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Biomarkers Used in Studying Air Pollution Exposure During Pregnancy and Perinatal Outcomes: A Review

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Abstract

Purpose—This review focuses on studies among pregnant women that used biomarkers to assess air pollution exposure, or to understand the mechanisms by which it affects perinatal outcomes.

Methods—We searched PubMed and Google scholar databases to find articles

Results and conclusions—We found 29 articles, mostly consisting of cohort studies. Interpolation models were most frequently used to assess exposure. The most consistent positive association was between polycyclic aromatic hydrocarbon (PAH) exposure during entire pregnancy and cord blood PAH DNA adducts. Exposure to particulate matter (PM) and nitrogen dioxide (NO₂) showed consistent inverse associations with mitochondrial DNA (mtDNA) content, particularly in the third trimester of pregnancy. No single pollutant showed strong associations with all the biomarkers included in this review. C-reactive proteins (CRPs) and oxidative stress markers increased, whereas telomere length decreased with increasing air pollution exposure. Placental global DNA methylation and mtDNA methylation showed contrasting results with air pollution exposure, the mechanism behind which is unclear. Most studies except those on PAH DNA adducts and mtDNA content provided insufficient evidence for characterizing a critical exposure window. Further research using biomarkers is warranted to understand the relationship between air pollution and perinatal outcomes.

Keywords

Biomarkers; pregnancy; mechanisms; oxidative stress; inflammation

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Declaration of interest

The authors report no declarations of interest.

Introduction

Exposure to environmental toxins during pregnancy is associated with various perinatal as well as adult health outcomes, as suggested by Barker's hypothesis (Bell et al., 2007, Gouveia et al., 2004, Hansen et al., 2007, Woodruff et al., 2009, Salam et al., 2005, Leech et al., 1999, Barker et al., 1989, Feldt et al., 2007). Fetuses are particularly susceptible to these toxins because they are still under physiological development (Perera et al., 1999). Human studies indicate that exposure to air pollution is associated with several adverse perinatal outcomes, including spontaneous abortions, intrauterine growth retardation, preterm births, and low birth weight (LBW) (Dejmek et al., 1999, Hannam et al., 2014, Liu et al., 2003, Poirier et al., 2015, Yorifuji et al., 2015). However, there is no clear understanding of the underlying mechanisms of these biologic processes.

Biomarkers have been widely used in animal models to gain insights into the mechanisms by which air pollution is associated with perinatal outcomes. Mouse models have shown that prenatal exposure to diesel exhaust is associated with placental hemorrhage, oxidative stress, inflammatory cell infiltration, as well as increased fetal brain cytokine and chemokine levels (Bolton et al., 2012, Weldy et al., 2014).

Recent studies have increasingly used biomarkers to measure personal exposure to specific pollutants, to understand the potential mechanisms by which air pollution exposure is related to various outcomes, and to predict health outcomes. This paper presents a comprehensive review of studies that utilized biomarkers to measure air pollution exposure and to explore the related mechanisms among pregnant women.

Clinical Significance

- Telomeres protect chromosomal degradation, and telomere lengths at birth predict those in adulthood
- mtDNA content is associated with low birth weight
- Oxidative stress is related to preeclampsia and miscarriage
- DNA methylation is a mechanism that relates air pollution exposure in early life to health events in adulthood
- Polycyclic aromatic hydrocarbons act as teratogens and are associated with reduced growth among children; PAH-DNA adducts are markers of PAH exposure
- The use of biomarkers of these processes help in understanding mechanisms underlying the associations between air pollution exposure during pregnancy and various health outcomes
- Trimester specific associations can indicate critical exposure windows

Methods

We searched PubMed and Google Scholar databases, and used several combinations of keywords specified in Table 1. We also used the ‘related articles’ option in Google Scholar, and the ‘similar articles’ option in PubMed, and searched the bibliography of the included articles. The inclusion criteria constituted studies (i) carried among pregnant women, (ii) involving the use of biomarkers, (iii) published after the year 2000, and (iv) published in English. We excluded studies linking biomarkers and clinical outcomes if they did not assess the association between air pollution exposure and biomarkers. We also excluded studies on smoking, tobacco, conference abstracts, review papers, and book chapters. We categorized the studies into two groups: those that used intermediate biomarkers, and those that used markers of exposure. This strategy yielded 21 articles in the category of intermediate biomarkers, and 8 in the exposure category.

Results and Discussion

The first section of the results includes studies using intermediate biomarkers such as telomere length, mitochondrial DNA (mtDNA) content, oxidative stress markers, inflammation markers, and DNA methylation. The second section includes studies using exposure biomarkers such as urinary PAH metabolites and PAH related DNA adducts. Tables 2 and 3 summarize the articles included in this review. Figure 1 illustrates trimester/pollutant specific findings.

I. Intermediate Biomarkers

1. Telomere length (TL)—Telomeres are protein structures at each end of chromosomes, which prevent their degradation and preserve genomic information (Shammas, 2011). TL at birth is hypothesized to predict the TL in adulthood (Heidinger et al., 2012). Exposure to cigarette smoke, under nutrition, and maternal stress is associated with LBW, and TL has been hypothesized to be involved in this fetal programming (Entringer et al., 2012, Ko et al., 2014, Lee et al., 2011a, Schulz, 2010, Torche, 2011). The degradation of telomeres occurs at a faster rate during the first four years of life, suggesting that environmental exposures during this phase may have more pronounced effects on health outcomes (Frenck et al., 1998).

Among non-pregnant populations, air pollution has been associated with TL shortening, while there is limited evidence of the same among pregnant women (Hoxha et al., 2009, McCracken et al., 2010, Bijmens et al., 2015). A cohort study among 231 Caucasian twins in Belgium reported that mothers living closer to major roads (<252m) had 14% (95% CI: 1%, 24%) lower placental TL compared to those living farther (≥ 252m) (Bijmens et al., 2015). Additionally, doubling the distance to the major road increased the placental TL by 5.32% (95% CI: 1.90%, 8.86%). Similarly, in a surrounding 5000m buffer, for an interquartile range (IQR) increase (22%) in greenness, TL increased by 3.62% (95% CI: 0.20%, 7.15%), while it decreased by 4.90% (95% CI: −9.35%, −0.22%) for an IQR increase (5%) in industrial areas. Although the study was conducted among twins, 62% (95% CI: 47%, 74%) of the variation in placental TL was attributed to environmental factors, whereas that attributed to genetic factors was minimal. Despite being the only study among pregnant

women, it provides strong evidence that air pollution exposure is associated with reduced placental TL.

2. Mitochondrial DNA (mtDNA) content—Mitochondria are intracellular structures involved in the production of energy in the form of adenosine triphosphate through oxidative phosphorylation (Montier et al., 2009). Mitochondrial respiration produces reactive oxygen species (ROS), which are hypothesized to be involved in the mechanism by which air pollution leads to adverse events (Li et al., 2008). mtDNA is prone to damage caused by ROS because it lacks the protective mechanisms present in nuclear DNA (Lee and Wei, 2000, Plaza, 2002). Altered mtDNA content has been associated with adverse pregnancy outcomes such as LBW (Gemma et al., 2006).

