Introduction

Environmental pollutants represent a health hazard of global concern because of their chronic exposure and the susceptibility of some groups, such as children. A critical window of vulnerability is the pregnancy period, because intrauterine life defines the health status of adulthood and many risks for developing diseases in late life could be prevented [1].

Epigenetics is the study of the heritable changes in gene expression that occur without a change in the DNA sequence. Epigenetic modifications, including DNA-methylation, regulate critical cellular functions from gene imprinting to gene reprogramming. The most studied epigenetic mechanism is DNA-methylation, which is the addition of a methyl group to carbon-5 of the cytosine in CpG dinucleotides to form 5-methyl cytosine (5 mC) through the action of DNA-methyl transferases (DNMT) [2]. Methylation of repetitive elements LINE1 and Alu have been widely used as surrogate markers of global DNA-methylation [3]. Most studies regarding epigenetic changes have been conducted in blood; however, blood cells as source for the DNA-methylation analysis has recently been discussed due to the differences in DNA-methylation levels among tissues and cellular types [4].

Epidemiological studies have postulated the hypothesis of the fetal origin of disease, which is mediated by epigenetic changes. Epigenetic marks are inheritable; they modulate the gene expression and can affect human health due to the presence of environmental factors.

Objective: This review focuses on DNA-methylation and its association with environmental-related diseases in children.

Methods: A search for studies related to DNA-methylation in children by pre- or post-natal environmental exposures was conducted, and those studies with appropriate designs and statistical analyses and evaluations of the exposure were selected.

Findings: Prenatal and early life environmental factors, from diet to exposure to pollutants, have been associated with epigenetic changes, specifically DNA-methylation. Thus, maternal nutrition and smoking and exposure to air particulate matter, polycyclic aromatic hydrocarbons, arsenic, heavy metals, persistent organic pollutants, and some endocrine disrupters during pregnancy have been associated with genomic and gene-specific newborns' DNA-methylation changes that have shown in some cases sex-specific patterns. In addition, these maternal factors may deregulate the placental DNA-methylation balance and could induce a fetal reprogramming and later-in-life diseases.

Conclusions: Exposure to environmental pollutants during prenatal and early life can trigger epigenetic imbalances and eventually the development of diseases in children. The integration of epigenetic data should be considered in future risk assessments.
shows the susceptibility windows of DNA-methylation to pollutants.

A literature search was done through PubMed, and those papers concerning pregnant women or children reporting changes in global or specific DNA-methylation associated with maternal influences including diet, smoking, and the exposure in utero or early in life to pollutants, such as air particulate matter (PM), metals, polycyclic aromatic hydrocarbons (PAHs), persistent organic pollutants (POPs), and some endocrine disrupters, were analyzed. Papers that followed adequate designs and statistical analyses with exposure data were eligible. The review is organized by type of pollutant, the influence on the DNA-methylation patterns (global or specific), and the susceptibility to develop diseases.

Diet as a Risk Factor for Epigenetic Modifications

Altered maternal nutrition and environmental factors during pre- and early post-natal development can trigger persistent changes that create a predisposition to the development of diseases in adulthood [8–11]. The major sources of methyl groups from food are methionine, folate, and choline, and the metabolic pathways of these molecules intersect at the formation of methionine from homocysteine. The impairment of the metabolism of these intermediates result in a disturbed availability of the methyl group from S-adenosylmethionine and, subsequently, in incorrect DNA-methylation [12].

Dietary folate is the most studied micronutrient in terms of global and gene-specific methylation changes. Genes such as the insulin-like growth factor 2 (IGF2, a maternal imprinted gene) are modified by folic acid intake before and during pregnancy, which is associated with lower methylation at DNA sequences regulating IGF2 expression [13]. Choline and betaine have demonstrated an influence on DNA-methylation in brain tissue in several in vivo models, because choline deficiency is associated with altered neurogenesis followed by decreased memory function [14]. Whereas, IGF2 methylation was inversely related to maternal serum B12 levels [15]. Maternal nutrition is one of the environmental factors most studied with regard to epigenetic changes, and information about it is well compiled in some reports [16, 17]. Therefore, this review will focus on toxic environmental pollutants as modifiers of the DNA-methylation status.

Effects of Maternal Smoking on Epigenetic Changes in the Offspring

Among the long-term risks associated with parental smoking is the development of cancer in the offspring through epigenetic mechanisms [18–20]. The most common epigenetic effect associated with prenatal tobacco smoke exposure is DNA-methylation (Table 1). One of the first reports was a longitudinal study performed in 272 children, where a hypomethylation of AluYb8 was associated with in utero tobacco smoke exposure [21]. However, an increase in LINE1 methylation was observed only in individuals carrying the GSTM1-positive genotype of the detoxifying enzyme glutathione-S-transferase M-type. This suggests that tobacco smoke could have different epigenetic effects depending on individual genetic diversity [21]. Regarding gene-specific methylations, a hypermethylation on gene promotors involved in the detoxification of tobacco smoke was detected in 272 children from the Children’s Health Study who had been exposed to tobacco in utero, specifically on AXL (AXL receptor tyrosine kinase), PTPRO (protein tyrosine phosphatase, receptor type O), KLK11 (kallikrein-related peptidase 11), and TGFB3 (transforming growth factor beta 3) genes [21]. Conversely, a study performed in 90 individuals from a birth cohort of women born in New York City did not find a correlation between prenatal

Figure 1: Susceptibility windows of DNA-methylation due to environmental pollutants. The epigenome undergoes reprogramming at two relevant stages, the gametogenesis and the early embryo preimplantation representing vulnerable stages to environmental exposures. During gametogenesis and fertilization, a general demethylation followed by a re-methylation process occur, with the exception of imprinted genes. DNA-methylation patterns are reestablished by DNA methyl-transferases DNMT3a and DNMT3b [1]. 5 mC, 5-methylcytosine. BPA, bisphenol A. Cd, cadmium. As arsenic. Pb, lead. Hg, mercury.
Alvarado-Cruz et al: Environmental-related Changes in Children's DNA-methylation

214

smoke exposure and LINE1 or Alu methylation but found a negative correlation with SAT2 (spnadia/spermine N1-acetyltransferase family member 2) [22].

A longitudinal study was conducted in 800 mother-newborn pairs from the ALSPAC Cohort in Southwest England where DNA-methylation were evaluated at birth, 7 years, and 17 years and showed that DNA-methylation changes were reversible for some genes related to development and metabolism, whereas some methylated CpG sites persisted for other genes like AHRR (aryl-hydrocarbon receptor repressor), MYO1G (myosin 1G), CYP1 A1 (cytochrome P450 family 1 subfamily A member 1), and CNTNAP2 (contactin

Table 1: Methylation changes associated with smoking, POP, phthalates, and BPA exposure during pregnancy and childhood.

