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Lead (Pb) and neurodevelopment: A review on exposure and biomarkers of effect (BDNF, HDL) and susceptibility

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ABSTRACT

Lead (Pb) is a ubiquitous environmental pollutant and a potent toxic compound. Humans are exposed to Pb through inhalation, ingestion, and skin contact via food, water, tobacco smoke, air, dust, and soil. Pb accumulates in bones, brain, liver and kidney. Fetal exposure occurs via transplacental transmission. The most critical health effects are developmental neurotoxicity in infants and cardiovascular effects and nephrotoxicity in adults.

Pb exposure has been steadily decreasing over the past decades, but there are few recent exposure data from the general European population; moreover, no safe Pb limit has been set. Sensitive biomarkers of exposure, effect and susceptibility, that reliably and timely indicate Pb-associated toxicity are required to assess human exposure-health relationships in a situation of low to moderate exposure.

Therefore, a systematic literature review based on PubMed entries published before July 2019 that addressed Pb exposure and biomarkers of effect and susceptibility, neurodevelopmental toxicity, epigenetic modifications, and transcriptomics was conducted. Finally included were 58 original papers on Pb exposure and 17 studies on biomarkers. The biomarkers that are linked to Pb exposure and neurodevelopment were grouped into effect biomarkers (serum brain-derived neurotrophic factor (BDNF) and serum/saliva cortisol), susceptibility markers (epigenetic markers and gene sequence variants) and other biomarkers (serum high-density lipoprotein (HDL), maternal iron (Fe) and calcium (Ca) status). Serum BDNF and plasma HDL are potential candidates to be further validated as effect markers for routine use in HBM studies of Pb, complemented by markers of Fe and Ca status to also address nutritional interactions related to neurodevelopmental disorders. For several markers, a causal relationship with Pb-induced neurodevelopmental toxicity is likely. Results on BDNF are discussed in relation to Adverse Outcome Pathway (AOP) 13 ("Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment of learning and memory abilities") of the AOP-Wiki. Further studies are needed to validate sensitive, reliable, and timely effect biomarkers, especially for low to moderate Pb exposure scenarios.

1. Introduction

Lead (Pb) is found in the environment mainly in its inorganic form. Most Pb in the environment originates from anthropogenic activities. Human exposure occurs mainly through food, water, tobacco smoke, air, dust and soil, and exposure can occur through inhalation, ingestion and skin contact. The fetus is exposed via placental transfer (EFSA 2010; WHO 2019).

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Pb absorption depends on the chemical form, particle size, exposure route, diet, health and age of the individual. Humans are mainly exposed to inorganic Pb. Most ingested Pb is excreted in urine and feces. Bioavailable Pb is more strongly absorbed by children than adults. (ATSDR 2017). In blood, >90% of the Pb is present in erythrocytes, and blood-Pb constitutes the most widely used biomarker of exposure. Although blood contains only a small fraction of the total Pb body burden, the matrix reliably reflects recent exposure. The half-life in the blood of adult humans ranges between 28 and 36 days. Plasma Pb can enter soft tissue compartments with a high exchange rate and hard tissues in which Pb is more strongly bound. Pb accumulates in the brain, liver, kidney and, over time, in bones and teeth. The calcified tissues contain 94% (adults) and 73% (children) of the total Pb body burden, where the metal can be stored in inert form for decades. From here, only a small labile proportion can be exchanged with the blood. Pb in the bone can, however, be mobilized into blood, a process that increases with age, physiological stress, certain diseases, pregnancy, lactation, menopause and calcium (Ca) deficiency. Therefore, the Pb pool in bones constitutes a particular risk as it is an endogenous source which, when activated, can increase blood Pb levels long after cessation of exposure (ATSDR 2017; Klotz and Goen, 2017; EFSA 2010; WHO 2019).

Developmental neurotoxicity in infants and cardiovascular effects, and nephrotoxicity in adults constitute the most critical health effects of Pb exposure (EFSA 2010). IARC has furthermore classified inorganic Pb compounds as probably carcinogenic to humans (Group 2A), while organic Pb compounds are not classifiable (Group 3) (IARC 2006). Even low Pb levels (<5 μg/100 mL blood) are known to exert adverse health effects in children and adults (Betts 2012). The dose-response relationship between Pb exposure and intelligence quotient (IQ) of children seems nonlinear, with lower exposures of Pb resulting in relatively greater loss of IQ than higher exposures. Hence, one study observed that increase in blood Pb from <1 to 30 μg/dL was associated with a deficit of ~9 IQ points, but with the largest fraction of the deficit (~6 IQ points) occurring below 10 μg/dL (Lanphear 2017; Lanphear et al., 2005). Primarily with regard to developmental neurotoxicity, the EFSA CONTAM Panel determined the $BMDL_{01}$ to 12 μg/L (EFSA 2010) but concluded that "there is no evidence for a threshold for critical lead-induced effects" and that "protection of children against the potential risk of neurodevelopmental effects would be protective for all other adverse effects of lead, in all populations" (EFSA, 2010). ECHA's Committee for Risk Assessment (RAC) recently evaluated the occupational exposure limits for Pb and its compounds. The biological limit value (BLV) was set to 150 μg/L blood for Pb and its inorganic compounds, while no BLV was set for organic Pb compounds. RAC notes, "*Neither the proposed BLV of* 150 μg/L *blood and the proposed air limit value of* 4 μg/m^3 … *protects from developmental toxicity*" and "*it is recommended to add a qualitative statement in the Chemical Agents Directive that the exposure of fertile women to lead should be avoided or minimized in the workplace because the BLV for lead is not protective of the offspring of women of childbearing age"* (RAC, 2020). Several health authorities are currently refraining from setting guideline values for Pb exposure, as it cannot be ruled out that Pb can affect neurological development even at very low levels of exposure (ATSDR 2017).

Neurodevelopment is characterized by decisive phases in which neuronal circuits are refined by interaction with the environment. When chemicals interfere with these critical developmental processes, neurological development can be irreversibly altered. Developmental exposure to Pb can alter neural structures, synapse formation, synaptic transmission and cell survival. Out of 214 human neurotoxicants, Pb was identified as the neurotoxicant that contributed the most to dysregulation of gene expression in the brains of mice during critical periods of plasticity (Smith et al., 2018). Accumulating data show that Pb can interfere with epigenetic regulation (Khalid and Abdollahi 2019). Low socioeconomic status and certain genetic factors can increase children's susceptibility to the neurotoxic effects of Pb (Lidsky and Schneider 2003).

The European Human Biomonitoring Initiative (HBM4EU), a joint effort of 30 countries, the European Environment Agency (EEA) and the European Commission, generates evidence on EU citizens' exposure to chemicals and their potential health effects to support policy. The main objective is to create an inventory of mechanistically-based effect biomarkers of utility in HBM programs (Baken et al., 2019; Mustieles et al., 2020). Pb was prioritised because of its hazardous properties, exposure characteristics, public concern and the strong need for an up-to-date health-based EU Biological Limit Value (BLV)[1] to protect workers, women of fertile age and children (Ougier et al., 2018). Of note, women have a high employment rate in the EU (63% on average in 2019) (Eurostat, 2020), which in combination with relatively high age at child birth (29.3 years at first child) (Eurostat, 2018), increases the risk of Pb exposure prior to conception, and hence the potential for induction of neurodevelopmental toxicity.

In recent decades, a downward trend in blood Pb levels has been observed in Europe and some other developed countries, due to the introduction of effective risk management measures, e.g. decrease and subsequent phasing-out of Pb in petrol, and removal of Pb from paints, ceramics, water pipes and food cans (EFSA 2012). Atmospheric emissions of anthropogenic Pb were reduced by 68% in European countries from 2000 to 2018 (EEA 2020).

Pb levels in food have declined significantly since 2003 (EFSA, 2012), but some foods, especially when grown at contaminated sites, still constitute non-negligible sources, alongside the use of herbal and traditional medicines (including geophagy), cosmetics, and water from leaded pipes in non-renovated houses (EFSA, 2010; EFSA, 2012; Saoudi et al., 2018; Parnia et al., 2018; Gundacker et al., 2017). Overall, cereals and grain-based foods are the most important dietary sources of Pb due to their high consumption, while milk and dairy products contribute the most to infants and toddlers. In 2012, EFSA estimated dietary intakes of 1.32 and 1.03 μg/kg b.w./day for toddlers and children, respectively (EFSA, 2012). These exceeded the dietary intake level of 0.50 μg/kg b.w./day corresponding to the EFSA $BMDL_{01}$ of 12 μg/L blood Pb for developmental neurotoxicity (EFSA, 2010). A recent study reported that the average Pb intake from food and tap water for infants, toddlers and children exceeded 0.50 μg/kg b.w./day (Kirinčič, 2019). Similar conclusions were made regarding women of child-bearing age (EFSA 2012).

The low to moderate exposure of the European population furthermore requires the identification of effect and susceptibility biomarkers that are sufficiently sensitive to reliably and timely detect Pb-induced health effects and sensitive populations, respectively. *Effect biomarkers* can be defined as measurable biochemical, physiological, behavioural or other alterations that are associated with an established or possible adverse health outcome (Zare Jeddi et al., 2021). *Susceptibility markers* can be considered inherent or acquired abilities of an organism to respond to the challenge of exposure to a particular chemical substance. Finally, some *other biomarkers* that do not fall into either of these categories can still be relevant as complementary markers in HBM studies. Fig. 1 provides a general overview of how the use of exposure, effect and susceptibility biomarkers can provide insight into the exposure-disease continuum.

In the present review, we focus on biomarkers associated with neurodevelopmental adverse effects following exposure to Pb, as recent reports indicate that effects might also be induced at the lower range of the exposure continuum. For this purpose, we conducted a systematic literature review to identify publications that addressed the endpoint of neurodevelopmental toxicity. The set of identified biomarkers is discussed in relation to current Pb exposure levels in Europe. We

[1] According to Ougier et al. (2018) "The Annex II of the Chemicals Agents Directive 98/24/EC mentions that a more up to date BLV will be recommended by the Scientific Committee on Occupational Exposure Limits (SCOEL). The binding BLV for lead indicated by the Directive 98/24/EC is 70 μg/100 mL blood, whereas the recommended BLV from SCOEL is 30 μg/100 mL blood".

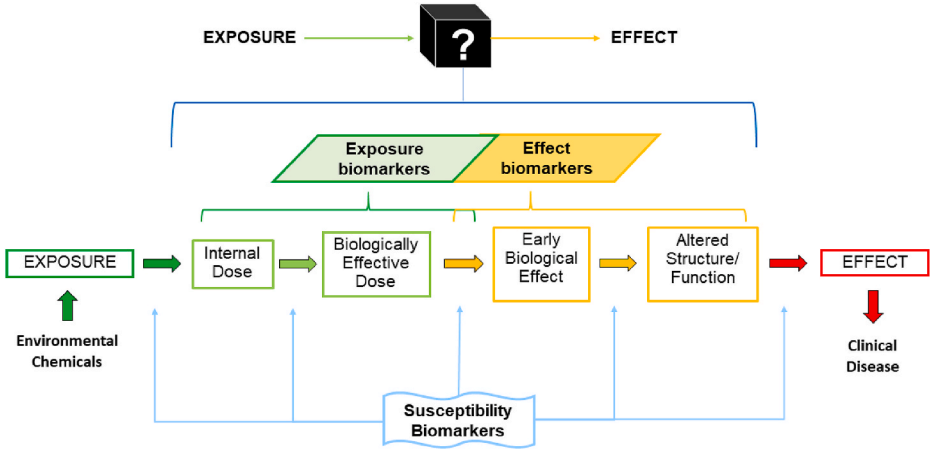

Fig. 1. Conceptual pathway representing the continuum between environmental chemical exposure and clinical disease. *Exposure biomarkers* measure the actual absorbed/excreted dose ("internal dose") and active dose at the putative target organ/tissue ("biologically effective dose"). *Effect biomarkers* measure early molecular or biochemical/cellular responses in target or non-target tissues ("early biological effect"), functional or structural changes in affected cells or tissues ("altered structure/function"), or actual clinical disease. *Susceptibility biomarkers* help to identify individuals with genetically mediated predisposition to xenobiotic-induced toxicity. Reprinted from Mustieles et al. (2020).

furthermore describe gaps in knowledge, in particular on the mechanisms of Pb-associated damage to the developing central nervous system, as well as the data needed to validate sensitive and reliable effect biomarkers in the future, with reference to existing AOPs.