PM and mtDNA content: A study in Belgium found that every 10 $\mu\text{g}/\text{m}^3$ increase in exposure to PM_{10} (PM_{10} 10 μm in diameter) in the third trimester decreased the placental mtDNA content by 17.40% (95% CI: -31.80%, -0.10%) (Janssen et al., 2012). Furthermore, doubling the residential distance to a major road increased the placental mtDNA content by 4% (95% CI: 0.40%, 7.80%). Cord blood mtDNA content was associated with neither PM_{10} exposure nor residential distance to a major road. Another study among 400 mother-newborn pairs in Belgium supported these findings and showed that placental mtDNA content decreased by 15.60% (95% CI: -23.92%, -6.38%) for every IQR increase in exposure to $\text{PM}_{2.5}$ ($\text{PM}_{2.5}$ 2.5 μm in diameter) during entire pregnancy (Janssen et al., 2015). This association was the strongest in the third trimester, -23.58% (95% CI: -36.27%, -8.37%).

NO_2 and mtDNA content: Prenatal NO_2 exposure has been associated with reduced fetal growth and adverse pregnancy outcomes (Ballester et al., 2010, Marozienne and Grazuleviciene, 2002, van den Hooven et al., 2012c). Analyses from two birth cohorts – the INMA (Infancia y Medio Ambiente; Environment and Childhood) and ENVIRONAGE (ENVIRONMENTAL influence ON AGEing) showed that for every 10 $\mu\text{g}/\text{m}^3$ increase in NO_2 exposure, the placental mtDNA content decreased by 5.50% (95% CI: -8.80%, -2.10%) in the INMA cohort during the entire pregnancy and by 10.1% (95% CI: -20.10%, 1.24%) in the ENVIRONAGE cohort during the second and third trimester (Clemente et al., 2015). Additionally, mediation analyses showed that placental mtDNA might explain 10% (95% CI: 6.60%, 13%) of the association between average NO_2 exposure and birth weight.

These studies provide consistent evidence that PM and NO_2 exposure particularly in the third trimester of pregnancy might alter mtDNA content. The mediation analysis supports the role of mtDNA content as a precursor to adverse pregnancy outcomes.

3. Oxidative stress—Oxidative stress refers to the disruption in the levels of free radicals in the body and the corresponding antioxidant defenses (Betteridge, 2000). Although oxidative stress can be assessed using various biomarkers, only a few have been used in relation to air pollution among pregnant women. Oxidative stress during pregnancy is a hypothesized mechanism related to miscarriage, preeclampsia, fetal growth restriction, preterm birth, and LBW (Al-Gubory et al., 2010, Jauniaux et al., 2006, Jauniaux et al., 2000). Air pollution exposure has been associated with oxidative stress in several non-

pregnant populations (Chahine et al., 2007, Kelly, 2003, Liu et al., 2003). In a study among pregnant women in South Africa, oxidative stress markers were found to be higher among women in high pollution areas compared to those in low pollution areas (Nagiah et al., 2015). In Belgian study, maternal mitochondrial 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels were found to increase by 18.30% (95% CI: 5.60%, 32.40%) and 13.9% (95% CI: 0.40%, 29.40%) for an IQR increase in PM₁₀ and PM_{2.5} exposure respectively during the entire pregnancy (Grevendonk et al., 2016). Additionally, cord blood 8-OHdG was positively associated with PM₁₀ exposure during the first (23% increase) and second trimester (16.60% increase). In another study in the same population, black carbon exposure in the first trimester, and PM_{2.5} exposure in the first and second trimester was associated with increased placental 3-nitrotyrosine (3-NTp) levels, indicating increased oxidative stress (Saenen et al., 2016). In a study in the Czech Republic, 8-oxo-7,8-dihydro-2-deoxyguanosine (8-oxodG), a marker of oxidative DNA damage, was found to be increased with increasing exposure to PM_{2.5} in the winter, and 15-F2t-isoprostane (15-F2t-IsoP), a marker of lipid peroxidation, increased with exposure to PM_{2.5} and benzo[a]pyrene (BaP) (Ambroz et al., 2016). While these studies show a positive association between air pollution exposure and oxidative stress, the specific etiologic time window of exposure needs to be identified through future longitudinal studies.

4. Inflammation markers—Inflammation is a critical mechanism through which air pollution is associated with various disease outcomes and is characterized by altered levels of cytokines, chemokines, and pattern recognition receptors (Brook et al., 2010, Brunekreef and Holgate, 2002, Demetriou et al., 2012, Hoek et al., 2013, Ruckerl et al., 2007, Challis et al., 2009). Inflammation among pregnant women is well studied, although not in relation to air pollution (Challis et al., 2009).

C-Reactive Proteins (CRPs): CRPs are markers of acute systemic inflammation that represent the presence and intensity of inflammation (Baiz et al., 2011, Delfino et al., 2008, Peters et al., 2001, Smith et al., 2008). Studies have shown that high CRP levels are associated with adverse pregnancy outcomes (Ernst et al., 2011, Guven et al., 2009, Lohsoonthorn et al., 2007, Pitiphat et al., 2005, Tjoa et al., 2003). In a US study, an interquartile increase in PM₁₀ exposure was positively associated with increased maternal CRP (>8 ng/mL) levels at 22-day [odds ratio (OR) = 1.23, 95% CI: 0.97, 1.57] and 29-day average periods (OR = 1.18, 95% CI: 0.91, 1.53) respectively (Lee et al., 2011c). Exposure to PM_{2.5} showed similar results (Lee et al., 2011c, Lee et al., 2011b). Another study in Netherlands reported that the highest quartile of PM₁₀ exposure during entire pregnancy was associated with elevated fetal CRP (>1 mg/L) levels at delivery (OR = 2.18; 95% CI: 1.08, 4.38) (van den Hooven et al., 2012a). Since CRP does not cross the placenta, the elevated fetal CRP levels are probably due to hepatic synthesis by the fetus (Jaye and Waites, 1997, Raio et al., 2003). The underlying mechanism relating maternal air pollution exposure to elevated fetal CRP levels is unclear. Further, maternal CRP levels are usually elevated because of inflammatory response to pregnancy, which makes it difficult to estimate the exact levels attributable to air pollution exposure (Thornton, 2010, von Versen-Hoeynck et al., 2009). However, studies were conducted among pregnant women, which increases

comparability and validates the positive associations between air pollution exposure and elevated CRP levels to some extent.

Immune response: A study in France found that PM₁₀ exposure before and during the entire pregnancy was associated with decreased levels of cord blood CD4+CD25+ T cells, and NO₂ exposure in the first and second trimester was associated with decreased CD8+ cells (Baiz et al., 2011). In a study in the Czech Republic, maternal CD3+, CD4+, and CD4+/CD8+ cells were found to be lower, and cord blood NK cell levels were higher in high pollution areas (Hertz-Picciotto et al., 2002).