| Xenobiotic | Population | Sample | Exposure association | Reference |
|------------|------------|--------|----------------------|-----------|
| Prenatal exposure to tobacco smoke | Women from USA (n = 90) | Peripheral blood granulocytes | – SAT2 | [22] |
| | Mother-offspring pairs from UK (n = 800) | Cord and blood cells | + GF11, ATP9 A, AHR, CYP1 A1, CNTNAP2 | [23] |
| | Pregnant women from USA (n = 34) | Placenta | – CYP1 A1 | [28] |
| PBDE47 | Mother-newborn pairs (n = 46) | Maternal and cord blood cells | – TNFα | [77] |
| o,p’-DDTs | Children at birth and/or age 9 (n = 358) | Blood cells | – Alu | [83] |
| o,p’-DDT, p,p’-DDE and PBDEs | Children from USA (n = 235) | Cord blood and blood cells | +/- LINE1, Alu α | [87] |
| | Children from USA (n = 336) | Cord blood | + IRAK4, ESM1, BRCA1, LASP1, IFT140 | [88] |
| Phthalates | Mother-newborn pairs from China (n = 119) | Blood cells | – LINE1 | [89] |
| | Mother-newborn pairs from China (n = 220) | Placenta | – IGF2 | [90] |
| | Mother-newborn pairs from USA (n = 179) | Placenta | – IGF2 | [91] |
| Bisphenol A | Preadolescent girls from Egypt (n = 60) | Buccal cells | – X chromosome | [92] |
| | Mother-newborn pairs from USA (n = 81) | Cord blood | + BDNF | [93] |
| | Fetuses from USA (n = 12) | Placenta | +/- LINE1 | [94] |

+ Increased methylation. – Decreased methylation. α Only in GSTM1 (Glutathione-S-transferase type M1)-present children.

+Methylation changes were reverted over time. αAt birth. αLow level of exposure to DDTs plus PBDEs, at birth. αHigh level of exposure to DDTs plus PBDEs at birth. LINE1 (Long interspersed nuclear element 1). AluYb8 (Alu element members of the Yb8 family). AXL (AXL receptor tyrosine kinase). PTPRO (Protein tyrosine phosphatase, receptor type O). SAT2 (Spermidine/spermine N1-acetyltransferase family member 2). GF11 (Growth factor independent 1 transcriptional repressor). ATP9 A (ATPase phospholipid transporting 9 A (putative)). AHR (Aryl-hydrocarbon receptor repressor). CYP1 A1 (Cytochrome P450 family 1 subfamily A member 1). CNTNAP2 (Contactin Associated Protein-Like 2, KLF13 (Kruppel like factor 13). MYO1G (Myosin 1G). TNFα (Tumor necrosis factor alpha). IRK4 (Interleukin-1 receptor-associated kinase 4). ESM1 (Endothelial cell-specific molecule 1). BRCA1 Breast cancer 1). LASP1 (LIM and SH3 Protein 1). IFT140 (Intraflagellar Transport 140). IGF2 (Insulin-like growth factor 2). BDNF (Brain-derived neurotrophic factor). PBDE (Polybrominated diphenyl ethers). DDT (Dichlorodiphenyltrichloroethane). DDE (Dichlorodiphenyldichloroethylene).
associated protein-like 2). Another relevant finding was that the maternal smoking impact was stronger than that of paternal smoking in the post-natal stage [23].

Regarding the epigenetic impact on the placental tissue, nicotine can induce changes in the methylation of more than 7000 fetal genes; these modifications can be preserved for more than three decades after exposure [24, 25]. According to Banik et al [26], some of these epigenetically modified placental genes could negatively influence neurodevelopment in the offspring, including the serotonin receptor gene HTR2 A [27], CYP1 A1 [28], the glucocorticoid receptor NR3C1, and the corticosteroid dehydrogenase isozyme (HSD11B2) [29] genes. Complementarily, a genome-wide scale study performed in 36 placental samples with equal proportions of smokers and nonsmokers showed a correlation between Cpg methylation changes and placental transcriptional regulation in 438 genes among smokers, mainly in genes related to oxidative stress [30]. This suggests that deregulation of the placental epigenetic balance could induce a fetal reprogramming.

In a cross-sectional study, cotinine levels were quantified in pregnant women from Valencia, Spain, as a biomarker of smoking, and a remarkable epigenetic effect was found in both maternal and umbilical cord blood, specifically in 31 Cpg sites of the exposed group, in the highly relevant adrenomedullin gene, which encodes for a pre-prohormone with important metabolic functions and antimicrobial activities [31].

Avant-garde total epigenome association studies allow for the broadening of the analysis spectrum and the recognition of new genome Cpg sites that contribute to a better understanding of the epigenetic mechanisms. Thus, the GECKO Drenthe Birth Cohort performed in cord blood from Dutch children exposed (n = 129) and not exposed (n = 126) to maternal smoking showed a differential methylation profile in AHRr (aryl hydrocarbon receptor repressor), MYO1G, CYP1 A1, CNNTAP2, LRPS5 (LDL receptor related protein 5), and GFI1 (growth factor independent 1 transcriptional repressor) genes, among others. Particularly, the GFI1 hypermethylation could explain the effect of prenatal tobacco smoke on birth weight [32]. In fact, there could be a relationship between in utero smoking exposure, epigenetic imbalances, and the risk of developing obesity and metabolic syndromes in adulthood [33, 34].

**Effects of Fetal and Infant PM Exposure on Global and Gene-specific DNA-methylation**

Recently, the IARC has established that air pollution and PM are human carcinogens [35]. PM is a complex mixture of organic and inorganic compounds, such as metals and PAHs. Size and composition determine the particle toxicity [36]. Several studies have shown associations between exposure to PM and DNA-methylation changes, and pregnant women and children are at risk (Table 2).

In a study conducted in 240 placenta samples of women from the ENVIRONAGE Cohort in Flanders, Belgium, DNA-methylation was inversely associated with PM$_{2.5}$ (PM with aerodynamic diameter ≤ 2.5 μm) exposure during the whole pregnancy and during the first trimester; a decrease of 2.13% in global methylation was observed for each 5 μg/m$^3$ of PM$_{2.5}$ in the air [37]. With regard to PM$_{10}$ (PM with aerodynamic diameter ≤ 10 μm), the relationship between DNA-methylation and fetal growth restriction was evaluated in 181 placental samples from Wenzhou, China; placenta LINE1 methylation showed a positive association with PM$_{10}$ during the first trimester, and the methylation of HSD11B2 (hydroxysteroid 11-beta dehydrogenase b2, a gene related to fetal growth) was negatively associated with air PM exposure during the first and second trimesters of pregnancy. These findings were more evident in newborns with fetal growth restriction [38].

On the other hand, Salam et al [39]. reported an association between 7-day average PM$_{2.5}$ exposure and lower methylation of the NOS (nitric oxide synthase) promoter in 940 American asthmatic and non-asthmatic children from 6 to 11 years old in New York City; 0.30% lower NOS methylation was observed per each 5 mg/m$^3$ increase in the 7-day average exposure. Breton et al. [40] evaluated the DNA-methylation in different NOS genes (NOS2 A, NOS3, and NOS1), which are involved in the proinflammatory status and related to PM$_{2.5}$ exposure. Changes in the methylation of specific Cpg sites of NOS2 A were associated with 7-day, 1-month, 6-month, and 1-year PM$_{2.5}$ concentration averages, and a positive association between 1-year PM$_{2.5}$ exposure and NOS3 methylation was observed at several Cpg sites. The mechanisms linking maternal PM exposure during pregnancy with adverse health outcomes is not clear, and some proposed hypotheses have been related to PM components, such as PAHs and metals, and reactive oxygen species (ROS) produced by air pollutants.