2. Methods

2.1. Search history

Three literature searches in peer-reviewed journals were performed in the bibliographic database PubMed (Fig. 2). Details can be found in the search histories listed in Suppl. Table 1. The term 'biomarker' was not integrated into any of the searches, as this was found to significantly reduce the number of hits (data not shown).

2.1.1. PubMed search 1 and identification of relevant papers

2.1.1.1. Search terms. ("lead"[Title/Abstract] OR "pb"[Title/Abstract] OR lead[MeSH Terms] OR "plumbum"[Title/Abstract] OR "7439 92 1"[EC/RN Number]) AND neurodevelopment.

The elements of the search strategy (**search 1**) comprised the term *lead* combined with the term *neurodevelopment*. A preliminary search was performed using these terms. The term *lead* was searched with a combination of Medical Subject Headings (MeSH) and chemical registration numbers (EC/RN) in the title/abstract fields using AND/OR terms in Boolean logic to cover as many publications as possible. However, the term presented some challenges in order to avoid the inclusion of publications that only contained the verb *lead*. Therefore, the resulting 596 abstracts (search #1) were manually sorted to identify the articles studying the metal *lead*. The selected 160 abstracts were evaluated, and 15 references met the inclusion criteria (i.e., studies of Pb-exposed humans in relation to neurodevelopment and epigenetic changes published before July 2019). Several articles that reported associations between gene sequence variants and neurodevelopmental outcomes were also included. The 15 eligible publications selected from the PubMed search were supplemented by one review found by handsearch (Keil and Lein 2016). From this, two additional original works were identified (Wright et al., 2003; Pilsner et al., 2010) and also included (i.e., 17 papers in total; Fig. 1). Because some relevant papers were not retrieved in the first search, a second literature search was performed. The list of search terms was extended with epigenetic and OMIC terms.

2.1.2. PubMed search 2 and identification of relevant papers

2.1.2.1. Search terms. ("lead"[Title/Abstract] OR "pb"[Title/Abstract] OR "lead"[Mesh] OR "plumbum"[Title/Abstract] OR "7439 92 1"[EC/RN Number]) AND ("DNA (Cytosine-5-)-Methyltransferase 1"[Mesh] OR "DNA (Cytosine-5-)-Methyltransferases/genetics"[Mesh] OR "DNA (Cytosine-5-)-Methyltransferases*"[Mesh] OR "Epigenesis, Genetic*"[Mesh] OR "DNA Modification Methylases*"[Mesh] OR "Epigenetic*"[tiab] OR "Epigenomics*"[Mesh] OR "DNA Methylation*"[Mesh] OR "Methylation"[Mesh] OR "DNA Methylation/drug effects*"[Mesh] OR "DNA Methylation/genetics*"[Mesh] OR "Genomic Instability/genetics"[Mesh] OR "CpG Islands/genetics"[Mesh] OR "Transcriptome/drug effects"[Mesh] OR "Transcriptome/genetics*"[Mesh] OR "Metabolomics"[Mesh] OR "metabolome"[Mesh] OR "Metabolomics"[Mesh] OR "Metabolome/drug effects*"[Mesh] OR "Metabolic Diseases/genetics"[Mesh]).

In **search 2** the term *lead* was searched in the same way as in search 1. The epigenetic and OMICS terms were searched using only MeSH terms (search #43 and #44). Terms not indexed in PubMed as MeSH were omitted. As in search 1, the combination with the term *lead* resulted in an exorbitantly high number of irrelevant hits (#46). To resolve this, further terms related to "neurodevelopment" were added to the search strategy (#49). The search (#50) retrieved 105 hits. These abstracts were manually sorted to identify articles addressing the metal *lead*. This was the case in 13 articles that were further screened for relevance. Seven articles were selected. These seven references, however, were already included in the final 17 references from search 1.

2.1.3. PubMed search 3 and identification of relevant papers

2.1.3.1. Search terms. ("lead"[Title/Abstract] OR "pb"[Title/Abstract]) AND ("human biomonitoring"[Title/Abstract] OR "human exposure"[Title/Abstract] OR "environmental exposure"[Title/Abstract] OR "biomarker of exposure"[Title/Abstract]) AND ("blood"[Title/Abstract] OR "urine"[Title/Abstract] OR "breast milk"[Title/Abstract] OR "cord blood"[Title/Abstract] OR "*teeth"[Title/Abstract] OR "nail"[Title/Abstract] OR "hair"[Title/Abstract] or "placenta" [Title/Abstract]); Filter: 2000–2021.

The strategy in **search 3** comprised three preliminary searches to identify the publications in which the terms *lead* or *pb* (#1), *human biomonitoring* or *human exposure* or *environmental exposure* or *biomarker of exposure* (#2), and *blood* or *urine* or *cord blood* or *breast milk* or *teeth* or

Table 1
Comparison of Pb concentrations determined in different matrices of fetuses, children and young people in European surveys carried out in the period of 2000–2018.

Biomarker of Pb exposure	Pb concentration (population)	No. of samples	Sampling year and country	Reference
Whole blood (venous or capillary) (μg/L)	6.9[b] (6–14 yr)	14	2018, Kosovo	Dehari-Zeka et al. (2020)
- *children and young people*	11.3[a] (20–29 yr)	995	2015–2016, Germany	Lermen et al. (2021)
	8.3[c] (20–39 yr)	158	2011–2015, Norway	Fløtre et al. (2017)
	11.7[a] (20–29 yr)	986	2013–2014, Germany	Lermen et al. (2021)
	9.5[a] (14–15 yr)	204	2013–2014, Belgium	Schoeters et al. (2017)
	23[b] (6–12 yr)	53	2012–2014, Kosovo	Kutllovci-Zogaj et al. (2014)
	2.4[a] (18–40 yr)	73	2011–2014, Denmark	Rosofsky (2017)
	9.7–11.0[c] (25–35 yr female - male)	477	2004–2014, Sweden	Wennberg et al. (2017)
	16.1[a] (14–15 yr)	174	2011–2013, Slovenia	Snoj Tratnik et al. (2013)
	17.3[a] (20–35 yr)	127	2011–2013, Slovenia	Snoj Tratnik et al. (2013)
	12.3[a] (20–29 yr)	968	2011–2012, Germany	Lermen et al. (2021)
	14.6[a] (14–15 yr)	207	2007–2011, Belgium	Schoeters et al. (2017)
	12.1[a] (20–29 yr)	5765	2009–2010, Germany	Lermen et al. (2021)
	14.9[a] (1–6 yr)	3831	2008–2009, France	Etchevers et al. (2014)
	19-22[c] (8–10 yr)	n.a.	2008, Czech Republic	Cerná et al. (2012)
	17.9[a] (7–11 yr)	46	2007–2008, Croatia	Hrubá et al. (2012)
	19.4[a] (7–11 yr)	57	2007–2008, Slovakia	Hrubá et al. (2012)
	14[a] (7–11 yr)	41	2007–2008, Sweden	Hrubá et al. (2012)
	13.4[a] (7–11 yr)	42	2007–2008, Slovenia	Hrubá et al. (2012)
	16.3[a] (7–11 yr)	27	2007–2008, Poland	Hrubá et al. (2012)
	18.7[a] (18–39 yr)	579	2006–2007, France	Falq et al. (2011)
	15.1[a] (20–29 yr)	856	2005–2006, Germany	Lermen et al. (2021)
	30[a] (4–15 yr)	253	2006, Hungary	Rudnai et al. (2009)
	16.9[c] (3–14 yr)	1560	2003–2006, Germany	Schultz et al. (2009)
	22.5[a] (14–15 yr)	1659	2003–2004, Belgium	Schoeters et al. (2017)
	31[c] (8–10 yr)	333	2001–2003, Czech Republic	Batáriová et al. (2006)
	42[c] (6 yr)	202	1997–2004, Poland	Barton (2011)
- *pregnant women*	10[c] (1st trimester)	48	2016–2017, Spain	Bocca et al. (2019)
	12[c] (at delivery)	40	2016–2017, Spain	Bocca et al. (2019)
	8.5[c,d] (at delivery)	100	2010–2012, Slovakia	Gundacker et al. (2021)
	12.5[c,d] (at delivery)	98	2010–2012, Austria	Gundacker et al. (2021)
	11.1[a] (at delivery)	235	2007–2011, Belgium	Baeyens et al. (2014)
	11[a] (2nd trimester)	210	2007–2011, Poland	Polańska et al. (2014)
	7.2[a] (2nd trimester)	211	2007–2009, Norway	Hansen et al. (2011)
	11.5[c] (at delivery)	50	2006, Germany	Kopp et al. (2012)
	24.9[c] (3rd trimester)	53	2005, Austria	Gundacker et al. (2010)
- *lactating women*	8.3[a] (2nd trimester)	2982	2000–2008, Norway	Caspersen et al. (2019)
	11[c] (n.a.)	100	2002–2003, Sweden	Gerhardsson. (2010)
	16.7[a] (6th wk. postpartum)	536	2008–2014, Slovenia	Snoj Tratnik et al. (2019)
	9.2[a] (3rd d. postpartum)	211	2007–2009, Norway	Hansen et al. (2011)
	13.2[a] (6th wk. postpartum)	253	2007–2009, Norway	Hansen et al. (2011)
Cord blood (μg/L)	7.9[c] (mother BL:12)	31	2016–2017, Spain	Bocca et al. (2019)
	6.4[a] (mother BL: n.a.)	281	2012–2015, Belgium	Schoeters et al. (2017)
	8.3[a] (mother BL: n.a.)	1968	2011, France	Saoudi et al. (2018)
	4.5[c,e] (mother BL: 8.5)	100	2010–2012, Slovakia	Gundacker et al. (2021)
	7.0[c,e] (mother BL: 12.5)	100	2010–2012, Austria	Gundacker et al. (2021)
	9.6[a] (mother BL: 9.9)	594	2007–2011, Poland	Polańska et al. (2018)
	8.6[a] (mother BL: 11.1)	241	2007–2011, Belgium	Baeyens et al. (2014)
	10.3[c] (mother BL: 11.5)	50	2006, Germany	Kopp et al. (2012)
	13.7[a] (mother BL: n.a.)	1196	2002–2004, Belgium	Schoeters et al. (2017)
	13.4[c] (mother BL: 24.9)	53	2005, Austria	Gundacker et al. (2010)
	14.5[a] (mother BL: 18.3)	145	2003–2004, Spain	García-Esquinas et al. (2014)
Placenta (μg/kg)	<6.5[c]	327	2000–2008, Spain	Freire et al. (2019)
	25.8[c]	53	2005, Austria	Gundacker et al. (2010)
	45.2[c]	36	2003–2004, Croatia	Klapec et al. (2008)
	13.8[c] (non-smokers)	109	n.a., Croatia	Stasenko et al. (2010)
	17.9[c] (smokers)	99	n.a., Croatia	Stasenko et al. (2010)
	51.6[c]	23	n.a., Poland	Zagrodzki et al. (2003)
Breast milk (μg/L unless otherwise indicated)	1.74[b] (1th-8th wk. postpartum)	27	2017–2018, Hungary	Ecsedi-Angyal et al. (2020)
	1.19 μg/kg[b] (n.a.)	50	2015, Cyprus	Kunter et al. (2017)
	0.23[c] (6th-8th wk. postpartum)	353	2008–2014, Slovenia	Snoj Tratnik et al. (2019)
	5.0 μg/kg[c] (colostrum[d]) (mother BL: 7 μg/L)	20	2012–2013, Croatia	Grzunov L. et al., 2016
	2.6 μg/kg[c] (3rd-4th wk. postpartum[d])	51	2012–2013, Croatia	Grzunov L. et al., 2016
	3.3 μg/kg[c] (3rd-4th wk. postpartum[e])	20	2012–2013, Croatia	Grzunov L. et al., 2016
	6.3[b] (1st-12th mo. postpartum)	320	2010, Poland	Winiarska-M. 2014
	2.6–6[b] (n.a)	52	2008–2009, Italy	De Felip et al. (2014)
	1.5[b] (2nd-3rd wk. postpartum)	60	2002–2009, Sweden	Björklund et al. (2012)
	<0.67 μg/kg[c] (3rd-8th wk. postpartum)	300	2002–2009, Norway	Vollset et al. (2019)
	15.5[a] (3rd wk. postpartum)	107	2003–2004, Spain	García-Esquinas et al. (2011)
	1.55[b] (colostrum) (mother BL: 20 μg/L)	34	2003, Portugal	Almeida et al. (2008)
	0.94[b] (4th wk. postpartum)	19	2003, Portugal	Almeida et al. (2008)
	0.48[b] (3rd d. postpartum)	95	2001–2002, Greece	Leotsinidis et al. (2005)
	0.85–1[b] (4th-8th wk. postpartum)	39	1999–2001, Italy	Abballe et al. (2008)