Cytokines: Cytokines are intercellular signaling polypeptides that play a central role in regulating tissue remodeling and cell signaling (Gabay and Kushner, 1999, Nathan and Sporn, 1991). A German study on maternal exposure to volatile organic compounds (VOCs) observed a positive association between IL-4 producing type-2 T cells and exposure to naphthalene (OR=2.9, 95%CI: 1.0, 8.2) and methylcyclopentane (OR=3.3, 95%CI: 1.1, 9.6), whereas tetrachloroethylene exposure was associated with decreased Interferon γ (IFN γ) producing type-1 T cells (Lehmann et al., 2002). Among pregnant women in Switzerland, PM₁₀ exposure during the third trimester was associated with elevated cord blood IL-1b levels, OR = 3.00 (95%CI 1.30, 6.91) (Latzin et al., 2011). Additionally, PM₁₀ exposure during the last three days of pregnancy was associated with decreased cord blood IL-10 levels, OR = 0.66 (95%CI: 0.45, 0.97). Adequate IL-10 levels are necessary during pregnancy, given their hypothesized function to suppress active maternal immunity to accept fetal allograft (Thaxton and Sharma, 2010). This study shows the changes in the cytokine levels in relation to PM₁₀ exposure during various time windows in late pregnancy.

A disruption of the delicate balance of innate immune responses during pregnancy induces parturition prematurely, the underlying biologic process, however, is not clearly understood (Challis et al., 2009, Peltier, 2003, Silver et al., 2004). Regulatory T cells play an important role in allergy development, and altered levels of CD4+CD25+ T cells in response to air pollution exposure, as seen in the above study, might increase the newborns' susceptibility to allergies (Smith et al., 2008). Overall, the above studies show an insufficient understanding of the relationship between air pollution exposure and inflammatory responses, necessitating further research.

5. DNA Methylation—DNA methylation plays a key role in maintaining genomic stability and expression of genes, and is hypothesized to be one of the mechanisms by which air pollution exposure in early life may be associated with adverse health outcomes in adulthood (Ohgane et al., 2008, Zhu et al., 2011, Koukoura et al., 2012).

Global DNA methylation: Air pollution exposure has been observed to be inversely associated with cord blood and placental global DNA methylation during pregnancy, starting from implantation stage (Herbstman et al., 2012, Janssen et al., 2013). In a prospective study among 240 mother-newborn pairs in Belgium, every 5 $\mu\text{g}/\text{m}^3$ PM_{2.5} exposure during implantation (6–21 days) was associated with decreased placental global DNA methylation at birth by 1.08% (95% CI: –1.80%, –0.36%) (Janssen et al., 2013). Furthermore, for every 5 $\mu\text{g}/\text{m}^3$ increase in PM_{2.5} exposure during the entire pregnancy, first trimester, and second

trimester, placental global DNA methylation decreased by 2.19%, 2.41%, and 1.51% respectively (Janssen et al., 2013). In a study among nonsmoking African-American and Dominican women in New York city, prenatal exposure to PAH during the third trimester was inversely associated with global methylation in the cord white blood cells (Herbstman et al., 2012). However, among newborns, high levels of genomic methylation were positively associated with detectable levels of cord blood BaP-DNA adducts, OR = 2.35 (95% CI: 1.35, 4.09). It is hypothesized that benzo[a]pyrene diol epoxide (BPDE) binds to DNA, which might encourage DNA methylation (Subach et al., 2006). However, the temporal sequence of the formation of BaP-DNA adducts and methylation is not clear (Herbstman et al., 2012). A potential mechanism is that air pollution exposure alters global DNA methylation which in turn influences BaP-DNA adduct formation (Herbstman et al., 2012).

mtDNA methylation: Another study by Janssen et al. (2015) in the Belgian birth cohort reported that an IQR increase in PM_{2.5} exposure in the first trimester was associated with an increase in mtDNA methylation of 1.27% (95% CI: 0.23%, 2.32%) in the MT-RNR1 region and 0.44% (95% CI: 0.12%, 0.75%,) in the D-loop respectively (Janssen et al., 2015). Further analyses showed that the inverse association between PM_{2.5} exposure and placental mtDNA content was mediated by placental MT-RNR1 methylation [54% (95% CI: 31%, 60%)]. Significant inverse association between placental mtDNA content and methylation levels were observed among newborns (MT-RNR1: $\beta = -0.04 \pm 0.002$, $p < 0.01$; D-loop: $\beta = -0.10 \pm 0.01$, $p < 0.01$), however, the mechanism behind this is unclear. One hypothesis is that abnormal methylation at MTRNR1 and D loop region interferes with mtDNA biogenesis (Janssen et al., 2015). This is the first study to report epigenetic mitochondrial modifications in relation to early environmental exposures.

Although there is limited evidence, global DNA methylation levels showed consistent inverse association, whereas placental mtDNA methylation showed a positive association with air pollution exposure. mtDNA methylation at the D-loop is hypothesized to influence its replication or transcription, whereas methylation of the MT-RNR1 region impacts mitochondrial ribosomes and halts translation of mtDNA-encoded RNAs into proteins (Aloni and Attardi, 1971, Janssen et al., 2015, Metodiev et al., 2009). Environmental effects on placental DNA methylation may lead to epigenetic alterations and fetal programming of diseases (Koukoura et al., 2012). Placental DNA methylation is prone to high variability compared to methylation in other tissues (Houseman et al., 2008). More research is warranted in this area to understand the role of DNA methylation in the relationship between air pollution exposure and adverse pregnancy outcomes.

6. Other studies using intermediate biomarkers—This review also includes studies using 25-hydroxyvitamin D [25(OH)D], placenta/birth weight ratio, placental growth factors, and micronuclei. 25(OH)D is formed when vitamin D undergoes hydroxylation in the liver, and is an efficient marker of vitamin D (Crew et al., 2009). Only one study conducted among pregnant women showed that for every 10 $\mu\text{g}/\text{m}^3$ increase in NO₂ and PM₁₀ levels, 25(OH)D levels decreased by 0.15 units ($p = 0.047$) and 0.41 units ($p = 0.037$) respectively (Baiz et al., 2012). This inverse association was the strongest during the third trimester of the pregnancy (NO₂: $\beta = -0.21$, $p < 0.01$; PM₁₀: $\beta = -0.43$, $p < 0.01$). It is hypothesized that maternal exposure to air pollution impedes the intestinal absorption of

vitamin D, thereby affecting the cord blood 25(OH)D levels (Baiz et al., 2011). Another plausible mechanism is decrease in levels of vitamin D binding proteins (DBP) as a result of air pollution exposure, which leads to reduced serum 25(OH)D levels (Baiz et al., 2011).

Placenta/birth weight ratio (PBWR) is a marker of placental oxygen and nutrient transport efficiency, and a high PBWR is associated with adverse pregnancy outcomes (Naeye, 1987). A study in Japan found that living within 200m to a major road increased the PBWR by 0.48% (95%CI: 0.15%, 0.80%) (Yorifuji et al., 2012). Exposure to traffic related air pollution might lead to impaired placental transport through inflammation or oxidative stress. As a potential compensatory mechanism, placental weight may increase relative to the weight of the fetus, thereby resulting in poor pregnancy outcomes (Barker et al., 1993, Yorifuji et al., 2012).