**Polycyclic Aromatic Hydrocarbons (PAHs)**

Maternal environmental exposure to PAHs has been demonstrated to have an association with DNA-methylation changes. White cord blood cells from 20 children (10 asthmatic and 10 nonasthmatic) belonging to the CCCEH in New York City were analyzed to identify changes associated with maternal exposure to PAHs through a genome-wide analysis (methylation sensitive restriction fingerprinting) [41]. Thirty sequences were differentially methylated, and six of them were located in Cpg islands; the most significant association was observed with the gene expression of acyl-CoA synthetase long-chain family member 3 (ACSL3), an enzyme that converts free long-chain fatty-acids into fatty-acyl-CoA esters and thereby plays a key role in lipid biosynthesis and fatty-acid degradation. These data were confirmed in 56 asthmatic children from the same cohort [41]. The relationship between ACSL3 expression and asthma and the mechanism by which PAHs modify DNA-methylation are not known. In another study performed in the same cohort, PAH exposure was evaluated in 164 women by personal air monitors and the quantification of PAH urinary levels and PAH-DNA adducts [42]. Children with the highest concentration of total PAHs, benzo[a] pyrene, and pyrene concentrations showed the highest DNA-methylation, while the highest PAH-DNA adduct levels were related to an increased global DNA-methylation. In a third report from the CCCEH Cohort, performed in
Table 2: Effects of air pollutants on DNA-methylation in newborns and children.

| Xenobiotic        | Population                                               | Sample          | Exposure association | Reference |
|-------------------|----------------------------------------------------------|-----------------|----------------------|-----------|
| PM$_{2.5}$        | Mother-newborn pairs from Belgium (n = 240)              | Placenta        | +                    | [37]      |
| PM$_{10}$         | Fetal growth restriction newborns from China (n = 101)   | Placenta        | +                    | [38]      |
| PM$_{2.5}$        | Hispanic and non-Hispanic children from USA (n = 940)    | Buccal cells    | +                    | [40]      |
| PAHs$^a$          | Dominican and African American pregnant women-newborn pairs from USA (n = 56) | Cord blood cells | +                    | [41]      |
| PAHs$^a$          | Dominican and African American pregnant women-newborn pairs from USA (n = 164) | Cord blood cells | –                    | [42]      |
| PAHs$^b$          | Dominican and African American pregnant women-newborn pairs from USA (n = 53) | Cord blood cells | +                    | [43]      |
| PAHs$^c$          | Atopic children from USA (n = 256)                       | Blood cells     | +                    | [45]      |
| Pyrene (benzo[b]fluoranthene) | Children from Mexico City (n = 150)                | Blood cells     | +                    | [46]      |
| Pyrene 8-OHdG     |                                                                                         |                 | +                    |           |

+ Increased methylation. – Decreased methylation. ETS (Environmental tobacco exposure). LINE1 (Long interspersed nuclear element 1). HSD11B2 (11-beta-hydroxysteroid dehydrogenase type 2). iNOS (Inducible oxide nitric synthetase). NOS3 (Nitric oxide synthase 3 expressed in endothelial cells). ACSL3 (Acyl-CoA synthetase long-chain family member 3). IL4 (Interleukin 4). IFNγ (Interferon gamma). IFT140 (Intraflagellar transport 140). FOXP3 (forkhead box protein P3). PARP1 (Poly (ADP-ribose) polymerase 1). $^a$PAH concentrations was obtained from personal monitoring. $^b$PAH concentration was obtained from daily at the EPA Supersite. $^c$PAH content in PM$_{10}$.

Cord blood leukocytes from 53 participants, IFNγ (interferon gamma) promoter methylation was associated with maternal PAH exposure [43]. This finding was corroborated in vitro in Jurkat and lung adenocarcinoma cell lines exposed to 0.1 and 1 nM of benzo[a]pyrene, in which a promoter hypermethylation and a reduced expression of IFNγ, but not interleukin 4 (IL-4), were observed [43].

A cross-sectional study was performed in the Czech Republic in 400 children, 200 with bronchial asthma and 200 healthy controls from two regions, one polluted (Ostrava, with high levels of benzo[a]pyrene, benzene, NO$_x$, PM, and metals) and one control (Prachatic). The results showed that methylation of 9916 CpG sites was significantly different in children from the polluted city, with 58 CpG sites showing lower methylation, suggesting a potentially higher gene expression. Among the altered genes are some involved in the immune response, DNA-protein binding, metabolism of xenobiotics, and signaling pathways. Differences between asthmatic and healthy children by city were observed in ACSL3 and ADRB2 (adrenergic beta-2 receptor surface) genes, but this study did not report associations with particular pollutants [44]. In another study performed in Fresno, California, the second-most polluted city in the United States in terms of PM, a group of 256 asthmatic and nonasthmatic children (10–21 years) were included. Exposure to PAHs with 4, 5, or 6 rings was assessed. Increased ambient PAH exposure (mean value of all PAHs) was associated with a hypermethylation and a decreased expression of FOXP3 (forkhead box P3, a locus involved in atopic or allergic hypersensitivity reactions) and its protein; these changes were associated with the 3-month to 1-year exposures to the PAH mixture with special impact in atopic children. Increments of DNA-methylation in FOXP3 were associated with IgE plasma levels [45].

Likewise, Alvarado-Cruz et al [46]. reported a positive relationship between PM-related PAH concentrations and LINE1 methylation in a cross-sectional study performed in schoolchildren from the north of Mexico City. This study also analyzed the methylation in Cpg sites of some DNA-repair genes and its relationship with the DNA damage. Results showed a higher specific methylation related to some PAH concentrations, particularly the methylation of two CpG sites in the PARP1 (poly [ADP-ribose] polymerase 1) promoter. Additionally, a 1% increase in the methylation of all CpG sites evaluated in the PARP1 gene resulted in a 35% increase in DNA oxidative damage (8-OHdG levels) in blood cells. PAH metabolism generates ROS and oxidative damage; however, the exact mechanism by which PAH alter the DNA-methylation is not clear. Experimental
evidence showed that oxidative DNA damage and single strand breaks generated by the oxidative stress can increase the de novo DNA-methylation [47].

**Epigenetic Alterations by Pre- and Post-natal Exposure to Metals**

Several epidemiological studies have shown that the carcinogenic potential of some toxic metals in adult populations may involve epigenetic changes [48–50]. However, there are few reports about prenatal or childhood exposures to metals and DNA-methylation (Table 3).

**Lead (Pb).** Lead, an ubiquitous heavy metal, can cross the placenta, representing a risk for the newborn [51]. It is one of the most studied metals in regard to epigenetic modifications. A cross-sectional study was conducted in 103 newborns from the ELEMENT Cohort in Mexico City, in which DNA-methylation was assessed in cord blood and Pb prenatal exposure was obtained by maternal bone Pb levels (mid-tibia shaft and the patella). A negative association between tibia and patella Pb levels and the cord DNA-methylation of Alu and LINE1 was reported [52]. A longitudinal study conducted in 43 children (3 months to 5 years old) and their mothers from a cohort in Michigan, United States, reported their 5 mC profiles [53]. The results showed DNA-methylation changes associated with Pb exposure in both females and males in genes associated with neurogenesis, neuronal differentiation, and ROS-metabolic processes, among others. Different sets of genes presented modifications in males.

