health Organization/ International Programme on Chemical Safety), 1989. Lead - environmental aspects. Environmental Health Criteria 85.

WHO/IPCS (World Health Organization/ International Programme on Chemical Safety), 1995. Environmental Health Criteria 165. Inorganic lead. Geneva.

WHO/IPCS (World Health Organization/ International Programme on Chemical Safety), 2008. Part 1: Guidance document on characterizing and communicating uncertainty in exposure assessment. Available from www.who.int/ipcs/methods/harmonization/areas/uncertainty%20.pdf.

Wilhelm M, Wittsiepe J, Schrey P, Hilbig A and Kersting M, 2005. Consumption of homegrown products does not increase dietary intake of arsenic, cadmium, lead, and mercury by young children living in an industrialized area of Germany. Science of the Total Environment, 343, 61-70.

Wright JP, Dietrich KN, Ris MD, Hornung RW, Wessel SD, Lanphear BP, Ho M and Rae MN, 2008. Association of prenatal and childhood blood lead concentrations with criminal arrests in early adulthood. PLoS Medicine, 5, e101.

Wu J, Basha MR, Brock B, Cox DP, Cardozo-Pelaez F, McPherson CA, Harry J, Rice DC, Maloney B, Chen D, Lahiri DK and Zawia NH, 2008a. Alzheimer's disease (AD)-like pathology in aged monkeys after infantile exposure to environmental metal lead (Pb): evidence for a developmental origin and environmental link for AD. Journal of Neuroscience, 28, 3-9.

Wu J, Basha MR and Zawia NH, 2008b. The environment, epigenetics and amyloidogenesis. Journal of Molecular Neuroscience, 34, 1-7.

Xie YX, Chiba M, Shinohara A, Watanabe H and Inaba Y, 1998. Studies on lead-binding protein and interaction between lead and selenium in the human erythrocytes. Industrial Health, 36, 234-239.

Yuan X and Tang C, 2001. The accumulation effect of lead on DNA damage in mice blood cells of three generations and the protection of selenium. Journal of Environmental Science and Health. Part A, Toxic/Hazardous Substances & Environmental Engineering, 36, 501-508.

Zawia NH, Crumpton T, Brydie M, Reddy GR and Razmiafshari M, 2000. Disruption of the zinc finger domain: a common target that underlies many of the effects of lead. Neurotoxicology, 21, 1069-1080.

Zawia NH, Lahiri DK and Cardozo-Pelaez F, 2009. Epigenetics, oxidative stress, and Alzheimer disease. Free Radical Biology & Medicine, 46, 1241-1249.

Ziegler EE, Edwards BB, Jensen RL, Mahaffey KR and Fomon SJ, 1978. Absorption and retention of lead by infants. Pediatric Research, 12, 29-34.

## APPENDICES

## APPENDIX A

### *Categorisation of occurrence data*

The table below reports the detail on how the food items, transmitted by the organisation providing the data has been mapped in the category/subcategory used for the occurrence analysis. Examples of food items are also reported for each food category. The list may not be exhaustive.

| Lead Occurrence – Food Category Name | Examples |
|---|---|
| 01. Cereals and cereal products | |
| 01A. Cereal-based mixed dishes | Pastry, cakes, croissants, paste, pizza, dumplings, sandwiches, waffles, egg pasta, noodles |
| 01B. Cereals and cereal products excl. cereal based mixed dishes | |
| 01B_1. Bran and germ | Only bran and germ when explicitly reported |
| 01B_2. Wheat products (Bread, pasta) | Bread, whole meal bread, bread of mixed cereals, bread with added seeds, potato bread, pumpkin bread. Rolls, rolls of mixed cereals, rolls with other non cereal products, Base mixture for bakery, Semolina products |
| 01B_3. Wheat grains and flour | Wheat grains and flower |
| 01B_4. Rice | Rice and rice products |
| 01B_5. Other cereal and cereal products excl. cereal-based mixed dishes | Muesli, oat, buckwheat, barley, mixed cereals products (pasta egg-free, biscuits, muesli bars, basic mixture, snacks, breakfast cereals) |
| 02. Sugar and sugar products including chocolate | |
| 02_1. Chocolate | Dark chocolate, milk chocolate, white chocolate |
| 02_2. Chocolate based products | Chocolate mixed products |
| 02_3. Other sugar and sugar products | Jam, marmalade, honey, toppings, chewing gum, toffees, fudges, candies, chocolate confectionery, liquorices, marzipan, sugar free confectionery, cocoa powder, meringue, nutritive sweeteners (e.g. fructose) List of items excluded: intense sweeteners |
| 03. Fats (vegetable and animal) | Mayonnaise, dressings, sauces (béchamel, hollandaise), low fat dressings or mayonnaise, goose fat, coconut extract. List of items excluded: vegetable sauces |
| 04. Vegetables, nuts, pulses including carrots, tomato and leafy vegetables | |
| 04A. Vegetable soups | Vegetable soups |
| 04B. Vegetables, nuts, pulses except vegetable soups | |
| 04B_1. Leafy vegetables | Lettuce, lollo, rocket salad, scarole, leafy vegetables excluding spinaches. |
| 04B_2. Fungi | Champignon, boletus, cultivated or wild mushrooms in general |
| 04B_3. Celeriac | Only celeriac |
| 04B_4. Stem and root vegetables | Artichokes, carrots, celery, etc with |