Placental growth factor (PGF) is a pro-angiogenic growth factor necessary for placental growth, however, its distinct role is unclear. Soluble fms-like tyrosine kinase (sFlt-1) is an anti-angiogenic protein that reduces the development of new blood vessels and halts the maturation of existing ones (Jacobs et al., 2011). Both PGF and sFlt-1 levels are used as biomarkers to predict pregnancy outcomes, particularly preeclampsia (Zhu et al., 2016). A prospective study found that PM₁₀ and NO₂ exposure during the entire pregnancy period was associated with higher sFlt-1 and lower PIGF levels in cord blood (van den Hooven et al., 2012b). This was the only study to assess the relationship between air pollution exposure and angiogenic factors.

Micronuclei (MN) are small, extranuclear bodies that are left behind in the anaphase stage of cell division, and are not included in the daughter nuclei in the telophase stage due to direct and indirect DNA damage (Mateuca et al., 2006). A study in Denmark reported increased cord blood MN frequencies among those residing near high traffic density areas (Pedersen et al., 2009). Another study provided similar evidence and reported a 53% increase (RR = 1.53, 95%CI: 1.02, 2.29) in maternal MN frequencies for every 5 µg/m³ increase in PM_{2.5} exposure (O'Callaghan-Gordo et al., 2015).

II. Exposure biomarkers

1. PAH metabolites and DNA adducts—PAHs are persistent organic pollutants that are released in the atmosphere through incomplete combustion of fossil fuels, wood, and charcoal (Bostrom et al., 2002). Urinary PAH metabolites are markers of recent exposure and can be chemically reduced to their parent compounds (Becher and Bjorseth, 1983, Strickland et al., 1996). In Hamilton, Canada, pregnant women living near the downtown area had significantly higher levels of urinary 9-hydroxyfluorene, 3-hydroxyphenanthrene, and 1-hydroxypyrene compared to those living in the suburbs (Nethery et al., 2012).

Being lipophilic in nature, PAHs can enter the placenta where they may act as teratogens (Kim et al., 2013). PAH DNA adducts are formed when PAH metabolites covalently bind to DNA, and act as markers of PAH exposure (Shuker, 2002, Tang, 2008). Bulky DNA adducts, like PAH DNA adducts, reflect individual differences in exposure, absorption, activation, metabolism, and the ability to repair DNA damage (Godschalk et al., 2002, Pedersen et al., 2013). Although PAH DNA adducts from maternal blood have been used as markers of fetal

PAH exposure, the adduct levels in newborns have been comparable or greater than the maternal levels (Perera et al., 2005, Tang et al., 2006, Whyatt et al., 2001). This is in spite of the transplacental PAH dose to the fetus being only 10% that of the mother (Yi et al., 2015). Cord blood PAH DNA adducts are markers of fetal PAH exposure of the preceding 4 months (Jedrychowski et al., 2013, Yi et al., 2015).

Previous studies have indicated that increasing levels of PAH exposure were associated with increased cord blood levels of PAH DNA adducts, BaP-DNA adducts, and bulky DNA adducts (Pedersen et al., 2009, Perera et al., 2005, Tang et al., 2014, Jedrychowski et al., 2013). Cord blood PAH DNA adduct levels have been associated with reduced head circumference and reduced physical growth among children, and maternal BaP-DNA adduct levels are a risk factor for missed abortions (Tang et al., 2006, Wu et al., 2010). The ratio of the fetal to maternal blood adduct levels (FMR) indicates susceptibility to fetal DNA damage, and has been used only in one study so far, which reported an FMR of 1.28 (95%CI: 1.10, 1.43) for high levels of PAH exposure (Perera et al., 2005). These results provide evidence of the teratogenic action of PAHs. The effects of PAH exposure on fetal growth are of particular concern because reduced head circumference and body growth are suggestive of poor neurodevelopmental and cognitive outcomes (Desch et al., 1990, Heinonen et al., 2008, Veena et al., 2010).

2. Other studies using exposure biomarkers—Methemoglobinemia is a condition characterized by increased methemoglobin levels, wherein hemoglobin cannot act as an oxygen transporter because the iron is in the ferric state instead of the ferrous state, thereby causing hypoxia (Mohorovic, 2003). In Croatia, ground level sulphur dioxide (SO₂) concentrations were found to be positively correlated with methemoglobin levels among pregnant women during the operation of a coal powered thermoelectric power plant, whereas this correlation was found to be negative when the plant was closed (Mohorovic, 2003).

Limitations

Responses from each group of biomarkers might depend on the critical window of exposure before and during pregnancy. Although we identified critical exposure windows for certain pathways, many studies measured exposure only during one specific time period during pregnancy. This limited our ability to draw definite conclusions regarding the critical windows of exposure. Furthermore, high collinearity among various pollutant exposures makes it difficult to tease out their individual effects. Finally, some studies had small sample sizes, which could have affected the power of detecting weak associations, if any.

Conclusion

From this review, we conclude that biomarkers are useful in understanding the biologic mechanisms underlying the relationship between with air pollution and perinatal outcomes. We found the most consistent positive association between PAH exposure and cord blood DNA adducts. We also found strong evidence of decreasing mtDNA content with increasing air pollution exposure, particularly in the third trimester. We observed that global DNA methylation levels decreased, while levels of oxidative stress markers increased in response

to air pollution exposure. However, no critical time window of exposure was identified for these pathways. Placental TL decreased with increasing exposure to traffic related air pollution. Although limited, this evidence was promising. Cytokine levels were altered in response to air pollution exposure throughout the entire pregnancy period, and also to third trimester exposure. Overall, we found that the use of biomarkers in relation to air pollution exposure among pregnant women is a promising but understudied area, with need for future research.

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Figure 1.

Association of biomarkers with air pollution exposure and clinical outcomes.

Abbreviations: 15-F2t-IsoP=15-F2t-isoprostane, 25 (OH)D=25-hydroxyvitamin D, 3-NTP=3-nitrotyrosine, 8-OHdG=8-hydroxy-2'-deoxyguanosine, BaP=benzo(a)pyrene, BC=black carbon, CD=cluster of differentiation, CO=carbon monoxide, CRP=C reactive protein, IFN-γ=interferon γ, IL=interleukin, MCP-1=monocyte chemoattractant protein-1, mtDNA=mitochondrial DNA, NK=natural killer, NO₂=nitrogen dioxide, O₃=ozone, PAH=polycyclic aromatic hydrocarbon, PGF=placental growth factor, PM₁₀=particulate matter (diameter 10μm), PM_{2.5}=particulate matter (diameter 2.5μm), sFlt-1=soluble fms-like tyrosine kinase 1, SO₂=sulphur dioxide, TNF-α=tumor necrosis factor α

Table 1

Keywords used in literature search

Air pollution related words	Pregnancy related words	Biomarker related words
Air pollution	Pregnant	Biomarker
Traffic	Pregnancy	Telomere
Particulate matter	Perinatal	Methylation
Sulfur dioxide	Newborns	Adducts
Nitrogen dioxide		Inflammation
PAH		Oxidative stress
Diesel		Folate
Phthalates		Mitochondrial
Naphthalene		DNA
		Hydroxyvitamin