**Table 3: Effects of metal exposure during pregnancy or childhood on DNA-methylation.**

| Xenobiotic | Population | Sample | Exposure association | Reference |
|-----------|------------|--------|----------------------|-----------|
| Pb        | Newborn-mother pairs from Mexico (n = 103) | Cord blood cells | − | Alu, LINE1 | [52] |
|           | Children (3 months to 5 years) and mothers from USA (n = 43) | Blood cells | + | 75 CpG sites | [53] |
|           | Children and mothers from USA (n = 35) | Cord blood cells | +/− | 564 CpG sites | [54] |
|           | Newborns from Bangladesh (n = 127) | Cord blood cells | − | Global DNA-methylation (newborns: boys) | [59] |
| Cd        | Mother-newborn pairs from USA (n = 17) | Cord and maternal blood cells | + | Genes related to transcriptional regulation and apoptosis (PRR13) | [61] |
| Hg        | Newborns from USA (n = 138) | Cord blood cells | + | 85 CpG sites² | [63] |
|           | Newborns from USA (n = 321) | Cord blood cells | − | PON1 | [65] |
|           | Newborns from Bangladesh (n = 101) | Cord blood cells | + | Global DNA-methylation | [69] |
|           | Newborns from USA (n = 134) | Cord blood cells | + | 75 CpG sites² | [70] |
|           | Newborns from Bangladesh (n = 44) | Cord blood cells | + | cg00498691 | [71] |
| As        | Newborns from Bangladesh (n = 127) | Cord blood cells | − | LRRC26v, HOXB9, BRSK2 | [72] |
|           | Newborns from Thailand (n = 71) | Cord blood cells | + | p53 | [73] |
|           | Newborns from Bangladesh (n = 113) | Cord blood cells | + | LINE1, p16 | [74] |
| Vb        | Children from USA (n = 163) | Buccal cells | − | IL4, IFNγ | [76] |
|           | Children from Mexico (n = 150) | Buccal cells | + | APEX, PARP1 | [46] |

+ Increased methylation. − Decreased methylation. *Not related to any gene. *V content in PM₁₀. HISTH4 L (Histone H4 family member). PAX9 (Paired box 9). APBB3 (Amyloid beta precursor protein binding family B member 3). GAP43 (Growth associated protein 43). PTPRN2 (Protein tyrosine phosphatase, receptor type N2). PRR13 (Proline-Rich Protein 13). PON1 (Paraoxonase 1). LRRC26 (Leucine rich repeat containing 26). HOXB9 (Homeobox B9). BRSK2 (BR serine/threonine kinase 2). p53 (Tumor protein p53). LINE1 (Long interspersed nuclear elements 1). p16 (Tumor protein p16). IL4 (Interleukin 4). IFNγ (Interferon gamma). APEX (Apurinic/apyrimidine endonuclease). PARP1 (Poly (ADP-ribose) polymerase 1).
or females, suggesting gender and exposure influences on DNA-methylation.

A second genome-wide methylation study was performed in 35 children and their mothers from the same cohort with PbB above and below 5 μg/dL. Samples from children and mothers at delivery were obtained from the Michigan Neonatal Biobank. The analysis at birth revealed 564 CpG sites that were differentially methylated in children with high PbB compared to low PbB; whereas, the analysis at 3 months to 5 years did not show associations with neonatal or childhood methylation patterns, suggesting that DNA-methylation returns to a normal pattern within the first years of life in low PbB children [54].

An analysis overlapping with the previous results [53] showed a common modification in 6 CpG sites, suggesting that pre- and post-natal Pb exposure can have similar effects on DNA-methylation. These results were corroborated in vitro using human embryonic stem cells acutely or chronically treated with Pb (1.5 μM Pb-acetate). Within the 6 CpG sites identified, the brain development-related molecule 1 or N-myc downstream-regulated gene (NDRG4) presented an increased methylation. The down-regulation of this gene was associated with reduced levels of the brain-derived neurotrophic factor (BDNF), leading to impaired spatial learning and memory [55]. A hypomethylation of the promoter of the H19 growth-inhibitory protein (H19) was observed; this protein is a cell adhesion molecule that acts as a promoter of neurite outgrowth in neurodevelopment [54, 56]. Some reports have shown DNA-methylation changes in animal models, which support the hypothesis of the fetal origin of disease. An interesting study conducted in aged primates (23 years old) exposed to Pb during the infantile stage showed an overexpression of the amyloid-protein precursor (APP) involved in Alzheimer’s disease (AD) and high oxidative DNA damage as well as a decrease in DNMT activity. This suggests that epigenetic imprinting in early development influences the expression of AD-related genes and may promote pathogenesis [57].

Cadmium (Cd). Cadmium is widely distributed in the environment and is considered a carcinogen for humans by the IARC [58]. Few epidemiological studies have evaluated alterations in DNA-methylation by pre- or post-natal Cd exposure. Kippler et al [59], analyzed the genome-wide DNA-methylation in 127 newborns exposed to Cd from rural Bangladesh. Cd exposure in pregnancy altered the fetal global DNA-methylation in a sex-specific manner. A positive association was observed in boys; whereas, an inverse relationship was observed in girls. Among the 500 CpG sites with stronger correlations between maternal blood Cd (CdB) and cord blood methylation in all newborns, the 5 top genes were histone H4 family member (HISTH4 L1), paired box 9 (PAX9), amyloid beta precursor protein-binding family B member 3 (APBB3), growth-associated protein 43 (GAP43), and protein tyrosine phosphatase receptor type N2 (PTPRN2). Additionally, DNA methylation of CpG sites in 6 genes was inversely correlated with birth weight. In contrast, a sex-specific analysis showed associations in 40 genes involved in embryonic and organ development in boys and in 105 genes related to cell death. These genes showed a change in fetal and maternal DNA, such as transcriptional regulation and apoptosis, containing proline-rich 13 (PRR13).

Mercury (Hg). Mercury has the ability to cross the placenta and is known for its toxicity on neurodevelopment [62]. Recently, Cardenas et al [63], evaluated the epigenome-wide methylation and its association with Hg exposure using Hg levels in toenails of 138 newborns from New Hampshire, United States. Among the top 100 CpG sites associated with maternal Hg levels, 85% were hypermethylated. In addition, a recent study reported a decrease of 1% to 3.8% in the CpG methylation of paraxoxonase 1 gene (PON1) in cord blood from males related to maternal blood Hg levels, after stratifying by sex, which persisted during early childhood. PON1 is considered an antioxidant protein involved in metal toxicity; in vitro studies have shown that Hg can inhibit its protective effect [64]. Although no significant changes were observed in DNA-methylation in females, an increase in DNA-methylation of PON1 was associated with lower cognitive test scores in both sexes. These results suggest that maternal Hg exposure can influence the epigenome of newborns and cause neurodevelopment alterations in childhood [65].

Arsenic (As). Arsenic is a natural metalloid that contaminates air and water in many countries [66] and is considered a human carcinogen by the IARC [67]. Several studies have shown an association between As exposure and the susceptibility to cancer development in adults through out epigenetic mechanisms [68], while only few studies are available in children. A study performed in cord blood from a population in Matlab, Bangladesh, (n = 101) showed a positive association between global DNA-methylation and As maternal urine levels [69]. Likewise, Koestler et al [70] showed a hypermethylation in 75% of the top 100 CpG loci associated with As exposure in utero in 134 newborns of the New Hampshire Birth Cohort Study. In the same way, another study conducted in 44 newborns of a cohort from Bangladesh, selected according to a range of 15–10 μg/L. As in their mother’s drinking water, showed altered DNA-methylation across the epigenome; however, only one CpG site (cg00498691) showed a significant positive association with prenatal As exposure [71].