(*continued on next page*)

Table 1 (*continued*)

	7.7[c] (3rd-4th d. postpartum)	143	2000, Italy	Turconi et al. (2004)
	1.63[b] (2nd-14th d. postpartum)	138	1999, Austria	Gundacker et al. (2002)
	0.15[b] µg/kg (1st wk. postpartum)	102	n.a., Portugal	Matos et al. (2009)
	13.8[b] (n.a.)	72	n.a., Spain	Falcó et al. (2006)
	4.7[b] (4th d. postpartum)	158	n.a., Slovakia	Ursinyova and Masanova (2005)
Urine (µg/g creatinine unless otherwise indicated)	1.3[c] (pregnant women)	53	2016–2017, Spain	Bocca et al. (2019)
	0.54[a] (mean age of 28.9 yr)	50	2013–2016, Belgium	Bai et al. (2019)
	0.49[a] (6th wk. postpartum)	410	2008–2014, Slovenia	Snoj Tratnik et al. (2019)
	1.16[c] (6–11 yr)	n.a.	2010–2011, Spain	Roca et al. (2016)
	1.24 µg/L[a] (5–11 yr)	n.a.	2007–2008, Italy	Protano et al. (2016)
	0.8[a] (2–17 yr)	n.a.	2005, Germany	Heitland and Köster (2006)
Hair (µg/g)	0.6–1.3 (11–12 yr)[a]	n.a.	2011–2012, Greece	Evrenoglou et al. (2013)
	0.7 (0–18 yr)[c]	n.a.	2008–2009, Spain	Llorente et al. (2017)
	1.13[a] (6 yr)	n.a.	1997–2004, Poland	Barton (2011)
	0.56[b] (11–15yr.)	n.a.	2001, Italy	Sanna et al. (2008)
	1.6[c] (mean age of 9.9 yr)	217	1994–2001, Czech Republic	Benes et al. (2003)
	1.01[b] (0–18 yr)	n.a.	n.a., Italy	Dongarrà et al. (2011)
Primary teeth (µg/g)	1.6[a] (6 yr)	284	1997–2004, Poland	Barton (2011)

Abbreviations: BL: blood level; LOD: limit of detection; n.a.: not available.
[a] geometric mean.
[b] arithmetic mean.
[c] median.
[d] Median Ery-Pb values obtained in the study (Bratislava: 17 µg/kg, Vienna: 25 µg/kg) were converted to whole blood values for comparability.
[e] Median Ery-Pb values obtained in the study (Bratislava: 9 µg/kg, Vienna: 17 µg/kg) were converted to whole blood values for comparability.

nail or *hair* or *placenta* (#3) were indicated in the title/abstract. Then these three preliminary searches were combined (#4) and articles published before year 2000 were filtered out (#5). This search had 656 hits which were manually sorted to identify publications on human biomonitoring studies focusing on children's, pregnant women's and young people's exposure to lead. In total, 58 publications from European countries were included. Some relevant publications from non-European countries were also selected for comparison (22 references). It must be noted that the selection of non-European data was not based on any predefined criteria.

2.2. Flowchart

3. Results and discussion

We conducted a systematic literature search to identify publications addressing exposure to Pb relative to neurodevelopmental toxicity, identified as the most sensitive endpoint. The biomarkers that are linked to Pb exposure and neurodevelopment were grouped into three different types: *effect biomarkers* (Table 3), *susceptibility markers* (Table 4), and *other biomarkers* (Table 5). This classification of markers is not generally applicable. Several of the biomarkers discussed here, such as high-density lipoprotein (HDL) levels or epigenetic changes, could also be caused by mutations/polymorphisms (this was not investigated in the studies included here), which would thus make them also susceptibility markers. The set of identified biomarkers is discussed in relation to current Pb exposure levels in Europe.

Table 2
Comparison of maternal peripheral blood, placental and cord blood Pb levels in surveys carried out in non-European countries after 2000.

Maternal blood (µg/L)	Placenta (µg/kg w.w.)	Cord blood (µg/L)	N. of samples	Sampling year and country	Reference
6.2[a]	-	-	1282	1999–2016, USA (NHANES)	Watson et al. (2020)
10.3[a]	-	7.1[a]	104	2013, South Korea	Kim et al. (2015)
-	2.3[b]	-	1159	2008–2013, USA	Punshon et al. (2019)
41.6[b]	-	35.0[b]	1050	2007–2011, Mexico	Sanchez-Guerra et al. (2019)
8.9[a]	-	-	211	2009–2011, USA	Sanders et al. (2012)
5.6[c]	-	7.6[c]	2000	2008–2011, Canada	Arbuckle et al. (2016)
39.7[c]	-	-	1931	2010, China, Shanghai	Li et al. (2017)
36.4[c]	7.5[c]	24.6[c]	91	2009–2010, Turkey	Tekin et al. (2012)
55.9[b]	-	-	174	2009–2010, China, Nanjing	Liu et al. (2013)
26.5[c]	-	22.5[c]	240	2007–2008, Bolivia	Barbieri et al. (2016)
595.0[b]	-	-	214	2006–2008, Nigeria	Adekunle et al. (2010)
32.6[c]	-	22.9[c]	350	2006–2007, Iraq	Al-Jawadi et al. (2009)
-	-	16.6[b]	121	2006–2007, Turkey	Dursun et al. (2016)
64.3[c]	-	35.7[c]	130	2006–2007, China, Shanghai	Wang et al. (2008)
72.4[c]	600[c] (d.w.)	43.6[c]	109	2002–2007, China, Hubei	Tian et al. (2009)
25.5[a]	390[a] (d.w.)	21.4[a]	1572	2005–2006, Saudi Arabia	Al-Saleh et al. (2011)
17.1[b]	-	12.9[b]	308	2004–2005, Taiwan	Lin et al. (2010)
21.0[b]	-	-	43,288	2003–2005, USA	Zhu et al. (2010)
48.2[b]	-	35,2[b]	365	2003–2004, Iran	Vigeh et al. (2006)
10.8[c]	11.2[c]	9.9[c]	649	2001–2003, Japan	Iwai-Shimada et al. (2019)
159.0[a]	-	-	50	n.a., Pakistan	Kayama et al. (2016)
-	300[c]	-	60	n.a., India	Singh et al. (2010)

Abbreviations: n.a.: not available; w.w.: wet weight, d.w.: dry weight.
[a] geometric mean.
[b] arithmetic mean.
[c] median.

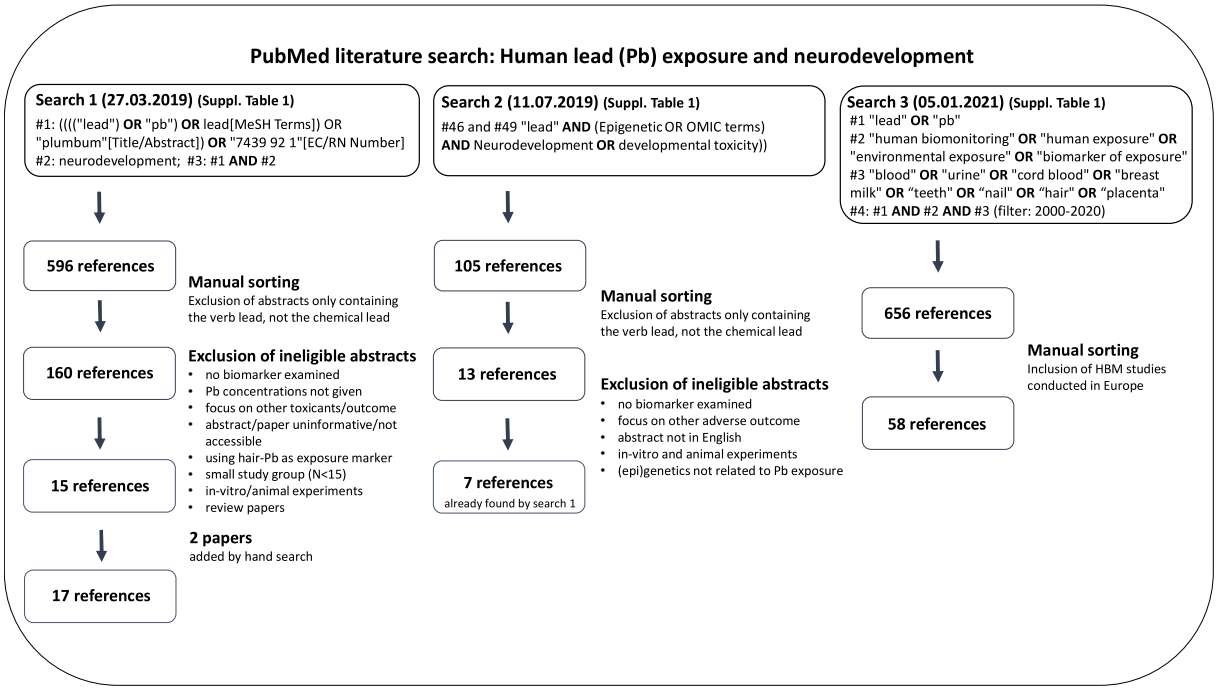

Fig. 2. The sequence of the literature search. Two PubMed searches on Pb exposure and neurodevelopment (search 1 and 2) identified 17 publications (for specific references, see Tables 3–5). PubMed search number 3 identified 58 publications focused on Pb exposure in Europe (Table 1). Several articles were reviewed in full length, if sufficiently specific information e.g., on biomarkers, was missing in the abstract.

Table 3
Summary of studies on **effect markers** linked to Pb exposure and neurodevelopment.