Table 2

Association between air pollution exposure and intermediate biomarkers

Author, year	Study design	Population	Pollutant	Exposure assessment	Biospecimen, collection time	Biomarker, assessment	Major Results: Estimate (95%CI)	Controlled in the analyses
Telomere length								
Bijns et al., 2015 ²⁷	Cohort-R	231 Caucasian twins, 1975–1982, Belgium	Traffic exp	Traffic densities	Placentas, 24h post delivery	Telomere length (TL):qPCR	<ul style="list-style-type: none">• Each doubling in distance to major road: ↑TL 5.32% (1.90, 8.86)• IQ (22%)↑ in greenness (5km buffer): ↑TL 3.62% (0.2, 7.15)	nb's sex, gsl age, BW, birth yr, zygosity and chorionicity, mat age, SES and smo during prg
mtDNA content								
Janssen et al., 2012 ³³	Cohort-P	178 nb, 02/05/2010–04/03/ 2011, Belgium	PM ₁₀	Kriging interpolation	Placentas, cord leukocytes at delivery	mtDNA content: qPCR	<ul style="list-style-type: none">□ ↑PM₁₀/10 µg/m³ -<ul style="list-style-type: none">- last mo prg: ↓ mtDNA 16.1% (–25.2, –6.0)- T₃- ↓ mtDNA 17.4% (–31.8, –0.1)□ Each doubling in distance to major roads: ↑ mtDNA 4.0% (0.4, 7.8)□ PM₁₀ and cord bl mtDNA: NA	nb's sex, mat and gsl age, parity, ethnicity, smo status, season, and time-sp apparent temp. Cord bl adj for bl cell count as well
Janssen et al., 2015 ³⁴	Cohort-R	381 mat-nb pairs, 2010–2013, Belgium	PM _{2.5}	Kriging interpolation	Placentas at birth	Placental mtDNA meth	<ul style="list-style-type: none">□ ↑PM_{2.5}/µg/m³ -<ul style="list-style-type: none">- T₃- ↓mtDNA 23.58% (–36.27, –8.37)- Total prg- ↓mtDNA 15.60% (–23.92, –6.38)- T₁, T₂: NA	Gender, mat and gsl age, smo, mat edu, parity, ethnicity, and season at conception
Clemente et al., 2015 ³⁸	Cohort-R	390 mat-nb pairs, 2004–2008, Spain; 556 pw, 2010 – 2013, Belgium	NO ₂	Spain: LUR passive samplers Belgium: Kriging interpolation	Placentas at/after delivery	mtDNA content: qPCR	<ul style="list-style-type: none">□ ↑NO₂/10 µg/m³: ↓ mtDNA 4.9% (–9.3, –0.3), ↓ birth wt 48g (–87, –9)□ NO₂- BW asso: Spain: –66g (–111, –23), Belgium: –20g (–101, 62)□ ↑QR mtDNA: ↑ BW overall 140g (43, 237)	nb's sex, mat age, mat smo status, gsl age, pre-pregnancy BMI, parity, ethnicity, season of birth, and edu, and interaction term sex and mtDNA content
Oxidative stress								
Nagiah et al., 2015 ⁴⁵	Cohort-P	Pw, T ₃ , 50 from South Durban (SD), 50 from North Durban (ND)	Air poln from industrialized locale	Not mentioned	PBMCs and serum: T ₃	MDA: TBARS assay: mt function, DNA integrity, SOD2, Nrf2, UCP2;GSH: GSH-Glo™ GSH assay	<ul style="list-style-type: none">□ SD vs ND (ref):<ul style="list-style-type: none">- Signi ↑: comet tail, UCP2 protein- Signi ↓: Nrf2 protein, GSH conc, OGG1 mRNA (2.78-fold) activity	Not applicable

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Author, year	Study design	Population	Pollutant	Exposure assessment	Biospecimen, collection time	Biomarker, assessment	Major Results: Estimate (95%CI)	Controlled in the analyses
Grevendonk et al., 2016 ⁴⁶	Cohort-R	293 cord bl, 224 mat bl samples, pw, 2010–2013, Belgium	PM ₁₀ and PM _{2.5}	Kriging interpolation	Mat and venous cord bl samples at delivery	8-OHdG: qPCR	- No diff: MDA, mt depolarization, ATP, UCP2 gene expression, SOD2, mRNA levels, mtDNA amplification, OGG1 mRNA activity	Models for mat bl: mat and gestl age, smo status, mat edu, alc intake during prg, season at conception. Models for cord bl : gender and date of delivery as well
							Mat bl - Each IQR	
							↑PM ₁₀ – ↑8-OHdG 18.3 % (5.6, 33.4)	
							↑PM _{2.5} –↑8-OHdG 13.9% (0.4, 29.4)	
Ambroz et al., 2016 ⁴⁸	Cross-Sec	Mat bl, mat urine, cord bl, nb's urine: Karvina : Mat bl and urine-288; Ceske Budejovice: 398 (CB) 2013–2014	PM _{2.5} , BaP	High volume air sampler	Mat bl, mat urine, cord bl, nb's urine at delivery	8-oxodG, 15-F2t-IsoP	Mat bl - Each IQ	Not applicable
							↑PM ₁₀ –↑8-OHdG 8.7% (–2.0, 20.6)	
							↑PM _{2.5} –↑8-OHdG 3.1% (–7.0, 14.46)	
							8-oxodG: - PM _{2.5} – mat 8-oxodG: OR=1.68 (1.05, 2.69) - PM _{2.5} – nb's 8-oxodG; BaP-mat and nb's 8-oxodG: NA 15-F2t-IsoP: - PM2.5, BaP-mat, nb's 15-F2t-IsoP: NA Summer vs winter in CB: - ↓mat 8-oxodG in summer (p<0.001) - nb 8-oxodG – NA with season - mat, nb 15-F2t-IsoP – NA with season Summer vs winter in Karvina: - ↓nb 8-oxodG in summer (p<0.05) - ↓nb 15-F2t-IsoP in summer (p<0.001) - mat 8-oxodG, 15-F2t-IsoP – NA with season	
Saenen et al., 2016 ⁴⁷	Cohort-R	330 mat-nb pairs, Feb 2010 – May 2013, Belgium	PM _{2.5} , NO ₂ , BC	Interpolation, monitoring stations	Placentas at delivery	3-NTp: Bio-Rad protein assay	↑PM _{2.5} / µg/m ³ - ↑ 3-NTp 35% (13.9, 60.0) ↑NO ₂ / µg/m ³ - ↑ 3-NTp 9.7% (–3.8, 25.0) ↑BC ₂ / µg/m ³ - ↑ 3-NTp 13.9% (–0.21, 29.9)	Gstl age, mat age, mat edu, pregestational BMI, smo status, nb's sex, nb's ethnicity, seasonality
Inflammation markers								
Lehmann et al., 2002 ⁷⁴	Cohort-P	85 mat-nb pairs, Dec 1997 – Jan 1999, Germany	VOC	Questionnaire, passive sampling	Cord bl at delivery	T cells: intracellular cytokine staining	↑naphthalene: ↑ IL-4 type 2 cells, OR=2.9 (1.0, 8.2) ↑methylcyclopentane: ↑ IL-4 type 2 cells, OR=3.3 (1.1, 9.6); ↓ TNF-α, OR=0.1 (0.0, 0.8)	Family atopy history, gender, mat smo