| Lead Occurrence – Food Category Name | Examples |
|---|---|
| | exclusion of starchy roots |
| 04B_5. Nuts | Cashew nuts, peanuts, almonds, pine nuts, hazel nuts, coconuts |
| 04B_6. Oil seeds[26] | Poppy seeds, sunflower seeds, sesame seeds, line seeds |
| 04B_7. Spinach | Spinaches |
| 04B_8. Legumes[11] | Butterbeans, peas, check peas, lens, this category includes also soybeans |
| 04B_9. Other vegetable not listed above except. Vegetable soups. | Brassica, capsicum, cucurbits, tomato, wine leaves |
| 05. Starchy roots or potatoes | |
| 05_1. Potatoes | Potatoes peeled and unpeeled |
| 05_2. Other starchy roots excl. potatoes | Tapioca, cassava, sweet potatoes. |
| 06. Fruits | |
| 07. Fruit and vegetable juices, soft drinks and bottled water | |
| 07A. Fruit and vegetable juices | |
| 07A_1. Fruit juices | Only fruit juice |
| 07A_2. Vegetable juices | Carrot juices, vegetable juices in general including mixed fruit and vegetables juices. |
| 07B. Soft drinks with percentage of fruits lower than nectar | |
| 07B_1. Soft drinks | Cola, tonic water |
| 07B_2. Other soft drinks | Prepared herbal tea, water based drinks |
| 07C. Bottled water | Bottled mineral water |
| 08. Coffee, tea, cocoa | |
| 08_1. Cocoa (Powder or bean) | Cocoa reported as cocoa powder or cocoa beans |
| 08_2. Coffee (Powder or beans) | Coffee reported as coffee powder or coffee beans |
| 08_3. Tea (Powder or dry leaves) | Tea reported as tea powder or tea dry leaves |
| 08_4. Infusions and herbal teas (powder or dry leaves) | Infusion and herbal teas reported as powders for infusions, dry leaves. |
| 09. Alcoholic beverages | |
| 09A. Beer and substitutes | Beer and substitutes including non-alcoholic beer, panache, cider, alcohol soft drinks |
| 09B. Wine and substitutes | Sangria, sparkling wine, "non-alcoholic" wine, fortified wine (e.g. porto, cherry wine, madeira, vermouth), fruit wine |
| 09C. Other alcoholic beverages and substitutes | Alcohol-free anise, spirits |
| 10. Meat and meat products, offal | |
| 10A. Meat and meat products and substitutes | |
| 10A_1. Soya meat substitutes | Soya products excluding soy beans, soya milk and soya sauce |
| 10A_2. Bovine, sheep and goat meat | Bovine, sheep and goat muscle meat |
| 10A_3. Poultry and rabbit meat | Chicken, duck, goose and rabbit |
| 10A_4. Pig meat | Pig muscle meat |
| 10A_5. Horse meat | Horse muscle meat |
| 10A_6. Game meat | Reindeer, dear, pheasant (where explicitly reported as livestock farming they are entered in 10A_7 "Not elsewhere classifiable meat and meat products") |

[26] Soybeans have been classified under the category 04B_8 while in Regulation No. 178/2006 they have been classified under oilseeds.