Author(s), Country	Marker(s) of Pb exposure	Effect Biomarker(s)	Neurodevelopmental testing	Summary
Brain derived neurotrophic factor (BDNF)				
Zhou et al., (2019) China	Whole blood, children, mean age: 5 yrs 6.7 μg/dL (6.5–7.0 μg/dL) (N = 561)	**Serum BDNF** (ELISA) 19.5 ng/mL	-	Serum BDNF negatively associated with blood Pb levels in all subjects and in boys, but not in girls. A negative interaction between blood Pb and Hg levels as well as a positive interaction between blood Pb and Al levels on serum BDNF concentrations was found in boys (not in girls).
Ren et al., (2016) China	Cord blood 7 ± 3 μg/dL[b] (low exp. group, N = 60) 13 ± 3 μg/dL[b] (high exp. group; N = 60)	**Cord serum BDNF** (ELISA) 2.5 ± 0.9 ng/mL (low exp gr) 3.5 ± 1.2 ng/mL (high exp gr)	Neonatal Behavioral Neurological Assessment (NBNA)	Cord serum BDNF positively associated with blood Pb, while NBNA sum score inversely correlated with blood Pb.
Serum cortisol				
Cai et al., (2019) China	Whole Blood (median), children, 3–6 yrs 4.9 μg/dL (Guiyu, N = 358) 3.5 μg/dL (Haojiang, N = 216)	**Children serum cortisol** (median) (Immunoassay) 452 ng/mL (Guiyu) 594 ng/mL (Haojiang)	Sensory Processing Measure-Hong Kong Chinese version (SPM-HKC). Note: Higher score means greater impairment.	Children from Guiyu (e-waste recycling town) had significantly lower concentration of serum cortisol than children in Haojiang (control site). Serum cortisol negatively correlated with blood Pb. All Sensory Processing Measure scores in Guiyu children were higher than in Haojiang children. Most sensory processing scores also positively correlated with blood Pb.
Tamayo et al., (2016) Mexico	Mat. whole blood and bone[a], N = 255 3.5 ± 2.5 μg/dL (Mat. Bl., 2nd trimester) 3.7 ± 2.9 μg/dL (Mat. Bl., 3rd trimester) 5.6 ± 5.8 μg/g (Mat. Tibia, postpartum) Maternal Pb levels refer to infants at the age of 12 months.	**Infant saliva cortisol** (Chemiluminescence assay) Cortisol levels not given	-	Early gestational Pb exposure alters diurnal cortisol rhythms of infants. Association is modified by infant age at 12 months and 18–24 months of age. Elevated prenatal Pb exposure of 12-month-old infants (2nd trim. Pb ≥ 10 μg/dL) is associated with 40% lower cortisol levels compared to infants with lower 2nd trim. Pb exposure (<5 μg/dL).

Pb concentrations are given as arithmetic or geometric mean values ± standard deviation, unless otherwise specified.
Abbreviations: exp.: exposure; Mat. Bl.: Maternal Blood; RBC: red blood cell.
[a] Bone Pb: in all studies midtibial shaft and/or patella Pb was analysed with a K-shell X-ray fluorescence instrument (KXRF).
[b] as converted from μM concentrations given in abstract.

Table 4
Summary of studies on **susceptibility markers** linked to Pb exposure and neurodevelopment.

Author(s), Country	Marker(s) of Pb exposure	Susceptibility marker(s)	Neurodevelopmental testing	Summary
Epigenetic modifications				
Wu et al., (2017) USA	Mat. RBC (2nd trimester) 1.2 ± 0.6 μg/dL (N = 268)	Cord blood leukocyte DNA (=268) ***CLEC11A DNHD1*** HumanMethylation450 Bead Chips	-	Prenatal low-level Pb exposure associated with newborn DNA methylation, particularly in female infants. CpG cg10773601, annotated to CLEC11A, showed an epigenome-wide significant negative association with maternal Pb exposure. CpG (cg24637308), which showed a strong negative association with maternal Pb exposure among girls, was annotated to DNHD1.
Pilsner et al., (2009) Mexico	Mat. bone[a], Cord blood (N = 103) 10.5 ± 8.4 μg/g (Mat. Tibia, N = 103) 12.9 ± 14.3 μg/g (Mat. Patella, N = 103)6.6 ± 2.7 μg/dL (Cord Blood)	Cord blood leukocyte DNA (N = 103) **LINE-1 methylation Alu methylation** EZ-96 DNA Methylation-Gold Kit	-	Prenatal Pb exposure (as indicated by maternal patella Pb) is inversely associated with genomic DNA methylation of the LINE-1 element. No association was found between cord blood Pb and cord genomic DNA methylation.
Candidate genes/Gene variants				
Wang et al., (2017) Bangladesh Mexico	Cord blood 5.1 ± 6.5 μg/dL (N = 390) 3.8 ± 2.7 μg/dL (N = 497)	***UNC5D SLC1A5*** Genome-wide gene-environment interaction study	Mental composite score and motor composite score (BSID-III) of infants 24 months of age	Top locus containing *UNC5D* associated with mental composite score. Two *UNC5D* SNPs had comparable main effects and GxE effects on both mental and motor composite scores in each cohort. By comparing GxE analyses and in-vitro transcriptome, glutamate transporter *SLC1A5* was identified as common gene/protein of interest with a *SLC1A5* variant being associated with mental composite score.
Wagner et al., (2017) Mexico	Mat. whole blood Pb, 2nd trimester 3.8 ± 2.6 μg/dL (N = 462)	***SPP1*** Genome-wide association study	Cognitive Development Index (CDI) score (BSID-III) of infants 24 months of age	Pb induces upregulation of SPP1 via NRF2 in human neural stem cells. *SPP1* SNP rs12641001 significantly associated with CDI score, however, no significant interaction with prenatal Pb exposure.
Wright et al., (2003) Mexico	Cord blood 6.6 ± 3.4 μg/dL (N = 311)	***APOE***	Mental Development Index (MDI) score (BSID-IIS) of infants 24 months of age	Negative effect of Pb exposure on MDI score at 24 months of age was 4-fold greater in APOE3/APOE2 carriers than in APOE4 carriers. Subjects with the E4 isoform of APOE may have advantages over those with the E2 or E3 isoforms with respect to early life neurodevelopment.
Pilsner et al., (2010) Mexico	Cord blood 6.7 ± 3.6 μg/dL (N = 255)	***MTHFR***	MDI score (BSID-IIS) of infants 24 months of age	Maternal MTHFR-677 genotype predicted MDI scores of infants at 24 months of age (BSID-IIS) but *MTHFR* genotype x Pb interaction was not observed. The maternal *MTHFR* 677T allele is an independent predictor of poorer child neurodevelopment at 24 months of age.
Liu et al., (2015) China	Whole blood, children, 3 yrs 11.3 ± 5.4 μg/dL (Guiyu, N = 120) 5.8 ± 2.5 μg/dL (ref group, N = 138)	***DRD2***	Cognitive and language scales (BSID-III)	Both scores lower in Guiyu children compared to reference group. No significant association between (DRD2) Taq IA polymorphism and neurodevelopment of Pb exposed children.

Pb concentrations are given as arithmetic or geometric mean values ± standard deviation, unless otherwise specified.
Abbreviations: Alu: Alu retrotransposons; APOE: Apolipoprotein E; BSID-III: Bayley Scales of Infant and Toddler Development, Third Edition; BSID-IIS: Spanish version of the BSID-II; CLEC11A: C-Type Lectin Domain Family 11, Member A; DNHD1: Dynein Heavy Chain Domain 1 gene; DRD2 (Dopamine receptor D2); GxE: gene-environment interaction; LINE-1: long interspersed nuclear element; Mat.: Maternal; MTHFR: Methylenetetrahydrofolate reductase; NRF2: Nuclear Factor Erythroid 2-Related Factor 2; p16: tumor suppressor gene p16; RBC: red blood cell; SLC1A5: Solute Carrier Family 1 Member 5 (alias ASCT2); SNP: Single Nucleotid Polymorphism; SPP1 (Secreted phosphoprotein 1); UNC5D: Unc-5 Netrin Receptor D.
[a] Bone Pb: in all studies midtibial shaft and/or patella Pb was analysed with a K-shell X-ray fluorescence instrument (KXRF).

3.1. Pb exposure in Europe

Whole blood Pb is the most widely used exposure parameter, as it is the most suitable indicator of the concentration of Pb in soft tissues, like the brain. There are limited recent European HBM data on Pb levels in other matrices than blood.

In the past two decades, more than 25 HBM surveys on blood Pb levels were performed in European countries, including Belgium, Czech Republic, France, Germany, Norway, Slovenia, and Spain. Nearly 20 studies included children (Table 1). The majority were carried out before 2015; thus data on the most recent exposure is lacking. As the developing central nervous system is at the highest risk for impairment due to Pb exposure, this review on the concentration of Pb in blood, placenta, breast milk, urine, hair, and teeth reflects the exposure of fetuses and children in Europe during the past two decades (Table 1). For surveys at contaminated sites, only data on the reference populations are reported. In addition, Pb levels in some populations of young adults of child-bearing age are provided as indicators of potential exposures of future fetuses.

3.1.1. Pb levels in children and young adults

The mean Pb concentrations of blood samples collected from children and young adults in European countries after the year 2000 were comparable (Table 1). Data show a decreasing trend in Pb blood levels of

Table 5
Summary of studies on **other markers** linked to Pb exposure and neurodevelopment.

Author(s), Country	Marker(s) of Pb exposure	Marker	Neurodevelopmental testing	Summary
High density lipoprotein (HDL)				
Ji et al., (2018) USA	RBC Pb, children (postnatal) 2.2 ± 1.6 μg/dL (whole gr; N = 1479) 2.1 ± 1.5 μg/dL (control gr; N = 1180) 2.4 ± 1.9 μg/dL (ADHD gr; N = 299)	**Maternal plasma HDL** (median) (nonfasting blood samples) 60.7 mg/dL (N = 1479) 61.5 mg/dL (N = 1180) 57.4 mg/dL (N = 299)	Attention Deficit Hyperactivity Disorder (ADHD) according to ICD-9 and ICD-10. Age of children's ADHD diagnosis not specified.	Mothers of children with any ADHD diagnosis had low HDL levels and high stress. 9% of the children had elevated Pb levels (5–10 μg/dL) in early childhood associated with a 66% increased risk of ADHD particularly for boys. The OR of ADHD associated with elevated Pb levels among boys was reduced by one-half if mothers had adequate HDL levels or low stress.
Iron (Fe), Calcium (Ca), Zinc (Zn), Copper Cu)				
Shah-Kulkarni et al., (2016) Korea	Whole blood (N = 765–790) 0.9 ± 1.5 μg/dL (Cord Blood) 1.3 ± 1.5 μg/dL (Mat. Blood, at delivery)	**Maternal Fe intake** (N = 891) 13 ± 4 mg/d	Mental Development Index (MDI) and Psychomotor Development Index (PDI), Korean version of Bayley Scales of Infant Development II (K-BSID-II), 6, 12, 24, and 36 months of age (N = 558–965)	Maternal Pb exposure in late pregnancy associated with lower MDI score in children 6 months of age with stronger effect in lower Fe intake group. No such associations found for Pb exposure in early pregnancy, in relation to cord blood, or PDI.
Ettinger et al., (2009) Mexico	Maternal Blood 3.8 ± 2.0 μg/dL (Ca suppl group, N = 283) 4.1 ± 2.0 μg/dL (Placebo group, N = 274)	**Maternal Ca intake**	-	Supplementation of 1200 mg dietary Ca associated with modest reductions in blood Pb (on average 11% reduction, i.e. 0.4 μg/dL) when administered during pregnancy.
Liu et al., (2014) China	Maternal Blood, 1st trimester, N = 415 3.98 ± 1.15 μg/dL	**Maternal Ca, Fe, Zn supplementation**	Neonatal behavioral neurological assessments (NBNA), postnatal day 3	Inverse associations between maternal Pb and neonatal behavioral neurological assessment (NBNA) scores. Ca, Fe, and/or Zn supplementation protects against high blood Pb levels.
Parajuli et al., (2013) Nepal	2.1 μg/dL (Cord Blood), N = 100	**Cord blood Zn**	Brazelton Neonatal Behavioral Assessment Scale (NBAS-III), postnatal day 1	Cord blood levels of Pb and As, but not Zn, showed significant inverse association with neurodevelopment of newborns (motor cluster).
Liu et al., (2018) Mexico	Maternal Blood, 2nd trimester PROGRESS (prospective prebirth cohort) study, Mexico City Metal levels (Mn, Pb, Co, Cr, Cs, Cu, As, Cd, Sb) not specified in this method paper	**Maternal blood Cu** Size of study group(s) not specified	BSID-III (cognition score, cognitive trajectories), 6, 12, 18, and 24 months after birth	Bayesian varying coefficient kernel machine regression was used to assess neurodevelopmental trajectories associated with exposure to complex metal mixtures. Positive associations between 2nd trimester exposure to Cu and cognition score at 24 months, and cognitive abilities across 6–24 months were found. Across 6–24 months, Pb was negatively associated with neurodevelopment. Overall, this resulted in negative interaction effect between 2nd trimester copper (Cu) and Pb exposures with 24-month neurodevelopment.