Author, year	Study design	Population	Pollutant	Exposure assessment	Biospecimen, collection time	Biomarker, assessment	Major Results: Estimate (95%CI)	Controlled in the analyses
Hertz-Picciotto et al., 2002 ⁷¹	Cross-Sec	Mat-nb pairs: 303-Teplice; 215-Prachatice, 1994–1996	PM ₁₀ , SO ₂ , NOx	Monitoring stations	Mat and cord bl at delivery	Lymphocyte subsets	<ul style="list-style-type: none">• $\uparrow$C₂Cl₄: $\downarrow$ IFN γ, OR=2.9 (1.0, 8.6)	Time of birth, mat alc intake, father's alc intake, employment status of father, father's edu, use of infertility treatments
							<ul style="list-style-type: none">• Teplice vs Prachatice (ref):<ul style="list-style-type: none">- Mat bl-CD3+: β= -2.4 (-4.3, -0.5), CD19+: β=-0.2 (-1.2, 1.7), NK: β=2.4 (-0.1, 5.0), CD4+: β= -2.6 (-4.4, -0.9), CD8+: β = 0.2(-1.3, 1.8), CD4+/CD8+: β= -0.1 (-0.3, -0.01)- Cord bl-CD3+: β=-2.3 (-5.3, 0.7), CD19+: β=-0.5 (-2.3, 1.3), NK: β=2.7 (-0.04, 5.4), CD4+: β= -0.8 (-3.0, 1.4), CD8+: β= -0.4(1.6, 0.8), CD4+/CD8+: β= -0.01 (-0.4, 0.4)	
Lee et al., 2011 ⁶⁴	Cohort-R	1696 pw, 1997–2001, Pennsylvania	PM ₁₀ , CO, PM _{2.5} , O ₃ , NO ₂ , SO ₂ ,	Kriging interpolation, monitoring stations	Mat nonfasting bl samples before 22 wks of gest	CRP: ELISA on the SpectraMax Me analyzer	<ul style="list-style-type: none">• Avg over 28 days before bl sample –<ul style="list-style-type: none">- $\uparrow$PM₁₀/9.2 g/m³: $\uparrow$CRP, OR 1.18 (0.91, 1.53)- $\uparrow$PM_{2.5}/5.0 g/m³: $\uparrow$CRP, OR 1.26 (0.97, 1.63)- $\uparrow$O₃/7.7 g/m³: $\uparrow$CRP, OR 1.05 (0.65, 1.71)• SO₂ and CRP, NO₂ and CRP, CO and CRP: NA	Gstl wk, season of sample collection, mat BMI at enrollment/bl draw, age, race, edu, cigarette exp in early prg, parity, household income, yr entering the study
Latzin et al., 2011 ⁷⁵	Cohort-P	265 neonates, 1999 – 2005, Switzerland	PM ₁₀ and indoor air poln	Monitoring stations, distance to major road, questionnaire	Cord bl serum after delivery	Cytokines, chemo: human Cytokine Multiplex Assay Kit	<ul style="list-style-type: none">• $\uparrow$PM₁₀/10 μg/m³(T₃):<ul style="list-style-type: none">- $\uparrow$IL-1b, OR 3.00 (1.30, 6.91)- PM₁₀ and MCP-1, PM₁₀ and IL-6, PM₁₀ and IL-10: NA	Gender, gstl wt and mat smo during prg, parental edu mat atopy and gstl age.
Baiz et al., 2011 ⁵⁵	Cohort-R	370 mat-nb pairs, Sept 2003–Dec 2005, France	PM ₁₀ and NO ₂	Monitoring stations; benzene, toluene, ethylbenzene and xylenes: diffusive air sampler	Venous cord bl at delivery	Lymphocyte subsets	<ul style="list-style-type: none">□ $\uparrow$PM₁₀/10 μg/m³:<ul style="list-style-type: none">- 3mo before and during prg: $\downarrow$CD4+CD25+T cells 0.82% (p = 0.01)- T₁: $\downarrow$CD4+CD25+T cells 0.71% (p = 0.04)- T₂: $\downarrow$CD4+CD25+T cells 0.88% (p = 0.02)- T₃: $\downarrow$CD4+CD25+T cells 0.59% (p = 0.04)□ $\uparrow$PM₁₀/10 μg/m³:<ul style="list-style-type: none">- T₁: $\downarrow$CD3+ cells 310% (p <0.05)□ NO₂-CD3+; NO₂-CD4+CD25+T cells; NO₂-CD4+/CD8+; NO₂-CD19+;NA	Mat age at delivery and BMI, mat history of allergy, active and passive smo, perinatal infections, mode of delivery, season of the birth, nb's sex, wt and gstl age at birth
van den Hooven et al., 2012 ⁶⁶	Cohort-P	5067 pw for mat and 4450 for fetal cord bl, 2001–2005, Netherlands	PM ₁₀ and NO ₂	Dutch national standard methods, monitoring stations	Mat venous bl: 4.5–17.9 wks gest; Venous cord bl: delivery	hs CRP: immunoturbidimetric assay on the Architect System	<ul style="list-style-type: none">□ Total prg period: $\uparrow$ fetal CRP (> 1 mg/L) at delivery:<ul style="list-style-type: none">- Q₄ PM₁₀: OR=2.18 (1.08, 4.38)	Gstl age at birth, season of birth, mat age, BMI, parity, ethnicity, edu, smo, alc consumption, and noise exp