| Lead Occurrence – Food Category Name | Examples |
|---|---|
| 10A_7. Other or not elsewhere classifiable meat and meat products | Mixed meat, reptiles, frog legs, ox and other meat of African animals |
| 10B. Edible offal and offal products | |
| 10B_1. Liver bovine, sheep, pig, poultry, horse | Pate and liver of bovine animals, sheep pig poultry and horse |
| 10B_2. Kidney bovine, sheep, pig, poultry, horse | Kidney of bovine animals, sheep, pig, poultry and horse |
| 10B_3. Liver and kidney of game animals | Reindeer, dear, pheasant (where explicitly reported as livestock farming they are entered in 10B_4 "Other) |
| 10B_4. Other offal products (Trip, lung, stomach, etc.) | Heart, tripe and offal not otherwise specified |
| 10B_5. Not specified offal products | Offal products which could not be identified because reported as "Offal", and therefore not classifiable in any of the 10B categories. |
| 10C. Meat based preparations | Mixed meat food |
| 11. Fish and seafood | |
| 11A. Seafood and seafood products | |
| 11A_1. Bivalve molluscs other then oysters | Scallops, mussels, cockles, clams |
| 11A_2. Crustaceans | Prawns, crayfish, langoustines, etc… |
| 11A_3. Cephalopods | Squids, octopus, cuttlefish. |
| 11A_4. Oysters | Oysters. |
| 11A_5. Snails and limpets | Soil and sea snails, limpets and gastropods in general. |
| 11A_6. Other or not elsewhere classifiable seafood products | Not elsewhere classifiable seafood, in particular only food category reported. |
| 11B. Fish and fish products | |
| 11B_1. Muscle meat of fish, excluding species listed in ML groups 3.2.6 and 3.2.7 | Muscle meat of all fish species not falling under categories 3.2.6 and 3.2.7 of the Annex to Regulation (EC) No. 1881/2006 in its version of 19 December 2006 |
| 11B_2. Muscle meat of fish matching ML group 3.2.6 | Only the species listed in the legislation, excluded processed fish. |
| 11B_3. Muscle meat of swordfish | Only when swordfish has been reported |
| 11B_4. Other or not elsewhere classifiable fish products | Process fish has been included here. Canned anchovy, anchovy in oil in salt etc., Fish liver and caviar and roe. Processed fish is included in this category |
| 11C. Fish based preparations | Fish soup, fish quenelle, etc. |
| 12. Eggs | Omelettes, fried eggs List of items excluded: fish eggs |
| 13. Milk and dairy based products | |
| 13A. Milk and dairy based drinks | |
| 13A_1. Soya milk | Milk substitute from soy processing |
| 13A_2. Milk | Cow milk and other milk from animal origin |
| 13B. Dairy based products | Chantilly, rice pudding, ices and sherbets, creams, desserts (e.g. mousse chocolate, Siberian omelettes, tiramisu, profiteroles), yoghurt, French fromage blanc, sour cream, custard. List of items excluded: drinkable yoghurt |
| 13C. Cheese | Mozzarella, spread cheese, cottage cheese, cheese substitutes (e.g. made of vegetable oil). Excluded: tofu. |

| Lead Occurrence – Food Category Name | Examples |
|---|---|
| 14. Miscellaneous / Food for special dietary uses | |
| 14A. Miscellaneous | |
| 14A_1. Herbs | All herbs dried and fresh or not specified. |
| 14A_2. Spices | All spices, ginger etc. |
| 14A_3. Soya sauce | |
| 14A_4. Other miscellaneous products | Additives, flavouring, sweeteners, Algae and seaweeds |
| 14B. Food for special dietary uses | |
| 14B_1. Supplements | Fish oil, multivitamins, herbal products, yeasts, supplements based on seaweeds |
| 14B_2. Other food for special dietary uses excluding food supplements | Food for infants, Food for diabetics, Material for food production, dietetic food |
| 15. Tap water | |

## APPENDIX B

### *Methods applied for the calculation of BMD and BMDL values as reported in Table 34 for cardiovascular effects observed in five studies for the risk characterisation of lead*

This appendix explains the derivation of $BMD_{01}$ and $BMDL_{01}$ values from the six slopes estimates reported in chapter 8.6.1.2 of the opinion based on the five studies of Glenn et al. (2003), Vupputuri et al. (2003), Nash et al. (2003), Glenn et al. (2006), and Cheng et al. (2001) shown in Table 34 of the Opinion. This table shows the slopes and their 95 % confidence intervals used for the calculation of the $BMD_{01}$ and $BMDL_{01}$ values.

The authors of the papers cited above investigated the association between increases in blood lead concentration (B-Pb) and/or tibia bone lead concentration (TB-Pb) versus increases of systolic and/or diastolic blood pressure (SBP and/or DBP). The authors usually quantified that relationship mostly in a multivariate regression adjusted for confounders and reported the linear relationship between B-Pb (and/or TB-Pb) and SBP (and/or) DBP. The derivation of $BMD_{01}$ and $BMDL_{01}$ values concentrated on SBP only. A slope estimate can be obtained from that linear relationship from which a $BMD_{01}$ value can be calculated directly by determining the dose that corresponds to an increase of SBP by 1.2 mmHG on a straight line. The five cited papers above had also reported either the standard error (s.e.) of the slope estimate or the two sided 95 % confidence interval (CI) of the slope. When the s.e. was available, we calculated an approximate upper one-sided 95 % confidence limit for the slope using the quantile of 1.645 of the standard normal distribution (± 1.645 s.e.). From the upper limit of the slope a $BMDL_{01}$ value can then be calculated in the same way as the $BMD_{01}$ value. When the two sided 95 % CI of the slope was available, an approximate s.e. was calculated as the ratio of width of the two sided 95 % CI divided by 7.84 (4 times the 97.5 quantile of the standard normal distribution) since ±1.96 s.e. is the width of the approximate two sided 95 % CI.