Pb concentrations are given as arithmetic or geometric mean values ± standard deviation.
Abbreviations: BSID-II: Bayley Scales of Infant Development, Second Edition; BSID-III: Bayley Scales of Infant Development, Third Edition; Mat. Bl.: Maternal Blood; NBAS-III: Brazelton Neonatal Behavioral Assessment Scale, Third Edition.

children and young adults after 2000. In surveys implemented before 2013, all reported mean blood Pb levels of children and young adults were above the BMDL01 of 12 μg/L set by EFSA for developmental neurotoxicity (EFSA, 2010). Moreover, a percentage of individual blood Pb concentrations exceeded this level even in populations characterized by a low mean exposure value. However, all values were substantially below the reference levels of 50 μg/L and 35 μg/L set by the CDC and the German Environmental Agency, respectively (CDC, 2010; Schulz et al., 2007). It must be noted that the risk for neurodevelopmental disorders due to Pb exposure cannot be ignored. Importantly, developmental neurotoxicity due to Pb exposure has been reported at exposures as low as 20 μg/L (Gilbert, 2006; EFSA, 2012; Rocha, 2019), and there is likely no safe threshold for Pb neurotoxicity (Lanphear et al., 2005; Skerfving et al., 2015). Although the information on Pb levels in children and young people after 2015 is limited, in some countries with regular nationwide surveys, the decreasing trend has not been observed to continue since 2010, and the concentration values levelled out at approximately 10 μg/L (Lermen et al., 2021; Wennberg et al., 2017).

3.1.2. Pb exposure of fetuses

Due to particular concern about neurodevelopmental effects, studies often included pregnant women and mother-neonate pairs. Pregnant and lactating women were investigated in at least nine European studies, and a similar number analysed cord blood with or without a sampling of maternal blood. The data show a decreasing trend in both maternal and cord blood Pb levels. In the European surveys implemented after 2005, mean maternal and cord blood Pb concentrations did not exceed the EFSA BMDL01 of 12 μg/L.

A significant correlation between maternal and cord blood Pb concentrations was found in most surveys, with ratios ranging between 0.55 and 0.97. Hence, Pb concentrations were only slightly lower in cord than in maternal blood, indicating that the placenta does not constitute a barrier for Pb transfer from the maternal to the fetal compartment. Maternal smoking and alcohol consumption were associated with enhanced maternal and cord blood Pb levels, while maternal Ca and Vitamin D intake seems to be protective against the mobilization of Pb from bones (Saoudi et al., 2018; Taylor et al., 2013; Gulson et al., 2016).

Some selected data on maternal and cord blood levels from non-European populations are listed in Table 2. The comparison shows that higher blood Pb levels were measured in developing countries (e.g. Nigeria, Pakistan) than in developed countries. Pb concentrations in maternal and cord blood observed in European surveys are similar to those reported from other high-income countries outside Europe, while they are much lower than Pb blood levels of populations living in

polluted as well as deprived areas.

3.1.3. Pb exposure of breastfed infants

Breast milk Pb level provides a direct indicator of Pb exposure of breastfeeding infants. Breast milk shows a higher variation in Pb levels than maternal blood (Table 1). In general, lower mean Pb concentrations in breast milk were measured in studies conducted after the year 2000 compared to previous studies (WHO, 1989). Due to the high variability in milk Pb levels, even within studies, this decreasing trend in breast milk Pb levels is not observed to continue after 2000. The opposite trend is even observed according to some studies (Abbale et al., 2008; De Felip et al., 2014).

Several studies showed higher breast milk Pb levels in current, passive or ever smokers than in never smokers (Gundacker et al., 2002; Grzunov Letinic et al., 2016; Winiarska-Meiczan 2014; Almeida et al., 2008). The high variation in breast milk Pb levels among the included studies suggests that besides differences in external exposures and genetic predispositions of the mothers, other individual maternal factors (e.g. age, past exposures, previous lactations and pregnancies, stage of lactation, Ca and D-vitamin intake during lactation) are also important.

The reported breast milk Pb concentrations in some studies (Winiarska-Meiczan 2014; García-Esquinas et al., 2011) suggest that infant Pb intake could approach the EFSA $BMDL_{01}$ of 0.5 μg/kg of body weight/day (EFSA 2010). Breast milk constitutes the optimal nutrition for young infants, but the potential for high Pb intake of infants via mother's milk cannot be ignored considering (i) the high Pb content of breast milk samples reported in some studies, (ii) other potential sources (e.g. complementary food or water) relevant at this period, and (iii) the fact that infants may absorb dietary Pb more efficiently than adults, especially if there is a concurrent deficit in Ca and other essential elements.

Overall, Pb exposure could still be associated with risk for developing fetuses, infants and children. Therefore, a new Pb action level for blood should be established. Larger nationwide surveys are needed to reliably assess recent exposure to Pb. To facilitate large campaigns, non-invasive sampling methods with harmonized methodology and adequate reference levels should be applied in HBM surveys among non-occupationally exposed populations, especially in the case of children (Esteban and Castano, 2009). Except for maternal and cord blood sampled in departments of obstetrics, blood is not an easily collectable biological matrix. Urinary Pb levels usually correlate with those measured in plasma as well as with the external exposure (Bai, 2019). Urine Pb therefore presents a valid and non-invasive alternative to blood Pb. Nail and hair Pb levels can serve as indicators of exposure to Pb; however, they are not considered reliable biomarkers of internal dose (Klotz and Goen, 2017; Olympio et al., 2020). Deciduous teeth Pb levels could also be easily applicable for biomonitoring of Pb exposure in children over the years of the preschool and early school period, since good correlation with blood levels has been reported (Barton, 2011). Placental tissue might be a suitable matrix for investigation of fetal exposure, although the comparability of the results requires standardization of collection, tissue preparation (e.g. washing and homogenization of whole placenta or biopsies from specific parts), and analytical methods (Esteban et al., 2012). Due to heterogeneity of the methods applied in published studies, data on placental Pb levels show great variability.

3.2. Biomarkers

Data sets from each of the 17 studies (Fig. 2) were grouped by type of marker and ranked in ascending order according to blood Pb concentrations in the populations measured for these groups of biomarkers (Tables 3–5).

3.2.1. Biomarkers of the effect associated with Pb exposure and neurodevelopment

Classical effect biomarkers of Pb exposure include markers related to heme biosynthesis and pyrimidine nucleotide metabolism (IARC 2006). However, these effects are not specifically related to neurodevelopment and have mostly been applied in settings of high (occupational) Pb exposure. The following section describes the studies found on effect biomarkers (Table 3).

3.2.1.1. Brain-derived neurotrophic factor (BDNF). As a member of the neurotrophin family of growth factors, brain-derived neurotrophic factor (BDNF) constitutes a key regulator of brain development and neural plasticity and is involved in the pathophysiology of diverse psychiatric disorders, including depression, anxiety, ADHD and autism (Kowiański et al., 2018).

We identified two studies on Pb exposure, neurodevelopment and BDNF showing apparently opposite results (Table 3). In the first study, 561 pre-school children at 5 years of age in China were assessed for blood concentrations of Pb (geometric mean of 6.7 μg/dL), Hg (0.10 μg/dL), Al (5.2 μg/dL), and Mn (1.8 μg/dL) as well as for BDNF concentration in serum (Zhou et al., 2019). Adjusted multivariable linear regression models were used to evaluate the possible interactions between metal co-exposure, taking into account several covariates. Blood Pb concentrations correlated significantly and inversely with serum BDNF concentrations in boys but not in girls. There was furthermore a negative interaction between blood Pb and Hg levels and positive interaction between blood Pb and Al levels in relation to serum BDNF concentrations in boys.

In the second study, cord blood total BDNF levels were determined in neonates from China grouped according to high (Pb > 10 μg/dL; N = 60) and low exposure (Pb < 10 μg/dL; N = 60) relative to their scores in the neonatal behavioral neurological assessment test (Ren et al., 2016). As described in the English abstract (article in Chinese), the high Pb exposure group showed significantly lower NBNA scores and higher serum levels of BDNF compared to the low Pb exposure group. Notably, higher cord serum levels of total BDNF were associated with poorer NBNA summary scores. Information on the number of male and female neonates, statistical analyses and the influence of infant sex on NBNA scores and BDNF levels missing from the abstract.

The physiology of BDNF is complex. In the brain, the precursor pro-BDNF is synthesized and stored in dendrites or axons. While pro-BDNF preferentially binds the p75 neurotrophin receptor leading to apoptosis, mature BDNF activates tyrosine kinase receptors (TrkB) to promote cell survival and synaptic plasticity (Miranda et al., 2019). Moreover, the ratio between the BDNF and its precursor may vary throughout development and hence vary between life-stages such as neonates, children, adolescents, adults and elders (Miranda et al., 2019).

Data from the paper by Ren et al. (2016) is only available from an abstract, and this obviously hampers the interpretation of data. However, the inconsistency between studies could possibly be ascribed to the different life stages of the children (neonates vs children) at the time of BDNF assessment, but it does not explain the difference in sensitivity between boys and girls at birth. Rather, these findings could be explained in terms of the BDNF isoform (pro- or mature) driving the association. The association between Pb exposure and higher serum BDNF levels thus could be explained by higher levels of the pro-BDNF isoform, which exerts proapoptotic effects, and could explain the association between higher serum BDNF levels linked to poorer neonatal development (Ren et al., 2016).