Author, year	Study design	Population	Pollutant	Exposure assessment	Biospecimen, collection time	Biomarker, assessment	Major Results: Estimate (95%CI)	Controlled in the analyses
DNA methylation								
Herbstman et al., 2012 ⁸²	Cohort-R	164 African-American and Dominican pw, New York	PAH	PAH in air: personal monitors Dietary PAH exp: questionnaire	Cord bl at delivery; urine specimens of some women to assess PAH conc	DNA adducts: HPLC-F; DNA meth: Methylamp/TM, Global DNA Meth Quant Kit	<div>- Q₄ NO₂: OR=3.42 (1.36, 8.58)</div> <div><div><div>□ ↑ PAH exp: ↓ global meth in cord WBCs (p = 0.05)</div><div>□ ↑ global meth levels: ↑ detectable cord bl adducts (p = 0.01)</div><div>□ Total PAH level in air correlated with urinary pyrene (r=0.69) and BaP (r=0.96)</div></div><div>□ Overall prg ↑PM_{2.5}/5 µg/m³: ↓placental global DNA meth 2.19% (−3.65, −0.73)</div></div>	No covariates were found to be confounders
B. G. Janssen et al., 2013 ⁸³	Cohort-P	240 mat-nb pairs, 02/05/2010–01/21/2012, Belgium	PM _{2.5}	Kriging interpolation	Placentas at birth	DNA meth levels		Nb's gender, mat and gssl age, parity, mat edu, smo, prenatal acetaminophen use, season at conception and trimester-sp apparent temp
B. G. Janssen et al., 2015 ³⁴	Cohort-R	381 mat-nb pairs, 2010–2013, Belgium	PM _{2.5}	Kriging interpolation	Placentas at birth	Placental mtDNA meth	<div>□ ↑IQR PM_{2.5}, entire prg:</div> <div>- ↑mtDNA meth: MT-RNR1: 0.91% (p= 0.01), D-loop: 0.21% (p= 0.05)</div> <div>- ↓ mtDNA content (relative change 15.60%, p=0.001)</div>	Gender, mat and gssl age, smo, mat edu, parity, ethnicity, and season at conception
Other biomarkers								
Pedersen et al., 2009 ⁹⁷	Cross-Sec	75 pw, 12/13/2006–12/20/2007, Denmark	Traffic poln, indoor NO ₂	Traffic: database, NO ₂ ; passive diffusive sampler cartridges	Mat peripheral bl and paired cord bl at C section	Micronuclei	<div>• High-traffic-density homes (>2500 vehicle km/24 h) – ↑ micronuclei freq (p=0.02) among newborns</div>	ETS exp, use of open fireplace, pre prg wt, folate, vit B12 levels, mat edu, season of delivery, dietary & genetic factors modulating adducts and micronuclei
Baiz et al., 2012 ⁸⁹	Cohort-P	375 mat-nb pairs, Feb 2003 – May 2005, Sept 2003 – Dec 2005, France	PM ₁₀ and NO ₂	Atmospheric Dispersion Modelling	Cord bl serum at delivery	25(OH)D cord bl serum level: ICLI	<div>□ ↑NO₂/10 g/m³: ↓ log 25(OH)D by 0.15 U (p = 0.05)</div> <div>• ↑PM₁₀/10 g/m³: ↓ log 25(OH)D by 0.41 U (p = 0.04)</div>	Mat age and BMI, mat history of allergy, active and passive smo, number of siblings, household income, city, nb's sex and wt, PTB, and season of birth
Yorifuji et al., 2012 ⁹¹	Cohort-R	14,189 pw+nb singletons (22 wks), Jan 1997 – Dec 2008, Japan.	Traffic exp	Major roads: >50,000 vehicles, Road Traffic Census	Placentas at birth	Placenta/birth weight ratio (PBWR)	<div>• Living within 200m of road:</div> <div>- ↑PBWR 0.48% (0.15, 0. 80)</div> <div>- ↓ BW 81.9 g (−120.4, −43.3)</div> <div>- ↓ placenta wt. 12.9 g (−21.4, −4.4)</div>	Mat age, mat BMI, mat occupation, mat alc consumption, paternal smo, area level SES, and mat smo.
E. H. van den Hooven et al., 2012 ⁹⁵	Cohort-P	7,801 pw, 2001–2005, Netherlands	PM ₁₀ and NO ₂	Dutch national standard methods for air quality modeling	T ₁ and T ₂ mat bl; cord bl samples at delivery	PGF and sFlt-1	<div>• ↑PM₁₀/10 µg/m³ – ↑ sFlt 35.8% (25.6, 45.9)</div> <div>• ↑NO₂/10 µg/m³ – ↑ sFlt 8.9% (0.6, 17.3)</div> <div>• 2mo prior delivery:</div> <div>- ↑PM₁₀/10 µg/m³ – ↓placenta wt 11.8 g (−20.9, −2.7)</div>	Mat age, BMI, parity, ethnicity, edu, smo, alc, infant's sex, folic acid suppln, gssl age at measurement, noise exp, season of conception, mat height

Author, year	Study design	Population	Pollutant	Exposure assessment	Biospecimen, collection time	Biomarker, assessment	Major Results: Estimate (95%CI)	Controlled in the analyses
O'Callaghan-Gordo et al., 2015 ⁹⁸	Cohort+P	181 mothers, 183 nb, Feb 2007–Feb 2008, Greece	PM _{2.5} , PM ₁₀ , NO ₂ , NO _x	Land use regression	Cord bl at delivery	Micronuclei	<ul style="list-style-type: none">- NO₂/10 µg/m³† - ↓placenta wt 10.7 g (-19.0, -2.4).• Mat bl micronuclei:<ul style="list-style-type: none">- PM_{2.5} - RR=1.53 (1.02, 2.29)- PM₁₀, NO₂, NO_x: NA• Cord bl micronuclei:<ul style="list-style-type: none">- PM_{2.5}, PM₁₀, NO₂, NO_x: NA	Mat age, mat educ, mat residence, mat origin, season of delivery, gestl age

Abbreviations: 25 (OH)D=25-hydroxyvitamin D, 8-OHdG=8-hydroxy-2'-deoxyguanosine, 8-oxodG=8-oxo-7,8-dihydro-2'-deoxyguanosine, 15-F2t-IsoP=15-F2t-isoprostane, 3-NTp=3-nitrotyrosine, adj=adjusted, alc=alcohol, asso=association, ATP=adenosine triphosphate, avg=averaged, BaP=benzo(a)pyrene, bl=blood, BMI=body mass index, BC=black carbon, BW=birth weight, C2Cl4= tetrachloroethylene, chemo=chemokines, CI=confidence interval, CO=carbon monoxide, cohort-P=prospective cohort, cohort-R=retrospective cohort, conc=concentration, cross-sec=cross sectional, CRP=C reactive protein, Dec=December, diff= difference, ECOD=7-ethoxycoumarin O-deethylase, edu=education, ELISA=Enzyme Linked Immuno sorbent Assay, ETS=environmental tobacco smoke, exp=exposure, Feb=February, gest=gestation, GSH=glutathione, GST=glutathione-S-transferase, gstl=gestational, HPLC-F=high performance liquid chromatography-fluorescence, hs=high sensitivity, ICLL=immunohemiluminescent immunoassay, IFN-γ=interferon γ, IL=interleukin, IQ=interquartile, IQR=interquartile range, Jan=January, LUR=land use regression, mat=maternal, MDA=malondialdehyde, meth=methylation, mo=month, mRNA=mitochondrial RNA, mtDNA=mitochondrial DNA, MT-RNR I=mitochondrial RNRI sequence, NA=no association, nb=newborn, NK=natural killer, NO2=nitrogen dioxide, nrf2=nuclear factor erythroid 2-related factor, O3=ozone, OGG1=oxoguanine glycosylase, OR=odds ratio, PAH=polycyclic aromatic hydrocarbons, PBMC=peripheral blood mononuclear cells, PBWR=placenta/birth weight ratio, PEMS=Personal Environmental Monitoring Sampler, PGF=placental growth factor, PM2.5=particulate matter 2.5µm diameter, PM10=particulate matter 10 µm diameter, poln=pollution, pig=pregnancy, PTB=preterm birth, pw=pregnant women, Q4=4th quartile, qPCR=quantitative polymerase chain reaction, quant=quantification, ref=reference, Sept=September, SES=socioeconomic status, sFlt-1=soluble fms-like tyrosine kinase 1, signi=significant, smo=smoking, SO2=Sulphur dioxide, SOD2=superoxide dismutase 2, sp=specific, suppln=supplementation, T1=1st trimester, T2=2nd trimester, T3=3rd trimester, TBARS=thiobarbituric reactive substances, temp=temperature, TEOM= tapered element oscillating microbalance, TNF-α=Tumor Necrosis Factor α, UCP2=uncoupling protein 2, vit=vitamin, VOC=volatile organic compounds, WBCs=white blood cells, wks=weeks, wt=weight, yr=year