Note that in the case of longitudinal studies the annual increase of SBP is related to the B-Pb or the TB-Pb, respectively, whereas in the case of cross-sectional studies observed increase of SBP in the population investigated is related to increases in B-Pb or TB-Pb. 95 % CIs of the slopes are given in brackets. The $BMD_{01}$ and $BMDL_{01}$ derived from these slope and relate to an increase of SBP by 1.2 mmHg corresponding to a BMR=1 % extra risk. (Note that the symbol * is used below to denote multiplication). Next are shown the calculations of the $BMD_{01}$ and $BMDL_{01}$ values using the results of Cheng et al. (2001), Glenn et al. (2003), Nash et al. (2003), Vupputuri et al. (2003), and Glenn et al. (2006).

a) The dose-response analysis of SBP as a function of tibia bone lead concentration (TB-Pb) of **Cheng et al. (2001)** yielded a slope estimate 0.1 mmHg/µg/g TB with 95 % confidence interval (0.0015 to 0.20) mmHg/µg/g TB. The slope 0.1 mmHg/µg/g translates to the **$BMD_{01}$ = 1.2/0.1 = 12 g/g.** From the width 0.2 to 0.0015 = 0.1985 mmHg /µg/g of the two-sided 95 % confidence interval on gets an approximate value of 0.052 mmHg /µg/g for the standard error (s. e.) of the slope. The one sided 95 % confidence bound is then 0.1 +1.645*0.052 =0.185 mmHg /µg/g, using the 95$^{th}$ percentile value 1.645 of an approximating Gaussian distribution. From there results

**$BMDL_{01}$ = 1.2/0.185 = 6.5 µg/g.**

b) **Glenn et al. (2003)** report a linear dose-response relationship between SBP and B-Pb of the form

SBP [mmHg] = 120 [mmHg] + 0.025 [mmHg/µg/L Blood] * 1 µg/L Blood.

with two-sided 95 % confidence interval (0.005 to 0.044) mmHg/µg/L interval which is approximately symmetric around the slope estimate of 0.025 mmHg/µg/L, roughly +/- 0.020. Therefore, **$BMD_{01}$ = 1.2/0.025 = 48 µg/L**. Using the 97.5$^{th}$ percentile 1.96 of the 97.5$^{th}$ percentile of the approximating Gaussian distribution one obtains an approximate estimate of s.e. through 0.020/1.96 =0.0102

mmHg/µg/L and the one sided 95 % confidence bound is 0.025 +1.645*0.0102 =0.042 mmHg/µg/L, when using the 95$^{th}$ percentile of the approximating Gaussian distribution of 1.645; and from there

**$BMDL_{01}$ = 1.2/0.042 = 29 µg/L.**

These authors investigated also the dose-response analysis of SBP as a function of TB-Pb and yielded a slope estimate 0.078 mmHg/µg/g TB with two sided 95 % confidence interval (0.024 to 0.13) mmHg/µg/g TB. From that slope results the **$BMD_{01}$ = 1.2/0.078 = 13 µg/g**. From the width of length 0.106 mmHg/µg/g of that two-sided 95 % confidence interval follows the approximate value of 0.028 mmHg/µg/g for the s. e. of that slope and as above a one sided 95 % confidence bound of 0.078+1.645*0.028 =0.124 mmHg/µg/g. Hence

**$BMDL_{01}$ = 1.2/0.124 = 9.7 µg/g**

c) **Nash et al. (2003)** derived from their cross-sectional data the slope estimate 0.032 mmHg/1 µg/L Blood with 95 % confidence interval (0.0006 to 0.0634) mmHg/µg/L. One gets such a **$BMD_{01}$ = 1.2/0.032 = 38 µg/L**. Using the s. e. of the slope of 0.016 mmHg/µg/L Blood one obtains a one-sided 95 % upper confidence bound as 0.032 +1.645*0.016 =0.058 mmHg/µg/L and a

**$BMDL_{01}$ = 1.2/0.058 = 21 µg/L.**

d) **Vupputuri et al. (2003)** observed in their analysis of cross-sectional data the slope 0.047 mmHg/µg/L corresponding to **$BMD_{01}$ = 1.2/0.047 = 26 µg/L**. From the length 0.066 mmHg/µg/L of the two-sided 95 % confidence interval (0.014 to 0.080) mmHg/µg/L on gets an approximate value of 0.0165 mmHg/µg/L for the s. e. of that slope. The one sided 95 % confidence bound is then 0.047 +1.645*0.0165 =0.074, when using the 95$^{th}$ percentile of the approximating Gaussian distribution of 1.645. Hence

**$BMDL_{01}$ = 1.2/0.074 = 16 µg/L.**

e) Another study of **Glenn et al. (2006)** report an average annual increase of 0.9 (95 % CI: 0.1 to 1.6) mmHg for every 10 µg/dL increase in B-Pb per year. This gives a slope estimate of 0.9 mmHg/100 µg/L or 0.009 mmHg/µg/L B-Pb with two-sided 95 % confidence interval (0.001 to 0.016) mmHg/µg/L. One obtains **$BMD_{01}$ = 1.2/0.009 = 133 µg/L.** From the width of 0.015 of the two-sided 95 % confidence interval one obtains an approximate s. e. of the slope estimate as 0.00375. The one sided 95 % confidence bound is then 0.009 +1.645*0.00375 =0.015, using the 95$^{th}$ percentile of the approximating Gaussian distribution of 1.645. The $BMDL_{01}$ is therefore obtained as

**$BMDL_{01}$ = 1.2/0.015 = 80 µg/L.**

The four BMDLs for B-Pb 29, 21, 16 and 80 µg/L give an average of **37 µg/L B-Pb.** The two BMDLs 6.5 and 9.7 µg/g tibia bone derived for tibia blood concentration give an average value of **8 µg/g tibia bone.** Note that the four BMDLs for B-Pb and the two BMDLs for TB-BP were obtained from independent studies, respectively. An average BMDL was calculated to summarize the information of those studies.