Another study conducted in a population of 127 newborns from Matlab, Bangladesh, reported that among the 500 CpG sites with the strongest association with urinary As levels in early pregnancy, 277 (55%) CpG sites showed a decreased DNA-methylation, whereas 223 (45%) sites showed an increase. The authors suggest that early
exposure may interfere with de novo DNA-methylation. Also, these authors showed that As prenatal exposure influenced changes in sex-specific DNA-methylation. For instance, a hypomethylation was observed in CpG sites associated with cancer-related genes: leucine-rich repeat-containing 26 (LRRC26), homeobox B9 (HOXB9), and BR serine/threonine kinase 2 (BRSK2) only in boys, which correlated with an increase in As levels [72]. More studies are needed to evaluate DNA-methylation changes due to As exposure and risk of cancer.

On the other hand, few studies have evaluated alterations in gene-specific DNA-methylation. A study performed in 71 newborns in contaminated areas of Ron Pibul, Thailand, revealed that a high level of As in nails was correlated with DNA-methylation of the p53 promoter; while no changes were observed in LINE1 methylation [73]. Alterations in tumor suppressor genes can represent an important mechanism for the development of cancer in adult stages.

In contrast, Kile et al [74]. performed a study in 113 newborns from a prospective birth cohort recruited in Sirajdikhan Upazila, Bangladesh, and showed a 1.3% increase in umbilical cord LINE1 methylation in the group with the highest As maternal urine levels and a hyper-methylation in the p16 promoter; however, no differences were found in the methylation of the p53 promoter.

Vanadium (V). Vanadium is a toxic element that has been associated with high mortality [75]. Some studies have shown the potential effect of V exposure on DNA-methylation. In a cohort of 727 African American and Dominican children diagnosed with asthma, Jung et al [76] demonstrated that V exposure decreases the DNA-methylation of IL4 and IFNγ promoters, and when stratified by overweight status and asthma, a relationship was observed between V exposure and hypomethylation of NOS2 A. These effects can lead to gene low expression, which could alter the allergic immune response.

Finally, in a cross-sectional study conducted in 150 children from the north of Mexico City, a positive relationship between V content in PM_{2.5} and the methylation at apurinic/apirymidine endonuclease (APEX) and PARP1 CpG sites was found [46]. These genes are involved in DNA-repair, so changes in DNA-methylation patterns may contribute to genetic damage and be a possible mechanism for cancer development.

Exposure to Persistent Organic Pollutants (POPs) and Epigenetic Marks During Pregnancy and Infancy

POPs include the large families of polychlorinated biphenyls (PCBs), organochloride pesticides, such as DDT (dichlorodiphenyltrichloroethane) and its metabolite DDE (dichlorodiphenyldichloroethylene), as well as polybrominated diphenyl ethers (PBDEs), a family of flame-retardant compounds. The high exposure to PBDE is a consequence of the enormous distribution of these compounds in diverse commercial products, and the exposure is greater in the infant population than in the adult population of the United States [77].

Epigenetic alterations due to POP exposure have been primarily studied in adults. For example, a cross-sectional study that included 253 men and 191 women found significant correlations between some POPs in plasma and Alu and LINE1 methylation, depending on the sex. In the case of men, p,p’-DDE, cis-Heptachlor epoxide, and several PCBs negatively correlated with Alu, while p,p’-DDE, PCB153, and PCB180 had positive correlations with LINE1 methylation. On the other hand, for women, only PCB153 and PCB180 showed a negative association with Alu, but p,p’-DDE and almost all PCBs were positively associated with LINE1 methylation. In general, POPs could have a DNA hypomethylating effect on Alu in men, while an increase in LINE1 methylation could be occurring in women, taking into consideration age, body mass index, and smoking and alcohol drinking habits [78].

The relevance of POP exposure during adulthood lies in the impact on germinal cells and the consequent epigenetic footprint in the offspring. Although there is no clear evidence of this, a preliminary study performed by Consales et al [79]. in nonoccupationally exposed fertile men from Greenland, Warsaw (Poland), and Kharkiv (Ukraine) found a subtle association between blood levels of PCB-153 and p,p’-DDE and a decreased global methylation in sperm.

Few reports have been published where epigenetic effects are associated with POP exposure during pregnancy or childhood. Because POPs can cross the placenta and reach the newborn through breast milk, exposure in the early stages of life becomes crucial [80, 81]. Even neurodevelopmental damage could be influenced by epigenetic mechanisms in scenarios of POP exposure [82]. One study was carried out in 46 mother-newborn binomials from the Boston Birth Cohort where measured blood maternal PBDE concentrations found an effect on tumor necrosis factor-alpha (TNFα) expression in cord blood through its promoter methylation. Therefore, these compounds could induce a proinflammatory process through epigenetic changes [77].

On the other hand, another study performed in the birth cohort CHAMACOS from Mexico evaluated the association between global methylation and exposure to POPs in newborns and at nine years of age. The main observation was that Alu methylation was associated with prenatal DDT exposure, while LINE1 methylation changes were observed with the co-exposure to DDTs/PBDEs. POPs showed a hypomethylating effect on LINE1 and Alu during prenatal exposure, and that effect increased at nine years of age, depending on the sex [83]. Both outstanding reports are summarized in Table 4.

Effects of Some Endocrine Disrupters on DNA-methylation in Children

Phthalates and phenolic substances, such as bisphenols, are widely used in the plastics industry [84, 85]. However, they are classified as potential endocrine disrupters, and as such, they have been associated with epigenetic changes (Table 1).

Phthalates. In a study performed in 256 Taiwanese children from the CEAS, the methylation of the TNFα promoter was lower in children with higher urine 5OH-MEHP (mono (2-ethylhexyl)phthalate values, main metabolite of
Global methylation profiles in the Long (LINEs) transposable elements from the kidney, liver, and placenta were obtained; a similar but weaker association with LINE1 methylation was observed [87]. In 336 mother-newborn pairs from the same cohort, five individual CpG sites hypermethylation was associated with maternal phthalate metabolite concentrations in genes involved in inflammatory response, cancer, endocrine function, and male fertility [88].

Two studies in Chinese cohorts have been reported. In 119 mother-newborn pairs (55 FGR-cases and 64 normal controls), placental LINE1 methylation was higher in FGR-cases, and it was negatively associated with urinary MEHP and the sum of phthalate metabolites [89]. In another sample of mother-newborn pairs (110 fetal growth restriction-FGR-cases and 110 healthy controls) in Wenzhou, China, the authors found that urinary concentrations of MEHP and mono (2-ethyl-5-oxohexyl) phthalate (MEOHP) were inversely associated with placental IGF2 promoter methylation. This effect was more evident in FGR newborns [90].

In 176 samples from mother-child pairs from two cohorts in Boston, Massachusetts, the authors evaluated some urinary phthalate metabolites in the first pregnancy trimester and the placental methylation in CpG sites of IGF2 and H19 genes (both genes play major roles in embryonic and placental growth). Inverse significant associations were found between some metabolites, and the sum of phthalate metabolites and the methylation of both genes and the IGF2 hypomethylation showed gender differences with some urine metabolites [91].

Bisphenol A (BPA): Regarding BPA exposure and DNA-methylation, urinary concentrations of BPA and genome-wide methylation were evaluated in saliva samples from 60 Egyptian preadolescent girls (10 to 13 years old). DNA-methylation varied widely among the girls, and higher urinary BPA concentrations were associated with decreased methylation in genes involved in the immune function, transport activity, metabolism, and caspase activity. In particular, hypomethylation of X chromosome was associated with higher urinary BPA, suggesting that BPA exposure may affect human health through epigenetic modification of relevant genes [92].