BDNF can be assessed in different biological matrices and at different levels of biological complexity. Until now, most ELISA kits have been designed to measure both pro-BDNF and mature BDNF in human serum/plasma. However, more specific measures that allow discriminating between the pro- and mature BDNF serum forms are progressively available and will facilitate the interpretation of divergent results in the

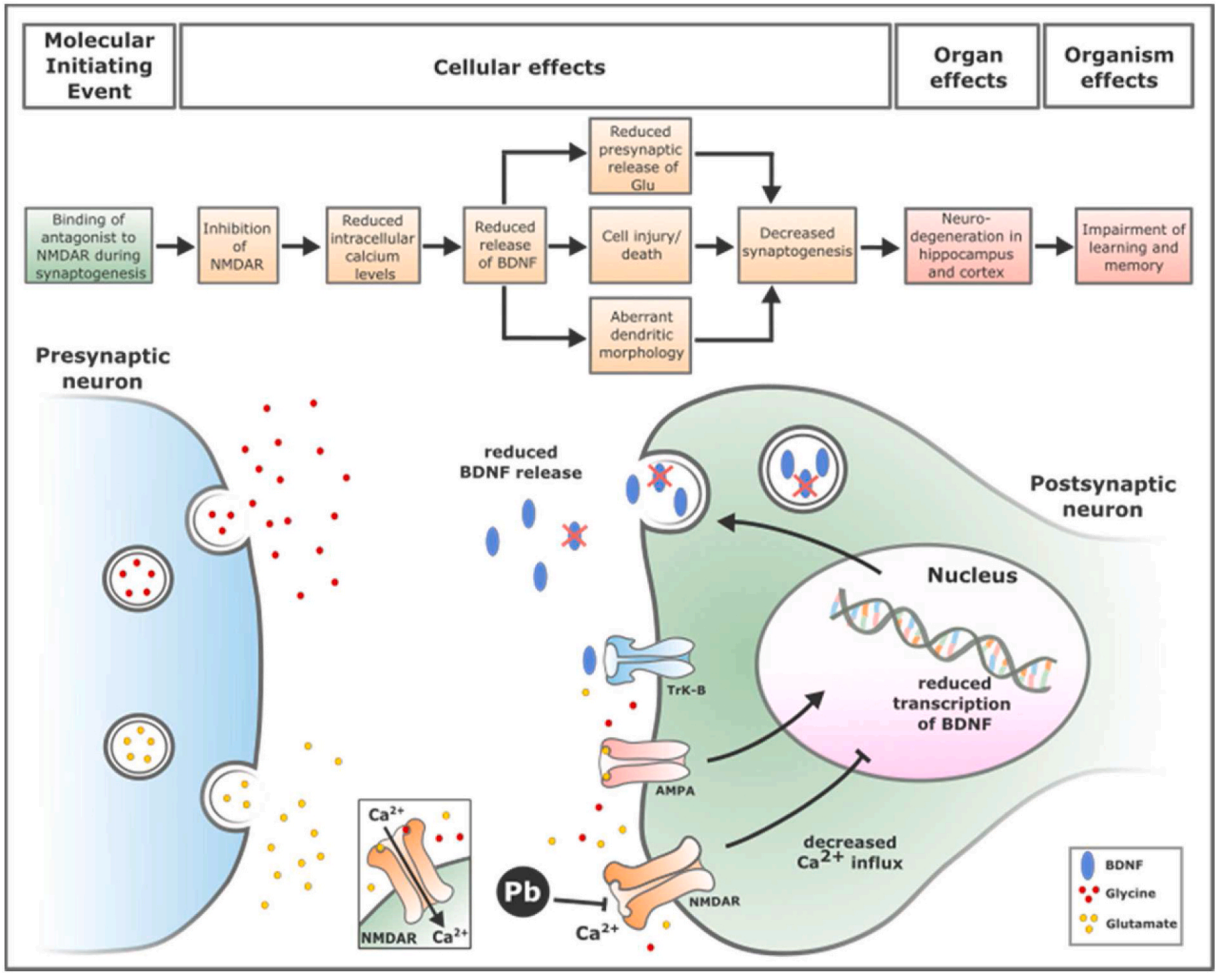

Fig. 3. Inhibition of the NMDA receptor (NMDAR) by Pb is followed by reduced Ca influx into the nucleus, which reduces transcription of BDNF. The reduced BDNF release eventually leads to neurodegeneration in the hippocampus and cortex and impairs learning and memory as described in AOP13 (https://aopwiki.org/aops/13).

literature (Bharani et al., 2020; Mizoguchi et al., 2020).

The potential for the use of BDNF biomarkers in relation to Pb exposure in HBM studies is supported by toxicological and AOP data (Fig. 3). Also, other environmental chemicals, including bisphenols, phthalates and polycyclic aromatic hydrocarbons, have been shown to interfere with BDNF signaling (Mustieles et al., 2020; Perera et al., 2015; Ponsonby et al., 2016). Therefore, there is recent interest in assessing BDNF at different levels of biological complexity (e.g. DNA methylation, gene expression, protein levels) as biomarkers of brain function with promising application in HBM studies (Mustieles et al., 2020). Indeed, epigenetic mechanisms, including DNA methylation, influence BDNF expression and regulation (Ikegame et al., 2013). Additionally, the DNA methylation status of the BDNF gene seems consistent across tissues, including the brain and blood, supporting its use as a valid and useful peripheral biomarker of psychiatric disorders (Kundakovic et al., 2015; Stenz et al., 2015). Another advantage is that peripheral blood BDNF DNA methylation could be more stable over time compared to serum/plasma BDNF protein levels, providing information on a longer period of time (Mustieles et al., under review).

Future HBM studies should ideally investigate a related set of BDNF biomarkers at different levels of biological organization, testing both pro- and mature BDNF protein isoforms in combination with infant/child neuropsychological tests to achieve the most accurate picture possible (Mustieles et al. under review).

3.2.1.2. Cortisol. Endocrine disrupting properties of Pb, one of which relates to cortisol, are thought to contribute to the metal's neurotoxicity. The stress hormone cortisol, synthesized from cholesterol, is the major glucocorticoid produced by the human adrenal cortex and the end product of the hypothalamic-pituitary-adrenal (HPA) axis. In the fetal brain, glucocorticoids are involved in several central aspects of development. The hippocampus, a known target of Pb toxicity and a central player in memory function, has the highest concentration of glucocorticoid receptors in the central nervous system (Lee et al., 2015; Tamayo et al., 2016; Graham et al., 2019).

Infant salivary cortisol was measured as an index of HPA-axis functioning in a birth cohort in Mexico City (Tamayo et al., 2016). Saliva samples were repeatedly collected over 2 days at 12 (N = 255) or 18–24 months of age (N = 150). Mixed-effects regression models were applied to account for the nonlinearity of cortisol rhythms. Several covariates were taken into account. In age-stratified models, a statistically significant negative association between maternal blood Pb and cortisol levels in 12-month-old infants and a positive association for 18–24-month-old infants were observed, although the latter was not statistically significant.

Children aged 3–6 years were studied for Pb exposure and serum cortisol in China (Cai et al., 2019). Children from Guiyu (n = 358, median Pb level: 4.9 μg/dL), a town where electronic waste is recycled, and Haojiang (n = 216, median Pb level: 3.5 μg/dL), a nearby town with no such recycling activity, were included. Serum cortisol levels were obtained from peripheral venous morning blood samples from fasting children. The children's sensory processing (relating to vision, hearing,

touch, taste and smell, body awareness, balance and motion) were assessed with the Sensory Processing Measure-Hong Kong Chinese version (SPM-HKC) home form, filled in by parents. Multiple linear regression analysis was applied to blood Pb and serum cortisol levels, taking into account several covariates. Blood Pb exceeded 5 μg/dL in 47% of the Guiyu children, and their serum cortisol concentrations were significantly lower than those of the Haojiang children. All scores of the Sensory Processing Measure were higher in Guiyu than Haojiang children, indicating greater difficulties, especially for touch, body awareness, balance and motion, and total sensory systems. Most sensory scores were also positively correlated with blood Pb.

In these studies, elevated maternal Pb exposure was associated with reduced cortisol levels in the 12 months and 3–6 year old children, whereas the cortisol curve shifted upwards in the 18–24 month old children. The normal trend in cortisol levels from 12 to 18–24 months of age is a downward shift. Pb exposure, therefore, appears to produce a cortisol rhythm pattern in 12-month-old children that is similar to the pattern normally seen at 18-24-month. It was concluded that early prenatal Pb exposure is associated with dysregulated infant HPA axis function, perhaps representing a premature HPA axis maturation (Tamayo et al., 2016). In the study from China, Pb exposure was associated with both lower cortisol levels and an increase in child sensory integration difficulties, especially regarding touch, body awareness, balance and motion, and total sensory systems. Cai et al. (2019) concluded that cortisol might be involved in touch-related sensory integration difficulties.

The most commonly used biomarkers, serum and salivary cortisol reflect levels at a single point in time. Levels are highly variable due to circadian fluctuations and fast changes in response to stressors. Effects of long-term systemic cortisol exposure are therefore complicated to assess, even if a predefined protocol is strictly adhered to (Lee et al., 2015). If basal cortisol levels are to be determined in human studies, cortisol concentrations must not only be measured several times a day to capture the large diurnal variation but also be done with non-invasive sampling to avoid acute stress (Tamayo et al., 2016). These requirements rather speak against the use of serum cortisol for routine use in HBM studies, and even salivary cortisol sampling might provoke stress in children. Hair cortisol analysis, which has been suggested as a promising technique for retrospective global assessment of chronic stress (Lee et al., 2015) may be an alternative in some settings. Another design that has been previously applied in infants is collecting salivary samples before and after a stressful stimulus (e.g. blood draw), which may provide information on the acute stress response of the child (Giesbrecht et al., 2017). Of note, it would be interesting to investigate the influence of Pb exposure on the response of the HPA-axis.

3.2.2. *Susceptibility markers associated with Pb exposure and neurodevelopment*

3.2.2.1. Epigenetic modifications. DNA methylation, a fundamental epigenetic process, modulates the level of gene expression without altering the DNA sequence. Methylation programming patterns can be modified during development. LINE-1 and Alu elements are repetitive DNA retrotransposons that constitute approximately 17% and 25% of the human genome, respectively (Baba et al., 2014; Luo et al., 2014). Therefore, the extent of LINE-1 and Alu methylation is considered a surrogate marker for global DNA methylation levels (Ruiz-Hernandez et al., 2015).

Two studies have examined DNA methylation in relation to prenatal Pb exposure, albeit without the concomitant study of markers of neurodevelopment (Table 3) (Pilsner et al., 2009; Wu et al., 2017). An epigenome-wide association study examined 268 mother-infant pairs in the USA (Wu et al., 2017). Genome-wide DNA methylation levels of infants were determined at 482,397 CpG[2] loci in umbilical cord blood nucleated cells using HumanMethylation450 Bead Chips. Maternal Pb exposure was analysed in red blood cells in the 2nd trimester (mean level: 1.2 ± 0.6 μg/dL). After adjusting for batch effects, cell types, and several covariates, robust linear regression models were used to examine associations of prenatal Pb exposure with DNA methylation in cord blood at epigenome-wide significance levels (false discovery rate <0.05). Elevated maternal Pb levels were associated with decreased DNA methylation of most CpG sites, and the methylation pattern was more frequently associated with maternal Pb levels in female than male infants (Wu et al., 2017). A CpG site annotated to *CLEC11A* showed an epigenome-wide significant negative association with maternal Pb levels. CLEC11A stimulates proliferation and differentiation of primitive hematopoietic precursor cells (RBCs, lymphocytes, granulocytes, macrophages), and Pb is known to interfere with hemoglobin synthesis (Wu et al., 2017). A second CpG site showed a negative association with prenatal Pb exposure among female infants was annotated to *DNHD1*. Little is known about its function, but it has been reported as a novel candidate gene associated with intellectual disability (Anazi et al., 2017) and early human developmental diseases (Meier et al., 2019). Of note, in another study, CpG methylation in *DNHD1* was shown to match well between blood and brain (Hannon et al., 2015), suggesting that peripheral blood cells may serve as a surrogate for the brain with respect to certain key regulatory loci.

In the study by Pilsner et al. (2009), genomic DNA methylation within CpG islands of LINE-1 and Alu retrotransposons was analysed in 103 umbilical cord blood samples. Cord blood Pb and genomic DNA methylation were not correlated, but maternal patella Pb correlated inversely with umbilical cord LINE-1 methylation, and maternal tibia Pb correlated inversely with Alu methylation. In a mixed-effects regression model, only maternal tibial Pb remained negatively associated with Alu methylation.

In a systematic review on environmental chemicals and DNA methylation in adults (Ruiz-Hernandez et al., 2015), four studies addressed Pb exposure. All studies reported a trend toward inverse associations of Pb exposure and DNA methylation. One of the studies confirms to some degree the findings of Pilsner et al. (2009), as Pb in the patella of adult men was also inversely associated with LINE-1 methylation (Wright et al., 2010). Since the method does not provide a fine epigenomic mapping of DNA methylation patterns, it remains unclear to which extent reduced global DNA methylation actually alters gene expression and in which specific chromosomal regions.