## APPENDIX C

The methods applied for the BMD analysis for the NHANES (1999-2006) CKD data based on the publication of Navas-Acien et al. (2009) for the risk characterisation of lead

### A. Benchmark dose modelling

The US EPA's benchmark dose software[27] BMDS 2.1.1 was used for modelling the incidence of CKD in adults as reported by Navas-Acien et al. (2009).

The models available in the BMDS software used for this analysis, model fitting characteristics and goodness-of-fit statistics, and methods for comparing the models in order to decide which model to use for obtaining the benchmark dose lower confidence limit (BMDL) as reference point are outlined below. The following dose-response models see Table 3 in the Scientific Opinion of the EFSA (EFSA, 2009) were fitted to the exposure-incidence data:

- *Probit*
- *Log-Probit*
- *Logistic*
- *Log-logistic*
- *Weibull*
- *Multistage*
- *Gamma-Multihit*

In addition the *Quantal-Linear* model was also reported in cases where its outcome was different from that of the Weibull model. Note that for restricted modelling the Multistage, Quantal Linear and Gamma often coincide with their result with the outcome of the Weibull model.

The benchmark response level (BMR) was set as 10 % because of the incidence type of the data. The (benchmark dose) $BMD_{10}$ and $BMDL_{10}$ values for an extra 10 % risk were calculated using the BMDS software (version BMDS2.1.1) by fitting each of the above models. The Scientific Opinion of the EFSA (EFSA, 2009) on the use of the benchmark dose approach in risk assessment states that "ideally the BMR would reflect an effect size that is negligible or non-adverse" but also constraints this proposal by requiring " that the benchmark dose response (BMR) chosen should not be too small to avoid having to estimate a BMD by extrapolation outside the range of observation". The Scientific Opinion of the EFSA also proposed a BMR=10 % as default for quantal data (EFSA, 2009). That choice addresses, in particular, experimental animal data where the number of individuals per dose group would be usually not larger than n=50, often much smaller. More data were available for the four dose groups of the human data of Navas-Acien et al. (2009). For reasons of consistency the BMR =10 % was also used for these incidence data. The position of the BMD and the BMDL values were assessed for their location in the range of the dose groups. For the human study where exposure data were available in an aggregated form of four quartiles of the dose intervals this was not possible. Fortunately the authors had provided the medians of the quartile dose ranges such that those could be used to locate each of the four dose intervals.

[27] http://www. epa. gov/ncea/bmds/about. html

Use of Constraints. It has been noted in the comments to BMDS 2.0 software (see BMDS 2.0 Help or manual of BMDS2.0/2.1) that unconstraint modelling (e.g. a slope or power parameter allowed to be less than 1) would cause an infinite slope at dose zero in some models. BMDS allows therefore restricting those critical parameters of the dose-response model to avoid an infinite slope at dose zero. For the Log-logistic and Log-Probit model this translates into constraints of the slope parameter, for the Weibull and Gamma model into a constraint of the power parameter, and for the Multistage models into constraints of the so-called "beta" parameters. The BMDS Tutorial warns about numerical problems in calculating the confidence interval in models in which dose is raised to a power which is a parameter to be estimated (such as a Weibull model) and therefore recommends to use constraints n that case[28]. This issue was also commented on by WHO (2009) who used exclusively constraint models for deriving a BMDL values. Constraints of model parameters for avoiding infinite slopes of the fitted dose-response curve at dose zero are addressed in the Scientific Opinion of the EFSA, but without giving further advice. For transparency reasons, both the results of the constraint and the unconstraint fitting were shown for models where that option was available in BMDS software.

Model Acceptance. The general principle of the BMD approach adopted by the EFSA is to find all models that are compatible with the data, i. e. those with an acceptable fit. In the case of non-nested models, where no exact statistical test criterion can be recommended, an acceptable model should in principle provide a reasonable description of the dose-response data. Therefore, a goodness-of-fit is judged as sufficient if a goodness-of-fit of the model to the data shows a p-value larger than 0.1, e. g. using the likelihood ratio test. For the data of this opinion, acceptability of a model was assessed using the log- likelihood value associated with the fitted model tested versus the full model and versus the reduced model in each model fit.

- The full model is the model that does not assume any dose-response function (its parameters are simply the frequencies per dose level). Its log-likelihood is therefore identical for each model fit as long as the same data set us used

- The reduced model is the model with no dose-relationship (it is a straight line parallel to the dose axis representing mean exposure of the total sample). Its log-likelihood is therefore identical for each model fit as long as the same data set us used

This approach was used (also in the case of non-nested models) such that the fit of the chosen model:

a) should be statistically significantly better than the reduced model ($p<0.05$)

b) should be not significantly worse than the full model ($p>0.1$)

for being acceptable. When BMDS software was used the p-values were reported. When PROAST was applied the critical level was set at 0.05.