In a cohort of prenatal exposure to BPA (measured in maternal urine at the thirty-fourth week of pregnancy, n = 81), results showed that exposure to high BPA levels was associated with altered DNA-methylation of the BDNF (as a marker of brain development) promoter in cord blood in a sex-specific way (only males showed a significant increased methylation) [93]. Finally, twelve samples from the kidney, liver, and placenta were obtained from fetuses (voluntary pregnancy termination, second trimester), and BPA (free and total) concentrations were determined. Placental free BPA concentration showed a positive correlation with LINE1 methylation, while total BPA concentration negatively correlated with placental LINE1 methylation. No significant correlations were observed in the liver or kidney [94].

Final Remarks

We conclude that there is a susceptibility to DNA-methylation by the environment during intrauterine development and early life, representing a mechanism through which long-term health effects can be initiated. Maternal influences, including nutrition, smoking, and exposure to environmental pollutants during pregnancy can result in altered DNA-methylation profiles of gene regulatory regions in newborns, many of which translate in risks of developing diseases. Therefore, the methylome is highly sensitive to maternal factors during early development but with long-term impacts. The epigenetic changes can be considered as biomarkers of early exposure and effect, however more evidence is needed to clarify the risk of the epigenetic changes associated with maternal factors or with the childhood environment.

Acknowledgements

The authors thank to the National Council of Science and Technology (Conacyt-Mexico Grant #233710) and to the Environmental Child Health Network (Conacyt-Mexico Grant #293450-2018).

Competing Interests

The authors have no competing interests to declare.