Table 3

Association between air pollution exposure and exposure biomarkers

Author, year	Study design	Population	Pollutant	Exposure assessment	Biospecimen, collection time	Biomarker	Major Results: Estimate (95%CI)	Controlled in the analyses
PAH metabolites and DNA adducts								
Nethery et al., 2012 ¹⁰²	Cohort – P	9 pregnant women from high pollution and 10 pregnant women from low pollution area, Canada	PAH, PM _{2.5} , NO ₂ and NO _x	Personal Monitors, PM _{2.5} , PEMS, TEOMs, NOx: Ogawa samplers	urine samples once in each trimester	Urinary metabolites of PAHs	<ul style="list-style-type: none"> Correlation of urine metabolites with parent PAHs: <ul style="list-style-type: none"> phenanthrene ($r_{\text{pearson}} = -0.31 - 0.45$) fluorene ($r = 0.37 - 0.58$) High vs. low pollution area (ref): <ul style="list-style-type: none"> Mean metabolite levels high for 9-hydroxyfluorene, 3-hydroxyphenanthrene, 1-hydroxypyrene (each $p < 0.05$) 	Not applicable
Perera et al., 2005 ¹⁰⁸	Cohort – R	pregnant women: WTC area (268), Manhattan (468), Poland (191), China (136)	PAH	Monitoring stations	mat and cord blood at delivery	BaP DNA adducts	<ul style="list-style-type: none"> Mean adduct conc in mat and cord blood: <ul style="list-style-type: none"> Northern Manhattan < WTC area < Krakow, Poland < Tongliang, China consistent with trend in exposure ($p < 0.001$) 	Not applicable
Tang et al., 2006 ¹⁰⁹	Cohort – P	150 newborns, 03/04/2002–06/19/2002, China	PAH	month of pregnancy overlapping with the period of coal fired plant	mat blood within 1 day postpartum, cord blood at delivery	PAH-DNA adducts	<ul style="list-style-type: none"> ↑PAH-DNA adduct: <ul style="list-style-type: none"> ↓birth HC ($p = 0.06$) ↓wt at 18, 24 and 30 months of age ($p < 0.05$) Longer exposure: <ul style="list-style-type: none"> ↓BL ($p = 0.033$) ↓ht at 18, 24 and 30 months ($p < 0.001$) Dist to PAH source and PAH DNA adducts: NA 	ETS, sex, mat ht and wt, gestational age added for birth outcome analysis, and mat HC and cesarean status added for all analyses involving HC
Pedersen et al., 2009 ⁹⁷	Cross-sectional	75 pregnant women, 12/13/2006–12/20/2007, Denmark	Traffic pollution, indoor NO ₂	Traffic: database, NO ₂ : passive diffusive sampler cartridges	mat peripheral blood and paired cord blood at c-section	Bulky DNA adducts	<ul style="list-style-type: none"> Residence near medium-traffic-density areas (>400–2500 vehicle km/24h) – high adducts ($p < 0.01$) 	ETS exposure, use of open fireplace, pre pregnancy wt, folate levels, vitamin B12 levels, mat education, season of delivery,

Author, year	Study design	Population	Pollutant	Exposure assessment	Biospecimen, collection time	Biomarker	Major Results: Estimate (95%CI)	Controlled in the analyses
Wu et al., 2010 ¹¹⁴	Case-control	81 cases: mscg <14 weeks; 81 controls: requesting abortion, 04/2007–11/2007, China	PAH	Mat interviews	aborted tissue and mat blood one hour post abortion	BaP DNA adducts: HPLC-F method	<ul style="list-style-type: none"> Women with BaP-DNA adducts >median – ↑ risk of abortion, OR: 4.27 (95% CI: 1.41, 12.99) Aborted tissue adduct levels: NA with mscg Correlation between mat blood and aborted tissue BaP-DNA adducts ($r = -0.12$) 	dietary & genetic factors modulating adducts and MN Mat educational attainment and household income (and gestational age where applicable)
Jedrychowski et al., 2013 ¹¹²	Cohort – R	362 pregnant women, 01/2001–02/2004, Poland	PAH, BaP	PEMS	mat blood within 1 day postpartum, cord blood at delivery	PAH-DNA adducts	<ul style="list-style-type: none"> BaP teratiles and cord blood PAH-DNA adducts – nonpara trend $z=3.50$, $p<0.001$ ↑BaP: ↑fetal/mat blood adduct ratio ($p<0.05$) ↑BaP: ↑susceptibility to fetal DNA damage, OR: 1.27 (95% CI: 1.08, 2.03) 	Mat DNA adducts, season of birth
Tang et al., 2014 ¹¹³	Cohort – P	150 newborns, 03/2002–06/2002, China; 158 newborns, 03/2005–05/2005, same hospitals	PAH	Mini-Vol samplers	mat blood - 1 day postpartum, cord blood at delivery	PAH-DNA adducts: HPLC-F	<ul style="list-style-type: none"> 2005 vs 2002 (ref) cohort: <ul style="list-style-type: none"> ↓ cord bl PAH-DNA adducts in 2005 cohort ($p<0.001$) ↑ newborn HC of 2005 cohort ($p=0.001$) ↓ ambient PAHs in 2005 cohort ($p=0.01$) 	Models with BW and BL adjusted for ETS, gender, mat ht & wt before pregnancy and gestational age. Models with birth HC also adjusted for mat HC and caesarian status
Other biomarkers								
Mohorović, 2003 ¹¹⁸	Cohort – P	260 pregnant women, Croatia	SO ₂	Not mentioned	Blood & urine – 3× each in clean & dirty periods, 1 month between each test	mHb	<ul style="list-style-type: none"> Correlation between mHb levels and SO₂ conc: <ul style="list-style-type: none"> dirty period: $r = 0.72$ ($p < 0.01$) clean period: $r = -0.60$ ($p = 0.05$) Dirty vs clean period (ref): <ul style="list-style-type: none"> ↑ number of still births ($p<0.05$) 	Not applicable

Abbreviations: BaP=Benzo(a)pyrene, BL=birth length, BW=birth weight, CI=confidence interval, cohort-P=prospective cohort, cohort-R=retrospective cohort, conc=concentration, CPF=chlorpyrifos, dist=distance, ETS=environmental tobacco smoke, ht=height, HC=head circumference, HPLC-F=high performance liquid chromatography fluorescence, mat=maternal, mHb=methemoglobin, MN=micronuclei, mscg=miscarriage, NA=no association, NO₂=nitrogen dioxide, NO_x=nitrogen oxides, nonpara=non parametric, OR=odds ratio, PAH=polycyclic aromatic hydrocarbons, PEMS=Personal