If there were constraint models which would fulfil the two criteria, the BMD/Ls obtained from them were considered as acceptable models for the risk characterization.

If none of the constraint models would fulfil the criteria, the set of unconstraint models would be searched for acceptable models and BMD/Ls obtained from them would be considered as acceptable models for the risk characterization. That was the case for the human data of Li et al. (2009).

If none of the constraint as well as none of the unconstraint models would obey the criterion b) above the acceptance boundary for the p-value could be reduced from 0.1 to a lower value and the procedure described above would be applied with that boundary. That case did however not happen in this analysis.

[28] See http://www.epa.gov/ncea/bmds/bmds_training/methodology/intro.htm

In the BMD analysis of the data provided by Navas-Acien et al. (2009) none of the constraint models obeyed the criterion i) such that accepted models at the level 0.01 were searched among the unconstraint models only. One should , however, note, that due to the large sample size of the cross-sectional data from the NHANES III (1999-2006) study and due to the aggregation of the dose information in the median of each quartiles the four incidence rates were available at a very high precision. Therefore minor deviations of the fitted curve from those rates would cause a violation of the acceptability criterion.

The Akaike information criterion (AIC) has also been used as an approximate criterion for comparing the fits of non-nested models (Sand et al., 2006). Note, the AIC is not recommended for use in the EFSA Opinion on the BMD. Therefore, it was used as supportive criterion only (not shown below). Furthermore, the overall p-value of the chi-square goodness-of-fit was calculated based on the residuals describing the difference between estimated and observed frequencies only (not shown below). All relevant statistics for the suitability of the fit as provided by the BMDS software were calculated. Consistency in the outcome of those criteria supports confidence for having chosen the best model. Inconsistency is a sign of enlarged uncertainty of model fitting and consequently of the resulting BMD/BMDL values.

If a range of models can be accepted, the lowest BMDL of that range is determined as the overall BMDL for a critical endpoint, according to the EFSA Opinion on the BMD approach. However, the range of BMDL values from different accepted models should not exceed one order of magnitude.

The $BMD_{10}$ and $BMDL_{10}$ values, as well as the associated statistics for the models used, were presented in tables.

**Table A1:** $BMD_{10}$ and $BMDL_{10}$ calculations for the chronic kidney disease data of Navas-Acien et al. (2009) in a study population of 14 778 adults at least 20 years old who participated in the NHANES (1999-2006) study. Blood lead concentration measurements were categorized into four quartiles (larger than 11 with median 0.8, 11 to 16 with median 13, 16 to 24 with median 19, and, larger than 24 with median 32, in units of µg/L) with the respective incidences 147/3242, 274/3167, 468/3734 and 779/4635 (see Table 35).

| Model | BMR Extra Risk | Number of Doses, Model Parameters of Fitted Model | -Log likelihood | P-value | Accepted with p≥0.1, p≥0.01 | $BMD_{10}$ mg/kg b.w. per day | $BMDL_{10}$ mg/kg b.w. per day |
|---|---|---|---|---|---|---|---|
| Full model | | | 5038.8 | | | | |
| Reduced model | | | 5208.9 | | | | |
| CONSTRAINT | | | | | | | |
| Probit | 10 | 4,2 | 5058.2 | $<10^{-8}$ | no/no | 25.3 | 24.2 |
| Log-Probit | 10 | 4,2 | 5060.4 | $<10^{-9}$ | no/no | 26.7 | 25.1 |
| Logistic | 10 | 4,2 | 5060.4 | $<10^{-9}$ | no/no | 26.2 | 25.1 |
| Log-Logistic | 10 | 4,2 | 5044.7 | 0.003 | no/no | 16.5 | 15.8 |
| Weibull[#] | 10 | 4,2 | 5046.1 | 0.0007 | no/no | 17.8 | 17.3 |
| UNCONSTRAINT | | | | | | | |
| Log-Logistic | 10 | 4,2 | 5044.4 | 0.004 | no/no | 16.3 | 15.5 |
| Log-Probit | 10 | 4,2 | 5042.8 | 0.018 | no/yes | 16.1 | 15.3 |
| Weibull | 10 | 4,2 | 5045.0 | 0.002 | no/no | 16.4 | 15.6 |
| Gamma | 10 | 4,2 | 5043.5 | 0.002 | no/no | 16.4 | 15.6 |
| Multi-Stage | 10 | 4,2 | 5043.5 | 0.010 | no/yes | 15.9 | 15.0 |

BMR: benchmark response; BMD: benchmark dose; BMDL: benchmark dose lower confidence limit; CKD: chronic kidney disease; BMR: benchmark response; p-value: probability value; b.w.: body weight.
# Among the constraint model resulted in identical fits: Gamma = Weibull = Multistage= Quantal Linear.

**Statistical Results and Result of Model Selection**

All constraint models were acceptable neither at $p \geq 0.1$ nor at $p \geq 0.01$ when using the log-likelihood test. All non-constraint models were not acceptable at p at $p \geq 0.1$, but two models were acceptable at $p \geq 0.01$ (Log-probit and Multistage).