References

1. Dolinoy DC, et al. Metastable Epialleles, Imprinting, and the Fetal Origins of Adult Disease. Pediatr Res. 2007; 61: 30R–7R. DOI: https://doi.org/10.1203/pdr.0b013e3180457f77
2. Singal R and Ginder GD. DNA Methylation. Blood. 1999; 93: 40597–0.
3. Carnell AN and Goodman JI. The Long (LINEs) and the Short (SINEs) of It: Altered Methylation as a Precursor to Toxicity. Toxicol Sci. 2003; 75: 229–35. DOI: https://doi.org/10.1093/toxsci/kfg138
4. Koestler DC, et al. Blood-based profiles of DNA methylation predict the underlying distribution of cell types. Epigenetics. 2013; 8: 816–26. DOI: https://doi.org/10.4161/epi.25430
5. Reik W, et al. Epigenetic Reprogramming in Mammalian Development. Science. 2001; 293: 1089–93. DOI: https://doi.org/10.1126/science.10634435
6. Terry MB, et al. DNA methylation in white blood cells. Epigenetics. 2011; 6: 828–37. DOI: https://doi.org/10.4161/epi.6.7.16500
7. Wu H-C, et al. Global methylation profiles in DNA from different blood cell types. Epigenetics. 2011; 6: 768–5. DOI: https://doi.org/10.4161/epi.6.1.13391
8. Barker DJP. Intrauterine programming of coronary heart disease and stroke. Acta Paediatr. 1997; 86: 178–82. DOI: https://doi.org/10.1111/j.1651-2227.1997.tb18408.x
9. Barker DJP, et al. Trajectories of Growth among Children Who Have Coronary Events as Adults. N Engl J Med. 2005; 353: 1802–09. DOI: https://doi.org/10.1056/NEJMoa044160
10. Dolinoy DC and Jirtle RL. Environmental epigenetics in human health and disease. Environ Mol Mutagen. 2008; 49: 4–8. DOI: https://doi.org/10.1002/em.20366
11. Godfrey KM and Barker DJP. Fetal programming and adult health. Public Health Nutr. 2001; 4: 611–24. DOI: https://doi.org/10.1079/PHN2001145
12. Niculescu MD and Zeisel SH. Diet, Methyl Donors and DNA Methylation: Interactions between Dietary Folate, Methionine and Choline. J Nutr. 2002; 132: 2335S–35S. DOI: https://doi.org/10.1093/jn/132.8.23353
13. Hoyo C, et al. Methylation variation at IGF2 differentially methylated regions and maternal folic acid use before and during pregnancy. Epigenetics. 2011; 6: 928–36. DOI: https://doi.org/10.4161/epi.6.7.16263
14. Craciunescu CN, et al. Dietary Choline Reverses Some, but Not All, Effects of Folate Deficiency on Neurogenesis and Apoptosis in Fetal Mouse Brain. J Nutr. 2010; 140: 1162–66. DOI: https://doi.org/10.3945/jn.110.1122044
15. Ba Y, et al. Relationship of folate, vitamin B12 and methylation of insulin-like growth factor-II in maternal and cord blood. Eur J Clin Nutr, 2011; 65: 480–85. DOI: https://doi.org/10.1038/ejcn.2010.294
16. Anderson OS, et al. Nutrition and epigenetics: An interplay of dietary methyl donors, one-carbon metabolism and DNA methylation. J Nutr Biochem. 2012; 23: 853–59. DOI: https://doi.org/10.1016/j.jnutbio.2012.03.003
17. Geraghty AA, et al. Nutrition During Pregnancy Impacts Offspring’s Epigenetic Status—Evidence from Human and Animal Studies. Nutr Metab Insights. 2015; 8: 41–7. DOI: https://doi.org/10.4137/NMIS.29527
18. Esteller M. Aberrant DNA Methylation as a Cancer-Inducing Mechanism. Annu Rev Pharmacol Toxicol. 2005; 45: 629–56. DOI: https://doi.org/10.1146/annurev.pharmtox.45.120403.095832
19. Timms JA, et al. DNA methylation as a potential mediator of environmental risks in the development of childhood acute lymphoblastic leukemia. Epigenomics. 2016; 8: 519–36. DOI: https://doi.org/10.4227/epi-2015-0011
20. Franco R, et al. Oxidative stress, DNA methylation and carcinogenesis. Cancer Lett. 2008; 266: 6–11. DOI: https://doi.org/10.1016/j.canlet.2008.02.026
21. Breton CV, et al. Prenatal Tobacco Smoke Exposure Affects Global and Gene-specific DNA Methylation. Am J Respir Crit Care Med. 2009; 180: 462–67. DOI: https://doi.org/10.1164/rccm.200901-0135OC
22. Flom JD, et al. Prenatal Smoke Exposure and Genomic DNA Methylation in a Multiethnic Birth Cohort. Cancer Epidemiol Biomarkers Prev. 2011; 20: 2518–23. DOI: https://doi.org/10.1158/1055-9965.EPI-11-0553
23. Richmond RC, et al. Prenatal exposure to maternal smoking and offspring DNA methylation across the life course: Findings from the Avon Longitudinal Study of Parents and Children (ALSPAC). Hum Mol Genet. 2015; 24: 2201–17. DOI: https://doi.org/10.1093/hmg/ddu739
24. Burris HH, et al. Associations of LINE-1 DNA Methylation with Preterm Birth in a Prospective Cohort Study. J Dev Orig Health Dis. 2012; 3: 173–81. DOI: https://doi.org/10.1017/S2040174412000104
25. Johanes R, et al. Epigenetic Signatures of Cigarette Smoking. Circ Cardiovasc Genet. 2016; 9: 436–47. DOI: https://doi.org/10.1161/CIRCGENETICS.116.001506
26. Banik A, et al. Maternal Factors that Induce Epigenetic Changes Contribute to Neurological Disorders in Offspring. Genes. 2017; 8: 150. DOI: https://doi.org/10.3390/genes8060150
27. Paquette AG, et al. Placental HTR2A methylation is associated with infant neurobehavioral outcomes. Epigenetics. 2013; 8: 796–801. DOI: https://doi.org/10.4161/epi.25358
28. Suter M, et al. In Utero Tobacco Exposure Epigenetically Modifies Placental CYP1A1 Expression. Metab Clin Exp. 2010; 59: 1481–90. DOI: https://doi.org/10.1016/j.metabol.2010.01.013
29. Appleton AA, et al. Patterning in Placental 11-B Hydroxysteroid Dehydrogenase Methylation According to Prenatal Socioeconomic Adversity. PLOS ONE. 2013; 8:e74691. DOI: https://doi.org/10.1371/journal.pone.0074691
30. Suter M, et al. Maternal tobacco use modestly alters correlated epigenome-wide placental DNA methylation and gene expression. Epigenetics. 2011; 6: 1284–94. DOI: https://doi.org/10.4161/epi.6.11.17819
31. Ivorra C, et al. DNA methylation patterns in newborns exposed to tobacco in utero. J Transl Med. 2015; 13: 25. DOI: https://doi.org/10.1186/s12967-015-0384-5
32. Küppers LK, et al. DNA methylation mediates the effect of maternal smoking during pregnancy on birthweight of the offspring. Int J Epidemiol. 2015; 44: 1224–37. DOI: https://doi.org/10.1093/ije/dyv048
33. Han TS, et al. Contributions of maternal and paternal adiposity and smoking to adult offspring adiposity and cardiovascular risk: The Midspan Family Study. BMJ Open. 2015; 5: e007682. DOI: https://doi.org/10.1136/bmjopen-2015-007682
34. Lin X, et al. Developmental pathways to adiposity begin before birth and are influenced by genotype, prenatal environment and epigenome. BMC Medicine. 2017; 15: 50. DOI: https://doi.org/10.1186/s12916-017-0800-1
35. IARC. Monographs on the evaluation of carcinogenic risks to humans, outdoor air pollution. International Agency for Research on Cancer Monographs; 2015.
36. Valavanidis A, et al. Airborne Particulate Matter and Human Health: Toxicological Assessment and Importance of Size and Composition of Particles for Oxidative Damage and Carcinogenic Mechanisms. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev. 2008; 26: 339–62. DOI: https://doi.org/10.1080/10590500802494538
37. Janssen BG, et al. Placental DNA hypomethylation in association with particulate air pollution in early life. Part Fibre Toxicol. 2013; 10: 22. DOI: https://doi.org/10.1186/1743-8977-10-22
38. Cai J, et al. Exposure to particulate air pollution during early pregnancy is associated with placental DNA methylation. Sci Total Environ. 2017; 607: 1103–08. DOI: https://doi.org/10.1016/j.scitotenv.2017.07.029
39. Salam MT, et al. Genetic and epigenetic variations in inducible nitric oxide synthase promoter, particulate pollution, and exhaled nitric oxide levels in children. J Allergy Clin Immunol Pract. 2012; 129: 232–239.e237. DOI: https://doi.org/10.1016/j.jaci.2011.09.037
40. Breton CV, et al. Particulate Matter, DNA Methylation in Nitric Oxide Synthase, and Childhood Respiratory Disease. Environ Health Perspect. 2012; 120: 1320–26. DOI: https://doi.org/10.1289/ehp.1104439
41. Perera PF, et al. Relation of DNA Methylation of 5′-CpG Island of ACSL3 to Transplacental Exposure to Airborne Polycyclic Aromatic Hydrocarbons and Childhood Asthma. PLOS ONE. 2009; 4: e4488. DOI:https://doi.org/10.1371/journal.pone.0004488
42. Herbstman JB, et al. Prenatal Exposure to Polycyclic Aromatic Hydrocarbons, Benz(a)pyrene–DNA Adducts, and Genomic DNA Methylation in Cord Blood. Environ Health Perspect. 2012; 120: 733–38. DOI: https://doi.org/10.1289/ehp.1104056
43. Tang WY, et al. Maternal Exposure to Polycyclic Aromatic Hydrocarbons and 5′-CpG Methylation of Interferon-γ in Cord White Blood Cells. Environ Health Perspect. 2012; 120: 1195–1200. DOI:https://doi.org/10.1289/ehp.1103744
44. Rosnerova A, et al. Factors affecting the 27K DNA methylation pattern in asthmatic and healthy children from locations with various environments. Mutat Res Fundam Mol Mech Mutagen. 2013; 741–742: 18–26. DOI: https://doi.org/10.1016/j.mrfmmm.2013.02.003
45. Hiew KM, et al. Childhood exposure to ambient polycyclic aromatic hydrocarbons is linked to epigenetic modifications and impaired systemic immunity in T cells. Clin Exp Allergy. 2015; 45: 238–48. DOI: https://doi.org/10.1111/cea.12377
46. Alvarado-Cruz I, et al. Increased methylation of repetitive elements and DNA repair genes is associated with higher DNA oxidation in children in an urbanized, industrial environment. Mutat Res Genet Toxicol Environ Mutagen. 2017; 813: 27–36. DOI: https://doi.org/10.1016/j.mrgentox.2016.11.007
47. Christman JK, et al. 5-Methyl-2′-deoxycytidine in single-stranded DNA can act in cis to signal de novo DNA methylation. Proc Natl Acad Sci USA. 1995; 92: 7347–51. DOI: https://doi.org/10.1073/pnas.92.16.7347
48. Ali AHK, et al. Aberrant DNA methylation of some tumor suppressor genes in lung cancers from workers with chromate exposure. Mol Carcinog. 2011; 50: 89–99. DOI: https://doi.org/10.1002/mc.20697
49. Hanna CW, et al. DNA methylation changes in whole blood is associated with exposure to the environmental contaminants, mercury, lead, cadmium and bisphenol A, in women undergoing ovarian stimulation for IVF. Hum Reprod. 2012; 27: 1401–10. DOI: https://doi.org/10.1093/humrep/des038
50. Wright RO, et al. Biomarkers of Lead Exposure and DNA Methylation within Retrotransposons. Environ Health Perspect. 2010; 118: 790–95. DOI: https://doi.org/10.1289/ehp.0901429
51. Li P, et al. Transfer of lead via placenta and breast milk in human. Biomed Environ Sciences. 2000; 13: 85–9.
60. El-Maarri O, et al. Gender specific differences in levels of DNA methylation at selected loci from human total blood: A tendency toward higher methylation levels in males. *Hum Genet*. 2007; 122: 505–14. DOI: https://doi.org/10.1007/s00439-007-0430-3

61. Sanders AP, et al. Cadmium exposure and the epigenome: Exposure-associated patterns of DNA methylation in leukocytes from mother-baby pairs. *Epigenetics*. 2014; 9: 212–21. DOI: https://doi.org/10.4161/epi.26798

62. ATSDDR. Toxicological Profile for Mercury. Agency for Toxic Substances and Disease Registry. US Department of Human and Health Services; 1999.