3.2.2.2. Candidate genes and gene variants. A genome-wide gene-environment interaction study (GWIS) in two birth cohorts (390 children from Mexico, 497 from Bangladesh) aimed to identify genetic loci in the child that modified the effect of prenatal Pb exposure (cord blood Pb) on

[2] CpG is the abbreviation for 5′-C-phosphate-G-3′, i.e. cytosine and guanine that are separated by only one phosphate group along its 5′ to 3′ direction. This notation is used to distinguish the single-stranded linear sequence from the CG base pairing in double-stranded sequences. CpG islands (CGIs) of vertebrates represent a class of short DNA sequences that are GC-rich, CpG-rich, and predominantly unmethylated. Silencing of CGI promoters is achieved by, among other mechanisms, dense CpG methylation. CGIs are thus generally capable of affecting local chromatin structure and facilitating regulation of gene activity (Deaton and Bird 2011).

neurodevelopment at approximately 2 years of age (Wang et al., 2017). Neurodevelopment was assessed using the motor composite scores as determined with the Bayley Scales of Infant Development. In addition, in-vitro transcriptome data were generated from Pb-exposed human neural stem cells. Univariate and multivariate regression analyses were performed to estimate the effects of covariates (child sex and age of neurologic examination, gestational age at birth, parity, maternal education, environmental tobacco smoke exposure) on neurodevelopment. The study (GWIS) provided data on several genetic polymorphisms that modified neurodevelopmental outcomes in response to prenatal Pb exposure. All candidate genes were generally related to neurodevelopment. The functional role of UNC5D is largely unclear, but involvement in neurite growth and developmentally programmed death of neurons have been demonstrated (Zhu et al., 2013; Srikanth et al., 2018). Two *UNC5D* variants had main effects on the Bayley Scales of Infant Development mental score and additionally showed a GxE interaction on the score. This was in an unexpected way, as the minor alleles of these *UNC5D* SNPs were associated with lower mental scores, but the GxE interaction was positive. It was suspected that Pb somehow counteracted the negative effects on neurodevelopment in carriers of the minor alleles. Another candidate gene, *SLC1A5*, was identified when GWIS data were combined with in-vitro transcriptomics. This solute carrier transports the neurotransmitter glutamate and is involved in synaptic function, neuronal development, and excitotoxicity. Pb, in turn, can selectively block glutamatergic synapses. Five hub genes were identified in network analysis. *CHUK* and *TWIST1* play important roles in early neurogenesis. As a physiological analogue to Ca^{2+}, Pb can directly activate Ca-dependent PKCs (α, β1, β2, and γ). *HSPA5* and *XBP1* are key genes in ER stress signalling pathways. Taken together, the network provided by the study revealed that certain genetic polymorphisms in/near oxidative stress genes and neurodevelopmental genes can modify the effects of Pb-induced oxidative stress on neurodevelopment (Wang et al., 2017).

Another GWIS aimed to identify Pb-induced transcriptomic changes in neural stem cells (NSCs) and link these changes to neurodevelopmental outcomes of 462 Pb-exposed children in a birth cohort in Mexico (Wagner et al., 2017). Human NSCs were exposed to 1 μM Pb and subjected to RNA-seq-based profiling. Prenatal Pb exposure was determined from 2nd trimester maternal blood samples. Infant neurodevelopment was assessed at 24 months of age using the Bayley Scales of Infant and Toddler Development. Nineteen genes showed significantly altered expression, several regulated by NFR2, a transcription factor largely responsible for the oxidative stress response. *SPP1* exerted a neuroprotective role by reducing Pb toxicity in hNSCs. Certain *SPP1* SNPs were associated with cognitive abilities of Pb exposed children, but significant interaction between any of the *SPP1* SNPs and Pb exposure on neurodevelopmental outcome was not found. One possible link between SPP1 and neurodevelopmental disorders may be that the etiology of neurodevelopmental disorders is associated with the dysfunction of microglia. A cluster of white matter-associated microglia express a unique signature of genes, including SPP1, which are enriched at early postnatal stages, and share molecular features with disease-associated microglia (Thion and Garel 2020).

In three other studies, Pb-exposed populations of infants and children were investigated relative to functional gene polymorphisms for *APOE* (encoding Apolipoprotein E), *MTHFR* (encoding Methylenetetrahydrofolate reductase), and *DRD2* (encoding Dopamine Receptor D2), proteins that have been related to neurodevelopment.

APOE is thought to play a role in the maintenance of lipids and cholesterol, the major components of brain myelin, with carriers of the APOE4 polymorphism showing decreased cortical gray matter volume, different distribution of white matter and myelin development, but no cognitive or behavioral differences (Dean et al., 2014). In a cohort of Pb-exposed mother-child pairs from Mexico City, 311 infants were genotyped (Wright et al., 2003). The polymorphic *APOE* gene (rs429358) leads to three isoforms that differ by one or two amino acids. The *APOE* genotypes were analysed in association with the 24-months Mental Development Index of the Bayley Scales of Infant Development (MDI). After adjustment for covariates, APOE4 carriers showed a 4.4-point higher MDI-24 score as compared to APOE3/E2 carriers. In multiple linear regression analysis stratified by *APOE* genotype, the negative effect of cord blood Pb level on the MDI-score was 4-fold greater in APOE3/APOE2 carriers than in APOE4 carriers. It was stated that the protective role of the *APOE* genotype in neurodevelopment remains to be confirmed (Wright et al., 2003).

Another study from Mexico City genotyped 255 mother-child pairs for two non-synonymous SNPs in the *MTHFR* gene (Pilsner et al., 2010), encoding a key enzyme in folate metabolism. The genetically determined reduction of MTHFR enzyme activity leads to impaired methylation and folate deficiency associated with the onset of several psychiatric disorders, autism, and ADHD (Wan et al., 2018). Perinatal Pb exposure was determined in newborns (cord blood levels) and mothers (bone Pb during the first month after birth) (Pilsner et al., 2010). The Bayley's Mental Development Index scores were examined at 24 months of age. Linear regression models were used to describe the relationship between MTHFR genotype and infant Index scores, adjusting for several covariates, including cord Pb levels. Maternal and child *MTHFR* genotypes had no impact on the MDI-scores, and *MTHFR* genotype × Pb interactions were not detected. The authors concluded that the maternal *MTHFR* 677T allele independently predicted poorer child neurodevelopment at 24 months (Pilsner et al., 2010).

DRD2 is a G-protein-coupled receptor located on postsynaptic dopaminergic neurons, centrally involved in reward-mediating mesocorticolimbic pathways, and a known target of antipsychotic drugs. The *DRD2* Taq1 polymorphism is associated with reduced receptor density in the brain (Jönsson et al., 1999; Neville et al., 2004). In the study of Liu et al. (2015), children were genotyped for *DRD2* Taq IA polymorphism (rs1800497), which had no impact on the neurodevelopment of children exposed to Pb.

3.2.3. Other markers associated with Pb exposure and neurodevelopment

3.2.3.1. Maternal high-density lipoprotein (HDL). Cholesterol and triglycerides are insoluble in water. Therefore, these lipids must be transported in conjunction with proteins. Lipoproteins are complex particles, and HDL is the plasma lipoprotein with the highest protein to lipid ratio (Feingold and Grunfeld 2000). Due to its anti-atherogenic activity, HDL has been considered as 'good cholesterol', beneficial to the whole body and especially to cardio-vascular health (Jomard and Osto 2020). Cholesterol is also essential for neuronal physiology during development. It is a major component of cell membranes, a precursor of steroid hormones, and is involved in the regulation of ion permeability, cell shape, cell–cell interaction, and transmembrane signaling (Martín et al., 2014). The brain contains about 20% of the body's cholesterol in the entire body, making it the organ with the highest cholesterol content. Most of the cholesterol in the adult brain (>70%) is found in the myelin sheaths formed by oligodendrocytes to insulate axons, with the remainder integrated into plasma membranes of astrocytes and neurons (Zhang and Liu 2015). Hereditary diseases with mutations in cholesterol-related genes result in impaired brain function during early life (Martín et al., 2014).

In a study by Ji et al. (2018), 303 children (20%) from the Boston Birth Cohort (1479 mother-infant pairs) were followed up to the age of 15 years and diagnosed for attention deficit hyperactivity disorder (ADHD) according to ICD-9 and ICD-10 codes (Ji et al., 2017). Maternal HDL levels were analysed in non-fasting blood samples obtained 24–72 h after delivery. Maternal stress during pregnancy was analysed as a binary variable (not stressed, stressed). Low maternal plasma HDL levels (≤60 mg/dL) were associated with an increased risk of ADHD in the children, particularly among boys. The same year, the group presented data that took into account postnatal Pb exposure of 299 of these

children (Ji et al., 2018). Findings from the Boston Birth Cohort showed that high maternal HDL levels and low maternal stress during pregnancy could partially counteract the increased odds of ADHD associated with early life Pb exposure in boys (Ji et al., 2018). The significance of the marker as a modulator of Pb-associated neurodevelopmental outcome is unclear. According to Fujita et al. (2008), HDL would be a dominant cholesterol carrier in fetal blood; very high HDL cholesterol could also contribute to neurodevelopment because of its close relationship with APOE levels. This may fit with the fact that mothers of growth-impaired neonates with a higher prevalence of neurodevelopmental delays had significantly lower HDL levels (Miranda et al., 2018). Regarding the association between Pb exposure and HDL levels of adults, contradictory results have been reported (e.g. Kristal-Boneh et al., 1999; Ademuyiwa et al., 2005; Buhari et al., 2020).

As HDL levels can be relatively easily implemented as a routine marker in HBM studies, this marker merits further investigation in future studies.

3.2.3.2. Nutritional markers. Five studies that measured Pb exposure addressed dietary intake of or supplementation with Fe, Ca, Zn, and Cu during pregnancy, whereof four also assessed neurodevelopment in infants and children.

In a study by Shah-Kulkarni et al. (2016), 965 pregnant women and newborns were studied in Korea (Shah-Kulkarni et al., 2016). Pb exposure was examined in relation to maternal Fe intake and infant neurodevelopment at 6, 12, 24, and 36 months of age (N = 965, 732, 655, and 558, respectively). The Korean version of the Bayley Scales of Infant Development was used, from which the Mental and the Psychomotor Development Indices were derived. Prenatal Pb exposure was determined in maternal blood during early pregnancy (before gestational week 20) and at birth and in cord blood. Maternal Pb exposure during late pregnancy was associated with lower mental developmental index levels in 6 months old children. This effect was stronger in the group with lower maternal Fe intake. The results implicate that adequate Fe intake during pregnancy might reduce Pb-associated effects on neurodevelopment. Findings from in-vitro, animal and human studies suggest an interaction between Pb and Fe. Fe can inhibit Pb uptake into human intestinal cells (Bannon et al., 2003), and iron deficiency is accompanied by higher Pb body burden in mice (Flanagan et al., 1979) and humans (e.g., Kwong et al., 2004; Wright 1999; Kim and Park, 2014). Placental transfer of Pb is also lower in women who consume iron-rich diets and have higher hemoglobin levels, as well as in hemochromatosis, a disease of systemic Fe overload (Kordas et al., 2007; Karwowski et al., 2014).

Fe is essential for many processes of brain development. It is an essential component of intracellular metabolism, for instance, as an integral component of cytochrome C oxidase. In this way, Fe deficiency can interfere with the metabolically demanding processes of brain development. Early Fe deficiency can also lead to sustained decreased metabolic activity due to alterations in gene regulation resulting from mTOR (Mechanistic Target Of Rapamycin Kinase), BDNF, and MAP2 (Microtubule Associated Protein 2) signaling. The hippocampus and the process of myelination may be particularly vulnerable to Fe deficiency. Fe is also involved in the production of dopamine, epinephrine, norepinephrine, and serotonin, which means that socio-emotional development, executive functions, and memory processes that rely on these neurotransmitters may also be affected (McCann et al., 2020). However, evidence from human studies on the role of Fe in brain development is inconclusive. In a recent systematic review, no clear relationship was found between Fe status and developmental outcomes in all included time windows from pregnancy up to 2–4 years of age (McCann et al., 2020). Another systematic review found some evidence that low Fe in pregnancy, possibly especially in the 3rd trimester, may be associated with adverse neurodevelopment. Fe supplementation during pregnancy did not appear to affect neurodevelopment in the offspring (Janbek et al., 2019).