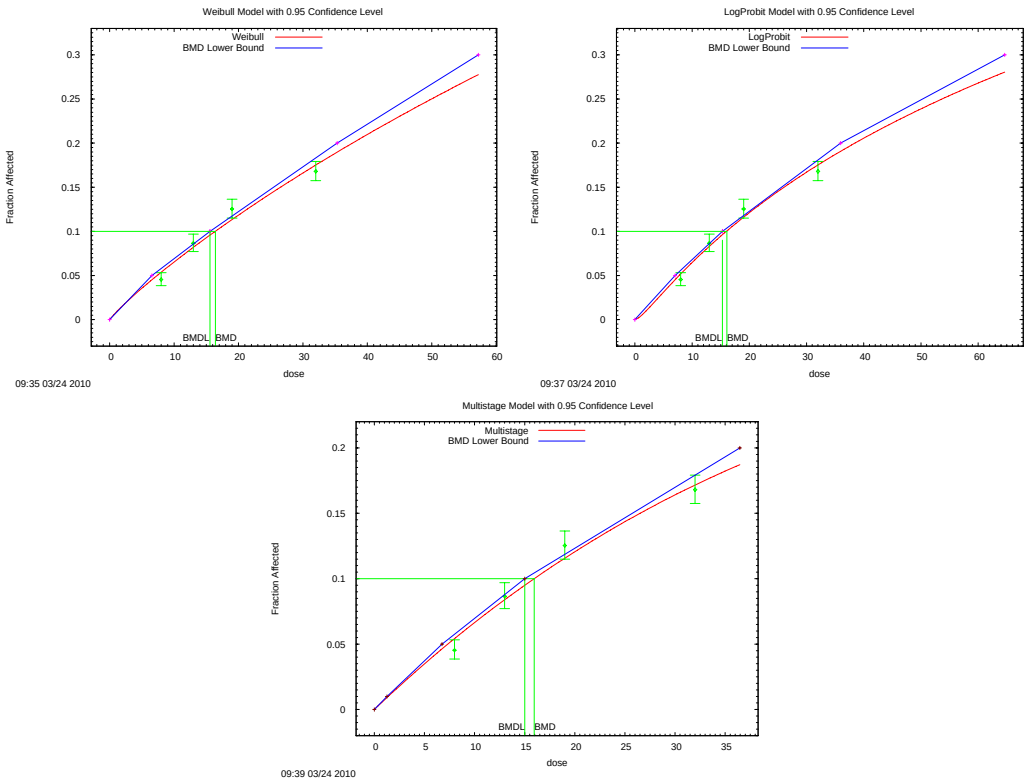
The goodness of fit of all unconstraint models was good and comparable by visual inspection although some of the models did not pass the acceptability criterion. The goodness of fit of two of the constraint models that of the unconstraint models although they did not pass the acceptability criterion. Due to the large sample size of the cross-sectional data from the NHANES III (1999-2006) study and due to the aggregation of the dose information in the median of each quartile the four incidence rates were available at a very high precision. Therefore minor deviations of the fitted curve from those rates cased a violation of the acceptability criterion.

The BMD values of the constraint Log-Logistic and the Weibull model (16.5, 17.8) were comparable with those of the unconstraint models ranging between 15.9 and 16.4 µg/L. The BMD values of the acceptable unconstraint Log-Probit and Multistage model were 16.1 and 15.9 µg/L, respectively.

The BMDL values of the constraint Log-Logistic and the Weibull model (15.8, 17.3) were comparable with those of the unconstraint models ranging between 15.0 and 15.6 µg/L. The BMD values of the acceptable unconstraint Log-Probit and Multistage model were 15.3 and 15.0 µg/L, respectively.

**For risk characterization the Panel chose a $BMDL_{10}$ value of 15** µg/L.

The graphs of the unconstraint Weibull and Log-probit model and Multistage models are shown below.