63. Cardenas A, et al. Differential DNA methylation in umbilical cord blood of infants exposed to mercury and arsenic in utero. *Epigenetics*. 2015; 10: 508–15. DOI: https://doi.org/10.1080/15592294.2015.1046026

64. Drescher O, et al. Methylmercury exposure, PON1 gene variants and serum paraoxonase activity in Eastern James Bay Cree adults. *J Exp Sci Environ Epidemiol*. 2014; 24: 608–14. DOI: https://doi.org/10.1038/jes.2013.96

65. Cardenas A, et al. Persistent DNA methylation changes associated with prenatal mercury exposure and cognitive performance during childhood. *Sci Rep*. 2017; 7: 288. DOI: https://doi.org/10.1038/s41598-017-00384-5

66. Chung JY, et al. Environmental Source of Arsenic Exposure. *J Prev Med Public Health*. 2014; 47: 253–57. DOI: https://doi.org/10.3961/jpmph.14.036

67. IARC. Arsenic and Arsenic compounds. International Agency for Research on Cancer; 2012.

68. Waalkes MP, et al. Transplacental carcinogenicity of inorganic arsenic in the drinking water: Induction of hepatic, ovarian, pulmonary, and adrenal tumors in mice. *Toxicol Appl Pharmacol*. 2003; 186: 7–17. DOI: https://doi.org/10.1016/S0041-008X(02)00224-4

69. Pilsner JR, et al. Influence of Prenatal Arsenic Exposure and Newborn Sex on Global Methylation of Cord Blood DNA. *PLoS ONE*. 2012; 7: e37147. DOI: https://doi.org/10.1371/journal.pone.0037147

70. Koestler DC, et al. Differential DNA Methylation in Umbilical Cord Blood of Infants Exposed to Low Levels of Arsenic in Utero. *Environ Health Perspect*. 2013; 121: 971–977. DOI: https://doi.org/10.1289/ehp.1205925

71. Kile ML, et al. Effect of prenatal arsenic exposure on DNA methylation and leukocyte subpopulations in cord blood. *Epidemiology*. 2014; 9: 774–82. DOI: https://doi.org/10.4161/epi.28153

72. Broberg K, et al. Arsenic exposure in early pregnancy alters genome-wide DNA methylation in cord blood, particularly in boys. *J Dev Orig Health Dis*. 2014; 5: 288–98. DOI: https://doi.org/10.1017/S2040174414000221

73. Intarasunanont P, et al. Effects of arsenic exposure on DNA methylation in cord blood samples from newborn babies and in a human lymphoblast cell line. *Environ Health*. 2012; 11: 31. DOI: https://doi.org/10.1186/1476-069X-11-31

74. Kile ML, et al. Prenatal Arsenic Exposure and DNA Methylation in Maternal and Umbilical Cord Blood Leukocytes. *Environ Health Perspect*. 2012; 120: 1061–66. DOI: https://doi.org/10.1289/ehp.1104173

75. Dominici F, et al. Does the Effect of PM(10) on Mortality Depend on PM Nickel and Vanadium Content? A Reanalysis of the NMMAPS Data. *Environ Health Perspect*. 2007; 115: 1701–03. DOI: https://doi.org/10.1289/ehp.10737

76. Jung KH, et al. Short-term exposure to PM(2.5) and vanadium and changes in asthma gene DNA methylation and lung function decrements among urban children. *Respir Res*. 2017; 18: 63. DOI: https://doi.org/10.1186/s12931-017-0550-9

77. Diao T, et al. Aberrant 5′-CpG Methylation of Cord Blood TNFa Associated with Maternal Exposure to Polybrominated Diphenyl Ethers. *PLoS ONE*. 2015; 10: e0138815. DOI: https://doi.org/10.1371/journal.pone.0138815

78. Lee MH, et al. Association between serum persistent organic pollutants and DNA methylation in Korean adults. *Environ Res*. 2017; 158: 333–41. DOI:https://doi.org/10.1016/j.envres.2017.06.017

79. Consales C, et al. Exposure to persistent organic pollutants and sperm DNA methylation changes in Arctic and European populations. *Environ Mol Mutagen*. 2016; 57: 200–09. DOI: https://doi.org/10.1002/em.21994

80. Fernández-Cruz T, et al. Perspective on pre- and post-natal agro-food exposure to persistent organic pollutants and their effects on quality of life. *Environ Int*. 2017; 100: 79–101. DOI: https://doi.org/10.1016/j.envint.2017.01.001

81. Park J-S, et al. Placental Transfer of Polychlorinated Biphenyls, Their Hydroxylated Metabolites and Pentachlorophenol in Pregnant Women from Eastern Slovakia. *Chemosphere*. 2008; 70: 1676–84. DOI: https://doi.org/10.1016/j.chemosphere.2007.07.049

82. Tran NQV and Miyake K. Neurodevelopmental Disorders and Environmental Toxicants: Epigenetics as an Underlying Mechanism. *Int J Genomics*. 2017; 2017. DOI: https://doi.org/10.1155/2017/7526592

83. Huen K, et al. Effects of age, sex, and persistent organic pollutants on DNA methylation in children. *Environ Mol Mutagen*. 2014; 55: 209–22. DOI: https://doi.org/10.1002/em.21845

84. Net S, et al. Occurrence, Fate, Behavior and Ecotoxicological State of Phthalates in Different Environmental Matrices. *Environ Sci Technol*. 2015; 49: 4019–35. DOI: https://doi.org/10.1021/es505233b

85. Rochester J and Bolden AL. Bisphenol S and F: A Systematic Review and Comparison of the Hormonal Activity of Bisphenol A Substitutes. *Environ
86. Wang IJ, et al. Effects of phthalate exposure on asthma may be mediated through alterations in DNA methylation. *Clin Epigenetics*. 2015; 7: 27. DOI: https://doi.org/10.1186/s13148-015-0060-x

87. Huen K, et al. Maternal phthalate exposure during pregnancy is associated with DNA methylation of LINE-1 and Alu repetitive elements in Mexican-American children. *Environ Res*. 2016; 148: 55–62. DOI: https://doi.org/10.1016/j.envres.2016.03.025

88. Solomon O, et al. Prenatal phthalate exposure and altered patterns of DNA methylation in cord blood. *Environ Mol Mutagen*. 2017; 58: 398–410. DOI: https://doi.org/10.1002/em.22095

89. Zhao Y, et al. Prenatal phthalate exposure, infant growth, and global DNA methylation of human placenta. *Environ Mol Mutagen*. 2015; 56: 286–92. DOI: https://doi.org/10.1002/em.21916

90. Zhao Y, et al. Third trimester phthalate exposure is associated with DNA methylation of growth-related genes in human placenta. *Sci Rep*. 2016; 6: 33449. DOI: https://doi.org/10.1038/srep33449

91. LaRocca J, et al. The impact of first trimester phthalate and phenol exposure on IGF2/H19 genomic imprinting and birth outcomes. *Environ Res*. 2014; 133: 396–406. DOI: https://doi.org/10.1016/j.envres.2014.04.032

92. Kim JH, et al. Bisphenol A-associated epigenomic changes in prepubescent girls: A cross-sectional study in Gharbiah, Egypt. *Environ Health*. 2013; 12: 33–33. DOI: https://doi.org/10.1186/1476-069X-12-33

93. Kundakovic M, et al. DNA methylation of BDNF as a biomarker of early-life adversity. *Proc Natl Acad Sci USA*. 2015; 112: 6807–13. DOI: https://doi.org/10.1073/pnas.1408355111

94. Nahar MS, et al. In utero bisphenol A concentration, metabolism, and global DNA methylation across matched placenta, kidney, and liver in the human fetus. *Chemosphere*. 2015; 124: 54–60. DOI: https://doi.org/10.1016/j.chemosphere.2014.10.071