In a study by Liu et al. (2014), 415 Pb-exposed mothers and their newborns (332 newborns with complete data sets) participated in a study undertaken to determine potential associations between Pb exposure and neurodevelopment of newborns in China, using the neonatal behavioral neurological assessment (NBNA) test scored in 20 items grouped in five clusters (behavior, passive tone, active tone, primary reflexes, general assessment) (Liu et al., 2014). An inverse association between maternal blood Pb in the first trimester and NBNA scores was observed. This was complemented by the finding that maternal supplementation of Ca, Fe, and Zn was associated with lower Pb exposure. However, the variable (Ca, Fe, or Zn supplements) was not further explained and was not used as a co-variate in regression analyses or examined for interaction with Pb. In another study from Nepal, NBNA scores were associated with Pb and As, but not with Zn, cord blood levels (Parajuli et al., 2013). In a review paper of Kordas et al. (2007), a similar finding was reported for children studied for the efficacy of supplementation of Ca, Fe alone, and Fe plus Zn. The only supplementation of Fe and Ca had some beneficial effect in lowering blood Pb levels.

Pregnancy and lactation are accompanied by physiologically upregulated bone resorption in response to Ca requirements of the developing fetus and for milk production. More than 95% of the maternal Pb is stored in bone. Mobilization of bone Pb into the bloodstream represents an endogenous source of exposure that can pose a significant risk to the fetus and infant in life after birth (Ettinger et al., 2007). Animal studies and human studies on adult women and children demonstrated a reduction in Pb concentrations when the diet was supplemented with Ca during pregnancy and lactation (Kordas et al., 2007). Finally, the aim of another study was to investigate whether Ca supplementation (1200 mg dietary Ca) can attenuate fetal Pb exposure (Ettinger et al., 2009). In a double-blind, randomized, placebo-controlled study conducted in Mexico City, 670 women were randomly assigned to receive Ca (n = 334) or placebo (n = 336) during the first trimester of pregnancy; it was found that Ca supplementation during pregnancy was associated with an average 11% reduction of maternal blood Pb (0.4 μg/dL) (Ettinger et al., 2009). Ca administration could therefore represent an important secondary preventive measure to reduce maternal Pb levels and, consequently, fetal exposure. These results agree with previous studies that showed dietary Ca supplementation to slightly reduce Pb content in the blood of lactating women (Hernandez-Avila et al., 2003) and in breast milk (Ettinger et al., 2006).

According to Liu et al. (2018), there is a lack of existing statistical models that can flexibly capture the longitudinal impact of exposure to chemical mixtures. The delineation of exposure-response relationships between mixed exposure to metals and neurodevelopment is complex, particularly in longitudinal studies. Potential interaction, correlation among mixture components, and potentially nonlinear and nonadditive mixture effects must be taken into account. Using Bayesian varying coefficient kernel machine regression, a negative interaction effect between 2nd trimester copper (Cu) and Pb exposures with neurodevelopmental scores at 24 months was found. This makes sense, as Cu is essential for myelin formation (myelin is formed by phospholipids whose synthesis depends on cytochrome C oxidase, a Cu-dependent enzyme) and is involved in ferroxidase activity and thus in Fe uptake into various tissues (González and Visentin 2016).

3.3. Selected biomarkers to be used in future HBM studies

The use of **BDNF** biomarkers in relation to Pb exposure in HBM studies is supported by toxicological/mechanistic data. AOP number 13 "Chronic binding of antagonist to N-methyl-D-aspartate receptors (NMDARs) during brain development induces impairment of learning and memory abilities" (https://aopwiki.org/aops/13) was constructed based on in-vitro and in-vivo studies, and on human data (Sachana et al., 2018). In this AOP, Pb can inhibit the N-methyl-D-aspartate (NMDA) receptor (the molecular initiating event - MIE) (Bal-Price and Meek 2017). This is followed by a cascade of key events (KEs), including

alteration of intracellular Ca homeostasis and reduced BDNF release. This, in turn favoring impairment of synaptogenesis and the neuronal network at the tissue level, leading to learning and memory problems as one possible adverse outcome (Sachana et al., 2018) (Fig. 3). The AOP has been endorsed by the OECD Task Force on Hazard Assessment/Working Group of the National Coordinators of the Test Guidelines Programme (TFHA/WNT).

Given the abundant evidence on the adverse effect of Pb on BDNF transcription and eventually decreased levels of extracellular mature BDNF, serum/plasma BDNF should be considered as a promising marker of effect (Gejl et al., 2019). Of note, Pb induced DNA methylation changes in the *BDNF* gene are consistent across some tissues, including peripheral blood and brain, in both adult rodents and in human post-mortem studies, supporting its use as a peripheral biomarker (Januar et al., 2015; Kundakovic et al., 2015; Zheleznyakova et al., 2016). Urinary BDNF protein concentrations have been investigated to a lesser extent in adult humans (Koven and Collins, 2014); therefore its use as an effect marker cannot be evaluated. Overall, the interpretation of BDNF findings should consider the biological matrix employed (serum, plasma, blood), the critical period of development (neonatal, infancy, childhood, etc.), as well as the BDNF form assessed (total, pro-BDNF or mature BDNF).

Additional information comes from a study in hippocampal neuron cultures from E18 rat embryos treated with 1 and 2 μM Pb acetate. Pb exposure was shown to decrease BDNF gene and protein expression and to alter BDNF vesicle transport to release sites, leading to decreased levels of extracellular mature BDNF. Moreover, it was shown that Pb exposure disrupted synaptic development and function by altering BDNF-TrkB transsynaptic signaling with subsequent changes in synaptic proteins and impairment of synaptic function. These effects likely alter synaptic maturation and disrupt neurodevelopmental processes that may underlie the cognitive and behavioral deficits in Pb-intoxicated children (Stansfield et al., 2012).

Cortisol levels have been shown to be inversely associated with Pb concentrations in infants and children (Tamayo et al., 2016; Cai et al., 2019); in one study, increased Pb levels in conjunction with decreased cortisol levels were also associated with reduced sensory abilities (Cai et al., 2019). However, the measurement of cortisol levels and the interpretation of the data obtained are complex, as described above. In our opinion, these requirements argue against serum cortisol for routine use in HBM studies. Hair cortisol as a marker of chronic stress could be an alternative in some situations but requires further validation. In prospective HBM cohorts with complex and repeated follow-ups, salivary cortisol, in addition to hair cortisol, may provide an important advantage.

Susceptibility markers, especially those based on genome-wide and epigenetic-wide studies, cannot be routinely examined in HBM studies. However, specific polymorphisms and targeted epigenetic measurements could be performed if sufficiently supported by the weight of evidence. (Prenatal) Pb exposure seems to reduce global DNA methylation (Pilsner et al., 2009; Ruiz-Hernandez et al., 2015), but global DNA methylation pattern provides little evidence for specific changes in gene expression. Data on Pb-induced methylation of certain loci (Wu et al., 2017) and on gene variants associated with Pb exposure (Pilsner et al., 2010; Wagner et al., 2017) or interacting with Pb to modulate neurodevelopmental outcome (Wright et al., 2003; Wang et al., 2017) increases our understanding of the complex gene-environment interrelations and may also serve as a starting point for further studies.

With respect to other markers, findings from one study in the Boston Birth Cohort showed that high maternal **HDL** levels during pregnancy could partially counteract the increased odds of ADHD associated with early life Pb exposure in boys (Ji et al., 2018). It is, therefore, possible that maternal HDL levels interact with Pb exposure in early life. It is of interest that a common *APOE* genotype (*APOE4* carriers) was shown to protect from Pb neurotoxicity during early life (Wright et al., 2003). This genotype is associated with lower HDL levels, as demonstrated in animal and human studies (Hopkins et al., 2002).

Some **nutritional markers** have been found to be associated with Pb exposure and neurodevelopment and are candidates for inclusion in studies of Pb-induced developmental neurotoxicity. Fe is essential for brain development, which may be reflected by the finding that low Fe intake during pregnancy aggravated the neurotoxic effects of Pb in children (Shah-Kulkarni et al., 2016). Because **Fe status** is comparatively easy to analyse, especially in the clinical setting, blood counts (hemoglobin, hematocrit, and other RBC parameters), serum Fe, and more specific Fe status markers (serum transferrin receptor saturation, serum ferritin along with C-reactive protein to assess inflammation) could be included in HBM studies of Pb-induced effects on neurodevelopment to identify populations at nutritional risk. A similar line of thinking applies to serum Ca. Ca supplementation have been found to reduce blood Pb levels in pregnancy (Ettinger et al., 2009; Liu et al., 2014). **Serum Ca** is easy to study and can aid the interpretation of blood Pb levels (Ettinger et al., 2007) relative to the risk for neurodevelopmental disorders.

Interestingly, Fe deficiency reduces the expression and function of BDNF and its receptor in certain areas of the brain (Estrada et al., 2014). Taken together, serum BDNF isoforms (if possible combined with targeted BDNF DNA methylation) constitute an interesting candidate to be considered as an effect marker for routine use in HBM studies of Pb exposed populations, complemented by markers of Fe and Ca status to also include nutritional risk for neurodevelopmental disorders. Further studies are needed to validate the markers, especially for the situation of moderate Pb exposure.

3.4. Literature search considerations

To simplify literature searches on publications dealing with the metal lead, it would be useful if the chemical abbreviation 'Pb' is included at least in the keywords. Our search posed a great challenge because the search term 'lead' will also retrieve all publications containing only the verb 'lead'.

4. Conclusions

A very thorough literature search revealed that relatively few biomarkers had been investigated for elucidating the potential effects of Pb exposure. In addition, the markers studied had been investigated in at most two studies, sometimes with divergent results. Based on the retrieved studies, it is therefore not possible to recommend 'mandatory' inclusion of specific effect markers in future HBM studies. However, some effect markers are highly interesting, especially when other knowledge is taken into account and merits further investigation in future HBM studies.

Evidence from mechanistic studies and molecular epidemiological studies point towards the key event of reduced BDNF release as being a potential biomarker of Pb-induced neurotoxicity that should be further studied as effective markers for routine use in future HBM studies of Pb-exposed populations. Indeed, we recommend combining BDNF biomarkers at different levels of biological organisation (serum BDNF pro- and mature isoforms, peripheral blood BDNF DNA methylation, etc.) to better map this key event. In addition, plasma HDL, Fe and Ca status are potentially very relevant markers that should be investigated not only in pregnant women but also in neonates. Further studies are needed to validate these biomarkers, especially for low to moderate Pb exposure levels.

The studies discussed here on biomarkers such as serum BDNF or

plasma HDL were conducted in populations exposed to Pb levels that may also occur in Europe. However, Pb exposure in Europe, particularly in vulnerable populations of fetuses, infants and children, and young women, has been studied only very sporadically since 2015. Standardized studies, including repeated measurements (and non-invasive methods), would be needed to investigate Pb concentrations in pregnant and lactating women and their children through adolescence. In summary, representative surveys examining both current Pb exposure in Europe and (effect) biomarkers related to neurodevelopment are undoubtedly needed to fill these knowledge gaps.

Health-based guideline values for blood and urine biomarkers are urgently needed for pregnant women, infants, and children. Since even low-level Pb exposure is associated with adverse neurodevelopmental effects, new efforts should be made to further reduce fetal and childhood exposure.

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Author's contributions

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

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