## ABBREVIATIONS

| | |
|---|---|
| 3-MCPD | 3-monochloropropane-1,2-diol |
| Aβ | β-amyloid peptide |
| AAS | Atomic absorption spectrometry |
| ACGIH | American Conference of Governmental Industrial Hygienists |
| AD | Alzheimer's disease |
| ADHD | Attention deficit hyperactivity disorder |
| AES | Atomic emission spectrometry |
| AIC | Akaike information criterion |
| ALA | δ-Aminolevulinic acid |
| ALAD | δ-aminolevulinate dehydratase |
| ALAS | δ-aminolevulinic acid synthetase |
| ATSDR | Agency for Toxic substances and Disease Registry |
| b.w. | Body weight |
| BBN | Beta binomial-normal |
| B-Cd | Blood cadmium |
| BfR | The German Federal Institute for Risk Assessment - Bundesinstitut für Risikobewertung |
| BMD | Benchhmark dose |
| BMDL | Benchmark dose level |
| BMI | Body mass index |
| B-Mn | Blood manganese |
| B-Pb | Lead in blood/ Blood lead |
| BMR | Benchmark response |
| CDC | United States Centers for Disease Control and Prevention |
| CEN | European Committee for Standardization |
| CHD | Coronary heart disease |
| CI | Confidence interval |
| CKD | Chronic kidney disease |
| CNS | Central nervous system |
| CONTAM Panel | Panel on Contaminants in the Food Chain |
| COPRO | Coproporphyrin |
| COT | Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment |
| CSTEE | Scientific Committee on Toxicity, Ecotoxicity and the Environment |
| d. w. | Dry weight |
| DATEX | Data Collection and Exposure Unit, European Food Safety Authority |
| DBP | Diastolic blood pressure |
| DNA | Deoxyribonucleic acid |
| EC | European Commission |
| EFSA | European Food Safety Authority |
| EMEP | European Monitoring Environmental Pollution |
| EOG | End-of-grade |
| EP | Erythrocyte protoporphyrin |
| ER | Emission rate |
| ETS | Environmental tobacco smoke |
| EU | European Union |
| FAAS | Flame Atomic Absorption Spectrometry |
| FEP | Free erythrocyte protoporphyrin |
| Fpg | formamidopyrimidine-DNA glycosylase |
| FSA | United Kingdom Food Standards Agency |
| GEMS/Food | World Health Organisation Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme |

| | |
|---|---|
| GFAAS | Graphite furnace atomic absorption spectrometry |
| GFR | Glomerular filtration rate |
| GRP | Glucose-regulated protein |
| HOME | Home observation for measurement of the environment |
| IARC | International Agency for Research on Cancer |
| ICP-AES | Inductively coupled atomic emission spectrometry |
| ICP-MS | Inductively coupled mass spectrometry |
| IL-6 | cytokine interleukin-6 |
| INRAN | Istituto Nazionale di Ricerca per gli Alimenti e la Nutrizione |
| IOM | Institute of Medicine |
| IPCS | International Programme on Chemical Safety |
| IQ | Intelligence quotient |
| IEUBK | Integrated Exposure Uptake Biokinetic Model |
| JECFA | Joint FAO/WHO Expert Committee on Food Additives |
| Kd | Affinity constant |
| L-NAME | L-N-(G)-nitro arginine methyl ester |
| LB | Lower bound |
| LD | Low dose |
| LH | Luteinizing hormone |
| LOD | Limit of detection |
| LOQ | Limit of quantification |
| MCRA | Monte Carlo Risk Assessment |
| MCV | Motor nerve conduction velocity |
| ML | Maximum level |
| M-LB | (Country) mean for the lower bound |
| MOE | Margin of exposure |
| MRL | Minimum risk level |
| MS | Mass spectrometry / Member states |
| MSCA | McCarthy Scales of Children's abilities |
| M-UB | (Country) mean for the upper bound |
| NAG | N-acetyl-D-glucosaminidase |
| NHANES | National Health and Nutrition Examination Survey |
| NHEXAS | National Human Exposure Assessment Survey |
| NO | Nitric oxide |
| NTP | National Toxicology Program |
| OECD | Organization for Economic Co-operation and Development |
| Ogg1 | 8-oxoguanine DNA glycosylase |
| OR | Odds ratio |
| OSPAR | The Convention for the Protection of the Marine Environment of the North-East Atlantic |
| oxo8dG | 8-hydroxy-2′-deoxyguanosine |
| P5N | Pyrimidine 5' nucleotidase |
| PBPD | Physiologically based pharmacodynamic model |
| PBPK | Physiologically based pharmacokinetic model |
| PBTK | Physiologically based toxicokinetic model |
| PBT | Persistent Bioaccumulative and Toxic |
| PKC | Protein kinase C |
| PNEC | Predicted No-Effect Concentration |
| PROTO | Protoporphyrin |
| PTFE | Polytetrafluoroethylene |
| PTTIL | Provisional total tolerable intake level |
| PTWI | Provisional Tolerable Weekly Intake |
| p-value | Probability value |
| RA | Release area |

| | |
|---|---|
| RfD | Reference dose |
| ROS | Reactive oxygen species |
| RSD | Relative standard deviation |
| RV | Room volume |
| SAF | Sampling adjustment factor |
| SBP | Systolic blood pressure |
| SCF | Scientific Committee for Food |
| SCOOP | European Commission Scientific Cooperation Project |
| s.e. | Standard error |
| TB-Pb | Tibia bone lead concentration |
| TDI | Tolerable daily intake |
| TDS | Total Diet Study |
| TML | Tetramethyl lead |
| UB | Upper bound |
| UNEP | United Nations Environment Programme |
| USA | United States of America |
| US EPA | United States Environmental Protection Agency |
| US FDA | United States Federal and Drug Administration |
| UV | Ultraviolet |
| VLD | Very low dose |
| w. w. | Wet weight |
| WG | Working group |
| WHO | World Health Organization |
| WISC | Wechsler Intelligence Scale for Children |
| WISC-R | Wechsler Intelligence Scale for Children Re-normalised |
| XRFS | X-ray fluorescence spectroscopy |